CONFERENCE PROCEEDING

Faculty of Agricultural Technology
in Collaboration with
Indonesian Association for Microbiology

2nd ICGAB 2018
International Conference on Green Agro-Industry and Bioeconomy

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Indonesian Biotechnology Consortium

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PERTETA

ICGAB 2018

PROCEEDING

THE 2nd INTERNATIONAL CONFERENCE ON GREEN AGRO-INDUSTRY AND BIOECONOMY

"Sustainable Development and Strengthening Tropical Resources for National Welfare"

18 – 20 September 2018

Widyaloka Convention Hall – Universitas Brawijaya, Malang

FACULTY OF AGRICULTURAL TECHNOLOGY
UNIVERSITAS BRAWIJAYA

PROCEEDING

THE INTERNATIONAL CONFERENCE ON GREEN AGRO-INDUSTRY AND BIOECONOMY
Faculty of Agricultural Technology
Universitas Brawijaya, Malang, Indonesia

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WELCOMING SPEECH FROM CAIRMAN AND DEAN FACULTY OF AGRICULTURAL TECHNOLOGY, UNIVERSITAS BRAWIJAYA

Assalamu'alaikum wr.wb.

Dear distinguished guests and ICGAB participants,

It is a great honor for me to cordially welcome you all in Malang, and to our campus Universitas Brawijaya. And as an organizing committee of this conference, Faculty of Agricultural Technology gratefully thanks the Rector of Universitas Brawijaya for his continuous support.

Last year, International Conference on Green Agro-Industry and Bioeconomy (ICGAB) was successfully held and attended by a total of 310 participants from 8 countries. This year event has brought together nearly 400 delegates (from 7 countries) coming from national and international universities, research intitutions, and industries. This is our second ICGAB as we are aiming to organise the event on a regular basis. This is because ICGAB is very relevant with the vision, mission and strategic planning of our faculty. The Faculty aims are becoming a centre of excellence in the field of Agricultural Technology both nationally and internationally and giving a significant contribution towards sustainable development for strengthening the national welfare in Indonesia.

As we know that the water-food-energy nexus is critical and central to sustainable development. A rising of global population, urban expansion, changing diets and consumption behaviours causes an increase for all three nexus. The complex linkages between these critical nexus need integrated aprocahes to sustain water and food security, and to ensure a sustainable agriculture and energy production. Bioeconomy may bring us new hope for fulfil those needs through various range of approaches that can be implemented in agriculture and forestry, food, renewable energy, chemical, and pharmaceutical, as well as in creating innovative materials.

Furthermore, as part of the local, national and global communities, our faculty have continuously been making significant contribution in finding solutions towards national problems through research, developing technology, machinery, and conducting community service to educate people outside university. We take very seriously national problems such food security and food safety, developing renewable energy resources, waste management, and environmental degradation. Our faculty has also contributed in participating and winning the international research and scientific competition aiming to tackling the global problems. In addition, all aspects of agricultural technology integrated within our six (6) undergraduate study program offered in our faculty are also represented in the ICGAB conference.

Therefore, it is an honour for our faculty to host ICGAB conference to disseminate knowledge, research results and technology advances, as well as to exchange ideas and share success stories among all of you. It is our hope that this conference will be inspiring and deliver fresh inspiration and motivation to all participants. Thus, we can contribute to foster our national welfare by developing and implementing green-agroindustry and bioeconomy based on local and tropical commodities, while sustaining the environmental sustainability.

Last but not least, we would like to sincerely thanks all of our speakers for contributing to the conference program. Furthermore, we would like to express our most sincere appreciation to all contributing organisations include PERMI, FKPT-TPI, KBI, SEARCA SEAMEO, PATPI, PERTETA and APTA. We would also like to express sincere gratitude to the conference organising committees who have been working hard and with full dedication to make this conference possible.

We wish you all to have a fruitful conference that can integrate holistic aproaches in tackling our national problems and can strengthen our collaboration nationally and internationally. Thus, we can contribute in creating a safe, healthy and eco-friendly world for our future generation.

Wassalamu'alaikum wr.wb.

Dr. Sudarminto Setyo Yuwono

Chair of ICGAB2018
Dean of Faculty of Agricultural Technology

WELCOMING SPEECH FROM RECTOR UNIVERSITAS BRAWIJAYA

Assalamu'alaikum wr.wb.

Excellency's, Distinguished Delegates, Ladies and Gentlemen,

On behalf of the University members, it is a great honour for me, to extend to you all, a very warm welcome to Universitas Brawijaya, to Malang – East Java, and to Indonesia.

I would also to take this opportunity to express my sincere gratitude to The Conference Committee and Faculty of Agricultural Technology for organizing The Second International Conference on Green Agro-Industry and Bio-economy.

This conference an important conference to address Sustainable Development and Strengthening Tropical Resources for National Welfare through implementation of circular bioeconomy and green agro-industry.

Both in global world and in Indonesia, sustainable development is critical to tackle problems of poverty, climate change, and environmental degradation. Therefore, as a major global key producer of various agricultural tropical products, Indonesian government commits to deal those global concerns and to increase development partnerships among relevant stakeholders to ensure sustainable development goals can be sucessfully achieved. Despite many intensive activities and collaboration have been implemented by the government with concerned bodies; however, a lots remains to be done.

Universitas Brawijaya, as one of the state universities in Indonesia is also committed to contribute in finding solutions for major problems faced by the nation and the world today.

Indonesia, as part of the global communities are in transitioning toward a more industrialized country. Thus, many natural resources exploitation, high demand for fossil fuel, green-house gas emission and deforestation are happening in the country, which damaging environment and impeding the sustainable development. Therefore, the creation of bioeconomy through adopting green agro-industries and industrial biotechnology may stimulating technological innovation, industrial competitiveness and sustainable development in Indonesia, and at the same time perserving the natural resources. Also, not to forget, for integrating the concept of green agro-industries 4.0. and society 5.0 to bring new values to industry and society.

Universitas Brawijaya plays an important role in supporting the sustainable development through various research findings and community service programs, which integrated within our roadmap of food security; energy security; good governance; afroforestry; and health, nutrition and medicine. We have also supporting the development of green agroindustries by providing assistance, training, and technical supports. However, we realized that our efforts for the better world will make a bigger impact with more collaboration involving various concerned stakeholders.

Therefore, it is an honour for Universitas Brawijaya to host the second ICGAB conference to disseminate knowledg, research results, and technology, exchange ideas and share success stories among us and stakeholders from around the globe.

Finally, I wish you all enjoying the conference, having a fruitful experience and networking from the conference, as well as having a pleasant stay in Malang.

Wassalamu'alaikum wr.wb.

Prof. Nuhfil Hanani

Rector of Universitas Brawijaya

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CONFERENCE COMMITTEE

Scientific Committee

Prof. Masahiro Ikeda (Pharmacology - University of Miyazaki - Japan)
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Prof. Teti Estiasih (Agricultural Product Technology - Faculty of Agricultural Technology, Universitas Brawijaya)
Prof. Sutiman (Biology - Faculty of Mathematics and Natural Sciences)

CONFERENCE PROGRAMME
PLENARY SESSION

DAY 1: 18 SEPTEMBER 2018, VENUE: WIDYALOKA CONVENTION HALL

TIME	PROGRAMME
07:30-08:20	Registration
08:20-08:30	**Opening:** Traditional Dance
08:30-08:33	**Welcome Ceremony** - Indonesian National Anthem
08:33-08.55	**Opening Speech:** • Chairman of the conference/ Dean of Faculty of Agricultural Technology Universitas Brawijaya/ FKPTTPI • SEARCA Representative • PERMI Representative • Rector of Universitas Brawijaya **Certificate award ceremony for Keynote Speakers**
08.55-09.00	Invocation
	PLENARY SESSION **Chair/Moderator: Prof. Dr. Ir. Harijono, M.App.Sc**
09:00-09:20	**Session 1** **Dr. Andi Amran Sulaiman (Minister of Agriculture of Indonesia)**
09:20-09:40	**Session 2** **Prof. Patricia Rayas Duarte (Oklahoma State University, USA)** Topic: Key Aspects of Food Innovation: Global and Local Cases
09:40-10.10	*Panel Discussion* (30')
10.10-10:30	Coffee break
	PLENARY SESSION **Chair/Moderator: Dr. Ir. *Joni Kusnadi*, M.Si**
10:30-10:50	**Session 3** **Prof Kazuhito Fujiyama (Osaka University, Japan)** Topic: Production of human β-glucocerebrosidase by root culture of *Nicotiana benthamiana*

10:50-11.10	**Session 4** **Prof Simon Bambang Widjanarko (Universitas Brawijaya)** **Topic:** Sustainable development of Konjac agroindustry for national welfare purposes
11.10-11.40	*Panel Discussion* (30')
11:40-13:00	Poster Presentation Lunch Break
13.00-17.30	**PARALLEL SESSION**
18.30-20.00	**GALA DINNER**

DAY 2 : 19 SEPTEMBER 2018, VENUE: WIDYALOKA CONVENTION HALL

TIME	PROGRAMME
07:30-08:30	Registration
08.30-08.50	**Session 1** **Prof Masahi Kato (Meijo University, Japan)** Topic: Study on The Biomass Degradation By Enzymes From Filamentous Fungi: Implications For The Sustainable Development Goals
08.50-09.10	**Session 2** **Swasmi Purwajanti, Ph.D (The Indonesian Agency for Assessment and Application of Technology)** Topic: Rational Design of Nanoparticles-Based Drug Delivery System For Prolonged Antibacterial Performance Of Vancomycin: Structure Matters
09.10-09.30	**Session 3** **Dr. Tamarath Pranamornkith (Mae Fah Luang University Thailand)** Topic: Appropriate postharvest management reduces losses of agricultural produce: a case study on pineapple CV. Phulae, a geographical indication of Chiang Rai, Thailand
09.30-09.50	*Panel Discussion (20')*
09.50-10.00	Coffee Break
10.00-12.00	**PARALLEL SESSION**
13.00-15.00	**Closing Ceremony** • Awards Announcement • Closing Speech • Lunch

PARALLEL SESSION DAY I

DAY I: TUESDAY, 18 SEPTEMBER 2018
WIDYALOKA CONVENTION HALL AND FACULTY OF AGRICULTURAL TECHNOLOGY – UNIVERSITAS BRAWIJAYA, MALANG

MODERATOR: Dr. Widya Dwi Rukmi Putri, STP., MP

TOPIC: AGRICULTURAL PRODUCT TECHNOLOGY

VENUE: WMH

No.	Time	Code	Presenter	Title
1	13.00-13.07	APT-01	Yusuf Hendrawan	Development of colour co-occurrence matrix (CCM) texture analysis for biosensing
2	13.07-13.14	APT-02	Siti Asmaniyah Mardiyani	The effect of spent mushroom compost and various composting starter combination on the growth and yield of kangkong (*Ipomoea reptans*)
3	13.14-13.21	APT-03	Pavalee Chompoorat	Using okara flour as a source of nutraceutical and functional food for gluten free hamburger
4	13.21-13.28	APT-04	Rosalina Ariesta Laeliocattleya	The effect of sodium bisulfite immersion to the potential of candi banana peel ethanol extract as radical scavenger and UV protection
	13.28-13.36	**Discussion Panel**		
5	13.36-13.43	APT-05	Erni Sofia Murtini	Characterization of carbonized rice straw as natural black colorant: comparison of various rice straw sections and rice varieties for production materials
6	13.43-13.50	APT-06	Diana Puspitasari	Characteristics of non-gluten biscuits from cowpea-purse composite flour
7	13.50-13.57	APT-07	Nur Lailatul Rahmah	Glucosamine production from palmyrah (*Borassus flabellifer* L.) seeds (a study of precursor type and concentration)
8	13.57-14.04	APT-08	Andi Rahmayanti Ramli	Formulation of premix for making the Indonesian empek-empek
	14.04-14.12	**Discussion Panel**		
9	14.12-14.19	APT-09	Dwierra Evvyernie	In vitro fermentability and digestibility of seedless noni waste (*Morinda citrifolia* L.) as a concentrate substitute in lactating dairy goat diet

10	14.19-14.26	APT-10	Andi Rahmayanti Ramli	Supplementation of snake-head fish bone powder for making cookies
11	14.26-14.33	APT-11	Dego Yusa Ali	optimization of sodium bisulfite application for producing sweet sorghum (*Sorghum bicolor* L. moench) concentrate by using falling film evaporator
12	14.33-14.40	APT-12	Tanto Pratondo Utomo	Feasibility study of small scale ginger essential oil agroindustry using indirect steam distillation
	14.40-14.46	**Discussion Panel**		
13	14.46-14.55	APT - 13	Ata Aditya Wardana	Physical, structural, and thermal properties of resistant starch III from taro (*Colocasia esculenta* L. Schott) flour
14	14.55-15.02	APT - 14	Anik Nur Habyba	An affective SMEs product packaging design for go online: a case study of jenang packaging design in Indonesia
15	15.02-15.09	APT - 15	Dharia Renate	Packaging materials of red chili puree
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16	15.15-15.22	APT - 16	Sri Maryati	Postharvest handling of the mengkuang leaf as handicraft product of tudung layah in area Ranto Panyang West Meureubo District, West Aceh District
17	15.22-15.29	APT - 17	Rakhmawati	Textural analysis, sensoris and finansial products snack bar flour ganyong (*Canna edulis* Ker.) and flour hunkwee
18	15.29-15.36	APT - 18	Elisa Julianti	The effect of pre-treatment in the making of orange fleshed sweet potato flour on dried noodles quality
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19	15.42-15.49	APT - 19	Satria Bhirawa Anoraga	Effect of extraction time and temperature on the organoleptic quality of cocoa powder
20	15.49-15.56	APT - 20	Teti Estiasih	Characteristics of crude palm oil from some palm oil refineries and their corresponding palm fatty acid distillates
21	15.56-16.03	APT - 21	Musthofa Lutfi	Innovation technology of local tubers and beans processing to increase value added and micro enterprises diversification
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25	16.36-16.43	APT - 25	Dian Histifarina	In-store dryingapplication on shallot postharvest handling
26	16.43-16.50	APT - 44	Widya D.R. Putri	Anti-diabetic properties and hypoglycemic action of annealed breadfruit flour in diabetic rats
27	16.50-16.57	APT - 27	Muhammad Aswan Syahputra	SenseHub: an integrated web application for sensory analyses
28	16.57-17.04	APT - 62	Subeki	Study of making siger rice from cassava (*Manihot esculenta*) in various harvest age on physical, chemical and organoleptic siger rice
	17.04-17.12	**Discussion Panel**		

MODERATOR: Dr. Eng. Akhmad Adi Sulianto, STP, MT, M.Eng

TOPIC: AGRICULTURAL ENGINEERING & AGROFORESTRY AND BIODIVERSITY

VENUE: WCR-1

No.	Time	Code	Presenter	Title
1	13.00-13.07	AEE-01	Yusuf Wibisono	Microstructure changes of taro (*Colocasia esculenta* L. Schott) chips and grains during drying
2	13.07-13.14	AEE-02	Mahmod Sidati Ali Abobaker	Design and performance of bioreactor for fermentative biogas production from marine microalgae
3	13.14-13.21	AEE-03	Yusuf Hendrawan	Development of discrete honey bees mating optimization (DHBMO) for solving bio-computation optimization problem using single and multi-objectives optimization
4	13.21-13.28	AEE-04	Mahayu Woro Lestari	Effect of electric shock on the media and foliar spray of $CaCl_2$ to the nutritional and bioactive content of lettuce
	13.28-13.36	**Discussion Panel**		
5	13.36-13.43	AEE-05	Azimmatul Ihwah	Forecasting of export demand of black tea (Case study in PT XYZ)
6	13.43-13.50	AEE-06	Fenty Ika Wardani	Designing small-medium scale groundnut (*Arachis hypogea* L.) shelling machine for local merchant in Tuban, East Java
7	13.50-13.57	AEE-07	Retno Rosariastuti	Soil bioremediation of lead (Pb) polluted paddy field using mendong (*Fimbristylis globulosa*), *Rhizobium* sp. I3, compost and anorganic fertilizer
8	13.57-14.04	AEE-08	Sonia Verent Yudi Santo Putri	Effect of hypobaric storage on the quality of hot chili peppers
	14.04-14.12	**Discussion Panel**		
9	14.12-14.19	AEE-09	Ifmalinda	System design grading gunung omeh siam citrus (*Citrus nobilis* Var. Microcarpa) based image processing in real time
10	14.19-14.26	AEE-15	Amnat Chaweram	Comparison of energy consumption rates in HFC-32 refrigerating systems under different humidity ratio conditions with heat exchangers
11	14.26-14.33	AEE-14	Pruetsapha Thipruetri	The experimental study of the flammable gas detecting system for the biogas engine and filler in an animal farm.
12	14.33-14.40	AEE-18	C.E. Vallejera-Corsiga	Assessment of major soil series grown to sugarcane under different land utilization types in Negros Occidental, Philippines

	14.40-14.46		**Discussion Panel**	
13	14.46-14.55	AFB-01	Enih Rosamah	Comparison of chemical components of teak (*Tectona grandis* Linn. F.) wood after usage of 2 years and 60 years
14	14.55-15.02	AFB-02	Ari Susilowati	Isolation and characterization of fungal endophytes that antagonist toward *Fusarium wilt* in melon plant (*Cucumis melo* L.)
15	15.02-15.09	AFB-03	Ayda Krisnawati	Morpho-chemical evaluation of soybean genotypes across tropical agroecosystem
	15.09-15.15		**Discussion Panel**	
16	15.15-15.22	AFB-04	Shreef Mahmood	Effects of rootstocks on the growth, yield and quality of summer tomato
17	15.22-15.29	AFB-05	Arinafril	Measurement of dragonflies‘ diversities with special reference to water quality parameters: study case in Palembang and Indralaya, Indonesia
18	15.29-15.36	AFB-06	Sri Dayuti	Characteristics of liquid smoke mangrove api-api (*Avicennia marina*) coated with different concentration of maltodextrin
	15.36-15.42		**Discussion Panel**	
19	15.42-15.49	AFB-07	Kiattisak Sonsri	The properties of soil as impacted by sea level rise in the dry season: a case study of Nonthaburi Province, Thailand
20	15.49-15.56	AFB-08	Thanakorn WANGSAWANG	Developing blast disease resistance of jasmine rice by phenotypic-genotypic simultaneous selection
21	15.56-16.03	AFB-09	pongpichai kladwang	Plant diversity in agroforestry system and food security of community in Mae Tha Sub-District, Mae On District, Chiang Mai Province
	16.03-16.09		**Discussion Panel**	
22	16.09-16.16	AFB-10	Nur Shafira Nisa Binti Shaharum	The utilisation of cloud computing and remote sensing approach to assess environmental sustainability in Malaysia
23	16.16-16.23	AFB-11	Siti Aisyah Ruslan	Development of geospatial model for elucidation of *Metisa plana*’s landscape ecology in Malaysian oil palm microclimate and prediction of its prevale
24	16.23-16.30	AFB-12	Blair Moses Kamanga	Assessment of the resistance and performance of pigeon pea *(Cajanus cajas)* varieties to selected major insect pests at Bunda, Malawi
	16.30-16.36		**Discussion Panel**	
25	16.36-16.43	AFB-13	Sarjiya Antonius	Study of plant growth promoting rhizobacteria and soil biochemical properties of three open post coal mining revegetations in South Kalimantan

26	16.43-16.50	AFB-25	Nic Oswald M. Borines	Ex situ conservation of agro-biodiversity of major food legumes in the Philippines
27	16.50-16.57	AFB-26	Gina D. Balleras	Entomopathogenic fungi as potential biocontrol agents against brown planthopper (*Nilaparvata lugens* Stål.)
	16.57-17.03	**Discussion Panel**		

MODERATOR: Dr. Panji Deoranto, STP, MP

TOPIC: BIOECONOMIY AND BIOBUSINESS & AGROINDUSTRIAL PRODUCTION SYSTEM MANAGEMENT AND REGULATION

VENUE: WCR-2

No.	Time	Code	Presenter	Title
1	13.00-13.07	BBS-01	Angga Nugraha	Livestock farmers motivation towards the practice of profit sharing system of beef cattle business in Maiwa District, Enrekang Regency, South Sulawesi Province
2	13.07-13.14	BBS-02	Sparisoma Viridi	Simulation of bioeconomy system using agent-based model in the case of smart, green, and conventional farming
3	13.14-13.21	APS-01	Siti Asmaul Mustaniroh	Strategy of quality process improvement in tofu stick SMEs cluster using fuzzy analytical hierarchy process
4	13.21-13.28	APS-02	Neti Yuliana	Potential agroindustry of banana in Lampung Province
	13.28-13.36	**Discussion Panel**		
5	13.36-13.43	APS-03	Endah Rahayu Lestari	The mediating role of customer value on innovation and firm performance: evidence from Indonesian SMEs
6	13.43-13.50	APS-04	Rizky Luthfian Ramadhan Silalahi	Integration of K-Means clustering and fuzzy AHP for establishing development strategy on cassava chips SMEs
7	13.50-13.57	APS-05	Imam Santoso	Green marketing strategy on souvenir agroindustrial development in supporting creative agroindustry: case study in Malang Strudle
8	13.57-14.04	APS-06	Delfitriani Delfitriani	Construction of business intelligence in dadih product affective design
	14.04-14.12	**Discussion Panel**		
9	14.12-14.19	APS-07	Luh Putu Wrasiati	Optimization of material concentration and length of extraction on rendement and content of α-Tocopherol of sea lettuce (*Ulva lactuca* L.) using response surface methodology
10	14.19-14.26	APS-08	Yudhi Mada	Purchasing behavior and store atmosphere-the comparison of batik stores and distro in Candi-Madura
11	14.26-14.33	APS-09	Harun AL Rasyid	Strategy of marketing and the development of beras siger (analog rice from cassava) in Way Kandis Village Tanjung Senang Bandar Lampung
12	14.33-14.40	APS-10	Miftakhurrizal Kurniawan	Risk management of shallot supply chain using failure mode effect analysis

				and analytic network process (case study in Batu, East Java)
	14.40-14.46	**Discussion Panel**		
13	14.46-14.55	APS-11	Ardaneswari Dyah Pitaloka Citraresmi	Inventory control of raw material on sweet bread production
14	14.55-15.02	APS-12	Danang Triagus Setiyawan	Production process analysis using value stream mapping at East Java sugarcane industry
15	15.02-15.09	APS-13	Ahmad Syihab	Process and the calculation of material handling cost in the ice cream production in PT. A B C
	15.09-15.15	**Discussion Panel**		
16	15.15-15.22	APS-14	M Fuad Fauzul Mutamar	The influence of halal product assurance laws on product development in Indonesia
17	15.22-15.29	APS-15	Millatul Ulya	Product development of black piper retrofractum Tea (Black PrV tea)
18	15.29-15.36	APS-16	Khoirul Hidayat	Potential development of Madura corn products
	15.36-15.42	**Discussion Panel**		
19	15.42-15.49	APS-17	Riska Septifani	Risk mitigation strategy of rice seed supply chains using fuzzy failure mode effect analysis (FUZZY-FMEA) and fuzzy analytical hierarchy process (FUZZY-AHP)
20	15.49-15.56	APS-18	Dhita Morita Ikasari	Analysis of fast food restaurant competition based on consumer perception using multidimensional scalling (MDS) (case study in Malang City)
21	15.56-16.03	APS-19	Andan Linggar Rucitra	Implementation of material handling on production process of the red snapper fish IN PT X
	16.03-16.09	**Discussion Panel**		
22	16.09-16.16	APS-22	Panji Deoranto	Productivity and environmental performance: an empirical evidence from a furniture factory in Malang City, Indonesia
23	16.16-16.23	APS-30	Aulia Bayu Yushila	Analysis of the labor, capital and machine production factors on micro-scale apple juice production in Batu City
24	16.23-16.30	APS-33	Danang Kumara Hadi[1]	Traceability implementation based on RFID at agroindustry: a review
	16.30-16.36	**Discussion Panel**		
25	16.36-16.43	APS-34	Cici Febriyanti	Study of feasibility investment and determination of optimal cpo production quantity using goal programming method on palm oil factory PT. Sandabi

				Indah Lestari in Central of Bengkulu
26	16.43-16.50	APS-29	Aldila Mawanti Athirah	Entrepreneurial skills and farming performance of organic agriculture
27	16.50-16.57	APS-28	Dwi Retno Mulyanti	The production efficiency and competitiveness of sugar cane farming in Pati Regency
	16.57-17.03	**Discussion Panel**		

MODERATOR: Tunjung Mahatmanto STP., M.Si., Ph.D

TOPIC: FOOD MICROBIOLOGY, FOOD SAFETY AND SECURITY & HEALTH, NUTRITION AND MEDICAL

VENUE: WCR-3

No.	Time	Code	Presenter	Title
1	13.00-13.07	FMB-01	Maria Erna Kustyawati	Who produces vitamin B12 in tempeh
2	13.07-13.14	FMB-02	Alifia Issabella Mulyawati	Diversity of lactic acid bacteria isolated from fermented mare milk products based on PCR-RFLP analysis
3	13.14-13.21	FMB-08	Agus Wijaya	The future of probiotic *Enterococci*
4	13.21-13.28	FMB-04	Hartati Kartikaningsih	Fatty acid profile alteration of catfish (*Clarias* sp) infected by *Aeromonas hydrophilla* by acid, salt and storage temperature variation
	13.28-13.36	**Discussion Panel**		
5	13.36-13.43	FMB-05	Firman Jaya	Microbiological properties of preparing facial mask cream from goat milk kefir
6	13.43-13.50	FMB-06	Rahmah Utami Budiandari	Purification and characterization of cellulase A7 cloned from a rumen fungus *Orpinomyces* sp. Y102
7	13.50-13.57	FMB-07	Yudiarti Turrini	Antioxidant and antimicrobial activities of functional feed produced from rice waste which fermented with *Monascus purpureus* and *Chrysonillia crassa*
8	13.57-14.04	FMB-03	Novia Rahmawati	Characterization of crude cellulase produced by cellulolytic bacteria isolated from Spring Water in Mount Merapi
	14.04-14.12	**Discussion Panel**		
9	14.12-14.19	HNM-01	Tika Widayanti	Production of monoclonal antibody anti-NS1 induced by DENV3 Indonesian clinical isolate for the development of diagnostic test
10	14.19-14.26	HNM-02	Alfan Danny Arbianto	Molecular docking and molecular dynamic studies of novel lipopeptide as transfection agent potentially capable for nuclear export
11	14.26-14.33	HNM-03	Dewi Kunthy Saraswati	The antibacterial activity of methanol extract of *Pleurotus flabellatus* towards multidrug resistance *Staphylococcus aureus* as the cause of ulcus in HIV sufferer based on in vitro
12	14.33-14.40	HNM-04	Laila Yum Wahibah	The influence of food dimension from Indonesian traditional-processed rice (steamed-rice, lontong, and ketupat) to the perception of satiety and consumer

				satisfaction level
	14.40-14.46	**Discussion Panel**		
13	14.46-14.55	HNM-06	Hasria Alang	Identification of lactic acid bacteria as antimicrobial from milk of Belang Buffalo Toraja, Indonesia
14	14.55-15.02	HNM-13	Jittraporn Bunta	A temperature distribution study in a heated bed component mode from butyl rubber for dust mite allergy patients
15	15.02-15.09	HNM-17	Ria Hayatun Nur	Glucose calibration modeling in blood with spline regression approaching to non-invasive tools
	15.09-15.15	**Discussion Panel**		
16	15.15-15.22	FSS-01	Ley Vasa Nursyafaat	Microplastic in salt production areas of Northern Coast of East Java
17	15.22-15.29	FSS-02	Laras Putri Wigati	Food losses and waste estimation of horticulture product along the supply chain (case study on tomatoes and red chili peppers in Sukabumi, West Java, Indonesia)
18	15.29-15.36	FSS-03	Mohammad Agus Junaidi	The affinity groups (AGs): local institutional strengthening to promote food safety and security in rural development in Indonesia
	15.36-15.42	**Discussion Panel**		
19	15.42-15.49	FSS-22	Anacorita O. Abasolo	Life cycle analysis of monocrop and multicrop in conventional and organic vegetable production systems in Tayabas, Quezon, Philippines
20	15.49-15.56	FSS-11	Molla Gebreyohannes Hailu	Introducing integrated mobile fish production on and off farm
21	15.56-16.03	FSS-12	Dinda Dewi Aisyah	Landscape integrated pest management as a tool to determine the risk of production of rice farming in Pliken Village Banyumas Regency
	16.03-16.09	**Discussion Panel**		
22	16.09-16.16	FSS-13	I Made Yoga Prasada	The potential loss of rice production due to wetland conversion in East Java
23	16.16-16.23	FSS-14	Herlin Natalia Dewi	Landslide hazard assessment based on human activity in the upstream area
24	16.23-16.30	FSS-17	Joshua M Santos	Impacts of agrarian reform on the productivity and technical efficiency of rice farms in the Philippines
	16.30-16.36	**Discussion Panel**		
25	16.36-16.43	FSS-18	Ruby A. Abao	The risks of parasitic helminths and protozoan infection in Philippine native swine: its implication to environmental health and food safety

26	16.43-16.50	FSS-20	Rizky Trisna Putri	Agriculture extension technique as an accelerator of adoption innovation
27	16.50-16.57	FSS-21	M Chrisna Satriagasa	Physical and anthropogenic characteristics-based landslide spatial pattern analysis in agricultural catchment
	16.57-17.03	**Discussion Panel**		

MODERATOR: Dr. Ir. Aji Sutrisno, M.Sc

TOPIC: FISHERIES AND MARINE RESOURCES, ANIMAL WALFARE AND TECHNOLOGY & INDUSTRIAL BIOTECHNOLOGY AND BIOPROCESSING

VENUE: WCR-4

No.	Time	Code	Presenter	Title
1	13.00-13.07	FMR-01	Astri Iga Siska	Profile of amino acid on *Pangasius djambal* fish finger processing
2	13.07-13.14	FMR-02	Irene Perina	Study on culture of seabass (*Lates calcarifer*, Bloch 1790) in hapa-in-pond environment
3	13.14-13.21	FMR-03	Umi Purwandari	The effect of seaweed (*Eucheuma cottoni*) flour and sago (*Metroxylon sagu*) starch formulation on characteristics of edible spoon
4	13.21-13.28	FMR-04	Ardiansyah Kurniawan	Domestication of *Osteochilus spilurus*: survival and growth in recirculated water
	13.28-13.36	**Discussion Panel**		
5	13.36-13.43	AWT-01	Sulastri	Evaluation of good breeding practices based on structure of population, survailans of disease, and performance test at Saburai Goat Breeding area
6	13.43-13.50	AWT-02	Hanny Indrat Wahyuni	Ecologycal changes of gastrointestinal digesta in broiler chicken fed ration with added extract glucomanan of porang (*Ammorphopallus onchophyllus*) tuber
7	13.50-13.57	AWT-03	Mohamad Agus Setiadi	Potential of sex sorting spermatozoa using snakehead fish albumin extract in Sumba Ongole cattle
8	13.57-14.04	AWT-04	Hamdani Maulana	Effect of season on feeding behavior of Bali cattle that kept in oil palm plantation with semi-intensive system
	14.04-14.12	**Discussion Panel**		
9	14.12-14.19	IBB-01	Siska Dwi Lestari	Actif (Bacteriocin Biopreservatif): bioassay bacteriocin in *Pediococcus acidilactici* from breast milk isolate as a biopreservative alternative (natural preserve)
10	14.19-14.26	IBB-02	Fitri	Luwak coffee in vitro fermentation: literature review
11	14.26-14.33	IBB-03	Evi Susanti	Potential of amobilized *Zymomonas mobilis* in silika from the rice husk ash
12	14.33-14.40	IBB-04	Haniyya Haniyya	*Bacillus halodurans* CM1 antibiotic resistance ability features

	14.40-14.46	**Discussion Panel**		
13	14.46-14.55	IBB-05	Dian Fajar Vitia Ningrum	Enzymatic analyses content of *Bacillus halodurans* CM1 supernatant cultures
14	14.55-15.02	IBB-06	Lina Mulyawati	Chromosomal integration of *Bacillus halodurans* CM1 xylanase (BhXyn) gene into *Bacillus subtilis* DB 104
15	15.02-15.09	IBB-07	Raida Amelia Ifadah	Change in chemical characteristics of kombucha from various cultivars snake fruit during fermentation
	15.09-15.15	**Discussion Panel**		
16	15.15-15.22	IBB-08	Estri Laras Arumingtyas	Is ethyl methane sulfonate induced mutation influence the KasI gene sequence and its expression?
17	15.22-15.29	IBB-09	Ahmad Wibisana	The effect of fermentation condition for cephalosporin acylase expression by *E. coli* mutant
18	15.29-15.36	IBB-10	Siswa Setyahadi	Screening microbe producing chitinase for inhibiting *Ganoderma boninense*
	15.36-15.42	**Discussion Panel**		
19	15.42-15.49	IBB-11	Abdul Aziz Jaziri	Transcriptional regulator of TK1285 in hyperthermophilic archaeon *Thermococcus kodakarensis* KOD1
20	15.49-15.56	IBB-12	Aji Sutrisno	Partial purification and characterization of a thermostable collagenolytic protease of a bacteria isolated from hot springs of Cangar Batu, East Java.
21	15.56-16.03	IBB-13	Asep Awaludin Prihanto	Endophytic fungi, *Aspergillus* sp. is a potential producer for L-Asparaginase from mangrove (*Avicennia germinans*).
	16.03-16.09	**Discussion Panel**		
22	16.09-16.16	IBB-14	Agustin Krisna Wardani	Isolation of lytic bacteriophage and its application for controlling bacterial pathogens
23	16.16-16.23	IBB-15	Asma Ilyani binti Kadar	Yield, physico-chemical and nutritional characteristics of MR219 mutants rice and their effects on glycemic index and responses in BALB/c mice
24	16.23-16.30	IBB-16	Siti Zulaikha Abd Ghafar	Antioxidant, α-glucosidase and nitric oxide inhibitory activities of *Phyllanthus acidus*.
	16.30-16.36	**Discussion Panel**		
25	16.36-16.43	IBB-24	R. Mendoza	Characterization of gold-nanoparticles (AuNP) produced from the bio-mediated synthesis by wildtype *Serratia marcescens* Bizio
26	16.43-16.50	IBB-18	Rinaldi Idroes	Identification and screening of plant-based antimicrobial potential in Meurah

				Village Ie Jue manifestation, Aceh Besar District
27	16.50-16.57	IBB-19	Edi Wahyu sri Mulyono	Recovery technique of xanthan gum from an aqueous fermented broth by addition of water-miscible-solvent
	16.57-17.03	**Discussion Panel**		

MODERATOR: Dr.Eng. Evi Kurniati STP, MT

TOPIC: RENEWABLE ENERGY AND BIOREFINERY & WASTE AND ENVIRONMENTAL

VENUE: SCR-1

No.	Time	Code	Presenter	Title
1	13.00-13.07	REB-01	Isran Mohamad Pakaya	Developing of *Calliandra calothyrsus* into biomass pellet as renewable energy resources in Indonesia
2	13.07-13.14	REB-02	Rian Christian Sondakh	Upgrading bio-oil using esterification approach
3	13.14-13.21	REB-03	Tanto Pratondo Utomo	Determination of cow dung biogas agroindustry based on bioreactor type case study: AAJJ agroindustry
4	13.21-13.28	REB-04	Ida Bagus Wayan Gunam	Isolation and characterization of cellulolytic bacteria from forest land and waste disposal places in Bali
	13.28-13.36	**Discussion Panel**		
5	13.36-13.43	REB-05	Trianik Widyaningrum	Screening and identification indigenous yeast from neera siwalan for bioethanol production
6	13.43-13.50	REB-06	Ribut Sugiharto	Application of response surface methodology to evaluate biodiesel production from spent bleaching earth by in situ transesterification process
7	13.50-13.57	REB-07	Sri Suhartini	Estimation of methane and electricity potential from canteen food waste
8	13.57-14.04	REB-08	Almira Nuringtyas Jayanti	Bioethanol production from sugarcane molasses by instant dry yeast (effect of pretreatment and fermentation temperature)
	14.04-14.12	**Discussion Panel**		
9	14.12-14.19	REB-09	Radite Raharja	Bioethanol production from sugarcane molasses by instant dry yeast
10	14.19-14.26	REB-10	Yanni Sudiyani	Characterization of glutathione from *Saccharomyces cerevisiae* as by product of second generation bioethanol from oil palm of empty fruit bunch fiber
11	14.26-14.33	REB-11	Siti Khotimah	Isolation of cellulose degrading fungi at various peat maturity in Teluk Bakung Peat Area, Ambawang District, Kubu Raya Regency
12	14.33-14.40	REB-12	Mohammad Azri Bin Azmi	Enhancing the utilization of agricultural by-product as livestock feed using biological pretreatment method
	14.40-14.46	**Discussion Panel**		

13	14.46-14.55	WEM-01	Ruth Octavia Enggaringtyas	Treatment of ammonia wastewater using membrane bioreactor: effect of activated sludge ratio and backwash
14	14.55-15.02	WEM-02	Tri Rahayuningsih	Exploration source of natural dyes for batik from fresh and fallen leaves
15	15.02-15.09	WEM-03	Evellin Dewi Lusiana	The application of Bayesian quantile regression to analyze the relationship between nutrients content and phytoplankton abundance in Sutami Reservoir
	15.09-15.15	**Discussion Panel**		
16	15.15-15.22	WEM-04	Prima Okta Adi Nugraha	Natural ingredient for pH and coagulation control in water treatment processes using palm oil mill boiler ash
17	15.22-15.29	WEM-05	Evi Susanti	Production of lignin peroxidase, manganese peroxidase and lakase by the indigenous rot fungus of KLUM1, KLUM2 and PnUM
18	15.29-15.36	WEM-06	Christia Meidiana	Design of experiments for waste constituents of municipal solid waste treatment.
	15.36-15.42	**Discussion Panel**		
19	15.42-15.49	WEM-07	Iwan Fajar Pahlawan	Hydrolysis of leather shavings waste for protein binder
20	15.49-15.56	WEM-08	Aditya Wahyu Nugraha	The environmental impact risk of cow-hide tanning small-medium industries
21	15.56-16.03	WEM-09	I Gede Herry Purnama	Study of factors relating to community behavior in disposing household waste in the illegal disposal site in West Denpasar District
	16.03-16.09	**Discussion Panel**		
22	16.09-16.16	WEM-10	Luhur Akbar Devianto	Biosurfactants production using glucose and molasses as carbon sources by *Azotobacter vinelandii* and soil washing application in hydrocarbon-contaminated soil
23	16.16-16.23	WEM-11	Udin Hasanudin	Estimation of energy and organic fertilizer generation from small scale tapioca industrial waste
24	16.23-16.30	WEM-12	Aulia Nur Mustaqiman	BOD, DO, and coliform condition on Metro River in Lowokwaru District of Malang City
	16.30-16.36	**Discussion Panel**		
25	16.36-16.43	WEM-14	Evi Kurniati	Bio-electricity production of constructed wetland system using plant microbial fuel cell (PMFC)
26	16.43-16.50	WEM-22	Mohd Kamarulzaman Ismail	Nutrient recovery of solid waste from spent bleaching earth and sewage sludge in ASEAN countries

27	16.50-16.57	WEM-24	Rita Susanti	Potential of rice husk silica as a source of lysine-modified for adsorption of Fe (III) ion
28	16.57-17.04	WEM-25	Sri Suhartini	Textile wastewater treatment: biodegradability on aerobic and anaerobic process
	17.04-17.10	**Discussion Panel**		

MODERATOR: Mokhamad Nur, STP., M.Sc, Ph.D

TOPIC: AGRICULTURAL PRODUCT TECHNOLOGY

VENUE: SCR-2

No.	Time	Code	Presenter	Title
1	13.00-13.07	APT-28	Eka Shinta Wulandari	Effect of mokapot brewing temperature on sensory profiling of Dampit coffee and Tulungagung Ijo coffee using rate-all-that-apply (RATA) method
2	13.07-13.14	APT-29	Anbarani Rachma Fristi	The physical properties of porang flour edible film made from different porang flour and glycerol concentration
3	13.14-13.21	APT-30	Adhian Dini Khoirina	The effect of various proportion on germinated brown rice and tempeh flour instant porridge
4	13.21-13.28	APT-31	Susilawati Susilawati	Optimization of the use of suweg (*Amorphophallus campanalatus* B) flour as stabilizer on organoleptic properties, overrun and melting time of goat milk ice cream
	13.28-13.36	**Discussion Panel**		
5	13.36-13.43	APT-32	Lisa Fitri Rahayu	The effect of materials to form viscous paste of carbonized rice straw (CRS) as colorant of noodle
6	13.43-13.50	APT-33	Mokhamad Nur	Tragacanth as an excipient in oral delivery of bioactive proteins and peptides: effect of different pH preparation and drying methods
7	13.50-13.57	APT-34	Mokhamad Nur	Fabrication, functional properties and applications of tragacanth
8	13.57-14.04	APT-35	Norberto N. Tadeo	Yield and composition of milk and detection of putative plasma ghrelin in dairy buffalo fed with *Moringa oleifera* leaf meal (MoLM) supplement
	14.04-14.12	**Discussion Panel**		
9	14.12-14.19	APT-36	Umi Purwandari	Pineapple fruit leather and pudding premix: textural and sensory characteristics
10	14.19-14.26	APT-37	Velmor M. Abellera	Effect of carbohydrase supplementationto broilers fed with reformulated diets based on corn-soybean meal
11	14.26-14.33	APT-38	Mahmuddin Ridlo	Extraction of bioactive compound from karamunting fruit (*Rhodyrtus tomentosa*) with microwave assisted extraction (MAE) method
12	14.33-14.40	APT-39	Muhammad Arwani	Nutrient and saponin content of *Moringa oleifera* leave under different blanching methods

	14.40-14.46	**Discussion Panel**		
13	14.46-14.55	APT-40	Joni Kusnadi	Effect of pretreatment with organic acids of bilimbi (*Averrhoa bilimbi* L) and *Japansche citroen* orange (*Citrus limonia* Osbeck) on the quality of chili powder (*Capsicum frutescens*)
14	14.55-15.02	APT-41	Amrina Rosyada	Characterization of chitosan nanoparticles as edible coating materials for Cavendish banana
15	15.02-15.09	APT-42	Nuke Hanasasmita	Effect of different aroma extraction methods combined with Gas chromatography-mass spectrometry (GC-MS) on the aroma profiles of medium-roasted coffee (Java Arabica and Java Robusta)
	15.09-15.15	**Discussion Panel**		
16	15.15-15.22	APT-43	Hera S Prasmita	In vitro starch hydrolysis and estimated glycemic index of cooked black, red and white glutinous rice in different cooling time
17	15.22-15.29	APT-26	Kiki Fibrianto	Brewing time and temperature optimization of Robusta Dampit coffee on several drip techniques
18	15.29-15.36	APT-45	Jhauharotul Muchlisyiyah	Effect of processing and cooling method on black potato (*Solenestemon rotundifolus)* in vitro starch digestibility
	15.36-15.42	**Discussion Panel**		
19	15.42-15.49	APT-46	Wenny B Sunarharum	Study on the effect of roasting temperature on antioxidant activity of early-roasted Java coffee powder (Arabica and Robusta)
20	15.49-15.56	APT-47	Dwi Setijawati	Usage of *Eucheuma* sp with various comparisons towards the quality of edible film
21	15.56-16.03	APT-48	Jaya Mahar Maligan	Optimization of fermented corn-based dry noodle formula which is fortified with corn flour and *Moringa* leaf flour as supplementary food for pregnant women.
	16.03-16.09	**Discussion Panel**		
22	16.09-16.16	APT-49	Dewi Yunita	flavour compounds profile of cacao beans from Pidie District, Aceh Province, Indonesia
23	16.16-16.23	APT-50	Jariyah	Glycemic index of biscuit from mangrove fruit flour (MFF) with porang and mocaf flour mixture
24	16.23-16.30	APT-51	Nur Bazilah Burhan	Polyphenols as potential prebiotic
	16.30-16.36	**Discussion Panel**		

25	16.36-16.43	APT-52	Kitisak Pathumwan	The experimental study to find conditions to process sago caterpillars for sago oil and meat.
26	16.43-16.50	APT-53	Ratih Kumala Dewi	Modification of cassava starch with combination of steaming and acid hydrolysis and its use as encapsulant in nanoenkapsulation of cocoa leaf crude extract
27	16.50-16.57	APT-54	Trimurti Habazar	Efficacy of indigenous selected endophytic bacteria as biocontrol against *Ralstonia solanacearum* to promote growth of chili
28	16.57-17.04	APT - 61	Mahrus Ali	Characteristic of acan: a traditional shrimp paste of Maduranese, Indonesia
	17.04-17.10	**Discussion Panel**		

PARALLEL SESSION DAY II

DAY II: WEDNESDAY, 19 SEPTEMBER 2018
WIDYALOKA CONVENTION HALL AND FACULTY OF AGRICULTURAL TECHNOLOGY – UNIVERSITAS BRAWIJAYA, MALANG

MODERATOR: Dr. Ir. Bambang Dwi Argo, DEA

TOPIC: AGRICULTURAL ENGINEERING

VENUE: WMH

No.	Time	Code	Presenter	Title
1	**10.00-10.30**	**INVITED SPEAKER**	**Samsuzana Abd Aziz**	**Determination of FFA content in palm olein during deep frying using raman spectroscopy**
2	10.30-10.37	AEE-11	Sudarminto Setyo Yuwono	Shelf life estimation of instan sauce of rujak cingur using accelerated shelf life testing (ASLT) method
3	10.37-10.44	AEE-12	Khandra Fahmy	The individual influences of high CO_2 on suppression chilling injury in 'Merah Delima' papaya fruit
4	10.44-10.51	AEE-13	Bambang Susilo	Study of sorption isotherm and isosteric heat of kepok banana (*Musa paradisiaca F.)* slice.
	10.51-10.57	**Discussion Panel**		
5	10.57-11.04	AEE-16	Ferisman Tindaon	Characterization of the volcanic ash and soils from the eruption of Mount Sinabung in North Sumatra
6	11.04-11.11	AEE-17	Shreef Mahmood	Effect of postharvest treatments on shelf life and quality of BARI Strawberry-1
7	11.11-11.18	AEE-10	Renny Eka Putri	Realtime measurement of human labor energy for primary tillage operation in rice cultivation
8	11.18-11.25	AEE-19	Delilah P. Dal-uyen	Developememt of microcontroller-based control system for safe grain storage in silo
	11.25-11.31	**Discussion Panel**		

MODERATOR: MANGKU PURNOMO, SP., M.Si., Ph.D (FP)

TOPIC: AGROFORESTRY AND BIODIVERSITY

VENUE: WCR-1

No.	Time	Code	Presenter	Title
1	10.00-10.07	AFB-16	Yunus Effendi	Metagenomic analysis of *Fusarium oxysporum* cv cubense-infected soil in banana plantation, Sukabumi Indonesia
2	10.07-10.14	AFB-17	Mulawarman	Control of *Ganoderma boninse* with consortium microba on oil palm plantations
3	10.14-10.21	AFB-18	Tutik Kuswinanti	*Phytopthora palmivora* in South Sulawesi: characterization, virulence and it's biocontrol
4	10.21-10.28	AFB-19	Baharuddin	Resistance improvement of banana against wilt disease by combination of tissue culture and antagonist application
	10.28-10.36	**Discussion Panel**		
5	10.36-10.43	AFB-20	Yuni Sri Rahayu	The role of Mycorhizae and Phosphate solubilizing bacteria to increase plant survival grown on calcareous soil
6	10.43-10.50	AFB-21	Eliyani	The superiority of fast growing teak for land rehabilitation and carbon mitigation
7	10.50-10.57	AFB-22	Henry Barus	Mycorrhiza status and tree diversity at different elevations of tropical rainforest in Central Sulawesi
8	10.57-11.04	AFB-23	Suseno Amien	Performance analisys of M1V4 stevia (*Stevia rebaudina* Bertoni) generation induced by gamma ray and ethil methane sulphonate
	11.04-11.10	**Discussion Panel**		
9	11.10-11.17	AFB-24	Azri Kusuma Dewi	Grain yield stability analysis of Jembar local rice mutant lines generated from mutation breeding
10	11.17-11.24	AFB-14	Yusran	Diversity of macro fungi at some land use types around Pangi Binangga Nature Reserve, Central Sulawesi Indonesia
11	11.24-11.31	AFB-15	Widyah Budinarta	A novel bacterial antifungal with strong antifungal activity against *Ganoderma boninense*
	11.31-11.37	**Discussion Panel**		

13	11.37-11.44	AFB-27	Erni Mukti Rahayu	Strategy for development of agroforestry system in agricultural land in Argosari Village Jabung District Malang Regency
14	11.44-11.51	AFB-28	Tsaniyah Nur Kholilah	Effectiveness of the sloping agricultural land management on the buffer zone of Bromo Tengger Semeru National Park in Sumberejo, Malang, East Java, Indonesia
15	11.51-11.58	AFB-29	Rose Novita Sari Handoko	Pesticides and chemical fertilizer are not negatively impact the diversity of entomopathogenic fungi on rice plant in Malang Indonesia
	11.58-12.06	**Discussion Panel**		

MODERATOR: Putri Setiani, ST, MES, Ph.D

TOPIC: RENEWABLE ENERGY AND BIOREFINERY & WASTE AND ENVIRONMENTAL

VENUE: WCR-2

No.	Time	Code	Presenter	Title
1	10.00-10.07	REB-13	Tri Yuliana	Potential of wood-degrading basidiomycetes *Marasmiellus* sp and *Ganoderma lucidum* in xylanase enzyme production and its activity using agro-industry waste
2	10.07-10.14	REB-14	Souvia Rahimah	Effects of substrate concentration on bioethanol production from oil palm empty fruit bunches with simultaneous saccharification and fermentation (SSF)
3	10.14-10.21	REB-15	Oo Abdul Rosyid	Study of palm oil waste and other renewable energy potentials for power generation at Pelalawan District
4	10.21-10.28	REB-16	Asep Ginanjar Arif	Potent of chicken manure as matter of gas production with simple and cheap gas reactor
	10.28-10.36	**Discussion Panel**		
5	10.36-10.43	REB - 17	Irnia Nurika	The pattern of lignocellulose degradation from Cacao pod using the brown rot (*Serpula lacrymans*) and white rot (*Schyzophylum commune*) fungi.
6	10.43-10.50	WEM-13	Muhammad Arif Kamal	Development of municipal solid waste optimization based on region clustering and vehicle routing of Pontianak City West Kalimantan
7	10.50-10.57	WEM-15	Dwi Haryanta	Study of onion growth (*Allium ascalonicum* L.) on medium sediment soil and urban waste compost
	10.57-11.03	**Discussion Panel**		
9	11.03-11.10	WEM-16	Akhmad Adi Sulianto	The cellulose-chitosan membrane for the removal of heavy metals from tannery industrial wastewater
10	11.10-11.17	WEM-17	Ika Atsari Dewi	Characterization of essential oil from baby Java orange solid waste
11	11.17-11.24	WEM-18	Michael Villaos Capina	Viability of utilizing arrowroot starch by-products into flour and pastries
	11.24-11.30	**Discussion Panel**		
13	11.30-11.37	WEM-19	Suharti Suharti	Isolation and characterization of a newly keratinase producing *Bacillus* sp N1 from *Liquid Tofu Waste*

14	11.37-11.44	WEM-20	Adhian Khoironi	Community behavior in plastics consumption to mitigate increasing plastic pollution
15	11.44-11.51	WEM-21	Siti Khodijah Chaerun	Biohydrometallurgy: role of microbes in extracting metals from low-grade ores and mine wastes
16	11.51-11.58	WEM-23	Mohamad Amin	Identification of indigen bacteria from waste water of regional general hospitals in Pacitan
	11.58-12.05	**Discussion Panel**		

MODERATOR: SUPRAYOGI, STP, MP, PhD

TOPIC: HEALTH, NUTRITION AND MEDICAL

VENUE: WCR-3

No.	Time	Code	Presenter	Title
1	10.00-10.07	HNM-08	Claudia Gadizza Perdani	Total phenols content of green coffee (*Coffea arabica* and *Coffea canephora*) in East Java
2	10.07-10.14	HNM-09	Tri Dewanti Widyaningsih	The effect of Indonesian herbal drink Mbah Jayus on hyperglycemic rats
3	10.14-10.21	HNM-10	Gusti Ayu Kadek Diah Puspawati	Hypoglycemic potency of crude extract of red tamarillo (*Solanum betaceum* Cav) on type 2 diabetes sprague Dawley
4	10.21-10.28	HNM-11	Syarif Hamdani	Isolation and identification of proteolytic bacteria from pig sludge and protease activity determination
	10.28-10.36	**Discussion Panel**		
5	10.36-10.43	HNM-12	Siti Nurdjanah	Sweet potato greens 'neglected vegetables rich in bioactive compounds' (part I): radical scavenging activity, inhibitory effect on α- amylase, total phenolic and flavonoid contents of local sweet potato (*Ipomoea batatas*) leaves
6	10.43-10.50	HNM-07	Dian Widiyanti	Comparison of microorganisms before and after disinfection using alcohol-based solution fogging method in operating room of private hospital in Jakarta
7	10.50-10.57	HNM-14	Dian handayani	Antimicrobial activity screening of the marine algal-derived endophytic fungi extracts isolated from marine brown algae *Padina* sp.
	10.57-11.03	**Discussion Panel**		
9	11.03-11.10	HNM-15	Elmi Nurhaidah Zainuddin	Prospects of green seaweed *Ulva reticulata* as a candidate for antibiotic against pathogenic microbes in humans
10	11.10-11.17	HNM-16	Sang G. Purnama	Quality of hygiene, sanitation and identification of *Eschericia coli* O157: H7 in Sate Languan related with traveler's diarrhea in Bali.
11	11.17-11.24	HNM-05	Dini Ryandini	Antibacterial ability of *Streptomyces* sp. E404 in different media formula and incubation time and molecular characterization of antibacterial compound encoding gene
	11.24-11.30	**Discussion Panel**		

MODERATOR: Dr. DODYK PRANOWO, S.TP, M.Si

TOPIC: FOOD SAFETY AND SECURITY

VENUE: WCR-4

No.	Time	Code	Presenter	Title
1	**10.00-10.30**	**INVITED SPEAKER**	**Fakhrul Zaman Rokhani**	**Design of portable wireless impedance spectroscopy for sensing lard as adulterant in palm oil**
2	10.30-10.37	FSS-04	Endrika Widyastuti	Paper-Based Colorimetric Immunosensor for Optical detection of Salmonella thypimurium
3	10.37-10.44	FSS-07	Nikmatul Khoiriyah	ANIMAL FOOD DEMAND INURBAN POOR HOUSEHOLD IN EAST JAVA: A Quadratic Almost Ideal Demand System Approach
4	10.44-10.51	FSS-15	Adelina Siregar	Roles of Rhizobacteria from Bamboo and Elephant Grass on Soil Nutrients Availability and Yield of *Brassica juncea*
	10.51-10.57	**Discussion Panel**		
5	10.57-11.04	FSS-16	Faridatul Mukminah	Adaptation of sweet potato (*Ipomoea batatas* (L) Lam.) varieties Cilembu in South Sumatra
6	11.04-11.11	FSS-10	Choirul Anam	The preparation technique in production of the trash fish fillet from Pantura Lamongan’
7	11.11-11.18	FSS-09	Robelyn Tortillas Piamonte	Enhanced polymerase chain reaction detection sensitivity for abaca bunchy top viruses
	11.18-11.24	**Discussion Panel**		
8	11.24-11.31	FSS-19	Catherine Roween C Almaden	A meso-level analysis on rural farmers' adaptive measures for slow onset hazard: the case of seawater intrusion in rice farms in the Philippines
9	11.31-11.38	FSS-05	Endrika Widyastuti	Optimization of high voltage sterilization machine based on dielectric barrier discharge plasma against *Salmonella* sp and total bacteria with responses surface methodology on (*Gallus gallus domesticus*) chicken egg
10	11.38-11.45	FSS-06	Erdi Suroso	Development strategy of palas rice in South Lampung Regency
11	11.45-11.52	FSS-08	Dewi Ermawati	Pesticides removal of fruit and vegetables by using ultrasound ozone
	11.52-11.58	**Discussion Panel**		

MODERATOR: INDRIA PURWANTININGRUM, STP., M.Si

TOPIC: AGRICULTURAL PRODUCT TECHNOLOGY & INDUSTRIAL BIOTECHNOLOGY AND BIOPROCESSING

VENUE: SCR-1

No.	Time	Code	Presenter	Title
1	10.00-10.07	APT-55	Endang Lukitaningsih	Optimization of microemulgel formula of combination extracts of strawberry (*Fragaria x ananassa* (Duchesne ex Weston)), langsat fruit (*Lansium domesticum* Corr), pomelo peel (*Citrus maxima* L.) and antiaging activity studies
2	10.07-10.14	APT-56	Robi Andoyo	Phyicochemical properties of milk ozonated against *Salmonella* sp at various ozone exposure time
3	10.14-10.21	APT-57	I Made Agus Gelgel Wirasuta	Prediction total anthocyanin content in *Ipomoea batatas* L, as cyanidin-3-glucoside equivalent, by using thin layer densitometry
4	10.21-10.28	APT-58	Nurhayati	Nutrient and energy content of black garlic in different time of fermentation
	10.28-10.36	**Discussion Panel**		
5	10.36-10.43	APT-59	Sudarminto Setyo Yuwono	Effect of withering time and chopping size on properties of pucuk merah (*Syzygium oleana*) herbal tea
6	10.43-10.50	APT-60	Qonitatillah	The potential of green coffee (*Coffea arabica* and *Coffea canephora*) as a source of natural antioxidant in East Java
7	10.50-10.57	IBB-20	Triana Hertiani	Exploration of target assay as guidance for potencial cytotoxic compound isolation from *Streptomyces* sp. GMY01 bacteria using in silico study
8	10.57-11.04	IBB-21	Yanti Rachmayanti	Genetic markers and biopigment composition of an Indonesian marine red microalgae *Porphyridium* sp.
	11.04-11.10	**Discussion Panel**		
9	11.10-11.17	IBB-22	Adek Zamrud Adnan	Agarose isolation from agar and its application as tissue culture medium component of *Eleutherine palmifolia* L. (dayak onion)
10	11.17-11.24	IBB-23	Arief Budi Witarto	Production of recombinant-mutated glucose dehydrogenase for glucose sensor

11	11.24-11.31	IBB-17	Anto Budiharjo	Potential application of *Bacillus altitudinis* as a biopesticide against phytopathogen in organic farming system
12	11.31-11.38	IBB-25	Anisa Nurfitriyah	Protein derived from plant pest (*Erionata thrax*) for growth medium of antagonistic bacteria
	11.38-11.46	**Discussion Panel**		

MODERATOR: MAS'UD EFFENDI, S.TP, MP

TOPIC: AGROINDUSTRIAL PRODUCTION SYSTEM MANAGEMENT AND REGULATION

VENUE: SCR-2

No.	Time	Code	Presenter	Title
1	**10.00-10.30**	**INVITED SPEAKER**	**Kongkiti Phusavat**	**Medical to wellness tourism in Thailand: agriculture's contributions**
2	10.30-10.37	APS-26	Sofihar Sinansari	Opportunity and business challenge of marine ornamental fishes in Indonesia as a potential commodity of fisheries
3	10.37-10.44	APS-27	Winda Amilia	Risk analysis on supply chain activities: a case of post harvest loss on raw material procurement of edamame product in Indonesia
4	10.44-10.51	APS-25	Neza Fadia Rayesa	A conceptual framework of a decision support system for developing catfish bussiness
	10.51-10.57	**Discussion Panel**		
5	10.57-11.04	APS-24	Mas'ud Effendi	Corn quality identification using digital image processing based on color and texture features
6	11.04-11.11	APS-20	Wendra G Rohmah	Integration of cluster analysis and fuzzy analytical hierarchy process (FAHP) in formulating cluster development strategy of tempeh chips SMEs
7	11.11-11.18	APS-31	M Saadah	Analysis of institutional paprika supply chain in Pasuruan Regency
	11.18-11.24	**Discussion Panel**		
8	11.24-11.31	APS-32	Media Rahmawati	Claim IBNR estimation using tweedie distribution
9	11.31-11.38	APS-21	Evy Latifah	Technical and economic advantages of starter solution technology applied to production chili (*Capsicum frutescens* L.): two field trials in Kediri – East Java
10	11.38-11.45	APS-23	Usman Effendi	Evaluation of supply chain performance with green supply chain management approach (GSCM) using SCOR and DEMATEL method (case study in PG Krebet Baru, Malang)
	11.45-11.51	**Discussion Panel**		

KEYNOTE SPEAKERS

Key aspects of food innovation: global and local cases

P Rayas-Duarte

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Abstract. With the prospect of reaching close to 11 billion people by 2050, the present and future food production and technology industries face a great challenge: a new level of food innovation with affordable technology and a more sustainable environmental impact compared to the present state of affairs. Food innovations now need to take a leap into a systems approach to improve all aspects of food agriculture and food ingredients, processing and packaging. Case studies of global and local success will include the cranberries and kiwi, improving cassava processing, lactose free dairy products and gluten free products. Radical changes versus incremental changes in food innovation are needed and examples of what is accomplished in different countries will illustrate the point of what can be done locally. The development of a network of partners of different disciplines appears to be an underlining road for success. Winners of global competitions in food innovations platforms score examples of possibilities to inspire the future of food. An example includes protein-rich drinks and snacks.

Keywords: systems approach, future food, innovations in food

Study on the biomass degradation by enzymes from filamentous fungi: implications for the sustainable development goals

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Abstract. Filamentous fungi, such as *Aspergillus* species can use a wide variety of carbon compounds as a single carbon source for growth. These compounds include polymers, oligo- and disaccharides, hexoses, pentoses, organic acids, aromatic compounds, alcohols, polyols, and fatty acids. Therefore, a great many anabolic and catabolic pathways are amenable to study. Especially with *Aspergillus nidulans*, the life cycle is easy to manipulate and to allow fine-structure genetic analysis. For this reason, mutants have been used to elucidate the organization of various metabolic pathways and the physiological importance of several individual genes. We have been extensively elucidating the structural and functional features of some transcriptional factors, such as Hap complex, AmyR, XlnR and ManR, which regulate genes encoding polysaccharide-degrading enzymes (1,2).

Filamentous fungi produce high levels of polysaccharide-degrading enzymes and are frequently used for the production of industrial enzymes degrading polysaccharides such as cellulose, xylan, and β-mannan. Among polysaccharides, β-mannan including glucomannan, galactomannan, and galactoglucomannan are widely distributed in nature. They constitute the main components of plant cell walls and serve as storage polysaccharides in some plants. Mannanolytic enzymes have recently become important natural resources for industrial biorefinery processes to produce second-generation biofuels from plant biomass. As β-mannans have a complex structure, a set of mannanolytic enzymes with different substrate specificities is necessary for complete degradation. Filamentous fungi produce various mannanolytic enzymes including β-1,4-mannanase, α-galactosidase, β-mannosidase, acetylmannan esterase, and β-glucosidase, making these organisms excellent sources of these enzymes. *Endo*-β-1,4-Mannanases that randomly hydrolyze the internal β-1,4-linkage of the mannan backbone are ubiquitous in viruses, bacteria, and eukaryotes.

Recently, we investigated the enzymatic functions of a secreted hypothetical protein of which the production was induced by β-mannans (3-5). The protein shared no homology to extant β-mannanases but displayed hydrolytic activity toward β-mannan. Furthermore, the protein had no homology to any proteins with known functions, and thus we propose that the protein is a novel β-1,4-mannanase belonging to a new GH family. The β-1,4-mannanase reacted with β-mannan and manno-oligosaccharides with mannotriose recognition and had high catalytic efficiency (k_{cat}/K_m) toward

mannohexaose (M_6) compared with the *endo*-β-1,4-mannanase Man5C belonging to a GH5 family, indicating that the enzyme had a unique catalytic property. Moreover, the novel β-1,4-mannanase had a synergistic effect with Man5C toward glucomannan and galactomannan, suggesting that would be useful for diverse industrial applications including conversion technology of lignocellulosic biomass.

Our recent progress on the biomass degradation technology will be also discussed. Just recently we succeed to developed a new and effective pretreatment method using a radical generator based on non-thermal atmospheric pressure plasma technology (6). Cellulose is the most abundant polysaccharide found in nature, consists of a β-1,4-linked linear chain of glucose units, and is used in the biofuel, oil, food, textile, and pulp industries. Cellulolytic enzymes are important reagents in industrial biorefinery processes, such as the production of biofuels from plant biomass. The complete degradation of cellulose requires the synergistic action of a set of cellulolytic enzymes with various substrate specificities. The efficiency of cellulolytic enzymes is important in industrial biorefinery processes, including biofuel production. Chemical methods, such as alkali pretreatment, have been extensively studied and demonstrated as effective for breaking recalcitrant lignocellulose structures. However, these methods have a detrimental effect on the environment. In addition, utilization of these chemicals requires alkali- or acid-resistant equipment and a neutralization step. Bioethanol production from lignocellulose generally involves three steps: (i) pretreatment to break down the complex lignocellulose structures; (ii) enzymatic hydrolysis of polysaccharides (i.e., cellulose and hemicellulose) into fermentable sugars; and (iii) fermentation to convert sugars into ethanol. Various biological, chemical, and physical pretreatment methods have been developed. Our results showed that the viscosity of carboxymethyl cellulose (CMC) solutions was reduced in a time-dependent manner by oxygen-radical pretreatment using the radical generator. Compared with non-pretreated CMC, oxygen-radical pretreatment of CMC significantly increased the production of reducing sugars in culture supernatant containing various cellulases from *Phanerochaete chrysosporium*. The production of reducing sugar from oxygen-radical-pretreated CMC by commercially available cellobiohydrolases I and II was 1.7- and 1.6-fold higher, respectively, than those from non-pretreated CMC. Moreover, the amount of reducing sugar from oxygen-radical-pretreated wheat straw was 1.8-fold larger than those from non-pretreated wheat straw. Oxygen-radical pretreatment of CMC and wheat straw enhanced the degradation of cellulose by reducing- and non-reducing-end cellulases in the supernatant of a culture of the white-rot fungus *P. chrysosporium*. These findings indicated that oxygen-radical pretreatment of plant biomass offers great promise for improvements in lignocellulose-deconstruction processes.

Keywords: Cellulolytic enzymes, genes, β-mannans, filamentous fungi

Sustainable development of konjac agroindustry for national welfare purposes

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Abstract. Indonesia, particularly East Java Province, for long history, known to be exporting Yellow Konjac Chips (*Amorphophallus muelleri* Blume) for Japan and China destinations. As the chip supply becoming shortage, and still occurs nowadays, searching for other unutilized konjac, which is white flesh bulb of konjac (*A. Spp*) are increasing in the future. This paper reports on non-technical and technical handicaps in developing konjac agroindustry for national welfare in general and to support people needs, especially, lower income communities who live in remote areas or near to forest. As a result of growing population, increasing food demand, increasing numbers of middle traders, decreasing rupiah exchange rate, unpredictable climate change, increasing areas of unexploited land usage etc, the supply of yellow konjac or *Porang* (*A. Muelleri*) from known centres of production decline significantly. Whilst, the supply demand for *porang* manufactures or for chip exporters increase sharply. As results, the price of fresh bulb or *porang* chips become exaggerated price. If this situation cannot be controlled in some ways, domestic porang manufactures will be shut down. The demand for *iles-iles* from China now increase, this could be due to the shortage supply of *porang* chips. This situation will accelerate environmental damage. Therefore, expanding planted *porang* or *iles-iles* in unexploited land usage under teak tree forest or *Sengon laut* (*Albazia chinensis*) forest or other tree plantations are needed. This programme will sustain domestic konjac agroindustry and keeps the food manufacture industry remains competitive. In conclusion, the national welfare accomplished with the available jobs.

Keywords: Porang, food, demand, agroindustry

Production of human β-glucocerebrosidase by root culture of *Nicotiana benthamiana*

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Abstract. Plants are an attractive alternative host for recombinant protein production without risk of contamination by human pathogens and animal-derived materials. In addition, plant systems can give the low cost of large-scale production. Gaucher disease results from an inherited disorder of the enzyme glucocerebrosidase (GCase). Currently, recombinant GCase is produced in mammalian cells and used as enzyme-replacement therapy. Compared to whole plants, root cultures can be easily handled under physical control, such as temperature and light. So, in this presentation, production of recombinant GCase in a Nicotiana benthamiana root culture is introduced. Root culture of a GCase-producing transgenic plant was induced by indole-3-acetic acid, a phytohormone, at the concentration of 1 mg/L. Recombinant GCase was successfully produced in roots as an enzymatically active form. Crude proteins were extracted from the roots and used for purification of recombinant GCase over Concanavalin A and phenyl 650C chromatography. The productivity of GCase was about 1 µg/g of the root. N-glycan structures of the purified GCase were examined using nano LC-MS/MS, showing a dominant structure was Man3XylFucGlcNAc2 (Man, mannose; Xyl, xylose; Fuc, fucose), which has two plant-specific residues, Xyl and Fuc. This study shows a new, safe and efficient root culture system of recombinant GCase production that might be applied to other recombinant proteins, such as vaccine, IgG etc.

Keywords: recombinant protein, β-glucocerebrosidase, physical control, *Nicotiana benthamiana*

Appropriate postharvest management reduces losses of agricultural produce: a case study on pineapple CV. Phulae, a geographical indication of Chiang Rai, Thailand

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Abstract. Postharvest losses of fresh produce in many Asian countries are huge. Fresh fruits and vegetables are perishable. Decline in quality of fresh produce after harvesting leads to their short shelf life. Thailand is one of the major agricultural exporting countries including fruits and vegetables. Approximately 30 up to 60% of fruits and vegetables is lost after harvest and during distribution. Therefore, to accomplish good and sustainable postharvest management for reducing postharvest losses of fresh produce, the applicable postharvest assessment should be performed since harvesting and distribution of products. However, determination of postharvest losses of fresh produce is difficult. The postharvest losses involve degradation in both quantity and quality of fresh produce due to inadequate storage condition and time, physical damage, physiological changes and diseases. Generally, a suitable postharvest handling helps to reduce these losses. The appropriate postharvest management assists in avoiding possible mechanical damages e.g. impact, vibration and compression damages. Furthermore, a good range of storage condition could delay physiological changes of fresh produce e.g. respiration, ethylene production, color and texture changes, including changes in chemical attributes such as flavor and nutritional modification, etc. Thai government has recently encouraged the stakeholders in agricultural section, exporters, business owners, clusters of farmers, researchers, etc. to apply technologies to their products. This is in order to create innovative and value-added agricultural products for domestic consumption and export. However, postharvest loss assessment and reduction need to be considered as important means for improving quality of farmer's life. This presentation provides an example of postharvest losses assessment of 'Phulae' pineapple, a geographical indication of Chiang Rai, Thailand. The presentation also introduces an environmentally friendly postharvest technique called 'intermittent warming' as an alternative technique for extending storage life of pineapple.

Keywords: loss assessment, postharvest handling, intermittent warming, storage

Rational design of nanoparticles-based drug delivery system for prolonged antibacterial performance of vancomycin: structure matters

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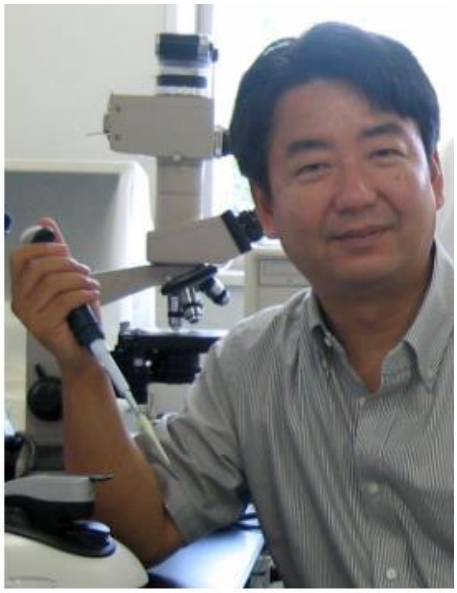
Abstract. Vancomycin (Van) is a glycopeptide which is one of the last resort antibiotics for the treatment of various bacterial infections. When delivered orally, Van is poorly absorbed or penetrated theinfection site and can be eliminated primarily (90%) via the renal route within 24 h. Van also has a short half-life and thus its administration requires long-term treatment. To maintain the antimicrobial activity, frequent administration is necessary in conventional formulations. Unfortunately, Van is toxic at a high dosage and upon exposure over a long time. Due to these limitations, research has turned towards developing delivery vehicles for Van.
Carbon based nanoparticles have recently received attention in cellular delivery due to their unique physical–chemical properties, adjustable nanostructures and excellent biocompatibility. In addition, carbon materials have extra advantages including chemical inertness and possess hydrophobic properties. In the present study, we have prepared mesoporous hollow carbon (MHC) nanospheres with carefully designed structural parameters with a focus on the study of the relationships between the structure, Van loading/release and antibacterial performance.
It is demonstrated that MHC materials possess a Van loading capacity of 861 mg g-1, much higher than that of any Van nano carrier in previous reports. Our results have shown that the hydrophobic attraction is responsible for the high loading and sustained release of Van in MHC materials. Moreover, while sustainable release is needed, a compromise in release kinetics which should not be too slow is also important to maintain an effectively high drug concentration in culture medium and to achieve long-term bactericidal activity. Our understanding paves the way for the design of novel nano-carriers for long-term bacterial inhibition.

Keywords: Van, nanoparticle, mesoporous hollow carbon, nano carrier

Prof. Masashi Kato



Prof. Masashi Kato is a professor in Faculty of Agriculture, Department of Applied Biological Chemistry, Meijo University, Japan. His research interests are mainly in applied microbiology and molecular biology. Fungi, including yeasts, molds, and mushrooms are organisms focused on. Production of useful enzymes, gene regulation, transcription factors, and also fermentation are major subjects of his study. He has many national and international professional experiences in academic and research area. For example, in 1997-1998, he worked in Nottingham University Department of Life Science Gest Recercher. In 2004-2010, he was also an Associate Professor in Nagoya University Graduate School. Since 2010, he obtained his full professorship in Faculty of Agriculture, Meijo University.

Prof. Patricia Rayas-Duarte

Patricia Rayas-Duarte is a tenured professor at Oklahoma State University (OSU), Department of Biochemistry and Molecular Biology. In 1982-1988, she obtained her M.S. and PhD at Food Science and Technology, Univ of Nebraska – Lincoln, NE. She also had her post doctorate in food science in 1988-1990 in University, W. Lafayette, IN. In 1997-2005, she became an Associate Professor in Department of Biochemistry & Molecular Biology, Oklahoma State University. Her research area is in adding value to cereal grains, rheological and sensory evaluation properties, development of novel probiotics for improvement of nutrient digestion in farm animals, gut microbiome of broiler chickens. Her extention focus is in-service training curricula in wheat quality, development of alliances with different community entities to deliver programs to decrease food waste at all levels, in cooperation with Lynn Malley, OSU solid waste management specialist. She has trained 26 undergraduates in research projects, three Wentz Scholars, and three Honor Theses. She has also participated in technical training and educational programs in Africa, Southeast Asia, and Latin America. Most of the programs included food science needs in countries including Mali, Thailand, China, Mexico, Guatemala, El Salvador, Venezuela and Brazil. She taught courses in Food Science such as Product Development I and II, Special Topics in Cereal Chemistry, Special Topics in Food Science and summer workshops in Cereal Rheology for industry and graduate students. Prof. Rayas-Duarte currently serves on the Editorial Board of Journal of Food Quality and Journal of Food Science (China), and is Ad Hoc Editor for Cereal Chemistry, Journal of Food, Agriculture and Environment, Lebensmittel-Wissenschaft und-technologie, Preparative Biochemistry & Biotechnology Journal, Food & Function Journal, Journal of Applied Polymer Science, Crop Science, Journal of Cereal Science, Journal of Agricultural and Food Chemistry, and Carbohydrate Polymer.

Dr. Andi Amran Sulaiman

Andi Amran Sulaiman (born 27 April 1968) is an Indonesian businessman and the Minister of Agriculture, who has served under Joko Widodo's Working Cabinet since his appointment on 27 October 2014. Prior to becoming minister, he was the leader of Tiran Group, a Makassar-based conglomerate operating mostly in Eastern Indonesia making him the wealthiest minister appointed to the new cabinet. Born in Bone, South Sulawesi, his education and the bulk of his career revolved around agriculture, with him being listed as a lecturer on agricultural sciences in the state-operated Hasanuddin University. Upon the completion of his basic studies, Sulaiman studied agricultural science in Hasanuddin University, starting in 1988 and obtaining his undergraduate degree in 1993. He would continue to obtain his masters and postgraduate degree from the same university on 2003 and 2012 respectively, all in the same subject. He graduated with the maximum GPA, and patented multiple inventions which covered pest control. He currently holds 5 patent rights, in addition to being listed as a lecturer at Hasanuddin University. Upon his graduation, Sulaiman worked for the Indonesian National Agricultural Company or PTPN (more precisely, PTPN XIV). He began his career as a head of field operations in a sugar factory in 1994, and was promoted 4 times throughout his first six years in the company, peaking as the chief of logistics. He resigned after 15 years. Later on, he founded his own business, beginning with his patent on rat poison (named "Tiran" as an acronym of Tikus diracun Amran i.e. Amran poisons rats) and expanded rapidly, covering 10 companies with combined annual revenues approaching USD 1 billion by 2014. He received a civil award Satyalencana Pembangunan from Indonesian president Susilo Bambang Yudhoyono in 2007. Joko Widodo announced his appointment as Minister of Agriculture on 26 October 2014, and he was sworn in the following day. His ministry's target was set as self-sufficiency in 4 key food commodities i.e. rice, corn, soybeans and sugar within 3 years in addition to improvements of irrigation systems in 11 Indonesian provinces.

Prof. Simon Bambang Widjanarko

Prof. Simon Bambang Widjanarko is a professor in Faculty of Agriculture Technology, Universitas Brawijaya, Indonesia. He was born on October 10, 1952. In 1990, He obtained his Ph. D in Food Technology, University of New South Wales, Australia. He teaches courses in food chemistry, advanced food analysis, food bio-chemistry, advanced food technology and research methodology. His research interest is in development and processing of porang (*Amorphophallus muelleri*). He is the head of porang research center (http://prc.ub.ac.id) tahta established since 2011, in Universitas Brawijaya. Porang Research Center (PRC) focus on the problems in developing porang and similar commodities (suweg, iles-iles and others) in Indonesia from various aspects including physiology of cultivation, cultivation of agriculture, processing and development product, marketing and socio-economics related to the development of people in Indonesia. Therefore, PRC brings together researchers from various fields of science from faculties in Brawijaya University, including Agriculture, Biology, Agricultural Product Technology, Engineering, and Medicine, which are expected to produce clear, measurable and provide benefits in various science applications in society.

Swasmi Purwajanti, Ph. D

Swasmi Purwajanti is a researcher in at The Indonesian Agency for Assessment and Application of Technology since 2009. She obtained his PhD on Advanced Functional Nanomaterial Engineering, Australian Institute for Bioengineering and Nanotechnology, The University of Queensland in 2017. Her research focus on developing metal oxide-based nanomaterial for water remediation application. She got some awards namely: Summer School Grant on Bio-Leaching and Metal Extraction Processes for Urban Mining at TU Dresden (2016) and UQ Graduate School International Travel Award (GSITA) for doing research internship in Lappeenranta University of Technology, Finland (2016). She is also a lecturer in Department of Chemical Engineering, Universitu of Muhammadiyah, Jakarta.

Dr. Tamarath Pranamornkith

Tamarath Pranamornkith is a lecturer in Postharvest Technology Program, School of Agro-Industry, Mae Fah Luang University, Thailand since 2015. He obtained his Ph.D in Food Technology from Massey University, Palmerston North, New Zealand in 2009. His research interest are Postharvest storage system (controlled atmosphere), ethylene flow-through treatment, Anoxia treatment, temperature conditioning and Intermittent warming treatment, pre and postharvest factors influencing fresh produce quality, postharvest physiology and technology of tropical or subtropical fruit, and fumigation - Methyl Bromide, Phosphine, EDN. He got some honors and scholarship/ fellowship awards, namely: Postdoctoral Scientist, The New Zealand Institute for Plant & Food Research Ltd, Palmerston North Research Centre, May 2013 to July 2014, Postdoctoral Fellow, Institute of Food Nutrition and Human Health (currently College of Health), Massey University, October 2011 to April 2013, Postdoctoral Fellow, School of Engineering and Advanced Technology, Massey University, July 2010 to August 2011 and the Clark Fletcher Memorial Citrus Bursary for the scholarship, New Zealand Citrus Growers Incorporated (NZCGI) in 2006. He is also a member of New Zealand Society of Plant Biologists (NZSPB) (since 2008) and The New Zealand Institute of Agricultural & Horticultural Science Inc (since 2012).

Prof. Kazuhito Fujiyama

Kazuhito Fujiyama is a professor in International Center for Biotechnology (ICBiotech, Osaka University) since 2009. He obtained his Ph.D in 1990 form Osaka University. He became asisstant professor in 1988 and associate professor in 2003. His research interest are glyco-engineering of hetelogously-produced recombinant proteins, plant glycobiology, and applied microbiology. He had many research experiences in foreign universities and institutes. He became a visiting scientist in some universities, namely: Univesity of California at Davis (1988), Boyce Thompson Institute for Plant Research at Cornell University (1998), Universtiy of Zurich (1999), Arizona State University (2003), and since 20014 until now, he became non-resident research faculty in The Biodesign Institute at Arizona State University.

INVITED SPEAKERS

Medical to wellness tourism in Thailand: agriculture's contributions

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Abstract. After 1997 economic crisis, the emphasis has focused on tourism, especially medical and now wellness tourism. For the ASEAN region, Thailand is the largest travel and tourism economy with $36.4 billlion. Tourism-trade constituting 9.3% of overall GDP. A frost and Sullivan research predict the market at around $50 billion to $65 billion dollars in 2014, growing at approximately 20%. Due to the competition and the need to evaluate the shift in consumers, Thailand has gradually incorporated medical into larger wellness tourism. Wellness tourism is healthy living, rejuvenation & relaxation, meaning & connection, authentic experiences, disease prevention & management. Wellness traveler donates high yield about 6.2% of all global trips and 14.6% of all expenditures. Trends and circumstances affecting wellness tourism's growth are productivity, aging population, digital penetration and generations Y and Z, globalization and mobility. Five new S-curved industries such as digital, robotics & automation, biofuels & biochemicals, aviation and logistics, and medical hub (consist of foods, herbal and others to support healthy life style) will be the main focus for Thailand in 2018.

Keywords: Wellness Tourism, Medical, Thailand

Determination of FFA content in palm olein during deep frying using Raman spectroscopy

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Abstract. Excessively use of frying oil will initiate the formation of undesirable compounds due to the degradation process during frying by series of complex chemical reaction. The frying oil degradation has to be monitored since consuming degraded oil will be detrimental to consumer health. Conventional methods for this purpose is usually time consuming, require skilled operator and involve significant amount of solvents. In this study Raman spectroscopy was used to monitor refined, bleached and deodorized palm olein (RBDPO) degradation in repeated deep-fat frying. French fries were intermittently fried for seven hours a day over five consecutive days (a total of 35 hours). The oil sample was taken once after every five batches of frying (30 batches/day). Raman spectra of the RBDPO samples were recorded over the range of 600 to 3000 cm^{-1} with a resolution of 1 cm^{-1}. For calibration purposes, free fatty acid (FFA) content in the frying oil samples were measured using Malaysian Palm Oil Board (MPOB) standard test method. Model was developed using partial least squares (PLS) analysis to predict FFA content. Results showed that there were significant differences in intensity of Raman spectra over five days of frying ($P<0.0001$). The PLS model showed significant prediction ability of FFA using full Raman spectral range. The model gave significant correlation, with $R2 > 0.90$ with low RMSECV of 0.02%. The results demonstrated that Raman spectroscopy provide good potential for frying oil quality monitoring.

Keywords: Deep-fat frying, Raman spectroscopy, free fatty acid, frying oil degradation, palm olein

Design of portable wireless impedance spectroscopy for sensing lard as adulterant in palm oil

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Abstract. This paper presents the design of portable wireless halal sensor using impedance spectroscopy technique for sensing lard as adulterant in palm oil. The impedance spectra of lard adulterated in palm oil (0.1%, 0.5%, 1%, 5% and 10%) at the frequency range of 5 – 100 kHz at 45°C by using AD5933 Evaluation Board, a custom-built Interdigitated Electrode (IDE) and a Bluetooth module. The impedance spectra show that the binary mixture of lard and palm oil decreases as frequency increases. It is also found that the impedance spectra decrease as the concentration of lard adulteration in palm oil increase. PCA classification shows that different concentration of lard adulteration can be grouped in different cluster. PLS analysis shows high R^2 value, 0.97, indicating reliable prediction of the percentage of lard adulteration in palm oil.

Keywords: Impedance spectroscopy, adulteration, lard, palm oil, prediction

Dr. Samsuzana Abd Aziz

Samsuzana Abd Aziz is a lecturer in Department of Biological and Agricultural Engineering, Faculty of Engineering, Universiti Putra Malaysia. She obtained her Ph. D in Agricultural Engineering from Iowa State University, USA in 2008. Her research interests are mainly in agricultural informatics and instrumentation, and precision agriculture. She is a member of Member of American Society of Agricultural and Biological Engineers (ASABE). She got several awards in national and international level, namely: Gold and Best Award (Agricultural, Environmental and Renewable Energy) in EYReC Recognised As Malaysia Invention & Design Society (MINDS) at International Engineering Invention and Innovation Exhibition (i-ENVEX) (2014), ISHS Medal (International Society for Horticulture Science) (2016), and Silver Medal in Advanced Innovation and Engineering Exhibition 2017, UMP. She was also an commitee member of Development Agricultural Mechanization Masters' Program, Department of Biological and Agricultural Engineering, UPM in 2017. She had become invited speaker in some scientific events: Consortium Unified Curriculum System (UCS) Among Sister Universities And University Of Tsukuba In Bioresources Engineering, University of Tsukuba (2017), Engineering a Successful Career for Women: Making a Difference of the Future, IEEE CASS Outreach, Women in Circuits and Systems (WiCAS) (2017), 9th International Conference on Computer and Computing Technologies in Agriculture (CCTA2015) and Information Processing in Agriculture(IPA) in Beijing (2015), etc.

Dr. Fakhrul Zaman Rokhani

Fakhrul Zaman Rokhani is a tenured senior lecture in Department of Computer and Communication Systems Engineering, Faculty of Engineering, Universiti Putra Malaysia (UPM). He obtained his Master and Doctoral degree in Electrical Engineering, University of Minnesota, USA. His research area is in VLSI Design – Digital VLSI circuits and systems, high-performance and low power custom circuit and interconnect design, System-on-Chip, Network-on-Chip, Embedded system design, Wireless Sensor Network, Energy harvesting, and Error Correcting Code. He is a member of Institute of Electrical and Electronics Engineers (MIEEE) and International Association of engineers (IAENG). Since 2010, he is Department CCSE Coordinator for Industrial and Society Networking. Since 2009, he became Research Associate for Institute of Advanced Technology, Head of Embedded System and Artificial Intelligence Lab, CCSE, UPM, and also Head of Common Lab for Software, UPM.

Kongkiti Phusavat, Ph. D

Dr. Kongkiti Phusavat is a Professor at Department of Industrial Engineering, Kasetsart University in Bangkok, Thailand. Dr. Phusavat earned his master and doctoral degrees from Department of Industrial and Systems Engineering, Virginia Polytechnic Institute and Sate University or Virginia Tech in the U.S. Dr. Phusavat attended Texas Tech University in the U.S for his undergraduate study in Industrial Engineering. His research and work interests include productivity measurement, quality improvement, performance management, acquisition logistics, design process and systems engineering, value chain management, pedagogical development for basic education, and networked government. Dr. Phusavat is the author of the book-the title of "Productivity Management in an Organization: Measurement and Analysis" and has contributed the chapters to several texts in the areas of process management and improvement. Dr. Phusavat has published over 100 referred journal articles for the past fifteen years. He is currently the Editor in Chief of International Journal of Innovation and Learning (Scopus-indexed journal) and serves several leading international journals and publisher as Senior Advisor, Associate Editor, Editor, Editorial Board Member, and Reviewer. He again received the recognition of 2015 Emerald Literati Award-Highly Recommended Paper. Currently, Dr. Phusavat is working with Thailand's Board of Trade in two capacities- the Chairman of Education and Skills Committee of Joint Foreign Chamber of Commerce in Thailand and a committee in Thai Chamber of Commerce's Education Committee. Dr. Phusavat has been actively working in the areas of education, especially pedagogical research and development in the past five years. Dr. Phusavat was granted the title of Honorary Professor in 2017 from Maria Curie-Skłodowska University, Poland due to his efforts on research and academic collaboration with Kasetsart University. Dr. Phusavat is a regular examiner for the universities in Australia, Finland, Malaysia, and United Arab Emirates. He has been frequently asked to evaluate research and project proposals from the funding agencies in Asia and Europe such as Austrian Science Fund. Finally, he has given the lectures and has conducted the workshops at several universities in Australia, Finland, Hong Kong SAR, India, Indonesia, Malaysia, Poland, South Korea, Taiwan ROC, Slovenia, and the U.S.

ORAL PRESENTATION

Effect of hypobaric storage on the quality of hot chili peppers

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Abstract. Hot Chili Peppers is a commodity that plays an important role in the economic, food and pharmaceutical aspects of the world seen from the demand level that always increases annually. These commodities are easy to have physically damage such as weight loss, color faded, and decay so it can degrade the quality. Futhermore, Hot Chili Peppers is rotten rapidly during storage and causes 30% damage to Hot Chili Peppers. Therefore innovation storage technology is needed to maintain quality and improve shelf life of Hot Chili Peppers. Hypobaric Storage for Hot Chili Peppers is an alternatives better than cold storage and room temperature. This technology consists of 5 main components are storage room, reservoir tank, vacuum pump, aerator, and control panel. The hypobaric system use controlled space under vacuum pressure and low temperatures so the oxygen rates reduced and respiration rate can be inhibited, also reduces ethylene gas, so the decaying of Hot Chili Peppers can be delayed, then the shelf life becomes longer. The results show an optimal conditions of Hot Chili Peppers with Hypobaric Storage is when using pressure -60 - (- 55) kPa, and temperature 22.9ºC. Hypobaric Storage for Hot Chili Peppers is able to keep the quality of Hot Chili Peppers about 4 times better than room temperature. This is shown by the data where hot chili peppers in Hypobaric storage have water content = 2.11% that lower than room temperature treatment = 3.96%. Vitamin C tests show that hot chili peppers in Hypobaric Storage has an increase in vitamin C = 13.8% and better than the others storage. In addition, hot chili peppers in Hypobaric Storage have weight loss = 3.28% and smaller than the other storage treatments.

1. Introduction

Insufficiency of Hot Chili Peppers supplies caused by inappropreiate process of storage and transportation. The distance between Hot Chili Peppers field to the market increasing the potential of physically damage; Pangidoan et al.[3] Transportation causes damage of Hot Chili Peppers about 30-50% so the Hot Chili Peppers can not to be marketed; Siswadi.[5] Hot Chili Peppers can be harvested 30 times, but through the rainfall season will affect the quality of harvested Hot Chili Peppers. Postharvest treatment is an effort to maintain the quality of Hot Chili Peppers and avoid the growth of mold that causes decay on Hot Chili Peppers due to weather factors and plants disease. Proper post-harvest treatment will maintain the quality and make the shelf life longer so the supply can be sufficient.

There are some existing storage technology of Hot Chili Peppers such as storage in room storage, refrigerator, and ozonation technology. Room storage can not kill microbes so the decomposition still occur, and the shelf life is about 10 days; Pradita.[4] The refrigerator is able to maintain Hot Chili Peppers and the shelf life is 14 to 21 days using low temperatures, but it can not kill Altenaria and Botrytis which cause wet decay on Hot Chili Peppers because the humidity of refrigerator storage can not be controlled; Vicente et al., 2005.[6] Asgar et al [1] found that ozonization is able to kill Alteraria and Botrytis so the Hot Chili Peppers decay can be delayed, and the shelf life becomes longer about 32 to 38 days, but the application of this method requires high cost and dangerous if the ozone be inhaled by humans.

Based on this problems, it is very necessary for innovation storage technology that can be applied to the transportation process. Hot Chili Peppers storage technology with hypobaric system is the solution of the problem. The hypobaric system uses an under atmospheric pressure treatment to reduce the controlled oxygen supply in the atmosphere. The optimal pressure that can be used is -60 - (- 55) kPa when the temperature is 22.9 ° C. This technology can be applied as an instrument to support the distribution of Hot Chili Peppers by integrating to the distribution transportation.

2. Materials and Methods

This programs done for 5 months from April to August 2018. Machine manufacture and testing is done at Lastrindo Engineering, Bioindustry Laboratory, and Food Quality and Food Laboratory of Brawijaya University.

2.1. Materials and tools

The tools used for the manufacture of Hypobaric Storage are drill, grinding, solder, hammer, ruler of iron, screwdriver, avometer, pliers, wrench, cable cutter, and welding machine. Materials used are glass tube, water tank, thermocouple, thermostat, box control, nut, bolt, stainless steel, plug-in, power button, cable, pressure control, pressure sensor, vacuum pump, pump tube, relay, and Hot Chili Peppers.

2.2. Components and Mechanism of Hypobaric Storage

2.2.1. Components of Hypobaric Storage

a. Storage Room Desain

The hardware of this instruments consists of storage room, reservoir tank, aerator, vacuum pump, temperature sensor, pressure sensor, control panel. Storage room is made using a glass tube which the capacity is 300 grams. The function of glass tubes is to optimize the work systems of the hypobaric. The function of a low pressure vacuum is to make the hypobaric system works only in a room which no angle in it. Storage room is a a place to store the Hot Chili Peppers during the treatment. This treatment use a vacuum condition in storage room to keep Hot Chili Peppers shelf life.

Figure 1. Storage Room

b. Reservoir Tank Desain

The function of reservoir tank is to store air reserves. This section is made by using a water tank which the capacity is 19 liters. Reservoir tank is connecting between the vacuum pump and storage room. The use of this component is intended to store air reserves from the vacuum pump, so that if the air pressure in the storage room is too low, the air will flow in the reservoir tank, and if the air pressure in the storage room is too high, the system will suck up the air of storage room and stored in a tank reservoir.

Figure 2. Reservoir Tank

c. Aerator Desain

Aerator is a component that maintain the temperature and humidity in the storage room. This component is made by using a water tank. The aerator works automatically, when the temperature inside the storage room exceeds the specified temperature of 22,9 ˚C, the aerator will drain the water vapor into the storage room. The principle of the aerator is water will flow into the tank and will be evaporated using a pump, then water vapor will be flow into storage room. This section will be connected with vacuum pump and storage space.

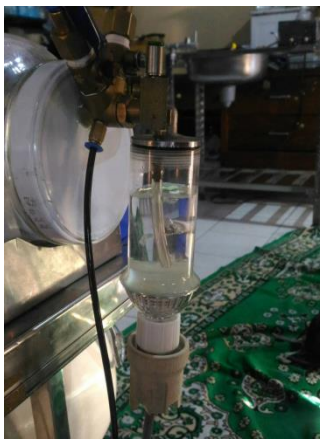

Figure 3. Aerator

d. Temperature and Pressure Sensor Desain

Temperature and pressure sensors are used to determine the temperature and pressure of the appliance. Temperature sensors are placed in storage room and connected by temperature control and aerator. The temperature of the storage room will be read the temperature sensor, then it will be displayed on the temperature control, if there is an inconsistent of room temperature with optimum temperature, then the aerator will work. This tool uses 2 pressure sensors. The first sensor is placed in the storage room and connected with the tank reservoir. This sensor reads the pressure on the storage room. While the second sensor is placed in the reservoir tank and connected with the vacuum pump. This sensor reads the pressure in the tank reservoir.

e. Control Panel Desain

The control panel function is to control the conditions contained within the tool. The control panel consists of ON / OFF button, temperature control, and 2 pressure controls. This control will displaying the temperature and pressure read by the sensor, so that other components of the aerator, and reservoir tank, and vacuum pump can work automatically if there was an inconsistent.

Figure 4. Control Panel

f. Additional Components Desain

The additional components are a support table. This table is made of stainless steel that serves to support the tool and the connected components. This component used to facilitate the transportation process easier.

2.2.2. Mechanism of Hypobaric Storage

Hot Chili Peppers can be stored in Hypobaric Storage in the following procedures. First, put Hot Chili Peppers into storage room. Second, connect the Hypobaric Storage with the power source. Third, turn on the power of pressure and temperature. Fourth, set the optimal temperature and pressure. Then, the system will automatically work and hot chili peppers can be stored inside until it will be used. The result of this storage treatment is make the hot chili peppers shelf life longer until 40 to 45 days.

These research have some tests on Hot Chili Pepper by comparing the treatment between Hypobaric storage, refrigerator, and room temperature for 4 days. The tests involve test of vitamin C, water content, and weight loss. Vitamin C test uses iodimetry titration method; Damayanti and Kurniawati, 2017.[2] Meanwhile, for the test of water content and weight loss is using the formula % = (*A-B*) / *A* x 100%.

3. Results and Discussions

3.1 Quality Effect of Hot Chili Peppers Storage on Treatments

The test was carried out for 4 days, where hot chili peppers was stored in 3 different treatments are Hypobaric storage, room temperature, and refrigerator. The results of the tests show that hot chili peppers stored in Hypobaric Storage have better quality than the other storage. This is shown by the data where hot chili peppers in Hypobaric storage have water content = 2.11% that lower than room temperature treatment which is 3.96%. Vitamin C tests show that hot chili peppers in Hypobaric Storage has an increase in vitamin C = 13.8% and better than the others storage which the room temperature does not increased nor decreased, and the refrigerator has a decreased on vitamin C to 0.1%. In addition, hot chili peppers in Hypobaric Storage have weight loss = 3.28% and smaller than the other storage treatments. Where chili in room temperature and refrigerator have decreased to 9,7% and 6.66%.

Table 1. Contents of Hot Chilli Peppers

	T = 4 days		
Parameter Tests	Hypobaric Storage	Room Temperature	Refrigerator
Water content	2.11 %	3.96 %	0.26 %
Vitamin C	13.8 %	0 %	-0.1%
Weight loss	3.28 %	9.7 %	6.66 %

3.2 Color and Textural Analysis

Based on the test showed that Hot Chili Peppers from Hypobaric Storage has a hard texture and looks fresh, from the color parameter Hot Chili Peppers has a relative low red mean value of 212, while red energy and red homogenity show the highest number compared to other treatments. Red mean indicates the mean red value, while red energy and red homogeneity indicate the homogeneity of red in chili. Based on the results of color tests using image processing concluded that Hypobaric Storage able to inhibit the ripening of Hot Chili Peppers.

Table 2. Results

T=4 days			
Parameters	Room Temperature	Refrigerator	Hypobaric Storage
- Color with Image Processing			
Red Mean	231	194	212
Red Energy	0.001622	0.001727	0.002144
Red Homogenity	0.4035193	0.410734	0.433484

4. Conclusions

Insufficiency of Hot Chili Peppers supplies caused by inappropreiate process of storage and transportation. Proper post-harvest treatment will maintain the quality and make the shelf life longer so the supply can be sufficient. There are some existing storage technology for Hot Chili Peppers, but it is not effective. So, the solution is use Hypobaric Storage that more effective than other technologies. The hypobaric system uses an under atmospheric pressure treatment to reduce the controlled oxygen supply in the atmosphere. The optimal pressure that can be used is -60 - (- 55) kPa when the temperature is 22.9°C. This technology can be applied as an instrument to support the distribution of Hot Chili Peppers by integrating to the distribution transportation. The results of these tests are hot chili peppers in Hypobaric storage have water content = 2.11% that lower than room temperature treatment = 3.96%. Vitamin C tests show that hot chili peppers in Hypobaric Storage has an increase in vitamin C = 13.8% and better than the others storage. In addition, hot chili peppers in Hypobaric Storage have weight loss = 3.28% and smaller than the other storage treatments. Based on the color and textural analysis showed that Hot Chili Peppers from Hypobaric Storage has a hard texture and looks fresh. Futhermore, from the color parameter we can conclude that Hypobaric Storage able to inhibit the ripening of Hot Chili Peppers.

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Formulation of premix for making the Indonesian Empek-empek

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Abstract. Empek-empek is one of authentic food from Indonesia which made from mackerel fish, tapioca starch and spices. The objective of this present work was to produce premix flour using mackerel powder, tapioca starch and spices that allow making empek-empek and to evaluate their chemical properties and sensory acceptability of the products. Mackerel was first dried, miled into powder form then mixed with tapioca starch and spices. According to the obtained results, empek-empek which made from Premix-D formula (25% mackerel powder and 75% tapioka starch) has the best result for all sensory attributes. It presented hedonic score like moderately for all sensory attributes. Ash, protein, carbohydrate, fat and moisture contents of Premix-D formula were 5.03%, 16.82%, 67.32%, 1.13%, 9.69%, respectively, which met the SNI requirements. Therefore, it can be concluded that empek-empek can be prepared using premix flour with less complicated process and the product did not show any negative results on sensory attributes.

1. Introduction

Empek-empek is widely known as an authentic food from South Sumatera, Indonesia, which made from fish paste, tapioca starch, and other additional ingredients. This product is served with sweet and sour sauce. Mackerel fish is commonly used as the main ingredient of empek-empek. The sharp flavor from mackerel makes empek-empek has a unique taste and flavor.

The preparation of empek-empek consists of several complicated steps and takes a quite long time. In addition, the availability of raw mackerel and product quality were also a problem in empek-empek production. Therefore, premix technology is one of an alternative solution to overcome the problems in empek-empek production.

Premix is a mixture of several different types of flour and generally used in baking industries [1]. Premix technology purposed is to make a product which consist a balanced formula, easy to use and has along self-life [2]. Determination of the premix formulation based on the ratio of amylose and amylopectin in starch. Premix technology itself can reduce the cost and the space of the raw material storage room. Moreover, optimization of labor and simply sanitazion can be reached also the quality and product standardization can be controlled [3]. In this study, we described the premix production using mackerel powder, tapioca flour and spices for making empek-empek and evaluated their chemical properties and sensory acceptability of the product.

2. Materials and Methods

2.1 Mackerel Powder Preparation

The mackerel fish was cleaned from skin, bones, fins, and gill. The meat fish was steamed for 10 minutes then pressed to remove water and oil. Steamed mackerel was dried using blower for 18 hours at 55^0C until the water content reach 5.37%. The dried mackerel then grinded using grinder and sieved using a 100-mesh sieve.

2.2 Premixes preparation

There are five premixes were formulated by mixing mackerel powder, tapioca flour and spices. Mackerel powder was mixed with tapioca flour with the following ratio displayed in Table 1. The spices then added into the mixture as much as 6% of the total volume of the mixture. The premix formulas and spices compositions could be seen in Table 1 and Table 2, respectively.

Table 1. Ratio of surimi powder and tapioca flour of premix formulas

Formula	Mackerel Powder (%)	Tapioca Starch (%)
Premix-A	10	90
Premix-B	15	85
Premix-C	20	80
Premix-D	25	75

Table 2. Spices composition of the premix

Spices	Quantity (%)
Garlic powder	2
refined salt	4

2.3 Preparation of Empek-Empek

Empek-empek was made by kneading each formulated premix and cold water (1:1) into dough. The dough was formed into cylinder form with a diameter of 2 cm and length 10 cm then boiled for 10 minutes at 90-100^{0}C and fried until the color changes into brownish yellow. Freshly prepared empek-empek was analyzed for sensory attributes.

2.4 Sensory Analysis

Empek-empek made by premix formulas were subjected to sensory analysis for attributes of aroma, texture, color, and taste using Hedonic Scoring Scale [5]. The scoring scale used was between 1-5 with the scores representing the hedonic attributes of 5,4,3,2,1 were “like very much”; “like”; “like moderately”; “dislike”; “dislike very much”, respectively. The samples were tested by 30 panelist.

2.5 Chemical Analysis

The chemical compounds of the premix best formula and empek-empek product were measured using AOAC methods [6]. Ash content was measured by weighing and furnace methods at 600°C for 3-5 h (942.05, 4.1.10). The protein content was measured using kjeldahl distillation and the nitrogent value was converted to protein value using conversion factors (960.52, 12.1.07). Oven drying and weighing methods (926.12, 41.1.02) were used to measure the moisture content. Fat extraction using sohxlet distillation and chloroform as a solvent was used to measure the fat content (948.22, 40.1.05). The carbohydrate content was measured by difference method.

3. Result and Discussion

3.1 *Sensory* analysis

Sensory analysis was an important test in the product development [2]. Because, the acceptability of a new product was always determinated by the consumer of point of view [7]. The best formula of the premix was evaluated by making empek-empek from each formula then subjected to sensory acceptability. The results was showed in Figure 1.

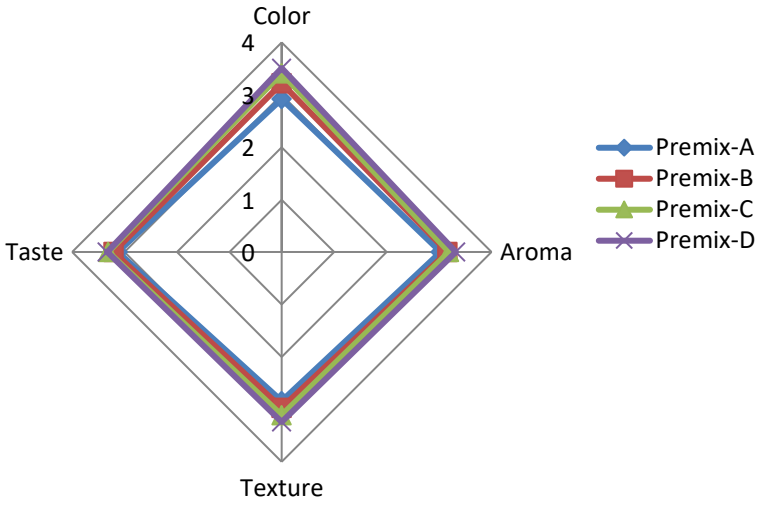

Figure 1. Sensory analysis of empek-empek

Figure 1 showed that empek-empek which made from Premix-D formula has the highest score for all sensory attributes while Premix-A has the lowest. Product with Premix-D formula was scored 3.5 for color attributes. On a scoring scale, these corresponded to near "like" or "like moderately". The score for aroma also 3.3 for Premix-D formula while Premix-A formula given the lowest score 3. This may be due to the ingredients used. The aroma was mostly contributed by mackerel powder and spices. Strong fishy aroma was a result from high amount of mackerel powder in Premix-D. On the other hand, the color attributes was also contributed by mackerel powder. The color of empek-empek turned into brownish yellow affected by Maillard reaction. This reaction caused by the reaction between reducing sugars and primary amine groups at high temperatures [8].

The score given for attribute taste and texture of Premix-D formula were 3.2 and 3.3, respectively. The texture of empek-empek was contributed by ratio of tapioca flour and mackerel powder. The ratio of amylose and amylopectin which contained in tapioca starch, also the protein which contain in mackerel powder could made a chewy texture which favored by panelist. Mackerel powder also provide a unique fishy taste which contributed to the taste of empek-empek.

3.2 Chemical composition of premix

The chemical composition of premix-D formula and empek-empek product were showed in Table 3. The moisture content was indicated the amount of water per unit weight of material and it was the most fundamental parameter because it affected the shelf life of the product [2]. The moisture content of the premix-D formula was 9.69% indicated that our products meet the SNI requirements. The standard of the premix flour referred to the standard of the fish flour. The water content of the fish flour was maximum of 10% according to the SNI number 01-3709-1995 [9]. Drying process of the mackerel was the key factors of the low moisture content of the premix flour. In this study, the mackerel was dried until the moisture content reached 5.37%. Moisture content from different premix also have been reported. The moisture content of the premix for preparing otak-otak was 4.28% [2] while premix for preparing flat breads and noodles were 4.05% [7]. The wide range of moisture content of the premix flour was caused by the moisture content of raw materials and drying process [2].

Table 3. Chemical composition of Premix-D and empek-empek products

Constituents	Premix-D (%)	Empek-Empek (%)
Moisture	9.69	37.80
Fat	1.13	1.62
Protein	16.82	10.82
Ash	5.03	2.38
Carbohydrate	67.33	47.38

The fat content of premix was low at 1.13% due to steaming and pressing process in mackerel powder production. Steaming and pressing process in mackerel powder production reduce the moisture and fat contents. High temperature could accelerate the fat molecules resulting the distance between fat molecules becomes large so the fat could be easily to remove [10]. The fat content of Premix-D formula also meet the FAO standard. According to the FAO, fat content of fish flour was maximum at 3% [11]. The fat content of the empek-empek mostly contributed from frying process. Otherwise, fish was known as the source of protein and this contributed to the high level of protein in Premix flour. Protein content of Premix-D formula was high at 16.82% which contributed by mackerel powder which has high level of protein. Mackerel fish has high protein content and low fat. The protein and fat contents of mackerel fish were 27.6 % and 6.5%, respectively [12]. Similary, the carbohydrate content was also high at 67.33% which influenced by tapioca starch.

4. Conclusion

It was concluded that the best formula of the premix for making empek-empek was Premix-D formula which consisted of 25% mackerel powder and 75% tapioca flour. It was presented the hedonic score "like moderately" for all sensory attributes. Premix-D formula contained ash, protein, carbohydrate, fat and moisture as much as 5.03%, 16.82%, 67.32%, 1.13%, 9.69%, respectively. This study clearly showed that it was possible to make empek-empek with less complicated process and the product did not have any negative results on sensory perception.

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Feasibility study of small scale ginger essential-oil agro-industry using indirect steam distillation

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Abstract. Indonesia is well known as one of main volatile-oil producers especially for patchouli, nutmeg, and clove, and also ginger (*Zingiber officinale* Rosc). The purpose of this research was to study the feasibility of small scale ginger essential oil agroindustry based on several aspects namely market, technical, and financial aspect. The method to be used in this research was descripive method. Information and data obtained would be processed and analyzed based on market aspect, processing aspect, and financial aspect. The results showed that small scale ginger essential-oil agroindustry using indirect steam was feasible due to the increasing trend of essential-oil demand in market aspect; increasing trend of the raw material availability and high yield of ginger essential-oil using indirect steam system in processing aspect; and Rp. 733,294,087.29 of NPV, 1.281 of Net B/C; 15% of IRR of 15%, and 1.69 years of PP in finansial aspect..

1. Introduction

Essential oils are one of the world's natural products that is widely used as basic ingredients for medicines, perfumes, food flavor and preservatives, aromatherapy, vegetable pesticides, and so on. The usefulness of various essential oils causes essential oils become one of the export commodities that generate high devisa for Indonesia that reached US$120,000,000, while the world essential oil trade value was estimated at USD 4,000,000,000 [1]

Essential oil products from Indonesia were known dominant in the world market. Indonesia has even become the main supplier of 3 commodities namely patchouli oil which is widely used in the perfume industry, nutmeg oil as one of the ingredients for making cola drinks, and clove leaf oil. Data from the Indonesian Essential Oil Council (DAI) showed that patchouli oil from Indonesia suplied 90 percent of world market, followed by nutmeg oil around 80 percent, meanwhile clove leaf oil supplied 70 percent [1].

In addition to these 3 commodities, ginger is popular in Indonesian society because ginger has health benefits because it contained around 2-3% of essential oil, starch resin, organic acids, malic acid, oxalic acid and gingerin. The main components of ginger essential oil that produced distinctive aroma of ginger were zingiberen, gingerol, shagaol, and resin [2].

Eventough ginger has good prospect to be further proceesed into essential oil using destillation process, the ginger farmer mostly sold in in form of fresh ginger or simple ginger products in form of sliced and dried. Therefore, this research conduct feasibility study of small scale ginger essential oil agroindustry using indirect steam based on market, processing, and financial aspects [3].

2. Materials and Methods

1. Research Design

Research design was used descriptive method and focused on problem solving using quantitative and qualitative data that were obtained from experts, questionnaire, and related *literatures.*

2.1. Market Aspect

Market aspect was conducted using essential oil market data from related sources and would be analyzed by appropriate forcasting method [3]

2.2. Processsing Aspect

Processing aspect was conducted using raw material availabilty data from related sources and ginger essential oil destilation yield data from destillation process of ginger using small scale destilation model using indirect steam (200 kg of raw material capacity).

2.3. Financial Aspect

Financial aspect was conducted using NPV, IRR, Net B/C, and PBP value of small scale ginger essential oil agroindustry [3}

3. Results and Discussion

3.1. Market Aspect

The result of ginger essential oil market aspect analysis showed that Indonesia export amount and value of essential oil, cosmetics, and perfumes were increase from US$ 637 in 2015 became US$ 694 in 2016 and then US$ 716 in 2017 [5].

The other condition that supported the market aspect of ginger essential oil was the price of ginger oil in the European market from China was quite high, ranging from US$ 42 per kilogram, meanwhile ginger essential oil from India was US$ 105 per kilogram [6]. With such a high price, it also gave an opportunity that ginger essential oil from Indonesia could match the value of the ginger essential oil from India.

Based on this condition, market potential for essential oil, included ginger essential oil, still available and there were opportunity to increase in the future.

3.2. Processing Aspect

The result of ginger essential oil processing aspect analysis showed that ginger as raw materials was relatively increased in amount based on harvest area (Tabel 1)

Tabel.1. Ginger harvest area in Lampung Province (Year 2012-2016) [7]

Year	Harvest area (ha²)
2012	127.45
2013	175.11
2014	245.31
2015	175.80
2016	103.66

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By using a model of small scale indirect steam distillation with 200 kg raw material of capaciy showed that the the average yield of ginger essential oil was 2.16 percent (0.8841 of density) (Figure 1).

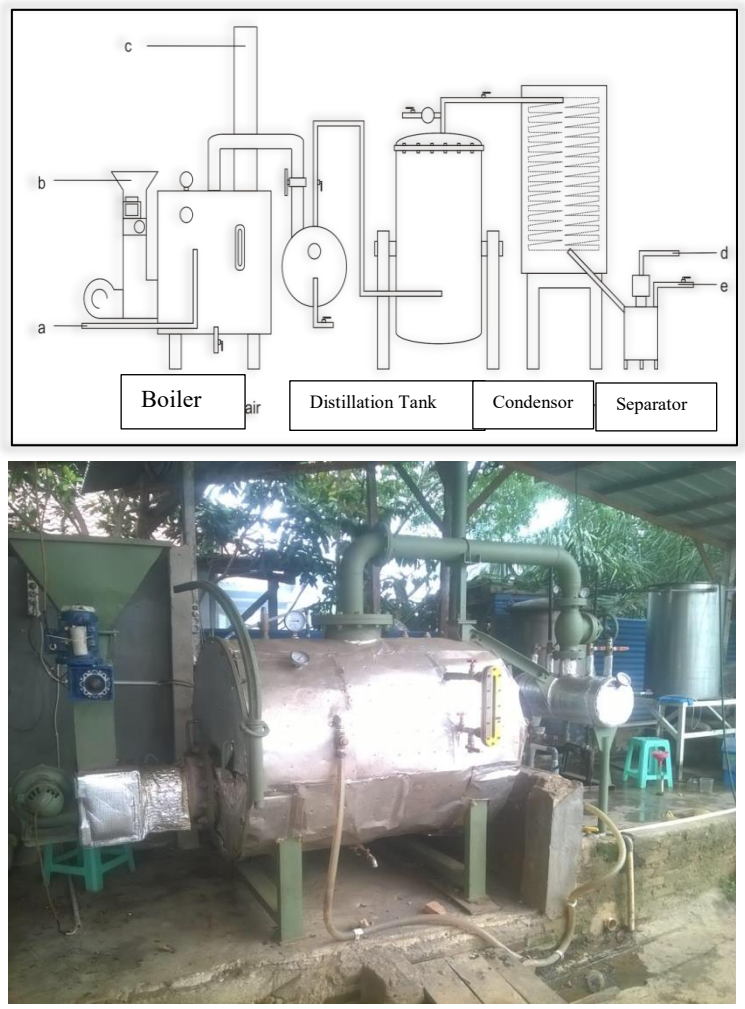

Figure 1. Schematic and Distillation Unit using Indirect Steam Model

3.3. Financial Aspect

The result of financial aspect analysis showed that small scale ginger essential oil agroindustry using indirect steam was feasible with Rp 736,665,862,00 of NPV; 1.28 of Net B/C, 14 percent of IRR, and 1.78 year of PP. The result of sensitivitas analysis showed that the project was feasible if raw material price increased up to 20 percent and product price decreased up to 14 percent.

4. Conclusion

Small scale ginger essential oil agroindustry using indirect steam was feasible based on market aspect namely increased of essential oil export value and high price of similar ginger essential oil; processing aspect namely availability of raw materials and relatively high yield of ginger essential oil produced, and financial aspect with value that meet the standard.

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The analysis of textural, sensory and financial of ganyong flour snack bar (*Canna edulis* Ker.) and hunkwee flour product

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Abstract. Indonesia is a country that has fertile land; various types of plants can grow in the region of Indonesia such as *ganyong* tuber. Processed products which similar to *ganyong* flour cakes are still rarely used. Most of *ganyong* starch is used as the basic ingredient for making *su'un* or *ganyong* vermicelli. The purpose of this study is to find out the textural characteristics as well as the overall preferred treatment on the snack bar and to know the financial analysis in production of snack bar of *ganyong* flour and *hunkwee* flour. The research method used is Randomized Block Design using 2 treatments; those are *ganyong* flour and *hunkwee* flour formulation with the addition of carrot and bark contents with repetition twice. The result of hardness of snack bar is in the range of 1993,77 up to 4553,85. The level of elasticity (springiness) is in the range of 0.855 up to 1.3353. The level of cohesiveness is in the range of 0.473 up to 0.5936. The overall sensory preference of snack bars shows that the most preferred treatment is a snack bar with 30% *ganyong* flour formulation and 70% *hunkwee* flour with 50% carrot stuffing and 50% zalacca and 30% *ganyong* flour formulations and 70% *hunkwee* flour with 30% carrot filling and 70%. Zalacca. The result of financial analysis of *ganyong* and *hunkwee* flour snack bar shows a value of HPP that is IDR 1,673, B / C Ratio 1.554, payback in period of 2 years 3 months with 1,921 units of BEP product.

1. Introduction

Indonesia is a country which has a fertile soil. Different types of plants can be grown in the region of Indonesia. Not only the types of fruit, vegetables, Indonesia is also suitable for planting different kinds of tubers. Types of tubers such as Amorphophallus, sweet coconut, gadung, dioscorea esculenta, ganyong, irut, and some types of talas-pronounced/kəˈleɪdiəm, all of which are potentially as a source of carbohydrate [1]. Tuber ganyong (*Canna edulis* Ker.) is one of the plants containing phosphorus, iron and calcium is high [2]. In addition we also have ganyong the content of carbohydrate and high fiber [3]. Processed products made from a kind of cake flour ganyong is still rarely used. Ganyong starch plumpness is used as raw material in the manufacture of su'un or the ganyong vermicelli. The snack bar is a product of the food interludes are ready to eat in the form of bars made from cereals or legumes [4]. Binding material in the manufacture of snack bars namely ganyong and flour hunkwee flour. The function of the binding material namely as an adhesive in the manufacture of snack bars. The purpose of this research is to know the characteristics of flour snack bars tekstural ganyong and hunkwee flour as well as knowing the preferred treatment of the whole snack bars hunkwee ganyong flour and flour and knowing the financial analysis in manufacture of snack bars and ganyong flour hunkwee flour.

2. Research Methods

2.1. Time and place of Research

This research was carried out in November 2017 until January 2018. Housed in the laboratory of agricultural Industry Technology Faculty of Agriculture University of Madura Trunojoyo Laboratory include waste management, Process Engineering Laboratory and laboratory Analysis Quality.

2.2. Tools and materials Research

Tools used in the manufacture of snack bars and ganyong flour hunkwee flour i.e. analytic scales, footbath, spoon, keeps, baking pan, oven, knives, and mixer. And tools for the manufacture of flour ganyong i.e. cabinet dryer, grinder and 100 mesh sieve. While the tools used in the measurement of texture that is texture analyzer. While the materials used in the manufacture of snack bars namely hunkwee ganyong flour, flour, carrots, fruit slak, eggs, skim milk, sugar, butter and salt.

2.3. The Process Of Making

2.3.1. The manufacture of Flour Ganyong

Process of making a tuber starch ganyong starting from peeling tuber ganyong and washed to clean then sliced thin with transverse. Heated cabinet into the dryer with a temperature of 60 ° C for 5 hours. After a wedge bulbs dry ganyong conducted the process of diminution of sizes using a grinder and then sifted with 100 mesh sieve.

2.3.2. Manufacture of Snack Bar

The process of loading a snack bar starting from mixing ingredients such as eggs, sugar, butter, salt and skim milk. Further mixing the dough and flour ganyong hunkwee. afterwards enter stuffing carrots and salak and then oven for 30 minutes.

2.3.3. Research Design

The experimental design used was Completely Randomized Block Design (using the 2 treatments namely ganyong flour and flour formulation hunkwee with the addition of stuffing carrots and salacca.

2.3.4. Parameters Research

As for test done on this research includes testing of the texture with the parameter test of hardness, cohesiveness and springiness. Test using color colour reader. Test of water activity aw free use or water meters. Test the moisture content and sensory tests as well as financial analysis is performed.

3. Results and Discussion

3.1. Hardness Test Texture, Cohesiveness and Springiness

Table 1. is the average value of hardness (hardness) snack bar on some formulation of stuffing. From the table it can be known that snack bar has the value of 4.169 × 103 to 2.893 × 103. The more the addition of stuffing carrots at snack bar make the texture violence snack bars being high. And the lower value of the hardness of a snack bar caused the lower addition of salak fruit.

Table 1. The average value of Hardness (Hardness) Snack Bar on some Formulations Stuffing

Carrot Stuffing formulations and *Salacca zalacca*	Hardness
50:50	3.464×103ab
70:30	4.169×103b
30:70	2.893×103a

The value of hardness (hardness) snack bar ganyong flour and flour hunkwee be between 1993.77 up to 4553.85. Escalating violence occurred on the snack bar the addition of flour and ganyong decline in green bean flour. According to Harzau and Teti [5] says that a comparison between the amylose and amylopectin in food can affect the texture of the cookies. Amylopectin in grocery produce adhesive ability which causes the structure of food become sturdy. In addition to the content of amylose and amylopectin, water levels can also affect the value of the product of violence caused the loss of the characteristic kerenyahannya. The higher the moisture content of snack bar then it will occur a violent debasement [6].

The value of the rate of elasticity (springiness) snack bar ganyong flour and flour hunkwee be between 0.855 up to 1.3353. The influence of degree of elasticity in the snack bar is affected by the addition of flour content of hunkwee. This is because the green bean flour or flour hunkwee protein. One of the properties of the protein is formed a gel, where increased levels of protein will increase maktriks and starch gel which causes texture more elastic [7]. Protein levels in mung bean is 22.2 g [8].

The value of the level of cohesiveness or cohesive power (cohesiveness) snack bar ganyong flour and flour hunkwee be between 0.473 until 0.5936. Indicate according to Firdausiyah [7] the rising value of the cohesive power caused by the large number of protein content in foodstuffs. This is due to the protein contained in the material can form gels and gel matrix and can increase the starch so that it can increase the power cohesive or cohesiveness. In research Saifudin et al. [6] says that the snack bar has the value-based power cohesive or cohesiveness of 0.27-37. While the research conducted says that the content of power kohesis-based snack bars contain a cohesive power of 0.98.

3.2. Moisture Test

Moisture content in snack bars and ganyong flour hunkwee flour has a higher value when compared to commercial products soyjoy (4.03%) and standard moisture content cookies (5%). Moisture content of snack bars and ganyong flour hhunkwee flour has a value between 31.5 up to 22.375%.

Moisture content of snack bars decreases if the addition of mung bean flour more out of flour ganyong. This is similar to research of Pradipta and Widya [9] that say water levels decrease due to the difference in content of amylose and amylopectin. Amylose have easily absorbs water and removing the water sedagkan it has difficult absorb water but water will be halted if absorbed. Ganyong flour contain 18.6% of amylose and amylopectin of 81.4% [3]. While the green beans have starch content of amylose and amylopectin 33% 67% [9].

3.3. Testing water activity (aw)

Water activity values are a snack bar and ganyong flour hunkwee flour is higher compared to the control bar snack is snack bar commercial value i.e. soyjoy 0.636. The value of water activity on the

snack bar range from 0.877 flour up to 0.833. Free moisture content (aw) at a snack bar ganyong flour and flour hunkwee with various additions of 4.

*3.4. Testing the color of L *, a * and b **

The result of the test color L * use colour reader shows the control value is higher than a snack bar and ganyong flour hunkwee flour. The value of the control that is the value of the test color L * commercial product from soyjoy i.e. 35.06 [10]. While the value of the test color L * snack bar hunkwee ganyong flour and flour has a value of L * range up to 11.775 12.475.

The value of the test color L * snack bar has no difference. This is allegedly due to the addition of flour ganyong probability comparison in the manufacture is not too extreme. Flour ganyong flour has a color Brown allegedly due to enzymatic Browning reaction and non-enzymatic. According to Ekafitri et al. [11] says that the reaction of enzymatic caused by a group of enzymes called polyphenol oxidases. Whereas a non enzymatic reactions caused by the process of roasting, caused by bereaksinya reducing sugar moieties with amin is free from amino acids.

3.5. Sensory Testing

Sensory testing at the snack bar ganyong flour and flour hunkwee includes colour, aroma, texture, flavor and overall favorite.

Table 2. Influence of formulation of sensory characteristics against flour snack bar

Flour formulations	colour	Aroma	Texture	taste	Overall Favorites
50:50	3.089^{b}	3.333^{ab}	3.006^{a}	3.328^{a}	3.267^{a}
70:30	2.872^{a}	3.183^{a}	3.211^{ab}	3.344^{ab}	3.222^{a}
30:70	3.450^{c}	3.457^{b}	3.433^{b}	3.722^{b}	3.572^{b}

In Table 2. be aware that the more hunkwee flour addition at the snack bar then the value of the fondness of colour, aroma, texture, flavor and overall favorite is getting high.

Table 3. The influence of IsianTepung against the Sensory Characteristic Snack bar

Formulation of Stuffing	Aroma	Taste
50:50	3.400^{b}	3.456^{b}
70:30	3.189^{a}	3.311^{a}
30:70	3.334^{ab}	3.628^{c}

In the parameter value Favorites scents ranging from 3.400 up to 3.189. The addition of more and more carrots then increasingly favored snack bars. Taste of favorite average snack bar ranges from 3.628 up to 3.311. So the more penambhan salak fruit at a snack bar then the higher sense of fondness. Overall in fondness snack bar hunkwee ganyong flour and flour have values between 3.1 to 3.634 of graphs can be seen more and more flour addition hunkwee and addition of stuffing salak.

3.6. Financial Analysis

The results of the financial analysis in snack bars and ganyong flour hunkwee flour then obtained the value of HPP i.e. IDR 1,673, B/C Ratio 1.554, payback period for 2 years 3 months with BEP 1,921 products unit. So from the table it can be known that effort a snack bar and ganyong flour hunkwee flour deserves to be executed because the value payback period does not pass the specified limit of 5 years.

4. Conclusions

Hardness snack bar ganyong flour and flour hunkwee be dikisaran between 1993.77 up to 4553.85. The level of elasticity (springiness) snack bar ganyong flour and flour hunkwee be dikisaran between 0.855 up to 1.3353. The level of cohesiveness snack bar ganyong flour and flour hunkwee be dikisaran between 0.473 until 0.5936. sensory Analysis the overall favorite snack bar shows that the most preferred treatment is a snack bar with the formulation of the flour ganyong flour hunkwee 30% and 70% with 50% carrot stuffing and salacca 50% and 30% ganyong flour formulation and flour hunkwee 70% with stuffing carrots 30% and 70% of salacca. The results of the financial analysis of business snack bars hunkwee ganyong flour and flour obtained value HPP i.e. Rp 1,673, B/C Ratio 1.554, payback period for 2 years 3 months with BEP 1,921 products unit.

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The effect of sugar cane (*Saccharum officinarum* L.) cut to mill delay time and natural anti-inversion concentration from Kesambi bark extracts (*Schleichera oleosa* Merr.) on characteristics of brown sugar

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Abstract. Sugar cane has a high sucrose, so it's sensitive to rottenness. Sugar cane rotting usually happens because of enzymatic inversion reaction. Adding anti-inversion from Kesambi bark of Kesambi tree *(Schleichera oleosa MERR.)* can inhibit inversion reaction. Kesambi contains saponin and tannin that can inhibit reaction of inversion by suppressing the growth of microorganisms. The study aims to determine the interaction between variation in cut to mill delay time of sugar cane *(Saccharum officinarum L.)* and concentration of extract Kesambi bark (*Scheicheraoleosa* MERR.) as a natural anti-inversion of characteristic in brown sugar. The study used a Randomized Block of Desaign (RBD) with 2 factors: cut to mill delay time consists of 3 levels such as (6 ± 0.5 hours), (18 ± 0.5 hours), and (30 ± 0.5 hours). As well as by adding exctract of Kesambi bark as the antural anti-inversion that consists of 3 levels (100 ppm, 300 ppm and 500 ppm). Determination of the best treatment uses Multiple Attribute method or Zeleny. The best treatment was obtained from the treatment of brown sugar with a cut to mill delay time 6±0.5 hours with adding concentration of Kesambi bark extraction 500 ppm which : sugar total (91.258%) reducing sugar content (0.681%), yield (10.087%).

1. Introduction

Brown sugar is made from collected sugar cane/*siwalan* which boiled and stirred slowly until it reached the specific thickness than moulded and cooled. One of the problems of this study is about the high water content in brown sugar which able to reduces the durability of brown sugar [1].

It might be the cause of inversion in sugar cane. Sugar cane has a high sucrose content it makes it so sensitive to rottenness. Microbial infection into the sugar cane happens in the harvesting process where there is contact between the sugar cane with knife or soil. Sugar cane rotting usually happens because of enzymatic inversion reaction by microorganism. There are two kinds of microorganisms that cause inversion reaction which is yeast and bacteria. *Saccharomyce cerevisiae* type of yeast at optimum condition will release the inverting enzyme to hydrolysed sucrose into D-glucose and D-fructose. The reaction mention before is called inversion reaction, the result of this reaction is called inverted sugar or reducing sugar. Inversion reaction might occur because of *Leuconostoc*

mesenteroides bacterial contamination. Inversion reaction is a hydrolysis reaction of sucrose by the inverting enzyme into D-glucose and D-fructose [2]. In the next stage the glucose will be dismantled into gum/mucus by the *Leuconostoc mesenteroides*. The damage on the sugar cane due to inversion reaction is characterized by decreased levels of sucrose on the sugar cane stems, increasing reducing sugar, dextran formation and the taste turns sour, white and slimy forms appearance. If the process carried on with an inverted sugar sap, the brown sugar will be difficult to crystallize and has a tender texture. This tender texture might shorten the durability of the brown sugar [3].

Therefore it is necessary to add anti-inversion in the brown sugar production process so the inversion in the sugar cane can be prevented. Kesambi bark (Schleicher oleosa MERR.) is a natural anti-inversion, which contains saponin and tannin compounds which able to suppresses the growth of microorganism [4].

2. Materials and Methods

The objects used in this study consist of the main objet which is sugar cane PS 862 with in cut to mill delay time of sugar cane (6±0.5 hours), (18±0.5 hours), (30±0.5 hours), and Kesambi bark extract (*Schleichera oleosa* MERR) as a natural anti-inversion divided into three levels (100 ppm, 300 ppm, and 500 ppm). Sugar cane PS 862 obtained from Malang City farmers and the tree bark obtained from Pamekasan, Madura. The material used for chemical analysis are Anthrone, Sodium oxalate, Pb-acetate, $CaCO_3$, standard glucose, filter paper, Nelson solution, Arsenomolibdate reagent, bugger 4, buffer 7, aquadest.

2.1. Tools

The tools used in this study are glassware, spectrophotometer (UV-VIS Brand: HITACI U-2810), analytic scales (Denver Instrument M-310), pH meter (Hana), color reader (Minolta), dry oven, vacuum oven, desiccator (Brand: Simax), electric furnace, porcelain exchange rate. The tools used in the process of brown sugar production are saucepan, blades, scales, wooden stirrers, thermometer, gas stove.

2.2. Design Experiment

The Design experiment used in this research is randomized block design (RBD) which was arranged factorially with two factors. Factor I consist of 3 levels and Factor II consist of 3 levels with 3 replications. These factors are:

Factor I : Cut-mill delay time variation (W)
Level W1 = Cut-mill delay time 6 ± 0.5 hours
W2 = Cut-mill delay time 18 ± 0.5 hours
W3 = Cut-mill delay time 30 ± 0.5 hours
Factor II : Natural anti-inversion concentration of the Kesambi bark (K)
Level K1 = 100 ppm
K2 = 300 ppm
K3 = 500 ppm

2.3. Research Stages

1. Random Selection of sugar Cane in land and cut off
2. Sprayed the solution of natural anti-inversion concentration of the Kesambi bark at the tip and base of sugar cane
3. Waiting Cut- mill Delay time according to experiment
4. Sugar cane grinding proses
5. Than the collected sugar sap filtered using filter cloth in order to separate the sap with fiber and waste.
6. Then the sap boiled using a gas stove at 70^0C for 20 minutes while stirring.

7. If there is brown foam while cooking the sap, the foam will be removed so that the color of the brown sugar doesn't go dark.
8. Than the sap will be thickened by increasing the temperature to 100-110^{0}C while stirring.
9. While the sap thick enough, the temperature will be decreased to 70^{0}C for 20 minutes while stirring.
10. Mould and cooled the brown sugar until it hardened.

3. Result and Analysis

3.1 Kesambi Bark Extract Analysis

Based on the result of the Kesambi bark extract with 95% ethanol with 48 hours maceration duration, the obtained % of tannin content is 19,8% and the saponin test was held quantitatively and the Kesambi bark extract contained saponin compounds which characterized by seeing the stable foam after the analysis.

3.2. Yield

The yield can be found by comparing the produced products with the amount of used material. The yield analysis is carried in order to determine the economic value of a product or material. The higher yield value, the higher economic value so the production is more effective. According to the analysis result, the yield of brown sugar is around 9.89-10.602%. The result of the study shows that the treatment in variation of cut-mill delay time gives significant effect p-value: 0.011 (α <0.05) to yield of brown sugar.

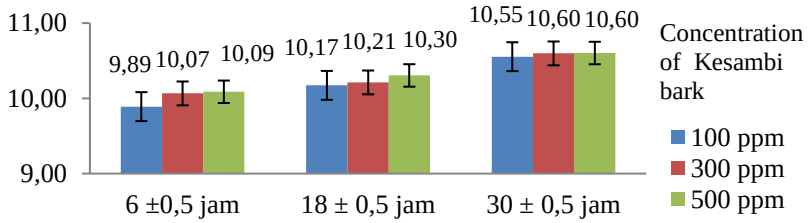

Figure 1. Yield of brown sugar on various combinations of cut-mill delay time variation and natural anti-inversion concentration of the Kesambi bark

The increasing of brown sugar yield might be caused by the cut-mill delay time. This cut-mill delay time makes the inversion happened in the sugar cane. Inversion reaction is a hydrolysis reaction of sucrose by the inverting enzyme into D-glucose and D-fructose. If the inverting enzyme got the optimum pH the reducing sugar will be increased. If the process using an inverted sugar sap (acid pH and high reducing sugar), quality of brown sugar produced will be hygroscopic (easily absorbs water). This hygroscopic quality will increase the yield of brown sugar [2]. The inversion reaction in sugar cane might decreases the pH and increases the reducing sugar. A bigger reducing sugar will result a lower quality of brown sugar. One of them is the high water content in the final product, so the resulting yield is higher [5].

3.3 pH or Acidity

According to the data analysis, pH or acidity in brown sugar is around 6.33- 6.07. The longer cut-mill delay time, the lower pH in the brown sugar and the pH will increases if the concentration of the addition from bark extract concentration increased. The result of the study shows that the treatment in variation of cut-mill delay time gives significant effect p-value: 0.000 (α <0.05) to pH of brown sugar.

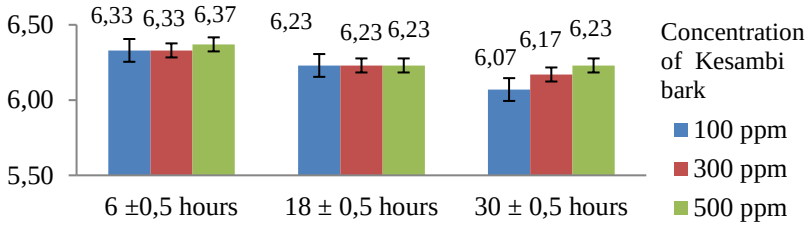

Figure 2. Acidity of brown sugar on various combinations of cut-mill delay time variation and Natural anti-inversion concentration of the Kesambi bark.

Decreasing in pH value due to treatment of cut-mill delay time. The existence of sugar cane cut-mill delay time makes the inverted sugar sap. Inversion process begins when post-harvest in the logging procedure when the sugar cane fiber opened. This opened fiber is contaminated by microorganism. Khamir type of Saccharomyces cerevisiae will hydrolyse sucrose into glucose and fructose [3].

In the second reaction, the formed glucose and fructose are consumed by microorganism and changed into ethanol and forms CO_2. This CO_2 makes foam in the sugar sap. Than the bacterium *Acetobacter aceti* oxidizing the ethanol until it forms acetic acid. This acetic acid makes the sour taste in the sugar sap [2].

3.4. Water Content

According to the analysis result, water content in the brown sugar is around 3.083-4.095%. The result of the study shows that the treatment invariation of cut-mill delay time gives significant effect p-value : 0.0033 (α <0.05) to water content of brown sugar.

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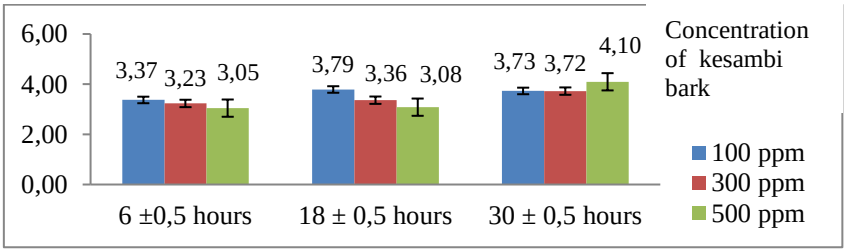

Figure 3. Water content of brown sugar on various combinations of Cut-mill delay time variation and Natural anti-inversion concentration of the Kesambi bark.

The reason of this increasing of water content in the brown sugar is because the cut-mill delay time. This cut-mill delay time making inversion in the sugar cane. Inversion reaction is a hydrolysis reaction of sucrose by the inverting enzyme into D-glucose and D-fructose. If the inverting enzyme got the optimum pH the reducing sugar will be increased. The high reducing sugar will increases more water content because the OH- component increases H+ from the air (hydrolysis process) [4]. If the process using an inverted sugar sap (acid pH and high reducing sugar), quality of brown sugar produced will be hygroscopic (easily absorbs water). This hygroscopic quality will increases the water content of brown sugar [6].

3.5. Total Sugar

According to the analysis result, the total of sugar content in the brown sugar is around 89.843-91.706%. The result of the study shows that the treatment variation of waiting time mill or

concentration of natural anti-inversion extractof Kesambi bark are not significantly toward total sugar in brown sugar.

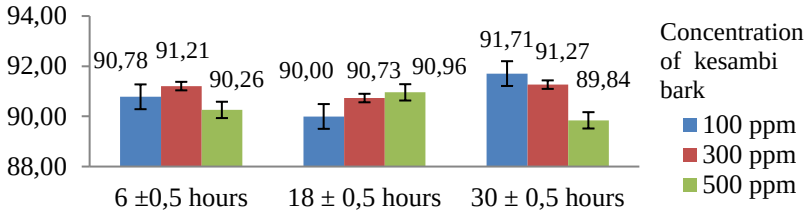

Figure 4. Total sugar of brown sugar on various combinations of cut-mill delay time variation and natural anti-inversion concentration of the Kesambi bark.

The high sugar value in this brown sugar value is because the detected sugar wasn't only the sucrose, but the reducing sugar also included in the calculation. Cut-mill delay time and the addition of Kesambi bark extract is not giving a significant effect towards the total amount of sugar value in the brown sugar, although the longer cut-mill delay time would affect the reducing sugar level in the brown sugar, but the reducing sugar value could not determine the total amount of sugar in the product.

3.6. Reducing Sugar

The result of reducing sugar data analysis on brown sugar can be seen at Figure 5. The average reducing sugar in the brown sugar is around 1.081-0.681%.. The result of the study shows that the treatment invariation of cut-mill delay time gives significant effect p-value: 0.000 (α <0.05) to reducing of brown sugar. Reducing sugar in the brown sugar increases if the cut-mill delay time is longer, and decreases if the Kesambi bark extract concentration increases.

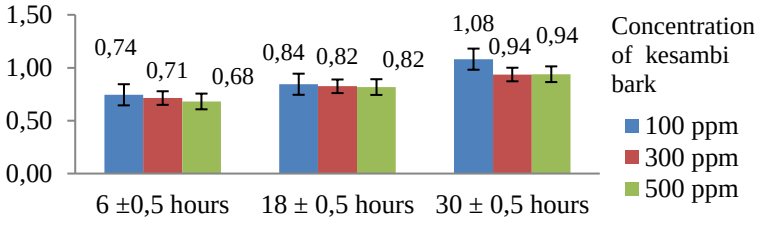

Figure 5. Reducing sugar of brown Sugar on various combinations of cut-mill delay time variation and Natural anti-inversion concentration of the Kesambi bark

The addition of Kesambi bark extract will prevent development of reducing sugar in the inversion on the sugar cane. This happened because the Kesambi bark extract has tannin and saponin component which functions as a natural anti-inversion. The addition of Kesambi bark extract will also hold the decrement of pH in the sugar sap. Tannin has an antibacterial ability done by precipitating proteins, because tannin has a similar effect as phenolic compound, this antibacterial effect happens through reaction with cell membrane, enzyme inactivation and reconstruction or inactivation genetic material function [7]. Saponin compound works as antimicrobial, when saponin reacts to porin (transmembrane protein) on the outer wall of bacterial cells, it forms a strong polymeric bond that damaged the porin. The damage on porin which is the entrance and the exit of the compound will reduces the permeability of the bacterial cell so the bacterial cells will be lack of nutrients and its growth will be hampered [8].

3.7 Ash Content

Ash content data result of brown sugar is around 2.127-2.403. The result of the study shows that the treatment variation of cut-mill delay time and concentration of natural anti-inversion extract of Kesambi bark are not significantly toward ash content of brown sugar.

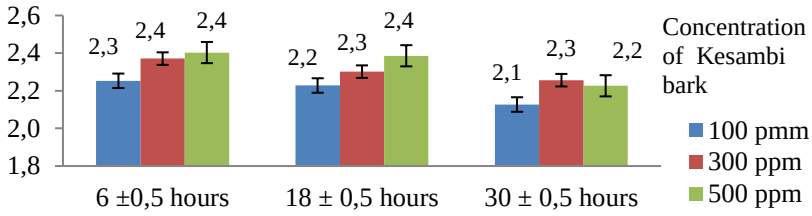

Figure 6. Ash of brown sugar on various combinations of cut-mill delay time variation and natural anti-inversion concentration of the Kesambi bark.

Figure 6 shows that ash content in the brown sugar is increased, then decreases as the cut-mill delay time increases, and increases along with the addition of Kesambi bark extract, the interaction between those two had no significance effect towards the ash content in the brown sugar, it is because the addition of Kesambi bark extract towards brown sugar only 100, 300, and 500 ppm, so It does not significantly affect the ash content of produced brown sugar.

3.8. Clarity (L)

The results of clarity analysis of brown sugar has an average clarity (L) around 45.64- 43.22. . The result of the study shows that the treatment invariation of cut-mill delay time gives significant effect p-value : 0.000 (α <0.05) to Clarity (L) of brown sugar. The aggregate clarity (L) of brown sugar in various combinations in variation cut-mill delay time and concentration of addition Kesambi bark extract can be seen in Figure 7.

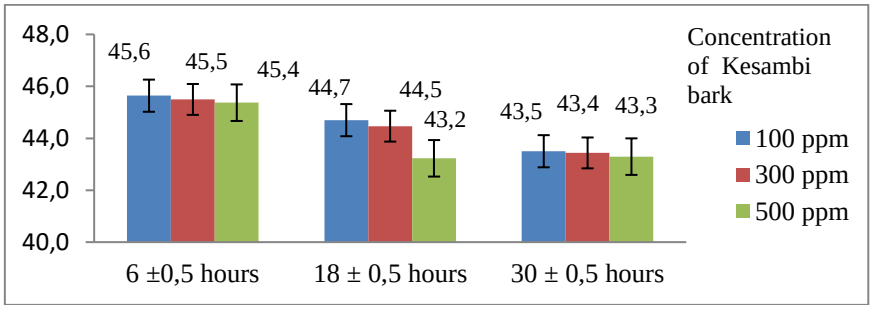

Figure 7. Clarity of brown sugar on various combinations of cut-mill delay time variation and Natural anti-inversion concentration of the Kesambi bark.

Figure 7 shows that the brown sugar clarity intensity decreases or getting darker along with a longer cut-mill delay time. The decreasing clarity (L) on the brown sugar happened because of the cut-mill delay time. The cut-mill delay time makes inverting reaction happen to the sugar cane. This inverting reaction decreases the sugar cane pH, the enzymatic Maillard reaction by microorganism increases reducing sugar, and decreases clarity of the sugar sap. In the processing stage, caramelization reaction will occur in either acidic or alkaline conditions. During the heating process, the caramelization process produces a brown pigment precursor at the dehydration stage [6]. High temperature cooking process is able to removes the water molecule from each sugar molecule to form

a glucose molecule, a molecule that is analogous to fructose. The more caramelized pigment, the darker color will occurs so the clarity value decreases [4].

4. Conclusion

According to physico-chemical parameters, the best treatment was obtained from the treatment of brown sugar with a cut-mill delay time variation of 6±0.5 hours with adding concentration of Kesambi bark extraction 500 ppm which : pH (6.37), sugar total (91.258%) reducing sugar content (0.681%), water content (3.045%), ash content (2.403%), yield (10.087%), clarity (L) (45.37).

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The effectivity of lytic bacteriophage FR38 to decrease *Salmonella* P38 indigenous on milk and chicken sausage

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Abstract. The ability of bacteriophage FR38 to lysis an indigenous *Salmonella* P38 from faeces of diarrhea patient has been studied, however its effects on food is not studied yet. This study was conducted to observe the effects of bacteriophage FR38 on milk. Lysis effectivity of bacteriophage FR38 on food was measured on milk. The total colony of *Salmonell*a P38 was counted by surface plate method. The result showed that indigenous bacteriophage FR38 had been able to decrease of indigenous *Salmonella* P38 on fresh milk ($alpha_{0.01}$). Bacteriophage FR38 was effective to decrease of *Salmonella* P38 on milk during 24 hours (940 cfu/ml), 48 hours (1200 cfu/ml) significantly than untreatment ($alpha_{0.01}$). Bacteriophage FR38 was effective to decrease of *Salmonella* P38 on sausages during 24 hours, 48 hours significantly than untreatment ($alpha_{0.01}$).

1. Introduction

Salmonella is a foodborne pathogenic bacteria that cause food borne diseases and water borne disease [1].*Salmonella* were used as an indicator of food hygiene and food safety [2].Contaminant of *Salmonella* on food had been analyzed on orange juice, fresh orange, apple cider product, beverage's product, milk, apple juice and fresh shrimp [3-8]. In Indonesia, decreasing microbe had been done with a chemical preservative. In the fact, the chemical preservatives not only expensive prices, but have a toxic effect. The high prices of the legal preservative, apparently a food producer was using un-legal preservative, such as, formaldehyde, aluminate and hydrogen peroxyde. Un-legal preservative, such formaldehyde, also cause a negative effect on organ and body cell. Base on presentation upon, its needs the other alternative to decrease microbe on food.

Bacteriophagelytic is a preservative alternative on food processing [9]; have an environmentally-friendly characteristic [10]; non-toxic and is easily to be isolated, such as, from humans, cattle, pigs, and chickens [11]; and can be produced [12, 13]. Bacteriophage lytic can be found on environment, earth, water, body, fermented food [14]; vegetable fermentation [15]; and food product. Isolate bacteriophage lytic can be taken from various food kind e.g. cheese, yoghourt [16]; salad, crispy, and lettuce [17].

Bacteriophage application as a biocontrol food, had been used to decrease a microbe contaminant on food, such as, *Bacillus cereus* bacteriophage in outbreaks of food poisoning [18]; psychrotrophic bacteriophage to prevent spoilage process on food [19]; *Xanthomonas* bacteriophage to prevent a spot on tomato [20]; *Listeria* bacteriophage [21] and *Salmonella enteriditis* bacteriophage on melon and apple slices [22]. According Greer [19] that *Staphylococcus aureus* bacteriophage also be applied on milk and *Salmonella enteritidis* bacteriophage on cheese. *E. coli* bacteriophage on beef steak [23]; *E. coli* bacteriophageon food processing [24]; *Flavobacterium columnare* bacteriophage on fish [25]; *Listeria* and *E. coli* bacteriophage on meat [26].

The others application of bacteriophage was as a microbe therapy, such as, by using *Salmonella enterica* bacteriophage [27];*Yersinia pestis* [28]; Mycobacterium bacteriophage [29]; *vibrio cholera* bacteriophage [30]; *Actinomycetes* bacteriophage [31]; bacteriophage of methicillin resistant *S. aureus* [32]; *Bacillus antrachis* bacteriophage [33]; *Listeria monocytogenes* bacteriophage [34]; bacteriophage of bacterial resistance to antibiotic [35]; and *Ecoli* O18:K1:H7 bacteriophage [36]. According to Sillankorva et al. [37], bacteriophage therapy on poultry had been done by using of *Salmonella enteriditis* bacteriophage. The result research of Budynek et al. [38], point out that bacteriophage therapy on cancer patient can decrease the incident of microbe infect significantly. Ghaemi et al. [39] reported that bacteriophage therapy on tumor can be done by use of □-bacteriophage. Budiarti et al. [40] reported that EPEC (Enteropathogenic *Escherichia coli*) can be degraded of bacteriophage isolated from environment.

On pre-study, Bacteriophage FR38 had been used to decrease of *Salmonella*P38 indigenous on nutrient broth media. The result of study to point out that Bacteriophage FR38 indigenous had been able to decrease *of Salmonella* P38 indigenous on nutrient broth media. Furthermore, the effectivity of lytic bacteriophage FR38 to decrease *of Salmonella* P38 on milk was unknown. The aim of this study was to observe the effectivityoflytic bacteriophage FR38 to decrease *Salmonella* P38 indigenous onmilk, sausage, and water.

2. Materials and Methods

2.1. Bacteriophage Production

Palette of *Salmonella* P38 indigenous culture (OD=1) are 10^{8} cfu/ml were dropped by bacteriophage FR38 (1 ml) (Sri Budiarti collection), then bed one *vortex* and were incubated at 37ºC for 30 minutes. The cocktail of *Salmonella* P38bacteriophage were cultivated in 49 ml of NB (Nutrient Broth) medium, were incubated at 37º C for 24 hours. After 24 hours incubation, bacteria-bacteriophage cocktail were centrifugated with 2800rpm speed (Backman GPR Centrifuge), at 4ºC for 20 minutes. Supernatan (3 ml) were took by use a syringe (vol. 5ml) and be done the filtration process by use amilipore's membrane 0.22 µm (Whatmann). The supernatant results from filtration process were moved into sterile tube [41]. After done the double overlay process, the bacteriophage were counted by use Clokie and Kropinski formula, which is, bacteriophage total = $1.59.\ 10^{7} \pm 2.449.10^{7}$pfu/ml.

2.2. Experimental Design

The milk processing with bacteriophage FR38 treatment was designed in Figure 1. The sausages processing with bacteriophage FR38 treatment was designed in Figure 2. The milk and sausages sample of treatment (control and bacteriophage FR38 treatment) were contaminated by indigenous *Salmonella* P38(4.3×10^{4}cfu). The bacteriophage treatment was added 3.8×10^{4}cfu of bacteriophage FR38.The research design were the randomized design. Experimental design for this research wererandomized group design, with model design as follows:

$$Y_{ij} = u + A_i + E_j.$$

2.3. Data Administration

After given the treatment for 0, 24, 48 hours, the total of *Salmonella* P38 and nutrient content of the milk was counted.

2.4. Statistical Analysis

Statistical analysis was carried out using student's t-test. The results are presented as the mean differences between individual groups with P (less than or equal to) 0.05 considered statistically significant.

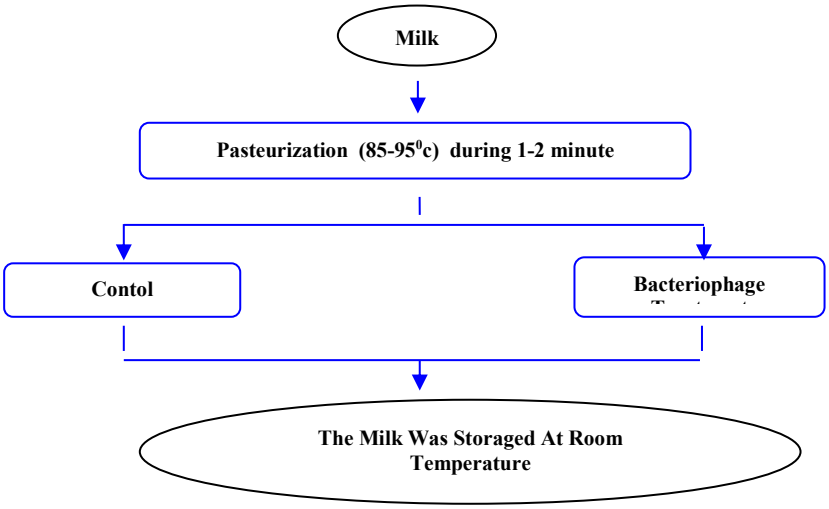

Figure 1. Application procedure of bacteriophage FR38 on milk

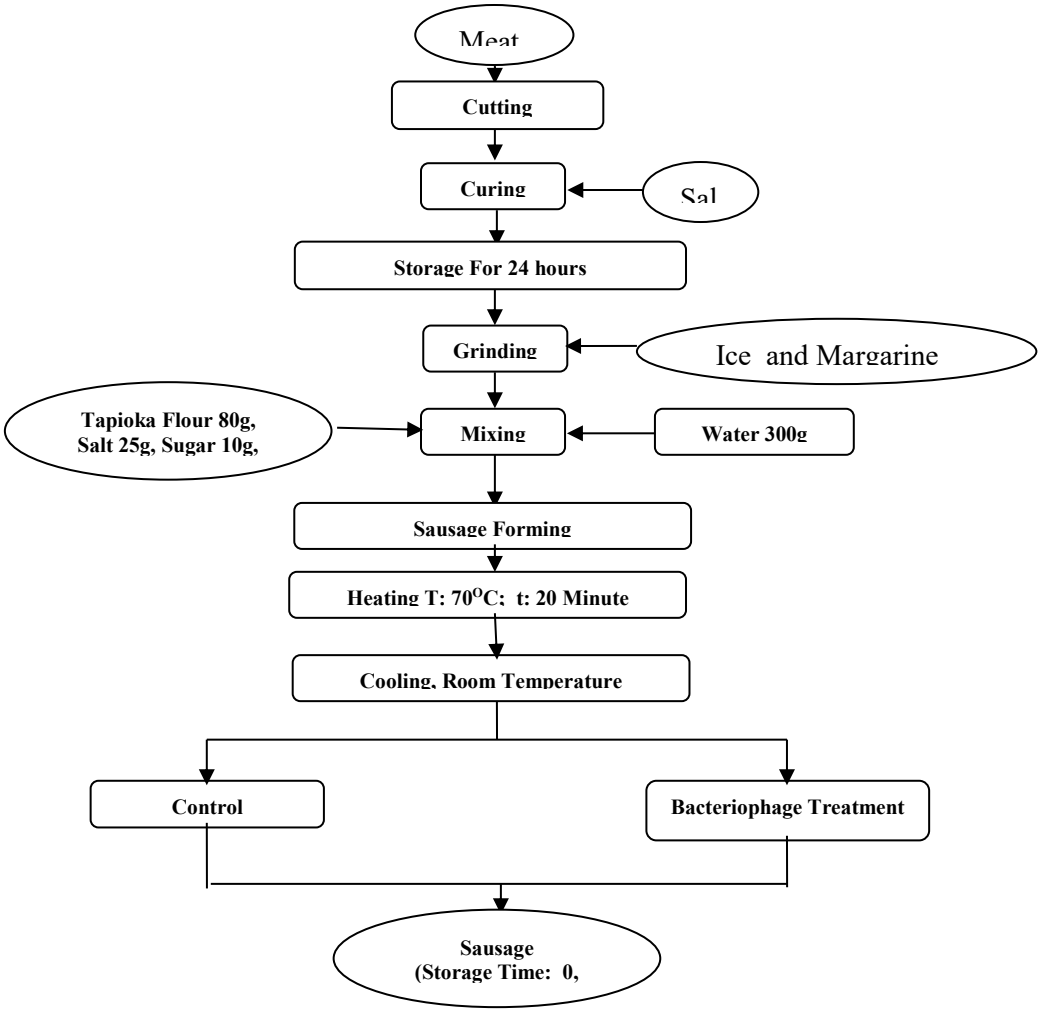

Figure 2. Application procedure of bacteriophage FR38 on sausage

3. Results and Discussion

3.1. Effectivity of Bacteriophage FR 38on Milk

3.1.1. Nutrition

The content of ash, protein and fat on milk that be given treat bacteriophage were not different than control significantly ($\alpha_{0.01}$) when milk was storage for 48 hours significantly (Table 1).It was s suspected that BacteriophageFR38 could inhibit Salmonella P38 action in denaturation of proteins and fats. The free Bacteriophage treatment (control) showed that the fat (1.76%), Abu (12:18%) and protein (1:09%) milk content was lower than Bacteriophage FR38 treatment significantly. The milk with Bacteriophage FR38 treatment had content characteristic was better than control, such as, of fat (3:32%), ash (0.25%), protein (2:20%) when milk was storage for 48 hours (99% confidence interval).

The composition of protein (mean = 2.58%) and fat (mean = 4:49%) in the milk sample is high. According Kluwer [42], the food that containing high fat and protein is a good growth medium for *Salmonella*. This case was same with this research, the control treatment was containing high *Salmonella* that could decrease on fat (1.76%) and protein content (1:09%) for 48 hours storages significantly. It was assummed that *Salmonella* has lipase and protease enzymes content that can break down fats and proteins (Figure 3).. Bacteriophage treatment was found to inhibit a break down process to content of fat, protein, moisture content, ash content and crude fiber of milk ($\alpha_{0.01}$)by *Salmonella* activity. This researchs proves that when applied to food eg. milk, the Bacteriophage will not affect to the nutritional content.

Table 1. The effect of bacteriophage FR38 treatment and incubation timetomilk nutrition content

Treatment	Storage time (hour)	Water Content	Ash	Fat	Protein
			(%)		
Negative control		62.92a	0.64a	4.57a	2.57a
Postive control (NB)	0	62.96a	0.68a	4.56a	2.59a
Postive control(NB + SM)		62.96a	0.67a	4.51a	2.57a
Salmonella P38		62.95a	0.68a	4.47a	2.59a
Salmonella P38 and Bacteriophage FR38		62.96a	0.64a	4.44a	2.58a
Negative control		62.33b	0.40b	3.86b	2.47b
Postive control (NB)		62.38b	0.49b	3.89b	2.40b
Postive control (NB + SM)	24	62.39b	0.41b	3.88b	2.46b
Salmonella P38		62.55c	0.29c	3.68c	2.30c
Salmonella P38 and Bacteriophage FR38		62.44d	0.36d	3.81d	2.40d
Negative control		87.11e	0.30e	3.47e	2.46e
Postive control (NB)		87.13e	0.32e	3.44e	2.41e
Postive control (NB + SM)	48	87.12e	0.31e	3.45e	2.47e
Salmonella P38		87.47f	0.18f	1.76f	1.09f
Salmonella P38 and Bacteriophage FR38		87.23g	0.25g	3.32g	2.20g

Note: a non different letter(s) in each column indicated a non significant difference on P > 0.05

(A) (B) (C) (D) (E)

Figure 3. The treatment effect for 48 hour storage: (A) bacteriophage FR 38 and *Salmonella* P 38*;* (B) *Salmonella* P 38; (C) Control; (D) Buffer SM and (E) Nutrient Broth

3.1.2. pH of milk

Different treatment also affected to the pH of the milk during 24 hours, and 48 hours storage (Figure 4). According to Winarno [43], decomposition of fats into fatty acids will release of a H^+ atom. The release of atom H^+ causes of a decrease process of the milk pH during storage. The addition of Bacteriophage turned will inhibit the microorganisms action in the fatrancidity, so, a pH of milk that was stored for 48 hours with Bacteriophage treatment was better (6.00) than with no bacteriophage treatment P38 (5:52) ($\alpha_{0,01}$) significantly with 99% confidence interval. It can be concluded that thebacteriophage addition can inhibit the growth of *Salmonella* destruction of milk by *Salmonella*.

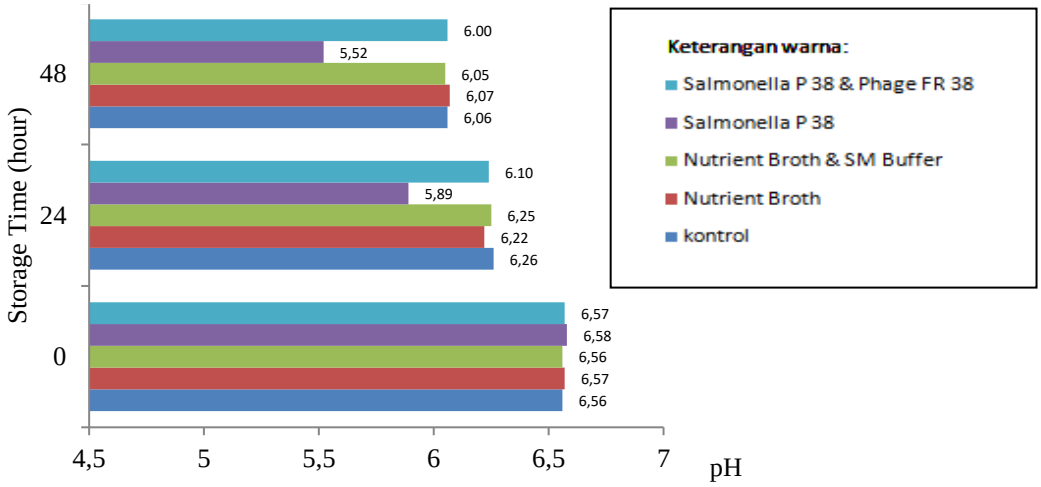

Figure 4. The Bacteriophage effect on pH milk

3.2. EffectivityBacteriophage FR38 on Sausages

The addition of Bacteriophage FR38 on the sausage also affects to decrease of *Salmonella* P38 growth during 0, 24 and 48 hours storage, at room temperature (Table 2). Bacteriophage FR38 able to reduce the *Salmonella* P38 number for 24 hours storage (6.9 x 10^1cfu /ml) and 48 hours (7.8 x 10^2cfu /ml) significantly than no bacteriophage treatment, at the 99% confidence level ($\alpha_{0.01}$). Different with unbacteriophage treatment, which increased the number of Salmonella on sausage,they are7.5 x 10^6cfu ml (24 hours storage) and 8.4 x 10^9cfu / ml (48 hours storage).

Table 2. The effect of bacteriophage FR38 treatment and incubation time to sausage nutrition content

Treatment	Storage time (hour)	Water Content	Ash	Fat	Protein	
				(%)		
Negative control		51.56a	2.68a	6.86a	14.12a	0.74a
Postive control (NB)						
Postive control (NB + SM)						
Salmonella P38	0	51.55a	2.67a	6.85a	14.13a	0.75a
Salmonella P38 and BacteriophageFR38		51.57a	2.68a	6.86a	14.12a	0.73a
Negative control		51.56a	2.68a	6.84a	14.12a	0.74a
Postive control (NB)						
Postive control (NB + SM)						
Salmonella P38		51.55a	2.68a	6.85a	14.13a	0.75a
Salmonella P38 and Bacteriophage FR38		53.49b	2.65b	6.63b	13.90b	0.71b
Negative control	24	53.48b	2.65b	6.64b	13.90b	0.71b
Postive control (NB)						
Postive control (NB + SM)						
Salmonella P38		53.49b	2.64b	6.63b	13.91b	0.71b
Salmonella P38 and Bacteriophage FR38		55.11c	2. 43c	6.45c	12.79c	0.68c
Negative control		53.70d	2. 55d	6.59d	13.85d	0.70d
Postive control (NB)						
Postive control (NB + SM)						
Salmonella P38		55.39 e	2.60e	6.51e	13.87e	0.68e
Salmonella P38 and Bacteriophage FR38		55.38e	2.61e	6.53e	13.88e	0.66e
Negative control	48	55.38e	2.61e	6.52e	13.87e	0.67e
Postive control (NB)						
Postive control (NB + SM)						
Salmonella P38		60.19f	2.49f	6.21f	11.06f	0.60f
Salmonella P38 and Bacteriophage FR38		57.61g	2.58g	6.49g	13.09g	0.63g

Note: a non different letter(s) in each column indicated not significant difference on P > 0.05

The milk samples with Salmonella treatment showed that a sample had an unlike performance, which was marked by the separation of dissolved solids and water during 24 hours storage. Bacteriophage are infectious only to target/specific host, for example *Salmonella* [2]. According to Winarno [43], denaturation of the protein was caused by the disintegration of the hydrogen bonds by external factors (such as, microbial). The disintegration of hydrogen bonds in a protein causes the protein denaturation. Denatured protein cause of solubility reduced, that give a bad effect, such as, the outside of proteins that have a hydrophilic characteristic will folded to inside part and hydrophobic parts will be folded out, so,it result a solids and liquids milk separated. The bad odor from the *Salmonella* P38 treatment was due to the decomposition processon fat components in milk, as a result of work by microorganisms. According to Winarno [43], the molecules that was broken down from fats will be oxidized, that result a hydroperoxide compound form, aldehydes component, and ketones, these reactions cause a bad odor (off-odor). This is in line with this observation.

7. 4. Conclusion

The Sausage has makro component, such as, protein and fat. Decreasing macro component on sausage showed that quality level of sausage. Low protein and fat content was a low quality sausage performance.The addition of Bacteriophage FR38 on the sausage inhibit growth *Salmonella* P38, during storage, at room temperature. Bacteriophage FR38 able to reduce the total of *Salmonella* P38 for 24 hours storage (6.9 x 10^1cfu /ml) and 48 hours (7.8 x 10^2cfu /ml) significantly.The result research showed that a bacteriophage FR38 able to decrease *Salmonella* P38.

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The physical properties of edible film made from different porang flour and glycerol concentration

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Abstract. Packaging materials used in the food industry are mostly synthetic. The synthetic packaging materials contribute to the environmental pollution. Edible film and packaging is one of the alternative packaging, because it is environmentally friendly and also able to maintain the quality of the food product. One of the agricultural products that can be used as the raw material in the edible film production is porang (*Amorphophalus muelerry* Blume) flour which has a high content of biopolymer of glucomannan with approximately 40 until 80%. This research aimed to determine the effect of porang flour and glycerol concentration to the properties of the edible film product. The result showed that the concentration of porang flour significantly (alpha = 0.05) affected the, thickness, water solubility, and tensile strength of the film. The concentration of glycerol treatment affected the physical properties of the film such as water vapor transmission rate, thickness, water solubility, tensile strength, elongation and seal strength, but there was no interaction between porang flour and glycerol concentration to the physical properties of edible film. The best treatment of edible packaging was obtain with 0.75% concentration of porang and 5% concentration of glycerol. The characteristics of the edible films had the thickness of 0.073 mm, water solubility of 88.775, water vapor transmission rate of 21.882 g / m^2 / hour, tensile strength of 5.41 N / cm^2 and percent of elongation of 12.222%.

1. Introduction

In the food industry, packaging is treatment to secure food products until it reach consumers in good condition and safe in terms of quality and quantity. The most used packaging is synthetic packaging, where synthetic packaging have a adverse impact risk on human health and the environment. Edible packaging (film) is one of the alternative food packaging because it was environmentally friendly and also able to maintain the quality of the product. Edible film is defined as a thin layer that can be used to separate layers of food, wrap or pocket it. On the other hand edible films or edible coatings can potentially extend shelf life and maintain the quality of fresh products [1].

One of the *film* former hydrocolloid material s is glucomannan derived from porang flour because of its properties that can form a good gel when interacting with water and plasticizer. Porang flour contain 40 – 80% of glucomannan [2]. To improve the physical properties of the film, plasticizer is needed. The presence of plasticizers is thought to reduce intermolecular interaction along the polymer chain, resulting in increased flexibility and decreasing permeability of film [4]. Plasticizer works by reducing the intermolecular strength in a polymer, the molecule of the plasticizer will stand between the bonds of each polymer so that it will make the polymer bonds not rigid and more plastic so as to reduce film fragility [4].

This research intend to determine the proportion of porang flour and glycerol concentration that can form a film with good physical properties.

2. Material and Method

2.1 Material

The main material that used in this reaserch is porang flour from PT Ambico Surabaya, West Java and Glyceroll pro-analysis from Amani, Malang, West Java. Aquadest (Hydrobat), ethanol 96%, HCl 37%, Aluminum sulfate, Isopropyl Alcohol, CaCl2, methyl red indicator, NH4OH, silica gel, Sodium azide, obtained from the Amani and Panadia chemical stores.

2.2 Methods

Factorial Randomized Block Design with 2 factor was used in this reaserch. Factor 1: concentration of porang flour (P), 0.50%; 0.75%. Factor 2: glycerol concentration (G): 5%, 15%; 25%; 35%, the experiment repeated 3 times for all combinations. The data were analyzed with ANOVA in 95% confidence interval and followed by honestly significance difference (HSD) as post-hoc test. The best treatment was determinated by Multiple Attribute methods (Zeleny).

2.3. Preparation Of Blend Film

Porang four from Ambico was leached with ethanol 40%,60% and 80% to carried out and to reduce oxalate content and other impurities from flour. To making edible packaging (film) is done with this step: Weighed porang flour with a concentration of 0.5%; and 0.75% (b/total), weighed glycerol with a number of 5%, 15%, 25% and 35% of the weight of porang flour, make a suspension from porang flour and glycerol with the addition of 200 ml of distilled water, heated in a water bath for 30 minutes at a temperature of ± 70ºC with occasional stirring, stirring with a magnetic stirrer for 5 minutes, the suspension was filtered using a vacuum filter to produce a clear filtrate, clear filtrate was poured on a glass plate and leveled with a stirrer, drained for 42 hours with a lamp dryer After the suspension was dry, it was cooled at room temperature for 10 minutes. Then the film was cut and analysis of the characteristics of the film was carried out.

Several characteristic analyzes were carried out on flour and film, that is: Water content analysis, Analysis of oxalate levels, glucomannan, thickness analysis , Solubility Analysis, Elongation and tensile strength analysis, Analysis of water vapor transmission.

3. Result and Discussion

3.1 Characteristics Of Raw Materials

Table 1. Porang Flour Analysis Result

Component	Average Value (%)	Literature (%)
Water Content	12.65 ± 0.17	10.2 [5]
Oxalate Level	0.337 ± 0.11	0.89 [6]; 0.095 – 6.022 [2]
Glucomannan Level	83.23 ± 2.55	36.58 – 87.49[2] ; 78.23[6]

Glucomannan is a major component in porang flour [5]. The high and low levels of porang are caused by many factors such as plant varieties, plant age, time after harvest and post-harvest treatment given [7]. Glucomannan is the most important component needed in making edible packaging, where one of the properties of glucomannan is that it can form a gel and a thin film (transparent). The result of glucomannan content analysis for the raw material is 83,23% and that is mean the porang flour that used in this reaserch is very high content of glucomannan.

The raw material is tested for oxalate content, where the oxalate content of the raw material is 0.337%, this value is still in the safe limit for consumption, where the safe limit for consuming oxalate is 2 g/person/day [8]. Oxalate levels are important to know because with the presence of oxalate in the material that is high enough it will reduce the bioavailability of calcium in the body and cause kidney stones [9].

Water content in porang flour is 12.65%, higher water content of raw materials can be caused by the high levels of glucomannan they contain. Glucomannan has high enough water absorbing properties so it can easily absorb water from the environment [6].

3.2 Edible Packaging Characteristics

This research conducted on edible packaging (film) with 6 parameters: brightness value, thickness, solubility, water vapor transmission rate, tensile strength and elongation. Test results data are presented in the following table:

Table 2. Edible Packaging Physical Characteristic

Combi-nation code	Value of Parameter				
	thickness (mm)	solubility (%)	Water Vapor Transmission Rate (g/m^2/hour)	Tensile strength (N/cm^2)	Elongation (%)
P1G1	0.052±0.02	100.00±0.00	21.84±2.49	4.77±0.78	8.89±1.57
P1G2	0.062±0.02	99.34±0.93	26.10±5.69	3.23±0.69	12.22±1.57
P1G3	0.063±0.02	54.87±0.51	32.86±5.12	2.18±0.41	13.33±0.00
P1G4	0.073±0.02	53.27±5.31	27.19±2.67	1.23±0.08	15.56±1.57
P2G1	0.073±0.01	88.77±9.05	21.82±1.70	5.41±0.80	12.22±1.57
P2G2	0.077±0.01	83.23±11.88	25.89±3.17	5.38±0.76	15.56±3.14
P2G3	0.078±0.01	47.00±14.55	29.28±2.41	3.96±1.47	16.67±2.72
P2G4	0.097±0.00	29.03±14.10	27.01±2.23	1.98±0.56	21.11±1.57

Description: Treatment of Porang Flour Concentration (P1: 0.50% and P2: 0.75%)
Treatment of Glycerol Concentration (G1: 5%, G2: 15%, G3: 25%, G4: 35%)

3.2.1 Thickness

Edible packaging (film) thickness is measured using a screw micrometer with accuracy of 0.01 - 0.025 mm. The thickness of edible packaging (film) obtained from the study ranged from 0.033 to 0.100 mm.

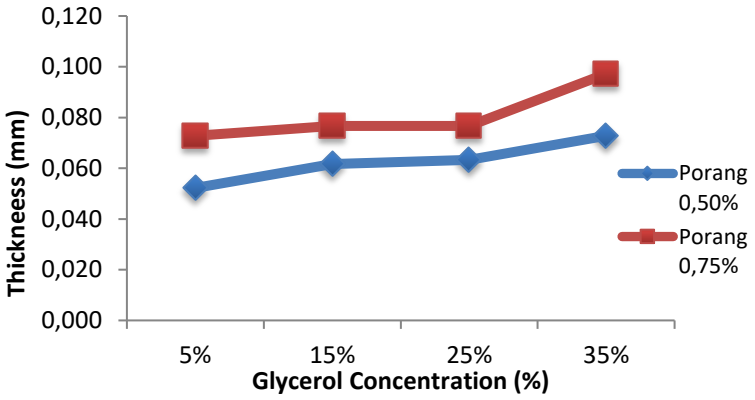

Figure 1. Thickeness Value of Edible Packaging (film) Due to the Effect of Suspension of Porang Flour and Glycerol Concentration

The results of analysis of variance (ANOVA), showed that the concentration of porang flour and glycerol concentration had a significant effect (α = 0.05) on the value of film thickness obtained while the interaction between the two factors did not show any significant differences. The thickness of the product is influenced by the main constituent material, porang flour. The higher the concentration of porang flour added, it will produce a film that has a higher thickness. The higher average film thickness was obtained from the film with higher addition of glycerol.

The higher the content of porang flour and glycerol will increase the viscosity, so the thickness will increase [10].

3.2.2 Water Solubility

The average value of water solubility of edible packaging (film) ranges from 29.036% - 100%.

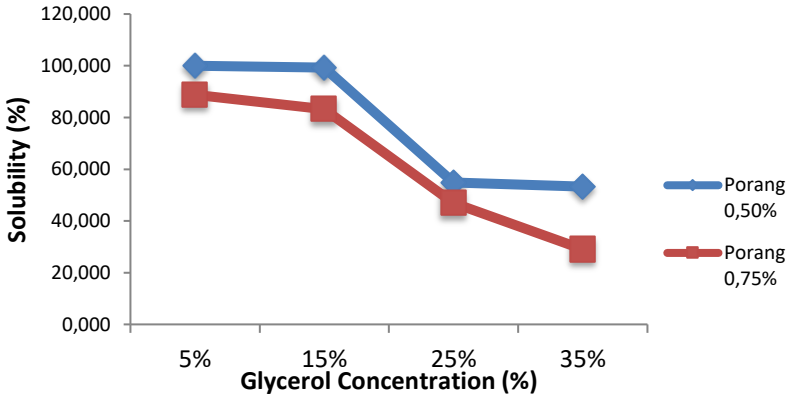

Figure 2. Water Solubility Value of Edible Packaging (film) Due to the Effect of Suspension of Porang Flour and Glycerol Concentration

The results of variance analysis (ANOVA) showed that the concentration of porang flour and glycerol concentration had a significant effect (α = 0.05) on the solubility value of the film, but there was no interaction between the two treatments.

Solubility of edible films in water indicates the film's hydrophilization process [11]. Glucomannan as the base material for edible packaging (film) products studied has the solubility in cold water and forms a thick mass on it [6].

With the addition of plasticizers it will increase the film matrix so that the film becomes stronger and not easily destroyed, which shows that if the glycerol concentration is higher, the percentage of solubility will decrease [12]

3.2.3 Water Vapor Transmission Rate

Average value of water vapor transmission rate in edible packaging (film) products ranges from 21.8225 - 32.8559 g / m2 / hour.

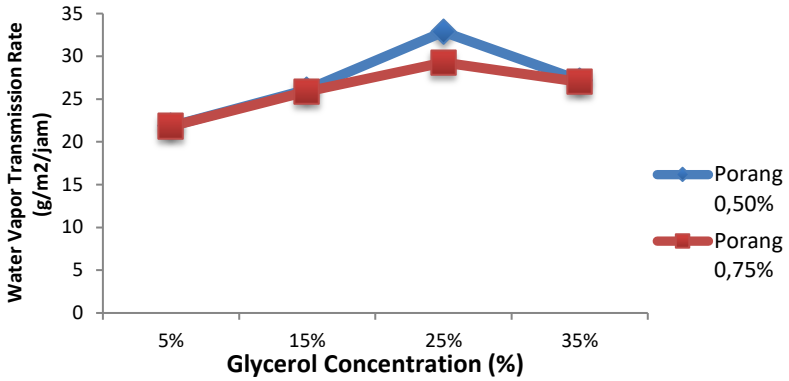

Figure 3. Water Vapor Transmission Rate Value of Edible Packaging (film) Due to the Effect of Suspension of Porang Flour and Glycerol Concentration

The results of variance analysis (ANOVA) show that the factors that have a significant effect (α = 0.05) on the value of the water vapor transmission rate of the product is the concentration of glycerol, porang flour does not give a real effect so there is no interaction between the two factors. The addition of glycerol can reduce the film's internal hydrogen bond to produce a film with a tighter pore so as to reduce the rate of water vapor transmission [3]

3.2.4 Tensile Strength

The average tensile strength of edible packaging (film) products ranged from 1.23 N/cm2 - 5.41 N/cm2. The higher the value of the tensile strength, film resistance to the pull, the stretch and pressure will be better.

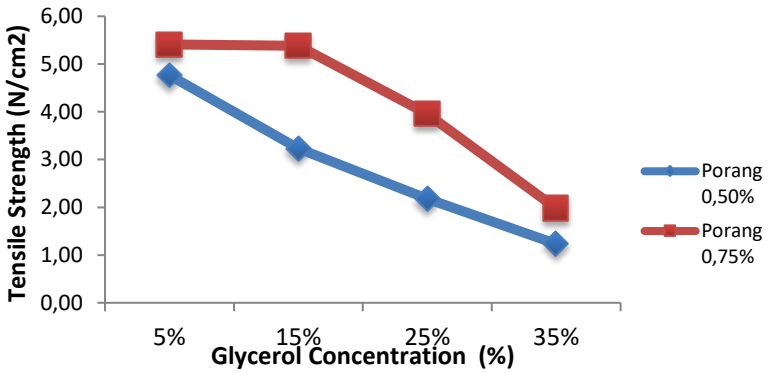

Figure 4. Tensile Strength Value of Edible Packaging (film) Due to the Effect of Suspension of Porang Flour and Glycerol Concentration

The results of analysis of variance (ANOVA) showed that the concentration of porang flour and glycerol concentration had a significant effect (α = 0.05) on the value of tensile strength of the product, but there was no interaction between the two treatments.

Glucomannan concentration has a large influence in determining the tensile strength of the film. The more addition of glucomannan to the film solution will make the matrix more robust so that the ability to withstand the pressure (N) of the film will increase [13].

To make edible film structures that are difficult to break, hydrocolloid needs to be added [14]. The tensile strength value will decrease with the increasing concentration of glycerol added. This is because glycerol as a plasticizer will reduce internal hydrogen bonds in intermolecular bonds and reduce the stability of the solid dispersion system.

3.2.5 Elongation

The average elongation value in edible packaging (film) products which was influenced by the concentration of suspension of porang flour and glycerol concentration ranged from 8.889% - 21.111%.

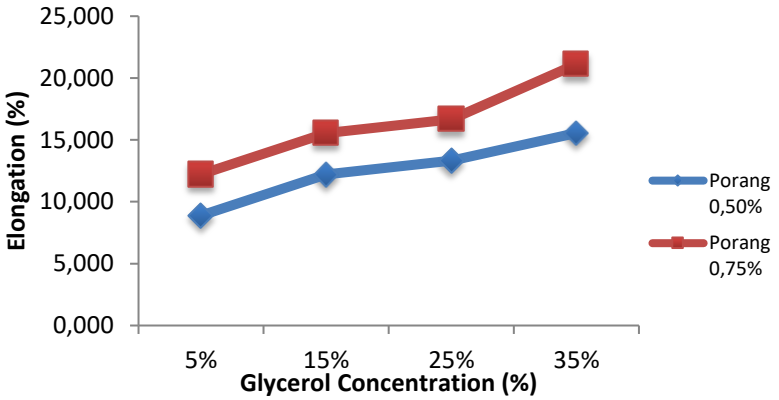

Figure 5. Elongation Value of Edible Packaging (film) Due to the Effect of Suspension of Porang Flour and Glycerol Concentration

The results of analysis of variance (ANOVA) showed that the concentration of porang flour and glycerol concentration had a significant effect ($\alpha = 0.05$) on the percent elongation value of the film, but there was no interaction between the two treatments.

Glucomannan has high water absorbing properties because it contains a lot of hydroxyl bonds and acetyl groups, where water can have a plastic effect on edible films so as to increase elongation [15]. This explains that with a higher concentration of porang flour, a stronger edible film will form. Stronger films will have greater range capability so that the percent elongation is higher.

Glycerol as a hydrophilic plasticizer will be able to interact with glucomannan in the film matrix, this ability will prevent the polymer chain from interacting strongly or crystallizing resulting in a rigid film matrix [16].

4. Conclusion

The results of analysis of variance (ANOVA) showed that the addition concentration of porang flour affected the thickness and solubility of edible packaging. With the higher concentration of porang flour will increase the thickness and tensile strength, but will reduce the brightness and solubility, while the addition of glycerol concentration affects the physical properties of water vapor transmission rate, thickness, solubility, tensile strength and elongation. the higher the concentration of glycerol will increase the elongation and thickness of the film but reduce the rate of transmission of water vapor, solubility, tensile strength. There was no interaction between porang flour and glycerol on the physical properties of edible packaging research results.

The best treatment of edible packaging was obtain with 0.75% concentration of porang and 5% concentration of glycerol (P2G1). The thickness of 0.073 ± 0.016 mm, water solubility of 88.775 ± 9.052%, water vapor transmission rate of 21.882 ± 1.700 g / m^2 / hour, tensile strength of 5.41 ± 0.769 N / cm^2 and elongation of 12.222 ± 1.571%.

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Optimization of the use of suweg (*Amorphophallus campanalatus* B) flour as stabilizer on organoleptic properties, overrun and melting time of goat milk ice cream

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Abstract. This study was aimed to find the optimal formulation for making goat milk ice cream using suweg tuber flour as stabilizer to substitute gelatine commonly used in making ice cream. In order to find the best formulation, ice cream were evaluated organoleptic for its color, texture, taste, aroma, and overall acceptance. While the melting time and overrun were determined objectively. The proportions of suweg tuber flour in the manufacture of goat milk ice cream were 0%, 2%, 4%, 6%, 8%, and 10%, as a comparison, 0.5% gelatine was used. The color and texture were evaluated using scoring test, while the taste, aroma and overall acceptance were evaluated using hedonic test (preference) performed by 30 trained panels for each test. The data were tested for their homogeneity using Bartlett test and the aditivity was tested using Tuckey test. Then the data were analysed for variance to find the effect of the treatments, and further analysed using Least Significant Difference (LSD) test. All tests were carried out at the level of 1% or 5%. The results showed the color, taste and aroma, and overall acceptance of ice cream was significantly affected by treatments. The most preferred The highest score for overall acceptance was found in goat milk ice cream treated with 2% suwed tuber flour which was 8.85 (most preferred) . Whereas the highest value for melting time and overrun were found in goat milk ice cream treated with 10% suweg tuber flour, which were 24.5 minutes, 80.5%.

1. Introduction

Ice cream is one of the Snack that is likes by society, from the baby until the old one. Ice cream is a product of Frozen food which is made by the combination of the frozen process and the agitation of the ingredient consist of milk, milk product, sweetest, stabilizer, thickener, gel amplifier, and flavor. The principle of ice cream manufacturing is to mold the cavity in the mixture of ingredient from the ice cream, so that there Will be produced the overrun which makes ice becomes lighter, not too solid and has soft texture. The good characteristic of ice cream is it has overrun value that is not less than 80% and 12%-14% fat level [1]. The Ice cream which has high quality is not quickly melt when it is served on room temperature. Meanwhile the texture that is wanted in ice cream is soft and creamy [2]. To produce ice cream which is soft and has stability of the crystals formation and also fast melting, the ingredient that must be added on making ice cream is the stabilizer. The stabilizer that is usually used on making ice cream they are: gelatine, CMC, and vegetable product which have polysaccharide[3]. The price of gelatin that is high enough, makes the result of product price of ice cream is also expensive. That is why it must be tried another ingredient stabilizer which include polysaccharide that has glucose and rich fiber, that is glucomannan from suweg tuber flour. The benefit of suweg tuber flour is food fiber content, protein and carbohydrates are quite high and also less fat content. Suweg tuber flour can be made as the Ingredient stabilizer because it has glucomannan that can be function as thickening agent, ini stabilize emulsion. The special purpose of this research is to optimize the formula of goat milk ice cream with uses suweg tuber flour, study about glucomannan content on stabilizing activity that is found in tuber, so that, Will give goat milk ice cream with chemical properties, organoleptic, and also the

criteria of ice cream that is meet the National Standard [4]. The result of Susilawati and Sartika's research [5] shows the concentrate of 5% suweg tuber flour can produce the characteristic of the ice cream that has protein nutritional value, fat and carbohydrate which have fulfilled the requirement [4]. The result of ice cream melting time test in 5 % concentrates of suweg tuber flour that has melting time internet 10 minutes/50 g. This condition indicates that the mechanism of glucomannan in suweg tuber flour can maintain the melting power of ice cream in room temperature but it is not still optimal. The 5% and 1% concentrate of suweg tuber flour can give the different of overrun increasing, and it also happens ink stabilize emulsion and melting time. But in 5% concentrate of suweg tuber overrun ice cream is only 22% and the store of the texture that is not optimal (still soft). Because of that in the second year of the research is used suweg tuber flour with the concentrate that is higher than addition of fat composition in basic Ingredient, it is hoped can increase overrun value and texture.

2. Materials and Methods

2.1. Material and Instruments

Suweg tuber variety *hortensis* used in the research was obtained from farmers in Purwosari, Metro Timur. Etawa goat milk was obtained from Sekampung Village, Lampung Timur District. Other ingredients were creamer, full cream powder milk (Frisian flag), skim milk (Tropicana Slim), sugar, ovallet stabilizer, egg yolk. Analysis chemicals were hexane, concentrated H_2SO_4, 1.25% H_2SO_4, 0.02N HCl, 50% NaOH, H_2BO_2, $Na_2S_2O_3$, and alcohol. This study used Tyler standard sieve 80 mesh, blender (Philips HR2115), thermometer, knife, basin, mixer (Philips HR1538), stove, pan, ice cream cup, scales, freezer (Frigigate F200), spoon, refrigerator, autoclave, petridisks, bottles, Soxhlet, desicator, furnance, cawan porselin, Buchner funnel, instruments and glassware used for analyses, and also intruments for organoleptic measurement.

2.2. Method

Analyses were conducted in Complete Randomized Design in triplicate with single factor consisted of 6 experimental levels of suweg powder concentrations (2%, 4%, 6%, 8%, 10%) and 0.5% gelatine as comparison. Statistical variance similarity was analysed using Bartlett test, while significant difference was measured using analysis of variance (Anova), followed by post-hoc *Tukey HSD* (Honestly Significant Difference) at 5% significance level.

2.3. Sample Preparation

2.3.1. Suweg powder preparation

Freshly harvested suweg (*Amorphophallus campanulatus* Bl) tuber was cleaned from dirt, peeled, washed in clean water, then soaked in solution contain 10% salt and 10% lime over the past 24 hours Tuber in thin slices then dried in 50°C oven for 18 hours, ground, and sieved to obtain 60 mesh powder.

2.3.2. Goat milk ice cream preparation

All ice cream ingredients were mixed, pasteurized at approximately 70°C for 30 minutes, and continuously stirred until homogenous. The mixture was then cooled in temperature below 5°C for 4 hours, and stirred again using hand mixer at high speed before freezing. The stirring and cooling was repeated 3 times before freezing for 24 hours at -30^{0}C. Ice cream was stored into -18°C freezer box before consumption.

2.4. Observation

2.4.1. Organoleptic Analyses

Texture and color of the ice cream was measured with scoring by 20 trained panelists for each replicate, taste, and aroma, while overall consumer acceptability was analyzed using hedonic test by 25 trained panelists for each replicate.

2.4.2. Volume Expansion (Overrun)

Overrun or % increase in volume of *ice cream* due to air bubble trapped into the mixture was measured using formula below [6].

$$\textbf{\%}\ \text{Overrun} = \frac{\text{Ice cream volume - ingredients mixture volume}}{\text{ingredients mixture volume}} \times 100\%$$

2.4.3. Melting time [6]

Melting time was measured by taking 100 ml packed ice cream previously stored at -20°C for 24 hours into room temperature. Melting volume was counted every 10 minutes until all ice cream melted.

3. Results and Discussion

3.1. Organoleptic test

3.1.1 Texture

It was indicated that suweg flour significantly affected goat milk ice cream texture, as also confirmed by HSD statistical analysis (Table 1).

Table 1. Texture of goat milk ice cream made by various suweg flour concentrations at 5% significance level HSD

	S1	Suweg concertation					$HSD_{0.05}$
	(0.5% gelatin)	S6 (10%)	S5 (8%)	S4 (6%)	S3 (4%)	S2 (2%)	
Texture mean	3.9^{a}	3.8^{ab}	3.6^{bc}	3.5^{c}	3.2^{cd}	3.1^{c}	0.406

Different superscript indicated significant difference.

Desired texture in ice cream is soft, creamy, and homogenous. Suweg flour concentration had significant effect on goat milk ice cream texture. The result of ice cream using 0.5% gelatine was significantly different to those of 8%, 6%, 4%, and 2% suweg flour, while those of 8% suweg flour was not significantly different to those of 6%, 4%, and 2%. It was indicated that ice cream with 10% suweg flour of 3.8 (soft) had almost similar texture to the highest score obtained by gelatine of 3.9 (soft). Glucomannan in suweg flour was able to form emulsion in goat milk ice cream, thus water in the mixture was fully incorporated in emulsion, in which micro-sized ice crystals were formed during freezing, resulted smooth and soft ice cream texture. Glucomannan also has gel strengthening, texture improving, and thickening property [7].

3.1.2 Flavor

Anova and HSD statistical analysis confirmed that suweg flour as stabilizer had significant effect on flavor of goat milk ice cream (Table 2).

Table 2. Taste and aroma of goat milk ice cream made by various suweg flour concentrations at 5% significance level HSD

	S1	Suweg concentration					$HSD_{0.05}$
	(0.5% gelatin)	S6 (10%)	S5 (8%)	S4 (6%)	S3 (4%)	S2 (2%)	
Flavor mean	4.0^{a}	3.9^{ab}	3.3^{b}	3.0^{c}	2.9^{bc}	2.9^{c}	0.406

Different superscript indicated significant difference

Significant difference was found among taste and aroma of goat milk made using gelatin and various concentration of suweg flour. Similar to previous analysis, the result of 0.5% gelatin was not significantly different than those of 10% suweg flour, indicated by the highest preferable taste and aroma of 4.0 (like) was similar to 10% suweg flour of 3.9 (like). The main component in the ice cream was

fresh goat milk, thus its unique strong odor remained. Another report mentioned that aroma and taste of ice cream is predominantly affected by milk and sugar [8]. As suweg flour has neutral aroma, it has no effect on product flavor when used as ingredient [9]. Suweg tuber has similar flavor to wild taro, locally known as talas, but with smother and softer texture. Previous research mentioned that suweg flour as wheat flour substitute in cookies had no effect on product color and taste [10].

3.1.3 Color

Anova and HSD analysis indicated that 0.5% gelatin and suweg flour significantly affected goat milk ice cream color (Table 3).

Table 3. Color of goat milk ice cream made by various suweg flour concentrations at 5% significance level HSD

	S1	Suweg concentration					$HSD_{0.05}$
	(0.5% gelatin)	S6 (10%)	S5 (8%)	S4 (6%)	S3 (4%)	S2 (2%)	
Color mean	3.7^{a}	3.4^{b}	2.9^{bc}	2.8^{bc}	2.5^{c}	2.4^{c}	0.406

Different superscript indicated significant difference

Goat milk ice cream color made using 0.5% gelatin was significantly different than those made using 2%, 4%, 6%, 8%, and 10% suweg flour in concentration dependent manner. This was due to suweg flour amount whose greyish and brownish color affected the final product, compared to white color of gelatin. Ice cream color index was 3.4 (greyish white) and 3.7 (white) for 2% suweg flour and gelatin, respectively. Pitojo [9] noted that suweg has physical property fine powder with greyish white or yellowish white color. But the color of suweg flour is broken white compare to sukun flour, cassava flour, and wheat flour. Different suweg flour concentration resulted different color. Addition of 10% suweg flour changed ice cream color. Al – Baarri [11] mentioned that goat milk's vitamin A contains no carotenoid like the yellowish white cow milk, and the previous has whiter color than the later. Combination of suweg flour with greyish white and the pure white goat milk was considered as the main factor determining color changing of the ice cream.

3.1.4 Overall acceptability

Anova and HSD analysis showed that gelatine and suweg flour utilization significantly affected panellist's overall product acceptability (Table 4).

Table 4. Panellist overall product acceptability of goat milk ice cream

	S1	Suweg concentration				
	(0.5% gelatin)	S6 (10%)	S5 (8%)	S4 (6%)	S3 (4%)	S2 (2%)
Acceptability	4.1^{a}	3.7^{b}	3.2^{c}	3.0^{cd}	2.9^{cd}	2.9^{cd}

Different superscript indicated significant difference

Overall acceptability of goat milk ice cream using gelatin 0.5% was different to those made using suweg flour. Addition of 0.5% gelatin had significantly different result than those of 10%, 8%, 6%, 4%, and 2% suweg flour. Based on organoleptic test, goat milk ice cream with 0.5% gelatine had the highest acceptability, but not significantly different than ice cream made with 10% suweg flour. Therefore, researcher selected 10% suweg flour to be used in this study as preferred ice cream stabilizer.

3.2. Overrun

Ice cream volume expansion known as overrun was calculated based on the difference between volume of ice cream and initial mixture at the same mass, or mass of ice cream and mixture at the same volume [12]. The highest overrun of modified goat milk ice cream of 80.5% was obtained by 10% suweg flour. Based on [4] released by National Statistic Agency, standard overrun for ice cream is in 70-80% range,

with 60-100% considered as good. Ice cream with good overrun of 80% has around 12-14% fat content [1]. Overrun is occurred due to trapped air in ice cream mixture during agitation [13]. Suweg flour addition increased solid material in mixture which resulted higher viscosity. Higher suweg flour concentration led to higher bound water, decreased free water, thicker mixture, and slower melting [14]. Another study also reported that higher overrun led to longer melting time [15].

3.3. Melting time

Goat milk ice cream made had various melting time at different suweg flour concentration. The lowest melting time or the longest period of 24.5 minutes was obtained by 10 % suweg flour, while the otherwise at 10.25 minutes was obtained by 0.5 % gelatine ice cream. Melting period is the time needed for ice cream to completely melting in room temperature. Ice cream is desired to melt longer in room temperature, but rapidly melt in body temperature. A short or a long melting period is undesirable. Short period rushed consumers to consume the ice cream immediately, whereas too long period indicated excessive solid ingredients. [16] explained that desirable ice cream melting time is 15-20 minutes.

4. Conclusions

Goat milk ice cream modified with 10% suweg flour as stabilizer to replace gelatin resulted texture, taste and aroma, color, and overall acceptability of 3.9 (soft), 3.9 (preferable taste and aroma), 3.7 (white), and 3.7 (likeable overall acceptability), respectively, which in accordance with Indonesian standard number 01-3713-1995. The highest overrun of 80.54% and the longest melting time of 20.5 minutes were obtained by goat milk ice cream made using 10% suweg flour.

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Effect of pretreatment with organic acids from bilimbi (*Averrhoa bilimbi* L) and Japansche citroen orange (*Citrus limonia* Osbeck) on the quality of chili powder (*Capsicum frutescens*)

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Abstract. Chili pepper (*Capsicum frutescens*) is one of the widely consumed commodities in Indonesia.Chili pepper has high moisture content which causes short shelf life. This problem can be solved by processing fresh chili into chili powder products to extend chili shelf life.The quality of chili powder needs to be improved by applyingpretreatment such as blanching and soaking. Chemical substances that commonly used in soaking process are citric acid and sodium metabisulfite. Thosechemical can be replacedby fruits that contain high acid such as Bilimbi (*Averrhoa bilimbi* L) and Japanschecitroen Orange (*Citrus limonia*Osbeck). This research used a Randomized Block Design with two factors: type of fruit (JapanschecitroenOrange and Bilimbi) and concentration of solution (5%, 10%, 15%) with four repetition. Data were analyzed using Analysis of Variance (ANOVA) followed by Least Significant Data (LSD) 5%. The best treatment was determined using multiple attributes Zeleny method.Different concentration of solution give significant effect (α = 0,05) to redness, extractcolor, vitamin c, total flavonoid, antioxidant activity IC50 of chili powder.The best result of this experiment is chili powder that soaked in 15%bilimbi solution with the moisture content of 8,14%, total colour (ASTA) of 191,26 ASTA value, vitamin C content of 0,64 mg / g, capsaicin content of 1316631,5 SHU, total phenol of 9,58 mg GAE / g, flavonoid level of 32,59 mg QE / g, antioxidant activity of IC50 207,5416 ppm, brightness level (L) of 57 , 98, redness level of 22,11, and yellowness level of 35,075

1. Introduction

Chili pepper (*Capsicum frutescens*) is one of the widely consumed commodities in Indonesia.Chili pepper has a high water content, it is around 80% [1], which is responsible to the short shelf life of chili. This shelf life influences the availability of chili. Short shelf life compounded with bad weather conditions that do not support the growth of chili pepper, reduced the availability of chili and caused price fluctuations of chili. Chili pepper production in 2016 was 843.99 thousand tons, reduced by lost during harvesting and processing about 16.88 thousand tons, national consumption of 350.18 thousand tons, seedlings purpose of 34 tons and supply for food industry 417.77 thousand tons [2]. The price of chili in Indonesia is not stable. According to the data on January 2017 the price of chilli reached IDR 89,000 per kg, then on February 2017 became IDR. 120,000 per kg, on April 2017 IDR 52,000 per kg, on November 2017 IDR 16,000 per kg, and lastly the average price of chili on January 2018 was IDR 34,000 per kg\ [3]. This condition is very detrimental to farmers, consumers and industry. Considering that, it is very necessary need to improve the quality and prices stability of chilli. One of possible effort to solve the problem is doing proper post-harvest handling and processing. One of the proper method is processing chili pepper into chili powder.

Maintaining the quality of chili powder is important. The quality parameters that is important to maintain are color and nutritional value of chili powder.The method to maintain the quality of chili powder is by adding sodium metabisulfite and citric acid [4] during the pretreatment proces (blanching

and soaking) of producing chili powder.Based on previous research [5], the addition of sodium metabisulfite and citric acid in the chilli soaking process can improve the quality of dried chili. Ascorbic acid, total carotenoids and total phenols in dried chili samples increased by increasing the concentration of sodium metabisulfite and citric acid in soaking solutions. A natural alternative to sodium metabisulphite and synthetic citric acid are fruits that contain high organic acids. Bilmbi and Japansche citroen (JC) orange are fruits that have high organic acid content. Soaking with the solution of these fruit is expected to replace the chemical subtances.

Bilimbi (*Averrhoa bilimbi* L) is a tropical plant that can be harvested throughout the year [6]. Bilimbi contains groups of oxalate compounds, phenols, flavonoids, and pectin. Bilimbi is easily found in Indonesia and has a cheap price. Japansche Citroen (Citrus limonia Osbeck) is one type of rootstock citrus varieties that is widely used in Indonesia. Until now, JC are traded only for its seeds and the juice is not used because it has a very sour taste [7]. In this research, the effect of addition of organic acids from Bilimbi (*Averrhoa bilimbi* L) and Japansche Citroen Orange (*Citrus limonia* Osbeck) during pretreatment process of chili powder production to the quality of Chili Powder (*Capsicum frutescens*) was studied.

2. Materials and Methods

2.1 *Materials*

The main materials used in this research were fresh chili pods (from Karangploso, Malang, East Java), JC orange (from Punten Balitjestro Experimental Garden), bilimbi, citric acid, sodium metabisulfite. The materials for analysis were Aquades, Hydrobatt, Ethanol Pro-Analysis (99%) Merck, Ascorbic Acid, Iodine, Amylum, DPPH powder, KI powder, Folin reagent, Quercentin, 7.5% Sodium Carbonate, $NaNO_2$ 5% , $AlCl_3$ 10%, Gallic Acid, NaOH 1 M, Acetone.The tools used in this research were 40 mesh shieve shaker, Microwave Assisted Extraction (MAE) (Anton Paar Multiwave PRO), UV-Vis spectrophotometer (Lan Optics), microplate 96 well (Costar), microplate reader (BNG Labtech GmbH), analytic scales (Denver Instrument), cabinet dryer, blender, micropipette, microplate shaker, microtube, vortex, nitrogen gas, color reader, pH meter, thermometer, stative, burette, vial bottles and other glassware.

2.2 *Methods*

This research used a Randomized Block Design with two factors: type of fruit (Japansche citroen orange , Bilimbi) and concentration of solution (5%, 10%, 15%) with four repetition of experiment. Data were analyzed using Analysis of Variance (ANOVA). The following test using Least Significant Data (LSD) with trust level 5%. The best treatment was determined using multiple attributes Zeleny method [8].

To make chili powder, first, chili were weighed and washed.Then the chili were blanched (90ºC, 3 minutes), and soaked in a solution of Japansche citroen or Bilimbi with a concentration of 5%, 10%, 15% in 200 ml of water (v / v). For the control chili was soaked with sodium metabilsulfite 0.3% and citric acid 1% (v / v). The parameter analysed were pH of the solution, color, capcaisin content, extract color, vitamin C, antioxidant activity, total phenol, flavonoid of chili powder. After that, the best treatment was determined using the multiple attribute method. The results of the best treatment were compared with control.

3. Result and Discusion

3.1Characteristic of Raw Material

The total capsaicin content, extract color, vitamin C content, total phenol, total flavonoids, antioxidant activity IC50, color (L, a, b) of fresh chili pepper presented in Table 1

Table 1. The Characteristic of Fresh Chili from Karangploso, Malang, East Java

Parameter	Analysis Result
Total Capsaicin (SHU)	536373.47± 28983.74
Extract Color (ASTA value)	199.98 ± 4.95
Vitamin C Content (mg/g)	1.12 ± 0.10
Total Phenol (mg GAE/g)	11.57± 0.67
Total Flavonoid (mg QE/g)	36.21 ± 1.14
Color L*	59.83 ± 2.94
Color a	26.80 ± 1.69
Color b	34.15 ± 2.61
Antioxidant Activity IC50 (ppm)	227. 41 ± 10.48

Explanation: 1) The number after ± is the standard deviation value
2) SHU (Scoville Heat Unit) is a unit of pungency
3) ASTA (American Spice Trade Association)

3.2 Acidity Degree of Soaking Solution

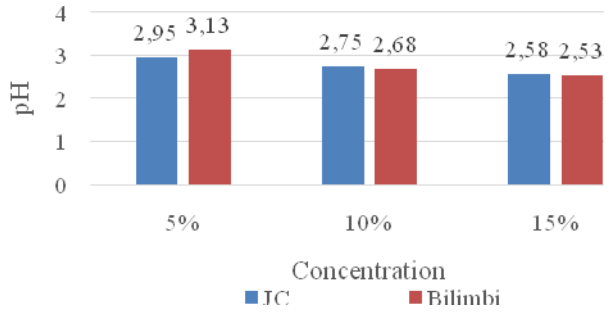

Figure 1. Effect of Solution Concentration and Fruit Type on the Degree of Acidity of Soak Solution

The acidity degree of japansche citroen and bilimbi solution were ranges between 2.53 - 3.13. Differences in fruit types did not give a significant effect on the pH value of the soaking solution while the concentration of the solution had a significant effect ($\alpha = 0.05$) on the pH value of the soaking solution.

The higher the concentration of fruit solution, the lower the pH value. The level of acidity is influenced by the content of organic acids which dominate in a substance. The most dominant organic acid in JC is citric acid, while the most dominant organic acid in bilimbi is oxalic acid. The lowest average pH value was on bilimbi solution with a concentration of 15%, which is 2.53. While the highest average pH value was onbilimbisolution5%, which is 3.13.

3.3 Chemical Characteristics of Chili Powder

The total capsaicin, extract color, vitamin C content, total phenol, total flavonoids, antioxidant activity IC50 of chili powder is presented in Table 2

Table 2. The Chemical Characteristic of Chili Powder

Treatment	Extract Color (ASTA Value)	Capsaicin (SHU)	Vitamin C (mg/g)	Total Phenol (mg GAE/g)	Total Flavonoid (mg QE/g)	Antioxidant Activity IC50 (ppm)
JC 5%	151.28 ± 8.01	1210788.95± 75754.95	0.59± 0.01	8.75± 0.24	26.00± 1.09	229.01± 7.66
JC 10%	172.21 ± 6.53	1291129.86± 95325.97	0.62± 0.03	8.97± 0.45	29.24± 2.88	218.34± 3.91
JC 15%	190.53 ± 8.34	1249276.08± 61028.11	0.67± 0.03	9.16± 0.84	32.03± 0.99	211.45± 7.86
Bilimbi 5%	149.33 ± 3.82	1208118.72± 37999.74	0.60± 0.03	8.74± 0.22	29.89± 2.40	227.34± 10.40
Bilimbi 10%	176.68 ± 5.69	1240888.37± 68702.84	0.61± 0.04	9.21± 0.46	31.94± 2.78	216.55± 8.61
Bilimbi 15%	191.26 ± 5.84	1316631.47± 75991.93	0.64± 0.03	9.53± 0.44	32.59± 0.,64	207.54± 6.75

Explanation: 1) The number after ± is the standard deviation value
2) Each data is an average of 4 repetitions

Capsaicin content of chili powder ranged between 1208118.72 - 1316631.47 SHU. Differences in fruit types and concentrations of solution did not have a significant effect on the capsaicin content of chili powder.Compared with capsaicin of fresh chili, capsaicin content in chili powder was increased. This could be due to the activity of the peroxidase enzyme and the heat temperature during the blanching and drying process. This because vanillyl is part of the capsaicin which is easily oxidized by enzymes peroxidise and these enzymes play a role in the degradation process of capsaicin [9-11].The chili that were used for production of chili powder has been blanched before drying, so the peroxidation enzyme is inactive, while in the fresh chilli is still active. Another possibility that causes the increase of capsaicin content in chili powder is the hydrolysis of glycosides from chili in the blanching and drying process. During the growth of chilli, not only free capsaicin can be formed, but free capsaicin can be bound with sugar and other compounds through the hydroxyl group in the capsaicin structure [11]. Glycocidic bonds in capsaicin can be damaged by blanching and drying, so that free capsaicin compounds are formed. In addition, reduced water content in chili can increase the yield of capsaicin extraction due to cell disruption during the extraction process with appropriate solvents [12-14].

Vitamin C content of chili powder ranged from 0.59 to 0.67 mg / g. Differences in fruit types did not give a significant effect on the vitamin C content of chili powder while the concentration of soaking solution had a significant effect on the vitamin C content of chili powder. The higher the concentration of the soaking solution, the higher the average vitamin C content. Compared with the vitamin C of fresh chilli 1.12 mg / g, vitamin C of chili powder decreased due to easily damaged of vitamin C by thermal processes. The decrease in ascorbic acid during drying is due to its irreversible oxidative process [15] which causes discoloration of dried vegetables with the formation of dehydroascorbic acid and diketogluconic acid from ascorbic acid which occurs in the later stages of the drying process [16]. A decrease in ascorbic acid content related to antioxidant properties helps prevent pigment oxidation [17]. Solutions that have a higher acidity level tend to be able to maintain vitamin C content in chili powder. This is confirmed by the previous study [5] which states that ascorbic acid of dried chili increases with increasing concentrations of sodium metabisulfite and citric acid in the soaking solution.

The total phenol of chili powder ranged from 8.74 to 9.53 mg GAE / g. Differences in fruit types and concentrations of soaking solution did not have a significant effect on the total phenolic ofchili powder.The higher the concentration of the soaking solution, the higher the average value of total

phenol. The previous study [5] stated that dried chili with sodium metabisulfite pretreatment and citric acid in a soaking solution produced a higher total phenol than chilli without pretreatment. However, when compared with fresh chili the total phenol was reduced. This was due to degradation by thermal processes. Similar results also occur in the previous research, the drying process affects the decrease in total phenols in red peppers [15].

Total flavonoid of chili powder ranged from 26.00 - 32.59 mg QE / g. Differences in fruit types did not give a significant effect on the flavonoids of chili powder. While the concentration of soaking solution gave a significant effect on the flavonoids of chili powder. The higher the concentration of the soaking solution, the higher value of flavonoids. Antioxidant compounds including flavonoids in chili are more stable in acidic conditions. The higher the concentration, the higher the acidity level in the solution. So the high concentration of the solution has the highest flavonoid content as well. This is confirmed by previous research [18] which states that flavonoid content is stable at pH 5 to 7 or in other words flavonoids are more stable at acidic pH. Compared with fresh chili the total flavonoids of chili powder was decreased. This is due to degradation by thermal processes [15].

The antioxidant activity IC50 chili powder ranged between 207.54 - 229.01 ppm. Differences in fruit types did not give a significant effect on the IC50 value of chili powder, while the concentration of soaking solution gave a significant effect on the IC50 value of chili powder.The higher the acidity level, the lower IC50 value, which means the higher the antioxidant activity of chili powder. The antioxidant compounds in chili tend to be more stable in acidic pH. Previous research [5] stated that the antioxidant activity of dried chilli increased with increasing concentrations of sodium metabisulfite and citric acid in the soaking solution.

Antioxidant activity of different extracts depends on DPPH radical scaveging activity at pH condition. Previous research [19] reported that methanol extract from peanut shells had higher antioxidant activity at neutral and acidic pH. Antioxidant activity of different extracts from cocoa products is higher at alkaline pH [20]. The phytochemical component in each plant depends on the type of species such as wax or non-waxed species, the material, fresh or dry, and parts used as leaves, fruit or bark, and selected compounds such as carotenoids, non-polar molecules and simple phenols, polar compounds are also a determinant factor of differences in phytochemical compounds [21]. Antioxidant activity is influenced by the functional properties of residuals (ie ascorbic acid, total carotenoids and total phenols, flavonoids) and when compared with fresh chili, decreased antioxidant activity is influenced by thermal processes [5].

3.4 Physical Characteristics of Chili Powder

The brightness level (L), redness level (a), and yellownes level (b) of chili powder is presented in Table 3

Table 3. The Physical Characteristics of Chili Powder

Treatment	Brightness Level (L)	Redness Level (a)	Yellowness Level (b)
JC 5%	58.86± 1.27	19.36± 0.15	35.85± 0.90
JC 10%	58.93± 0.89	20.75 ± 0.80	36.65± 1.04
JC 15%	59.09± 1.05	22.08± 0.51	36.34± 1.53
Bilimbi 5%	59.09± 1.60	19.32 ± 0.78	35.70± 2.06
Bilimbi 10%	59.25± 1.40	20.47± 0.85	35.57± 1.66
Bilimbi 15%	57.98± 1.33	22.11 ± 0.71	35.08± 0.88

Explanation: 1) The number after ± is the standard deviation value
2) Each data is an average of 4 repetitions

The brightness level of chili powder ranges from 57.98 - 59.25. Differences in fruit juices and concentrations of soaking solution did not have a significant effect on the brightness level of chili powder. The higher the L value, the brighter the colorof the sample [22]. Color brightness is influenced by the temperature and duration of the drying process. If the temperature is too high or the

chili drying time is too long, the color of the chili becomes dark. Natural pigments such as carotenoids in dried chili affect the bright colors of the final product due to oxidative degradation of the pigment [16].

The redness level of chili powder ranges from 19.32-22.11. Difference in concentration of soaking solution had a significant effect ($\alpha = 0.05$) on the level of redness of chili powder while the differences in fruit types did not give a significant effect on the redness level of chili powder. The higher the concentration of the solution, the higher the redness level of the chili powder. High concentration of solution has a lower pH or in other words has a higher level of acidity. Some natural color pigments stable at certain pH include caramel color (pH 3 to 10), carotenoids (pH 2 to 8) and turmeric (pH 2.5 to 8) [23]. The application of weak acids in pretreatment of fruits and vegetables is a technique that widely used in the food industry to prevent browning of dried products. Citric acid, the most widely used acid in the food industry, acts as a pH-lowering agent and is often used with other anti-browning agents [24] to prevent enzymatic and nonenzymic browning of dried fruits and vegetables [25].

In addition, the red color difference of chili powder is due to the carotenoid content of chili. Carotenoid is unstable when exposedto light, oxidizing agents and heat [26]. Bilimbi and JC orange solution has acidic content which is able to maintain the red color of the product. Both fruits contain ascorbic acid which plays a role in color stabilization. Ascorbic acid is an oxygen scavenger that helps prevent the fading of the product color [23].

The yellowness level of chili powder ranges from 35.08 - 36.65. Differences in fruit types and concentrations of soaking solution did not have a significant effect on the yellowness level of chili powder.The value of b + (positive) with the range 0 to +70 shows yellow color, while the value of b- (negative) with the range 0 to -70 shows blue color.

3.5 Best Result

The best result between various chili powder which had received different treatments determined by the Zeleny method [8] was chili powder treated with 15% bilimbi solution. This treatment resulted in chili powder with extract color of 191.26 ASTA value, vitamin C content of 0.64 mg / g, capsaicin content of 1316631.5 SHU, total phenol of 9.58 mg GAE / g, total flavonoid of 32.59 mg QE / g, antioxidant activity of 207.5416 ppm, brightness level (L) of 57.98, redness level (* a) of 22.11, and yellowness level (* b) of 35.075. The comparison between the best result of the experiment and control is presented in Table 4.

Table 4. The Comparasion Between Best Result and Control

Parameter	Chili Powder (J2K3)	Chili Powder (Control)
Extract Color (ASTA value)	191.26 ± 5.84[a]	182.39 ± 6.29[a]
Capsaicin	1316631 ± 87748[a]	1207960 ± 115099[a]
Vitamin C Content (mg/g)	0.6391 ± 0.03[a]	0.6816 ± 0.12[a]
Total Phenol (mg GAE/g)	9.531 ± 0.51[a]	9.300 ± 0.09[a]
Total Flavonoid (mg QE/g)	32.59 ± 0.74[a]	32.19 ± 2.73[a]
Antioxidant Activity IC50 (ppm)	207.54 ± 6.75[a]	208.98 ± 16.49[a]

Explanation: 1) Data from the analysis are the average of 4 replications ± standard deviation
2) J2K3 is pretreatment with soaked in bilimbi solution 15%
3) Control is pretreatment with Sodium Metabisulfite0.3% + Citric Acid1%
4) Values that share the same letter are not signifficntly different

Based on the paired t test, bilimbi solution 15% (J2K3) as the best treatment, can replace sodium metabisulphite and citric acid in the chilli soaking process. The two samples showed not significantly different chemical characteristics. Pretreatment with soaking in bilimbi solution 15% has the same ability with pretreatment with soaking in synthetic chemical subtances in maintaining chemical characteristics (total extracted color, capsaicin, vitamin C levels, total phenol, flavonoid levels, and antioxidant activity) of chili powder.

4. Conclusion

Difference of solution concentration give significant effect towards extract color, vitamin C, antioxidant activity IC50, flavonoid, redness level (a*) of chili powder. The best result of this experiment was pretreatment with soaking in bilimbi solution 15%. Soaking with bilimbi15% solution has the same ability as soaking with synthetic chemical subtances in maintaining chemical characteristics of chili powder.

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Polyphenols as potential prebiotic

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Abstract. Several hundred molecules that consist of polyphenols structure have been identified in a broad range of commonly consumed fruits, vegetables, and plant-derived products such as cocoa, tea, or wine. Hindered by the low bioavailability and hydrophobic aspect, these polyphenols are not absorbed in the small intestine but pass to the large intestine. Here they maybe degraded by the colonic microbiota, modulates the bacterial population and improves host's health; in which meet the definition of prebiotics: substrate that is selectively utilized by host microorganisms conferring a health benefit. However, there is still a limited study in investigating the influence of polyphenol and colonic microbiota interaction considering there is huge different type of polyphenols in our daily consume food and beverages.

1. Introduction

Normal gut microbiota. The gastrointestinal tract comprises of mouth, esophagus, stomach, small intestine and colon [1]. With up to 10^{11} to 10^{14} of more than 400 species of bacteria for every gram of gut contents in healty adults, the colon is by far the most heavily colonized region of the gastrointestinal tract [2-4]. The anaerobic bacteria, such as *Bacteroides*, *Bifidobacterium* and *Lactobacilli*, outnumbers the aerobic bacteria, such as *Clostridia*, *Enterococci* and multiple species of *Enterobacterium*, by 1000 fold [5], as seen in Table 1. These microbiota remains stable conditions with respect to the environmental factors which includes the nutrient availability, temperature, pH and the exposure to oxygen [1]. Knowledge about the impact of human gut microbiota on human health has been highlighted and stated that this massive and diverse microbiota has long been recognized as contributing to intestine development, host nourishment and pathogen resistance [6]. In addition to that, past studies proved the microbiota were able to modulate intestinal epithelial proliferation [7], boost host vitality mechanism [8] and regulates inflammatory immune responses [9].

Diseased gut microbiota. Gut microbiota are embroiled in diseases extending from allergies [10,19-21], autism [11, 22], inflammatory bowel disease [12,21], obesity [13], diabetes [14], Crohn's disease [15] and cancer [16, 23]. Study in 2011 showed he impact of the gut microbiota on the gut-brain axis in health and disease. Intestinal dysbiosis affect the gut physiology and hence caused to faulty gut-brain axis signaling and interferes with the central nervous system main functions. This results in stress or disease conditions such as alterations in behavior, emotion, cognition and nociception [24, 25].

Table 1 Composition of the human gastrointestinal microflora [5,17,18]

Gut Microbiota		Bacterial content (CFU/ml or CFU/g)				
		Mouth	Stomach	Jejunum	Ileum	Colon
Anaerobes	*Bacteroides*	$10\text{-}10^5$	-	$0\text{-}10^3$	$0\text{-}10^3$	$10^3\text{-}10^7$
	Bifidobacterium	$10\text{-}10^4$	-	$0\text{-}10^4$	$10^3\text{-}10^9$	$10^8\text{-}10^{11}$
	Lactobacilli	$0\text{-}10^3$	$0\text{-}10^3$	$0\text{-}10^3$	$10^2\text{-}10^5$	$10^4\text{-}10^9$
	Streptococci	-	-	$0\text{-}10^3$	$10^2\text{-}10^6$	$10^{10}\text{-}10^{12}$
Aerobes	*Clostridia*	-	-	-	$10^2\text{-}10^4$	$10^6\text{-}10^{11}$
	Eubacteria	-	-	-	-	$10^9\text{-}10^{12}$
	Enterobacterium	$0\text{-}10^4$	$0\text{-}10^2$	$0\text{-}10^3$	$10^2\text{-}10^7$	$10^4\text{-}10^9$
	Streptococci	$10^5\text{-}10^7$	$0\text{-}10^3$	$0\text{-}10^4$	$10^2\text{-}10^5$	$10^4\text{-}10^9$

Table 2 Changes in the Gut Microbiota Associated with Disease

Disease	Implicated Microbiota	Bacterial enumeration	Changes in microbiota count/function
Allergies	*Lactobacillus spp*	Decrease	early colonization with Lactobacillus associated w/decreased allergies [19]
	Bifidobacterium adolescentis	Decrease	early colonization with more diverse microbiota might prevent allergies [20]
	Clostridium difficile	Decrease	
	Helicobacter pylori	Decrease	H. pylori tolerance mediated by Tregs that suppress asthma [21]
Autism	*Bacteroids*	Increase	increased bacterial diversity in feces of autistic children compared to controls [22]
	Proteobacteria	Increase	
	Actinobacteria	Decrease	
	Firmicutes	Decrease	
Obesity	*Bacteroids*	Decrease	significant changes in gut microbiota are associated with increasing obesity [13]
	Lactobacillus spp	Increase	
	Firmicutes/Bac-teroids ratio	Decrease	
	Methanobrevi-bacter smithii	Decrease	
Diabetes	*Firmicutes*	Decrease	shifts in gut microbiota associated with increases in plasma glucose concentrations [14]
	Clostridia	Decrease	
	Bacteroides-Prevotella	Increase	
	Clostridia coccoides-Eubacterium rectale	Decrease	
	Betaproteobac-teria	Increase	
Crohn's disease	*Bacteroidetes/ Firmicutes ratio*	Increase	less diversity in patients with Crohn's disease compared to healthy patients (15)
	Bacteroides ovatus	Increase	
	Bacteroides vulgatus	Increase	
	Bacteroides uniformis	Decrease	
Cancer	*Helicobacter pylori*	Increase	important element in carcinogenic pathway for developing gastric adenocarcinomas (23)

As discovered by Louis et al. in 'The Gut Microbiota, Bacterial Metabolites and Colorectal Cancer', both presence of specific pathogens and the metabolic produced of the entire colon microbiota contributes to the progression of colorectal cancer. Microbial Short Chain Fatty Acid (SCFA)s, along with low pathogen count and high microbial diversity, plays significant roles in maintain the colon homeostasis as well as suppress the Gram-negative pathogens. Simultaneously, this will act as energy supply, promotes anti-inflammatory agent and also enhance apoptosis of cancer cells [25].

Regardless vast factors are known to influence gut microbiota composition, such as host genotype and microbial interactions, diet is the most obvious potential source of generating the diversity and individuality of these communities [27]. Hence, studies based on functional food such as prebiotics inclined within years as interest on its discovered effect towards gut microbiota.

2. Effect Of Prebiotic On Gut Microbiota

Prebiotics. Priorly defined *as a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health* by the its founder, Glenn R. Gibson in 1995 [28]. However, in 2017, The International Scientific Association for Probiotics and Prebiotics (ISAPP) discussed statements on the definition and scope of prebiotics. Currently, prebiotics are defined *as a substrate that is selectively utilized by host microorganisms conferring a health benefit* [29]. Non-digestibility, low energy value and able to modulate the gut microbiota such as promote *Bifidobacterium* and *Lactobacilli* and as well

repress *Clostridia* (30). Hence, it is well-known today that prebiotic effects probably extend beyond health-promoting gut microbiota such as *Bifidobacterium* and *Lactobacilli*. However, it has to meet the selectivity criteria of a prebiotic, the range of microorganisms affected must be limited [29, 30].

3. Undigested Polyphenols

Polyphenols or phenolic compounds are secondary metabolites with a widespread occurrence in the plant kingdom. For centuries, it has been included in human daily diet, as it is present in massive range of commonly consumed vegetables, fruits and plant-derived products such as cocoa, tea and wine. The effect of polyphenols on host health depends on their doses and bioavailability, which both can vary tremendously [36]. With the estimation 90-95% dietary polyphenols are not absorbed in the small intestine, these polyphenols will be fermented in the colon [37]. Undigested substances that reach the colon are mainly fermented by the anaerobic gut microbiota to produce a broad range of metabolites, which benefits both the chemical diversity of the available substrates and the distinguish biochemical capacity of the gut microbiota. Besides gases, organic acids, mainly the three SCFAs acetate, propionate and butyrate (commonly in a 3/1/1 ratio) are the main fermentation products that regulates a healthy colonic environment [31, 32]. With combined concentration of 50 to 150 mM in the colon [33], these acids are able to inhibit bacterial proliferation [34, 35]. Nevertheless, a range of studies was executed, through *in vitro, in vivo,* animal assays and human intervention, to determine the beneficial effect of polyphenols on gut microbiota and hence discuss the potential on undigested polyphenols as prebiotics, which is the main purpose of this review.

4. In Vitro Study

Using Batch Culture. A simpler method that involves a generally closed systems, mainly a sealed bottles, reactors or vessels that consist of feacal suspensions and maintained respectively to mimic the colon condtions. This method is cost-effective and easy to be carried out with minimum contact with the gut microbiota. Commonly, Flourescence in situ hybridization method will be carried out for the bacterial enumeration for this method. With this model, Mandalari et al., used feacal concentration of 10% of weight per volume, to observe the effect of predigested almond skins on gut microbiota growth within 24 hours. As a result, count *of Bifidobacterium* increases while *Clostridium histolyticum* group decreases [38]. The health-promoting gut microbiota which is also known as lactic acid bacterium (LAB) *Bifidobacerium* and *Lactobacilli* , increases when tested with blueberry extracts and water-insoluble cocoa fractions, though the incubation time was longer at 48 hours and 36 hours respectively [39, 40]. A number of studies were carried out using batch culture method and has outcome of a promising start to discover polyphenols as prebiotic potentials. However, batch culture method is more suitable for short-duration period which do not exceed more than 48 days.

Table 3 Studies using batch culture fermentation

Polyphenols / plant	Dose	Time of incubation (hour)	Microbial enumeration	Growth incline	Growth decline	No effect
Pomegranate extract and punicalagin [41]	10%	48	FISH	Total bacteria *Bifidobacterium* spp. *Lactobacillus* *Enterococcus* spp.		*C. coccoides-E. rectale* group *C. histolyticum* group
Grape seed extract [42]	300-450 mg/L	48	FISH		*C. histolyticum* group	*Lactobacillus* *Enterococcus* spp.
Red wine extract [43]	600 mg/L	48	FISH		*C. histolyticum* group	

(+)-catechin [44]	150 mg/L, 1000 mg/L	48	FISH	*Bifidobacterium* spp. *Lactobacillus Enterococcus* spp. *C. coccoides-E. rectale* group *E. coli*	*C. histolyticum* group

Using Gastrointestinal Modulation. In order to study the adaptation of the gut microbiota on a long term experiments, studies using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) are used in most studies. Similiar to batch culture, except this model can comprehends a longer incubation period which may exceed up to weeks. In 2013, Kemperman et al. used this method on black tea extract and red wine grape extract, with 1000 mg polyphenols as total daily dose for a fortnight. The bacterial enumeration using plate count and PCR-DGGE pyrosequencing were as tabulated in Table 4.

Table 4. Kemperman et al. studies using SHIME

Polyphenols / plant	Dose	Growth incline	Growth decline
Grape seed extract [45]	3 x daily dosing (total of 1000 mg per day intake)	*Klebsiella* spp. *Enterococci Akkermansia* spp.	*Bifidobacteria Blautia coccoides Anaeroglobus* spp. *Victivallis* spp.
Red wine extract [45]		*Klebsiella* spp. *Alistipes* spp. *Cloacibacillus* spp. *Victivallis* spp. *Akkermansia* spp	*Bifidobacteria Blautia coccoides* group *Anaeroglobus* spp. *Subdoligranulum* spp. *Bacteroides*

5. In Vivo Study

Animal Assays. With a degree of confidence from the preliminary studies, animal models were used for better understanding the mechanisms and biological effects that would likely be similar to human mechanisms. In order to assess the effects of polyphenols in the modulation of intestinal microbiota using animal assays (commonly rodent) are tabulated in Table 5.

Table 5. Studies using batch culture fermentation

Polyphenols / plant	Dose	Time of incubation	Microbial enumeration	Growth incline	Growth decline
Apple juice [46]	Free access	4 weeks	Plate count	*Lactobacilli Bifidobacteria*	
Resveratrol [47]	1 mg/kg/ day	25 days	Plate count	*Lactobacilli Bifidobacteria*	
Blackcurrant extracts [48]	13.4-30 mg/kg	4 weeks	FISH	*Lactobacilli Bifidobacteria*	
Blueberries [49]	20 g feed/day	6 weeks	Metagenomic sequencing	*Thermonospora* spp. *Corynebacteria* spp. *Slackia* spp.	*Lactobacillus* spp. *Enterococcus* spp.

Human Intervention. For best understanding of the interactions of polyphenols and human gut microbiota, the human model was used with variety number of volunteers. In 2010, Shinohara et al. uses apples, with daily intake of 2 apples per day for 8 volunteers which resulted increase population of *Lactobacillus* spp. *Streptococcus* spp. *Enterococcus* spp. and reduction of Enterobacteriaceae lecithinase-positive clostridia including *C. perfringens, Pseudomonas* spp. This fortnight experiment were one of the many positive feedbacks on the effect of polyphenols on gut microbiota using human interventions [50].

6. Conclusions

This review summarizes some of the current studies on effect of polyphenols on gut microbiota. With a vast positive feedbacks on the growth of health-promoting gut microbiota, it shows that the polyphenols does have potential in becoming a prebiotics. However, due difference in bioavailability and digestibility from a huge range of polyphenols from human daily intake, further research on the dose and impact on gut microbiota are still required.

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Study of making siger rice from cassava (*Manihot esculenta*) in various harvest age on physical, chemical and organoleptic siger rice

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Abstract. CASSAVA is a food crop commodity that can be processed into siger rice. Siger rice is the term of the Lampung community to mention artificial rice from cassava which has a white color with granular forms such as rice. Siger rice is made so that people psychologically consume siger rice with rice. This study aims to determine the age of cassava harvest which is appropriate in producing the best physical, chemical and organoleptic properties of siger rice. The treatment was arranged in a Complete Randomized Block Design with 4 replications. The treatments consisted of cassava aged 6, 7, 8, 9, 10, 11 and 12 months of harvest. Data were analyzed by variance to obtain variance estimation errors and significance test between treatments and further analyzed by Honestly Significant Difference Test at the level of 5%. The results showed that the difference in cassava harvest age significantly affected the swelling power and amylose levels of siger rice, as well as the hardness, texture, color, preference, and overall acceptance of siger rice. Cassava 6 months of harvest yield the best siger rice with swelling power value of 13.61 and amylose content of 18.61%, and rice hardness of siger 0.57 kg/(10x5mm), rice texture score of 3.29 (same as white rice), rice color score was 3.40 (slightly yellowish white), flavor preference and rice aroma 3.09 (somewhat like), overall acceptance score of rice 3.20 (somewhat like), water content 10.80%, ash content 0,23%, protein content 1.22%, fat content 0.88%, crude fiber content 1.18%, and carbohydrate levels 85.96%.

I. Introduction

Indonesia is a country with high rice consumption. The results of the 2015 National Socio-Economic Survey by the Central Statistics Agency (BPS) [1] stated that per capita rice consumption as of March 2015 was 98 kg per year. This number increased compared to the previous year which was only 97.2 kg per year. This situation proves that the culture of eating rice is difficult to change so that the need for rice is increasing every year in line with population growth. This is the cause of national food security has decreased. Data from FAO in 2016 states that as many as 19.4 million Indonesians are still experiencing hunger due to national food needs not being met. One solution to solve these problems according to Presidential Regulation No. 22 of 2009 is to diversify food by reducing people's dependence on staple foods derived from rice.

Cassava (*Manihot esculenta*) is one of the substitute for rice which is important enough to support food security. Cassava as an alternative food superior commodity in Lampung Province with a production level of 8,038,963 tons and an area of 301,684 ha makes the government develop it into a potential food source besides rice [2]. Cassava is the third food crop commodity in Indonesia after rice and corn. Cassava contains high levels of macro and micro nutrients that have the potential to be used as functional food [3].

Siger rice is an artificial rice product from cassava which adopts the process of making tiwul but with better appearance and taste. Siger rice is made from a mixture of cassava flour and tapioca in the form of granules such as rice. Siger rice grain size is made to resemble rice size so that psychologically the

community when consuming rice is the same as eating rice from rice [4]. The advantages of Siger rice products as staple foods for rice substitutes are that they have characteristics as functional foods, especially for someone who runs a diabetic diet. According to Subeki et al. [5] that the administration of siger rice in mice with a composition of 50% in the ration did not cause liver and kidney damage and could reduce blood glucose levels of normal mice again by 168.50 mg / dL on the 22nd day after alloxan induction. In addition, blood glucose levels of 2 hours post prandial after consuming rice siger is 96.43 mg / dL lower than consuming white rice of 119.37 mg / dL. Administration of siger rice in diabetic patients can stabilize blood glucose levels of less than 200 mg / dL [6].

Siger rice products currently produced still have drawbacks, namely physically cooked rice from siger rice has a sticky, chewy texture, and easily hardens after cold. These characteristics are not favored by the community because they do not give the same impression as rice from rice [7]. This happens because the amylose content in cassava starch is quite high. Amylose has an important role in the process of gelatinization and retrogradation of starch. The shape of the amylose linear chain facilitates the meeting of hydroxyl groups through hydrogen bonds and forms a matrix so as to increase the viscosity of the starch paste. The unstable amylose linear chain causes the gelatinized starch paste to easily retrograde, which is the process of re-forming the starch crystalline structure which causes the product to harden [8].

The characteristics of siger rice products are influenced by the amylose and amylopectin content of the material. The age of harvesting cassava can affect the content of the material, so selection of the right harvest age is important. The age of cassava harvest used as raw material for tapioca industry ranges from 9-12 months. At the age of harvest will produce high levels of starch [9]. According to Nurdjanah et al. [10] that the highest cassava starch content was found at the age of 10 months, which was 23.6%.

In making siger rice, cassava with high starch content is not a consideration in choosing raw materials to make siger rice. The selected raw material is cassava with low amylose content and high amylopectin content. According Susilawati et al. [11] that amylose and amylopectin levels will change in line with increased harvest age. At the age of 7 months, amylose levels of cassava were 12.07% and continued to increase to 20.26% at the age of 9 months. While cassava amylopectin at the age of 7 months was 87.93% and at the age of 9 months it decreased to 79.74%. This proves that harvest age affects the ratio of cassava amylose and amylopectin.

The time to harvest cassava as raw material for making siger rice which can produce the best physical, chemical and organoleptic properties is unknown. Therefore, there will be research on the manufacture of siger rice using cassava from various age levels of certain crops and their effects on the physical, chemical, and organoleptic properties of siger rice produced. This study aims to obtain the best physical, chemical, and organoleptic properties of siger rice from cassava at the right age of harvest.

2. Materials and Methods

2.1. Place and time of research

This research was carried out at the Agricultural Product Processing Laboratory and the Agricultural Product Analysis Laboratory, Department of Agricultural Product Technology, Faculty of Agriculture, University of Lampung. This research will be held from February to April 2018.

2.2. Materials and tools

The ingredients used to make siger rice are cassava harvesting age (6 months, 7 months, 8 months, 9 months, 10 months, 11 months, and 12 months), glycerol Monostearate (GMS), cooking oil, salt, acid ascorbate, and water. The ingredients for analysis are HgO, K2SO4, H2SO4, NaOH-Na2S2O, H3BO3, HCl 0.02 N, 1N NaOH, iodine, distilled water, hexane, water destilate, buffer Na-acetate, α-galactosidase, dinitrosalicylic, amylose, ethanol , acetic acid, acetone, and other ingredients for analysis. The tools used are extruder machines, ovens, scales, sieves, pans, basins, filters, grater machines, stoves, pans, soxhlet, furnaces, analytical balance, filter paper, and
glassware for analysis.

2.3. Research methods

This study uses a Completely Randomized Design (CRD) with 3 replications. The study was conducted with the treatment of age of cassava harvest U1 (6 months), U2 (7 months), U3 (8 months), U4 (9 months), U5 (10 months), U6 (11 months), and U7 (12 months) . The data obtained were tested for homogeneity by Bartlet test and data addition by Tuckey test. The data was then analyzed by variance to obtain variance estimation errors and significance test between treatments. Furthermore, to find out the differences between treatments the data was tested further with the smallest real difference test (LSD) at 1% and 5% real levels.

2.4. Research Implementation

2.4.1. Raw Material Preparation

The raw material used is cassava meal with a harvesting age of 6, 7, 8, 9, 10, 11, and 12 months. Cassava is peeled, washed and grated with a grater. The grated cassava is then soaked in water (1: 3) for 12 hours then squeezed until it is obtained filtrate and cassava pulp. The filtrate is allowed to stand for 1 hour until the tapioca precipitate is obtained. The tapioca precipitate is then dried in an oven at a temperature of 60 ° C until the moisture content is <13% and ground into tapioca. Cassava pulp is also dried in the oven at 60oC until the moisture content is <13% and ground into cassava pulp. The process of making cassava and tapioca pulp can be seen in Figure 1.

2.4.1. Making Siger Rice

Siger rice is made by using 1: 4 cassava and tapioca pulp mixed with additional ingredients such as emulsifier. Siger rice mixture is then homogenized using a mixer. The mixture is then steamed in a pan for 30 minutes at 90 ° C. The dough is cooled for 1 hour and then printed using an extruder. The material enters the movement of the rollers to be forced out in a 2 x 6 mm elliptical hole equipped with cutting blades. The rice granules obtained are then aerated and then dried using an oven at a temperature of 60 ° C until 8% moisture content is obtained. The rice grain formed is then sorted. The process of making rice can be seen in Figure 2.

Siger rice obtained was analyzed by swelling power using the method of Leach et al. [12], as well as amylose and amylopectin levels using the method of Apriyanto [13]. Siger rice is then cooked into rice and the organoleptic properties of color and texture will be analyzed using a scoring test. Organoleptic properties in the form of taste, aroma, and overall acceptance were analyzed using hedonic tests. The best siger rice from the results of organoleptic test was then analyzed proximate using the AOAC method [14].

2.5. Observation

2.5.1. Characteristics of Siger Rice

2.5.1.1. Sensory Test

Sensor tests are performed to see the characteristics of siger rice after being cooked into rice on the texture, color, taste and aroma, and overall acceptance. Assessment of texture and color using a scoring test, while the taste and aroma and overall acceptance using hedonic tests [15]. Sensory testing was carried out by 20 semi-trained panelists. The sensory test scale can be seen in Table 1.

2.5.1.2. Swelling Power

The ability to expand rice is determined by the method of Leach et al. [12]. Samples of 0.1 g of siger rice which have been mashed are put into a test tube. The sample is then added 10 ml of distilled water and heated in a water bath at a temperature of 70ºC for 30 minutes while stirring continuously. The supernatant was separated from the solution by means of a test tube containing a centrifuged sample at a speed of 2500 rpm for 20 minutes and then decanted. The resulting paste is then taken and weighed

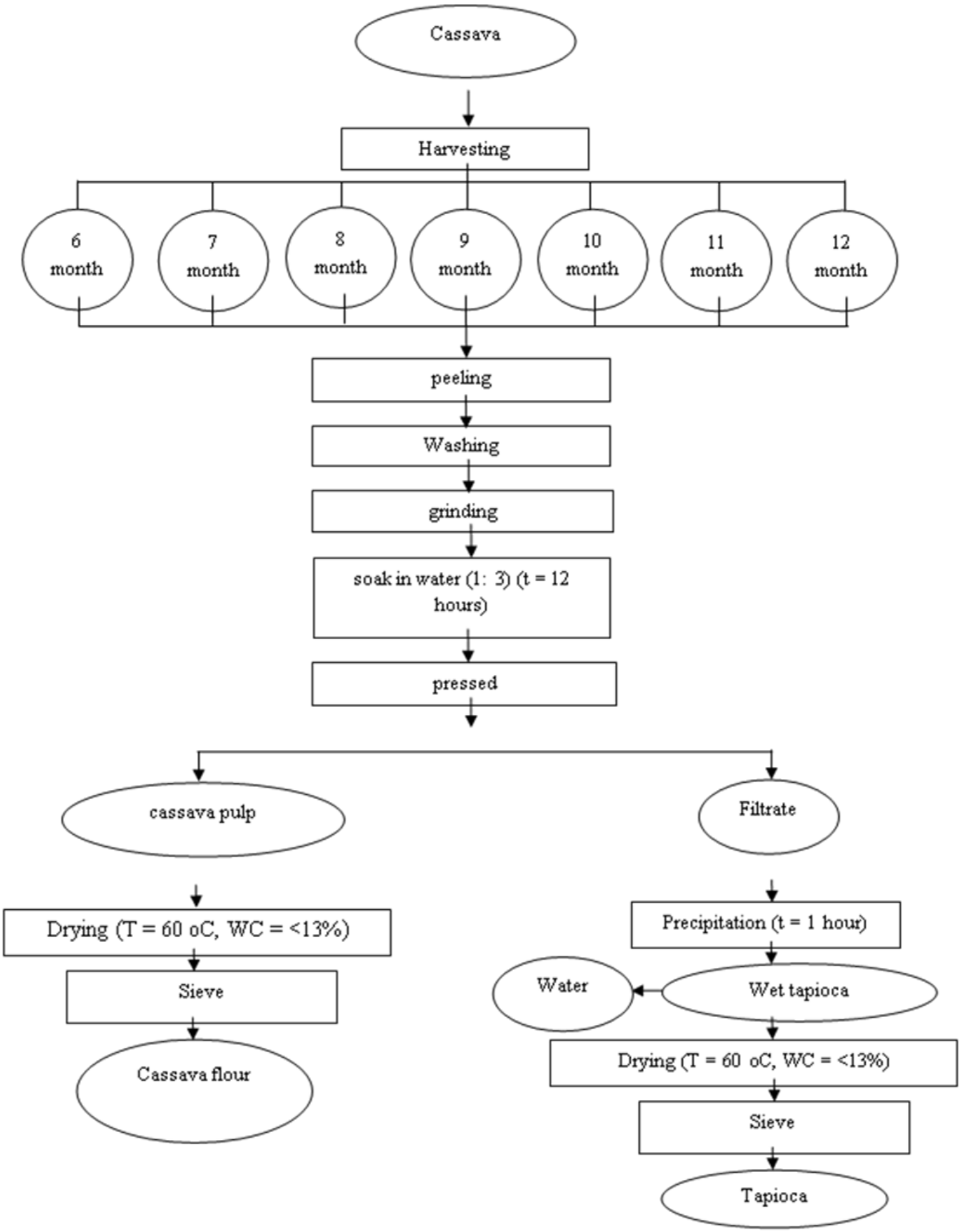

Figure 1. Making cassava and tapioca pulp [7]

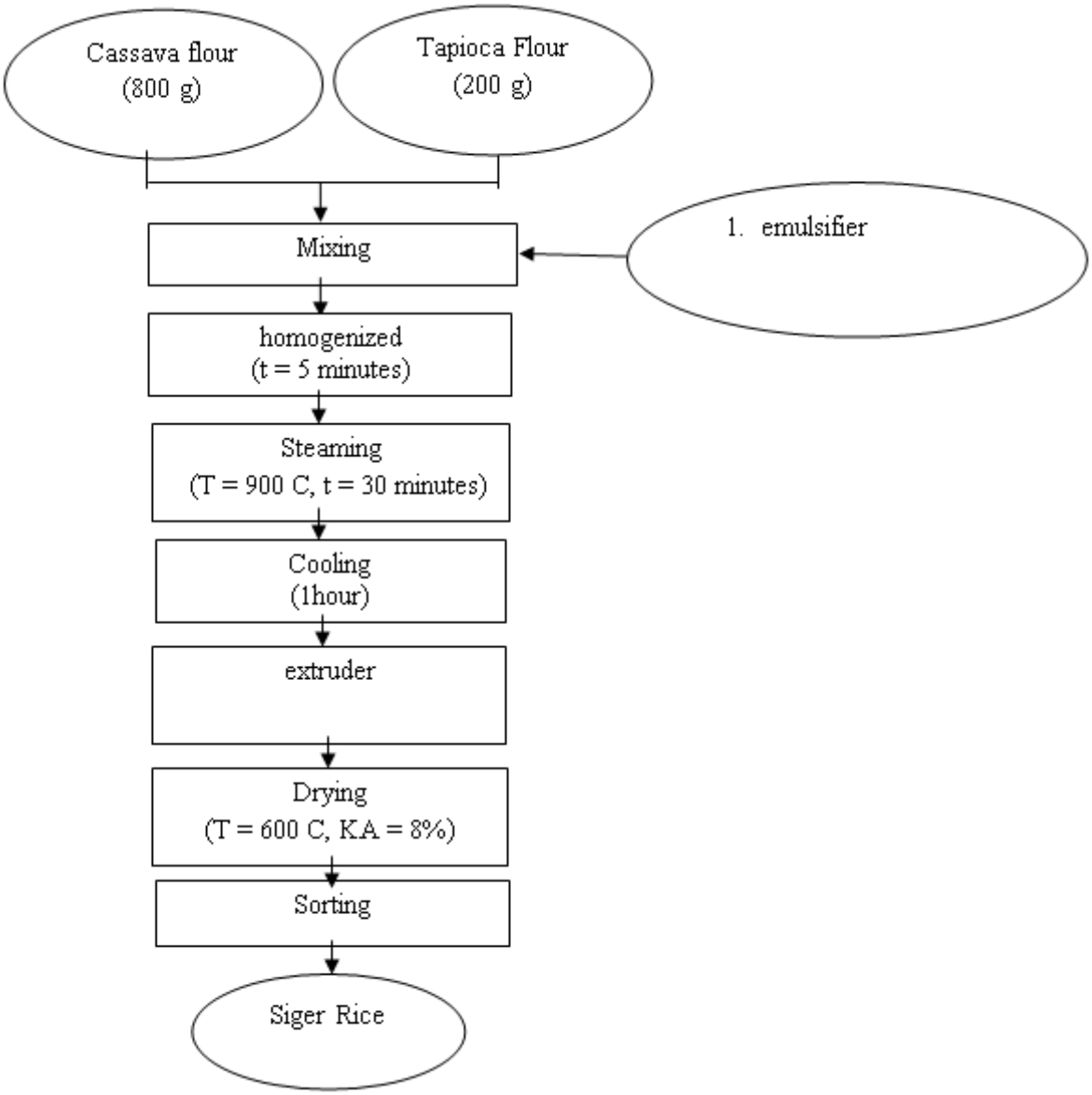

Figure 2. The process of making siger rice [7]

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Table 1. Scale of sensory test

Parameter	Criteria	Score
Texture	Very chewy	5
	chewy	4
	Rather chewy	3
	Not chewy	2
	Very not chewy	1
Color	White	5
	Brownish white	4
	Rather yellowish white	3
	Brownish yellow	2
Taste and Aroma	Chocolate	1
	Really like	5
	Like it	4
	Rather like	3
Overall reception	Do not like	2
	Very dislike	1
	Really like	5
	Like it	4
	Rather like	3
	Do not like	2
	Very dislike	1

2.5.1.3. Amylose and Amylopectin levels

Amylose content is analyzed based on Apriyanto method [13]. The analysis begins with the manufacture of a standard amylose curve, which is 40 mg of pure amylose put into a test tube and then added 1 mL of absolute ethanol and 9 mL of 1M NaOH. The mixture was heated in boiling water (100ºC) for 10 minutes and then transferred to a 100 mL measuring flask. Gel is added with destilated water and homogenized, then held up to 100 mL using distilled water.

The solution obtained was taken with pipettes of 1, 2, 3, 4 and 5 ml, respectively, then put in a 100 mL measuring flask and acidified with acetic acid 1 N as much as 0.2, 0.4, 0.6, 0, 8, and 1.0 mL. Each measuring flask was added with 2 mL of Iod and distilled water until the tera mark. The solution was homogenized by hand until evenly distributed and left for 20 minutes, then its absorption was measured by UV Vis spectrophotometer at a wavelength of 620 nm. The results obtained are then made a relationship curve between amylose levels and its absorbance.

Amylose content measurement in the sample was carried out by as much as 1 mL absolute ethanol, 9 mL 1N NaOH solution and 100 g of sample were mixed and heated for 10 minutes on boiling water bath. After 5 mL of cold the sample was added 2 mL of 1 mL iodine solution and 1 N HCL and then treated with destilated water in a 100 mL flask, then left for 20 minutes. The absorbance is measured at a wavelength of 620 nm. Amylose content is calculated based on the standard curve equation obtained. Amylopectin levels are obtained by by difference, namely by reducing the value of 100% with amylose content or can be written with the following equation:

$$\text{Amylopectin (\%)} = 100\% - \text{amylose content (\%)}$$

3.5.2. Proximate Analysis of the Best Siger Rice

Water, ash, fat, protein, carbohydrate content testing using the oven method [14].

3. Results and Discussion

3.1. hardness

The results of variance analysis (Table 2) show that the difference in cassava harvest age has a very significant effect on the hardness of siger rice produced.

Table 2. Effect of cassava harvest age on siger rice hardness based on BNJ test level of 5%

Treatment	Hardness value (kg / (10x5 mm)
A1: Cassava 6 months	0.565[c]
A2: Cassava 7 months	0.573[c]
A3: Cassava 8 months	0.695[b]
A4: Cassava 9 months	0.728[b]
A5: Cassava 10 months	0.798[a]
A6: Cassava 11 months	0.795[a]
A7: Cassava 12 months	0.733[b]
BNJ (0.05) = 0.049	

Remarks: The numbers followed by the same letter show no significant difference in the 5% Honest Honest Difference Test (BNJ)

The value of the Siger rice hardness test that was tested with penetrometer ranged between 0.798 to 0.565. Siger rice made from cassava with a 10-month harvest is rice with the highest hardness value, which is 0.798. The lowest hardness value is in Siger rice which is made from cassava in the age of 6 months of harvest, which is 0.565. Siger rice hardness is related to starch retrogradation that occurs during the cooling process after heating (gelatinization). Starch gel if left idle for a while, there will be an expansion of the crystal area and result in shrinkage of the gel structure followed by the release of water from the gel and make the texture of the rice hard. Gel hardness is also influenced by the crystallinity of starch which depends on the amount of amylose and amylopectin in starch [8].

Based on Table 7, it can be seen that the results of BNJ further test of 5% level on rice siger hardness showed that cassava treatment at 6 months of harvest was significantly different from the cassava treatment of harvesting ages 8, 9, 10, 11, and 12 months. The cassava treatment at 10 months of harvest was significantly different from the cassava treatment at 6, 7, 8, 9, and 12 months.

Increased harvest age can increase the hardness of siger rice, but the highest level of hardness is found in cassava aged 10 months, and after that the violence of rice has decreased. This is due to the fact that in this study, cassava siger rice was harvested with the highest amylose content and in the cassava treatment at 11 months of harvest the amylose content of siger rice had decreased, so that the level of violence of Siger rice decreased, although the value of the violence was not different. real with rice siger from cassava aged 10 months harvest. The amylose component plays a major role in the retrogradation process which causes siger rice to harden after cold. In the retrogradation process, free amylose forms hydrogen bonds with fellow amylose and some branching of amylopectin extends from the swollen granule [16]. The grains of the starch incorporated into a kind of nets form microcrystals and settle [17]. Amylose has the ability to form crystals because of its simple polymer chain structure. This simple structure can form strong molecular interactions. The formation of hydrogen bonds is easier to occur in amylose than amylopectin [18].

3.2. Swelling Power

Swelling power shows the ability of starch to expand in water. High swelling power indicates the higher the ability of starch to expand in water [19]. Swelling power value of siger rice from various harvesting ages ranged between 11,076 - 14,350. The results of the analysis of variance showed that differences in the age of cassava harvest had a very significant effect on the swelling power value of Siger rice, so it was necessary to do further testing of BNJ at the level of 5% to determine the differences between treatments. The effect of cassava harvest age on the swelling power value of siger rice based on BNJ test level of 5% is presented in Table 3.

Table 3. Effect of cassava harvest age on rice siger texture based on BNJ test level of 5%

Treatment	Swelling power score
A1: Cassava 6 months	13.612^{abc}
A2: Cassava 7 months	13.791^{ab}
A3: Cassava 8 months	14.350^{a}
A4: Cassava 9 months	14.195^{ab}
A5: Cassava 10 months	12.767^{bcd}
A6: Cassava 11 months	12.339^{cd}
A7: Cassava 12 months	11.976^{d}
BNJ (0.05) = 1.502	

Note: The numbers followed by the same letter show no significant difference in the 5% Honest Honest Difference Test (BNJ)

BNJ test results of 5% level on swelling power value of siger rice at various harvesting ages showed a significant difference in the treatment of cassava aged 6 months of harvest with cassava aged 12 months of harvest. Swelling power of cassava treatment at 6 months of harvest was the same as cassava treatment at harvesting ages 7, 8, 9, 10, and 11. The treatment of cassava for 10 months of harvest was significantly different from cassava treatment at 8 months of harvest. Siger rice made from cassava 11 months of harvest has a swelling power value which is significantly different from siger rice from cassava with a harvesting age of 7, 8 and 9 months. Swelling power of cassava treatment at 12 months of harvest was the same as in cassava treatment at 10 and 11 months of harvest, but it was significantly different from the age treatment of cassava 6, 7, 8, and 9 months.

The highest swelling power value was found in cassava siger rice with 8 months of harvest, which was 14.350. The lowest swelling power value is found in cassava siger rice with 12 months of harvest, which is 11.976. The swelling power value of a starch-based material is based on the amylose and amylopectin content of the starch. Amylose and amylopectin ratios affect the value of swelling power. High amylose causes the amorphous region of starch to be higher and makes water easier to enter the granule [20]. Increased amylopectin will increase the strength of the crystalline structure and inhibit granular swelling [21]. Research by Charles et al. [22] showed an increase in swelling power and solubility with increasing amylose levels. In mung bean starch also reported an increase in amylose levels can increase the solubility and power of starch blooms [23].

4.3. Amylose

The results of the analysis of variance (Table 4) showed that the differences in the age of cassava harvest had a very significant effect on the levels of siger rice amylose, so it was necessary to conduct further testing of BNJ at the level of 5%. Based on the BNJ further test the 5% level of the amylose content of siger rice from cassava in various harvesting ages showed significant differences between treatments. The cassava treatment at harvesting ages of 6 and 7 months had amylose content significantly different from the cassava treatment at 8, 9, 10, 11 and 12 months. Siger rice from cassava at 8 months of harvest was the same as cassava treatment at 11 and 12 months of harvest, but it was significantly different from the cassava treatment of 6, 7, 9 and 10 months of harvest. The cassava treatment of harvesting ages 8 and 9 months was significantly different from the cassava treatment at harvesting ages of 6, 7, and 8 months. The cassava treatment of harvesting ages 11 and 12 months was significantly different from the cassava treatment at 6 and 7 months of harvest.

Table 4. Effect of cassava harvest age on amylose content of siger rice based on BNJ test level of 5%

Treatment	Amilosa
A1: Cassava 6 months	18.605[c]
A2: Cassava 7 months	19.171[c]
A3: Cassava 8 months	22.691[b]
A4: Cassava 9 months	25.219[a]
A5: Cassava 10 months	25.351[a]
A6: Cassava 11 months	24.132[ab]
A7: Cassava 12 months	23.698[ab]
BNJ (0.05) = 2.339	

Note: The numbers followed by the same letter show no significant difference in the 5% Honest Honest Difference Test (BNJ)

The highest amylose content obtained in siger rice is made from cassava in the 10 months of harvest, which is 25.351%. The lowest amylose content is found in siger rice which is made from cassava in the age of 6 months, which is 18.605%. The difference in age of cassava harvest will affect the amylose content in the tuber. Sriroth et al. [24] stated that the levels of amylose and starch in cassava will generally be lower in plants that are still in the growth phase (not ready for harvest). Susilawati et al. [11] stated that, the high levels of amylose in cassava at a certain harvest age was caused because at that age cassava had a high starch content. The starch is thought to have a longer α 1,4 D-glycoside chain compared to cassava at other harvesting ages. The longer the α 1,4 D-glycoside chain contained in the starch, the higher the amylose content contained in it [25].

In the growth phase the growing amylose molecule with a glucose unit having a C-4 reaction group at the end joins the C-1 glucose added from ADPG, while the branch on amylopectin between C-6 in the main chain and C-1 in the branch chain is formed by various isoenzymes of several enzymes which are concisely called branching enzymes or Q enzymes [26]. According to Thomas and Atwell [27], the formation of amylopectin occurs due to the cutting of the amylose chain which is then connected to the α-1.6 bond in one of the amylose chain D-glucose molecules. At the beginning of starch synthesis, amylose molecules have a longer chain and along with the age of the plant, the amylose chain will experience branching to form amylopectin so that the amylose content in starch will decrease.

Based on Table 4. it can be seen that increasing the age of cassava harvest can increase amylose levels in siger rice, but at the age of harvesting cassava that is too old can reduce levels of siger rice amylose. In the cassava treatment of 6 to 10 months of harvest, the amylose content of siger rice had increased respectively from the ages of 6.7.8.9, and 10 months at 18.605%, 19.171%, 22.691%, 25.691%, and 25.351%. In the cassava treatment at 11 months of harvest, the amylose content of siger rice decreased to 12 months of cassava treatment to 24,132% and 23,698%. Increased levels of amylose siger rice are influenced by amylose content in the raw material for making siger rice, namely cassava. This is supported by research by Susilawati et al. [11] stated that at 7 to 8 months of harvest, cassava has increased amylose levels, from 12.07% to 20.82%. At higher harvesting ages, ie 9 to 10 months of harvest, cassava has decreased amylose content to 20.26% and 18.03%.

4.4. Organoleptic Test

Organoleptic test for rice siger from cassava in various harvesting ages using scoring, hedonic and multiple comparison tests. Parameters observed by scoring method include color, while the parameters of aroma and taste, as well as overall acceptance of rice siger are tested by hedonic. Multiple comparison tests are used to determine the organoleptic value of texture parameters.

4.4.1. Texture

Siger rice texture is assessed based on the level of hardness of rice when chewed. Assessment of the texture of rice siger using the multiple comparison organoleptic test with reference samples (R) in the form of white rice from rice rice. The texture score obtained is 4.875 (worse than R) – 3.288 (equal to

R). The rating scale is based on rank, so the high value indicates that Siger rice has a worse quality than R. The results of the analysis of variance showed that differences in the age of cassava harvest gave a very significant effect on rice siger texture scores so that further testing of BNJ was needed with a 5% confidence interval. The effect of cassava harvest age on rice siger texture based on BNJ test at 5% level is presented in Table 5.

Table 5. Effect of cassava harvest age on rice siger texture based on BNJ test level of 5%

Treatment	Texture score
A1: Cassava 6 months	3.288^{d}
A2: Cassava 7 months	3.363^{cd}
A3: Cassava 8 months	3.438^{cd}
A4: Cassava 9 months	3.913^{bc}
A5: Cassava 10 months	4.088^{b}
A6: Cassava 11 months	4.375^{ab}
A7: Cassava 12 months	4.875^{a}
BNJ (0,05) = 0,618	

Note: The numbers followed by the same letter show no significant difference in the 5% Honest Real Difference Test (BNJ). Texture score (1) Very better than R, (2) better than R, (3) equal to R, (4) worse than R, (5) very worse than R.

Table 5 presents the results of the BNJ texture score test at the 5% level which shows the real differences between treatments. Siger rice made from cassava with a 6-month harvest has a different texture score with rice siger made from cassava with a harvest age of 9, 10.11, and 12 months. The cassava treatment at harvesting ages of 7 and 8 months was not significantly different from the cassava treatment at 6 and 9 months of harvest. The cassava treatment at 9 months of harvest was not significantly different from the cassava treatment at harvesting ages of 7, 8, 10, and 11 months, but it was different from the cassava treatment at 6 and 12 months of harvest. Siger rice from cassava for 10 months of harvest had a texture score that was not significantly different from the cassava treatment at 9 and 11 months of harvest. Treatment of cassava at 11 months of harvest is the same as cassava treatment at 12 months of harvest.

The highest texture score is owned by cassava treatment at 12 months of harvest, which is 4,875 and is a worse score with criteria worse than R. The best score from the assessment of rice texture with criteria equals R is found in siger rice made from cassava harvesting age 6 month, which is 3.288. The difference in the results of panelists' assessment of the texture of Siger rice made from cassava of various ages is affected by the retrogradation process of Siger rice. Siger rice is cooked and undergoes gelatinization to Siger rice. After the gelatinization process, the cooled siger rice will undergo a process of retrogradation and cause the rice to turn hard due to amylose chains that re-bond. Amylose molecules will bind with each other and also with the amylopectin branch on the outer edges of the granule. These molecules connect the starch grains that were previously swollen during the gealtinization process. The grains of the starch incorporated into a kind of nets form microcrystals and settle [17].

Based on Table 10. it is known that the texture quality of Siger rice will be worse than the white rice of rice rice along with the age of cassava harvest. This occurs because cassava with a lower harvest age has a lower amylose content [24]. Amylose affects the retrogradation process of Siger rice. This is in accordance with the statement Noviasari et al. [28] that the amylose content contained in the raw material for making analog rice affects the nature of rice and rice produced, such as the level of crispness (texture) and functional properties. The higher the amylose content found in rice, the more rice it will produce with low pulses, and vice versa. The higher the composition of starch in analog rice, the higher the amylose content, and the more dry or hard texture of rice [29].

4.4.2. Color

Color is the first factor in human consideration in choosing food. A food with high nutrient content, good taste, and good texture, will likely not be chosen if it has an unattractive or distorted color. Organoleptic color test results showed a score ranging from 1.66 (brownish yellow) - 3.25 (yellowish white). The scale used is suspension so that the higher the value, the better the quality of the color of Siger rice.

The results of the variance analysis showed that the difference in the age of cassava harvest was significantly different for the Siger rice color score made from cassava, so that further BNJ testing was needed with a 5% confidence interval. The effect of cassava harvest age on rice siger color based on BNJ test level of 5% is presented in Table 6.

Table 6. The effect of cassava harvest age on rice siger color based on BNJ test level of 5%

Treatment	Colour score
A1: Cassava 6 months	3.400[a]
A2: Cassava 7 months	3.250[a]
A3: Cassava 8 months	3.325[a]
A4: Cassava 9 months	3.350[a]
A5: Cassava 10 months	2.713[b]
A6: Cassava 11 months	1.613[c]
A7: Cassava 12 months	1.663[c]
BNJ (0.05) = 0.360	

Note: The numbers followed by the same letter show no significant difference in the 5% Honest Real Difference Test (BNJ). Color score (1) brown, (2) brownish yellow, (3) yellowish white, (4) yellowish white, (5) white.

Based on the results of the BNJ 5% further test, it is known that the color of siger rice made from cassava at 6 months of harvest is not significantly different from the color of rice siger from cassava in the age of 7, 8 and 9 months, but it is significantly different from the harvested cassava. 10, 11 and 12 months. The treatment using cassava for 10 months of harvest was significantly different from all other treatments. The color of Siger rice made from cassava 11 months of harvest is not significantly different from rice siger from cassava with a 12-month harvest, but significantly different from rice siger from cassava with a harvesting age of 6, 7, 8, 9, and 10 months.

The highest color score in this study is owned by rice siger made from cassava with a 6-month harvest age, which is 3.400 and is the best score with yellowish white criteria. The lowest color score in this research is owned by nasi siger which is made from cassava 11 months of harvest, which is 1.613 and is the worst score with the criteria of brownish yellow. The color of siger rice is influenced by the raw material of siger rice. Siger rice is made from yellow cassava flour and tapioca which tends to be white. This color is produced because the results of the process of drying the material into flour [30]. Siger rice also undergoes a heating process in order to experience gelatinization into Siger rice. High heating temperature has an impact on the brightness level of Siger rice [31].

Based on Table 6, the increase in the age of harvesting cassava makes the siger product color score decreases. This is influenced by the starch content in the material. These chemical components can cause changes in color in the material due to reaction with oxygen and water vapor [31]. Increasing the age of cassava harvest causes an increase in starch levels [10]. High starch levels increase carbohydrate content. Siger rice which contains high carbohydrates will experience discoloration during heating due to browning reactions. The browning reaction that occurs is a non-enzymatic Mailard reaction that involves reducing sugars with amines from amino acids or proteins. Amino acids which are the main constituent of peptides and proteins will react with reducing sugars which contain aldehyde and ketone groups, resulting in a brown color [31].

4.4.3. Taste and Aroma

Taste and aroma are one of the parameters in determining the quality of a food product. The taste and aroma of food can be felt by the human senses in the sense of smell and taste senses (tongue). The organoleptic score of the taste and aroma of Siger rice ranged from 1.613 (not like) – 3.400 (rather like) with the assessment criteria very like to very dislike.

The results of variance analysis showed that the difference in cassava harvesting age was significantly different from the flavor and aroma score of siger rice made from cassava, so that further BNJ testing was needed with a 5% confidence interval. The effect of cassava harvest age on the taste and aroma of rice siger based on BNJ test level of 5% is presented in Table 7.

Table 7. Effect of cassava harvesting age on the taste and aroma of Siger rice based on BNJ further test at 5% level

Treatment	Aroma and taste Scores
A1: Cassava 6 months	3.085[a]
A2: Cassava 7 months	2.800[ab]
A3: Cassava 8 months	2.675[bc]
A4: Cassava 9 months	2.508[bc]
A5: Cassava 10 months	2.400[cd]
A6: Cassava 11 months	2.163[d]
A7: Cassava 12 months	1.810[e]
BNJ (0.05) = 0.294	

Notes: The numbers followed by the same letter show no significant difference in the 5% Honest Real Difference Test (BNJ). Taste and aroma scores (1) very dislike, (2) dislike, (3) rather like, (4) likes, (5) really like.

BNJ further test results at 5% level on the taste and aroma of siger rice in Table 12. shows that rice siger from cassava aged 6 months is not significantly different from the cassava treatment at 7 months old, but significantly different from the cassava plant age of harvest 8, 9, 10, 11 and 12 months. The level of panelists' preference for the taste and aroma of cassava rice treated with cassava at the age of 7 months was the same as that of cassava rice with cassava age of 8 and 9 months of harvest. The cassava treatment of harvesting ages 8 and 9 months was significantly different from the cassava treatment of harvesting ages 6, 11, and 12 months. The cassava treatment at 10 months of harvest had the same taste and aroma score as the cassava treatment at 8, 9 and 10 months of harvest. The taste and aroma score of the panelist's preference for cassava treatment at 12 months of harvest was significantly different from all treatments.

The highest flavor and aroma score in this study was obtained in cassava treatment at 6 months of harvest, which was 3.085 and was the best score with the criteria rather like. The lowest taste and aroma score in this study was obtained in cassava treatment at 12 months of harvest, which was 1,810 and was the worst score with criteria of dislike. The taste and aroma of Siger rice depends on the ingredients of the product. Siger rice is a product made from a mixture of cassava flour and cassava pulp that has a distinctive taste and aroma. The taste and aroma of cassava can be influenced by the content of volatile compounds in cassava. The specific aroma of rice siger made from cassava and tapioca pulp and other additives such as emulsifire and glycerol can occur due to oxidation or due to Mailard reaction during the process of making rice. Oxidation can occur against lipids and proteins in the ingredients [32]. The Mailard reaction occurs from the reaction of reduced sugar carbonyl groups with amino acid (amino groups) formed from nitrogen substituted by glycosylamine or fructosylamine [33]. This reaction will produce scented volatile compounds such as furan, pyridine, and pyrazine [34]. These compounds are the cause of the distinctive aroma of siger rice which is less preferred by consumers.

Based on Table 7 Increasing age of cassava harvest makes the taste and aroma score of siger rice products lower. This is influenced by the content of chemical components in the material. Harvest age differences affect the nutritional content of tubers [35]. Nutrient content such as carbohydrate, protein

and fat content in cassava can increase or decrease depending on variety, harvest age, climate, and soil fertility [36]. In the growth phase (not ready for harvest), generally some of the plant's nutrient content is lower than plants that are ready for harvest [24]. The higher carbohydrate, protein and fat content in cassava in a certain harvest age can affect the volatile formation reaction in the tubers, so that their distinctive taste and aroma are stronger and it turns out that the taste is less preferred by consumers.

4.4.4. Overall acceptance

Analysis of variance results shows that the difference in age of cassava harvest has a very significant effect on the overall score of siger rice, so it is necessary to do further testing of BNJ with a 5% confidence interval. The effect of cassava harvest age on the overall acceptance of siger rice based on BNJ test level of 5% is presented in Table 8.

Table 8. Effect of cassava harvest age on overall acceptance of siger rice based on BNJ test level of 5%

Treatment	Score for overall acceptance
A1: Cassava 6 months	3.200[a]
A2: Cassava 7 months	3.050[ab]
A3: Cassava 8 months	2.900[ab]
A4: Cassava 9 months	2.588[bc]
A5: Cassava 10 months	2.513[bcd]
A6: Cassava 11 months	2.063[cd]
A7: Cassava 12 months	1.950[d]
BNJ (0.05) = 0.567	

Notes: The numbers followed by the same letter show no significant difference in the 5% Honest Real Difference Test (BNJ). Overall acceptance scores (1) very dislike, (2) dislike, (3) rather like, (4) likes, (5) really like.

The results of the BNJ further test of the 5% level presented in Table 8 show that Siger rice made from cassava for 6 months of harvest did not differ significantly from the overall acceptance score with cassava age of 7 and 8 years old. The cassava treatment of harvesting ages 7 and 8 months was different from the cassava treatment of harvesting ages 9, 10, 11, and 12 months. The cassava treatment at 9 months of harvest was significantly different from the cassava treatment at 6 and 12 years of harvest, but the same as the cassava treatment at 7, 8, 10 and 11 months of harvest. The cassava treatment at 10 months of harvest was significantly different from the cassava treatment at the age of 6 months. Cassava treatment at 12 months of harvest has the same acceptance score as cassava treatment at 10 and 11 months of harvest.

The highest score was found in cassava treatment at 6 months of harvest, namely 3.200 with the category of rather like and the best score. The lowest score was found in cassava treatment at 12 months of harvest, which is 1.950 with the category of dislike and the worst score. The overall acceptance of the product is influenced by the organoleptic properties of other parameters. The panelist will assess the product as a whole. The results of the assessment of all organoleptic parameters in this study did show the best results in the treatment of cassava aged 6 months of harvest.

Based on Table 8, it is known that increasing the age of cassava causes the overall acceptance score to decrease. This is due to changes in other sensory parameters, such as color, taste and aroma, and texture. The longer the age of harvesting cassava, the raw material for making siger rice will also change its characteristics, such as the color of siger rice which is more brown when made from cassava with a higher harvest age. Cassava with an older harvest age tends to have a higher chemical content, so the reaction caused by the component is also getting bigger. Mailard reaction is a reaction that can affect the color, taste and aroma, and the texture of cooked siger rice [32].

4.5. Selection of the Best Treatment

This study aims to get the highest quality siger rice that consumers like. Determination of the best treatment from this study focused more on the results of organoleptic tests on the parameters of texture, color, taste and aroma, and overall acceptance. The determination is based on the assumption that if the panelist has liked a particular product because of its organoleptic properties, then the product can be well received by other consumers.

The best texture parameters are determined based on the lowest value of the treatment. The result of organoleptic texture showed that the best texture of Siger rice is Siger rice made from cassava with a 6-month harvest. The treatment produces a texture value of 3.288 with the same texture criteria as the reference (R). The reference used in the organoleptic test of this texture is white rice from rice rice. Color, taste and aroma parameters, and overall acceptance are best determined based on the highest value of each parameter. Organoleptic test results of the best color of Siger rice are on cassava treatment aged 6 months of harvest with 3,400 color criteria yellowish white. The results of organoleptic taste and aroma, as well as overall acceptance were best found in cassava treatment at 6 months of harvest with a flavor and aroma score of 3.085 with a rather favorable criteria, and an overall acceptance score of 3.200 with somewhat like criteria. Organoleptic test results showed that Siger rice made from cassava aged 6 months was siger rice which was the most preferred and accepted by panelists. The results of the recapitulation of the results of organoleptic test of rice siger from cassava in various harvesting ages are presented in Table 9.

Table 9. Recapitulation of organoleptic test results of Siger rice

Results	Harvest Age Treatment						
Observation	A1	A2	A3	A4	A5	A6	A7
Texture	**3.288d***	3.363cd	3.438cd	3.913bc	4.088b	4.375ab	4.875a
Color	**3.400a***	3.250a	3.325a	3.350a	2.713b	1.613c	1.663c
Taste and aroma	**3.085a***	2.675bc	2.800ab	2.508bc	2.400cd	2.163d	1.810e
Overall acceptance	**3.200a***	3.050ab	2.900ab	2.588bc	2.513bcd	2.063cd	1.950d

Notes: (*) The best treatment for these parameters, (A1) cassava with 6 months of harvest, (A2) cassava with 7 months of harvest, (A3) cassava with 8 months of harvest, (A4) Cassava with 9 months of harvest, (A5) Cassava is 10 months old, (A6) Cassava is 11 months old, (A7) Cassava is 12 months old.

4.6. Proximate analysis

Proximate analysis was carried out on the best treatment siger rice made from cassava with a 6-month harvest yielding nutrient content which can be seen in Table 10. Proximate analysis carried out included water content, ash content, fat content, protein content, crude fiber content, and carbohydrate levels.

Water is an important component in food that can affect the quality of materials, especially the durability of the product. Siger rice made from cassava pulp powder for 6 months of harvest has a water content of 10.8010%. The water content of siger rice still meets the standard specifications for rice quality requirements based on SNI 6128-2015, ie rice water content is less than 14%. Siger rice made from cassava pulp flour in the 6 months of harvest has ash content of 0.2346%. Ash content is closely related to the mineral content of a substance [37]. However, ash content is not always equivalent to all the mineral content available in the material, because there are some minerals that are lost during combustion and evaporation.

Table 10. Results of proximate analysis of the best treatment siger rice

Parameters	Content(%)
Water content	10.80 10
Ash	0.2346
Protein	1.2190
Fat	0.8787
Fiber	1.1764
Carbohydrate	85.6903

Siger rice made from cassava with a 6-month harvest has protein content of 1.2190%, fat content of 0.8787, crude fiber content of 1.1764%, and carbohydrate content of siger rice at 85.6903%. These results indicate that the value is not much different from the results of previous studies on the proximate content of siger rice added with ascorbic acid. Protein content, fat content and levels as well as coarse obtained greater value respectively were 3.82%, 2.42%, and 1.13%. Meanwhile, the previous carbohydrate research content is much smaller, namely 81.11% [7]. The difference in the results of this test can be caused by the type of cassava as a material for making different siger rice.

4. Conclusions

The difference in age of cassava harvest affects the quality of siger rice. Siger rice made from cassava 6 months of harvest produces the best quality with the same texture characteristics as white rice, yellowish white color, 10.80% moisture content, 0.23% ash content, 1.22% protein content, 0.88% fat content, 1.18% crude fiber content, and 85.69% carbohydrate content.

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Entomopathogenic fungi as potential biocontrol agents against rice brown planthopper (*Nilaparvata lugens* Stål.)

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Abstract. Entomopathogenic fungi (EPF) are known defense bioresource as biological control agents. Artificial inoculation of EPF on high value crops has been reported to effectively reduce economic losses due to insect pests. In the Philippines, however, the use of EPF strains has never been explored. Hence, this study has examined the biotic interaction between and among EPF strains (*Beauveria bassiana* and *Metarhizium anisopliae*), brown planthopper, BPH, (*Nilaparvata lugens* Stål.) and rice (*Oryza sativa* L.). Scanning electron microscopy analysis of 21-day old treated seedlings revealed successful endophytic colonization of the two distinct EPF strains. A higher percentage of colonization, as indicated by hyphal growth and appresoria formation, were recorded in the culm and leaf sheath than in the leaf lamina for all the treated seedlings without any pathogenic symptoms. A significantly higher mortality (50%) was observed on BPH continuously exposed to EPF-treated rice seedlings for 50 days as compared to those in the control group. Light microscopy revealed BPH exposed to EPF-colonized rice seedlings for 7 days showed remarkable hyphal growth in the gut, suggesting the direct transmission of the fungus from the EPF-colonized host plant to the insect. The endophytic colonization EPF in rice and its pathogenic effects on BPH was further confirmed using Koch's postulate tests. Our results showed the successful establishment of these EPF strains as rice endophyte and its biocontrol agent potential against BPH and perhaps to other rice insect pest species.

1. Introduction

The brown planthopper (BPH) commonly known as *Nilaparvata lugens* Stål (Hemiptera: Delphacidae), is one of the most economically important insect pests of rice (*Oryza sativa* L.) particularly in the tropics [1,2]. BPH directly damages the rice plant by sucking the sap from the mesophyll and ovipositing its eggs on the midribs. Heavy infestation results to drying of plants known as "hopperburn". Indirectly, BPH damages the crop by transmitting rice grassy stunt virus (RGSV) and rice ragged stunt virus (RRSV) which are widespread in Southeast Asia, including the Philippines. The absence of efficient ecological control strategies to regulate losses due to BHP pushed the rice growers to use hazardous synthetic pesticides. In response to the on-going campaigns on environmental and biodiversity conservation, rice researcher and development programs were focused on crop protection and management are geared towards identification, characterization and utilization of beneficial microorganisms as biological control agents against insect pests.

Entomopathogenic fungi (EPF) including *Beauveria bassiana* (Bals-Criv.) Vuill. (Hypocreales: Cordicypitaceae) and *Metarhizium anisopliae* (Metschn.) Sorokin (Hypocreales: Clavicipitaceae) are identified as efficient mycological control against herbivores. However, their effectiveness is limited by its susceptibility to abiotic factors that reduce viability of fungal conidia such as ultraviolet (UV),

temperature, and low humidity [3]. Recently, an alternative application method to inoculate plants with fungal entomopathogens has been reported in maize [4], potato [5], cocoa [6], date palm [7], coffee [8], banana [9], sorghum [10], tomato [11], jute [12] and common bean [13]. Despite these wide potential, application of EPPF as pest control agents has not been explored in rice.

Hence, this study aims to: (i) establish protocol on EFPF inoculation onto rice using seed immersion technique; (ii) examine the colonization pattern of EEPF in rice seedlings; and (iii) assess the resulting effects of EEPF-inoculated rice on BPH mortality under controlled condition.

2. Materials and Methods

2.1. Preparation of entomopathogenic fungi (EEPF) suspensions

Strains of *B. bassiana* and *M. anisopliae* isolated from *Leptocorisa acuta* (Thunberg) and *Scotinophara coarctata* (Fabricius), respectively were maintained in potato dextrose agar (PDA) and incubated at 25±2ºC under complete darkness. Under sterile conditions, conidia were scraped from the surface of the medium and suspended in 15 mL sterile distilled water with 1% Triton-X 100 and vortexed for 3 minutes. The fungal suspensions were filtered through sterile cheese cloth to remove hyphae and obtain the stock suspension. Conidial concentration was determined using improved Neubuer haemocytometer. The suspensions were adjusted to 1 x 10^8 conidia mL^{-1} in autoclaved distilled water containing 0.1% Triton X-100 and with 80% of fungal germination for each fungal isolate.

2.2. Establishment of rice plant samples

Rice seeds (NSIC Rc222) were inoculated with EEPF by seed immersion technique using the protocol published by Greenfield *et al.* [14] with modifications. Seeds were washed with concentrated liquid detergent, rinsed with tap water, and dipped in 20% NaClO (20 mL of NaClO and 80 ml of sterile water) for 20 minutes, rinsed with sterile distilled water, immersed in 70% ethanol and washed with sterile distilled water for five times. Under a laminar flow hood, 100 seeds were allowed to dry and placed on 1 x 10^8 conidia mL^{-1} sporulating *B. bassiana* and *M. anisopliae* fungal suspension in Petri dish replicated 5 times. After which, seeds were placed in a moistened sterile filter paper for seven days. For control, seeds were soaked in sterile distilled water with 1% triton X-100. Treated and untreated rice seeds were planted in autoclaved clay pots (10 cm diameter 10 cm height) with sterile growing substrate and maintained in a screenhouse for three months.

2.3. Evaluation of EEPF colonization success

2.3.1. Fragment plating method

After 21 days of plant growth, *B. bassiana* and *M. anisopliae* were tested as an endophyte following the protocol of Vega [15] with minor modification. Leaf and leafsheath and culm were Leaf and stem samples were first surface sterilized with washed with clean water, disinfected by dipping in a solution of 1% Sodium hypochlorite (NaClO), followed by 70% ethanol and rinsed with sterile distilled water. The samples were cut into 5 mm pieces and transferred aseptically onto petri dishes containing PDA medium. The plates were maintained at 25ºC under complete darkness. The fungal growth on plated leaf blade, leaf sheath and culm fragments were examined under light microscope to determine percent colonization of *B. bassiana* and *M. anisopliae*. The percent colonization was calculated following the formula of Greenfield *et al.* [14].

2.3.2. Scanning Electron Microscopy

Leaf sheath and culm and leaf samples were incubated in 3% glutaraldehyde and 2% formaldehyde in 0.1 M phosphate buffer, pH 7.2 for a minimum of 24 hours at room temperature. Samples were washed with 0.1M phosphate buffer (pH 7.2) three times and dehydrated by passing through a graded ethanol series. After which, samples were mounted onto aluminum

specimen support stubs sputter coated with gold and observed using field electron and ion scanning electron microscope. (FEI Quanta 200; FEI Co., Hillsboro, OR, USA).

2.3.3. Pathogenecity Test

A total of 1,500 3rd instar *N. lugens* collected from the laboratory cultures were used in the experiment. Ten individuals were aspirated and transferred in the test tube then directly released onto EEPF-treated and non-treated rice seedlings. Immediately after release, each treatment was covered with mylar cage and maintained under greenhouse condition. *N. lugens* cadavers were collected daily. The collected cadavers were transferred individually onto sterile petri dishes lined with moistened sterile Whitman #1 filter paper, sealed with parafilm and incubated under dark room with ambient temperature for 21 days. Morphological features of fungal outgrowth were documented and characterized following the procedure cited by Humber [16].

3. Results and Discussions

Scanning electron microscopy analysis revealed that endophytic colonization of *B. bassiana* and *M. anisopliae* on rice leaf lamina and leaf sheath and culm was evident at 21 days after inoculation (DAI). Successful colonization was significantly varied among rice plant parts wherein highest recovery rate was recorded in leaf sheath, next in culm and lowest in leaf lamina for both fungal strains without any pathogenic symptoms. Hyphal growth of *B. bassiana* showed a typical conidium produced in a long zig-zag rachis comprised of white short-globose to flask-shaped conidiogenous cells (Figure 1). On the other hand, hyphal growth of *M. anisopliae* is characterized cylindrical or elongate to short or long parallel chains which become densely packed to form green to light green palisade-like masses (Figure 2).

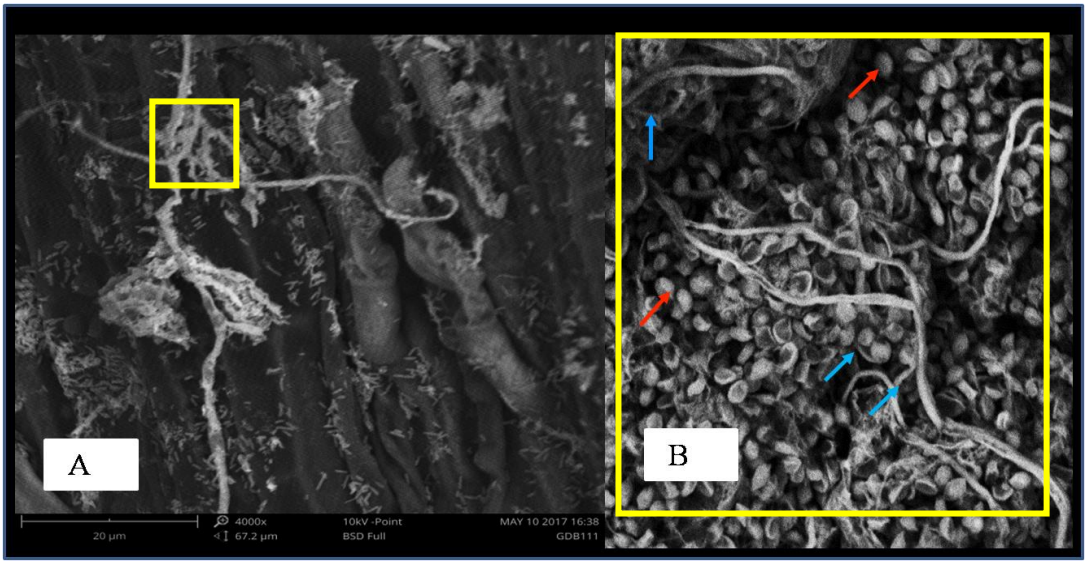

Figure 1. Growth morphological traits of *B. bassiana* as EEPF in rice: A) SEM-generated micrograph showing the portion of leaf sheath of *B. bassiana*-treated rice seedling and B) enlarged portion of A showing the elongate rachis in a long zig-zag form (Blue arrow); and spore ball composed of a cluster of short-globose to flask-shaped conidiogenous cells (Red arrows)

Results of pathogenicity test showed that mortality of the 3rd instar *N. lugens* nymphs was 50% higher in both *B. bassiana* and *M. anisopliae*-inoculated seedling than the control recorded 20 days after exposure (Figure 3). Hence, both *B. bassiana* and *M. anisopliae* can be established as an

endophyte in rice seedlings fusing seed immersion techniques. Increased in mortality rate of BPH in EEPF-treated seedlings was attributed to the EEPF-rice plant symbiotic association. Such observation was also reported in maize [4], potato [5], cocoa [6], date palm [7], coffee [8], banana [9], sorghum [10], tomato [11], jute [12], common bean [13], and faba beans [17].

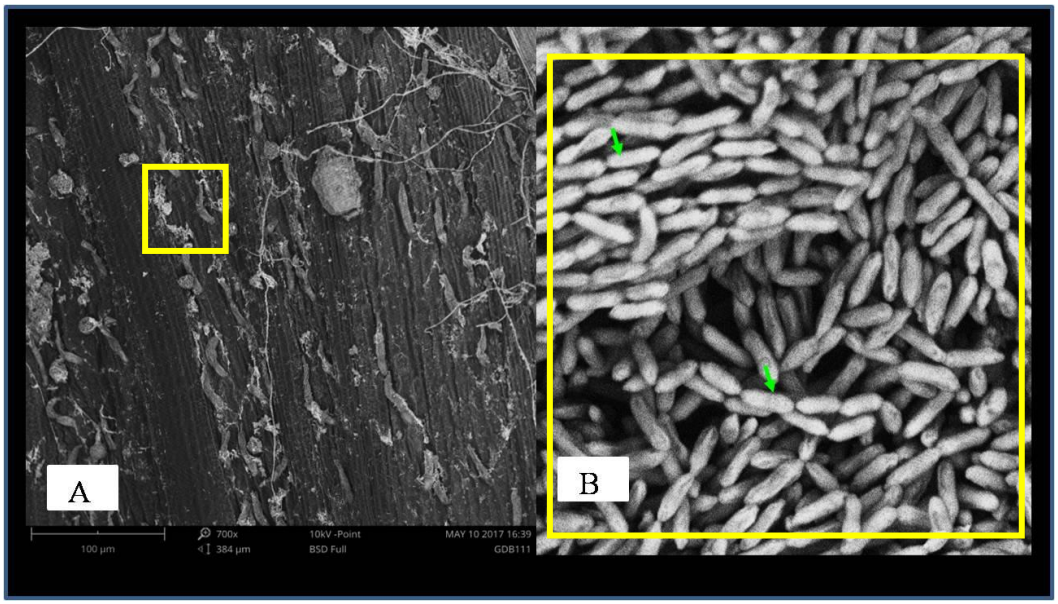

Figure 2. Growth morphological traits of *M. anisopliae* as EEPF in rice: A) SEM-generated micrograph showing the portion of leaf sheath of *M. anisopliae*-treated rice seedling and B) enlarged portion of A showing the chain of densely packed conidia forming palisade-like masses (Green arrows).

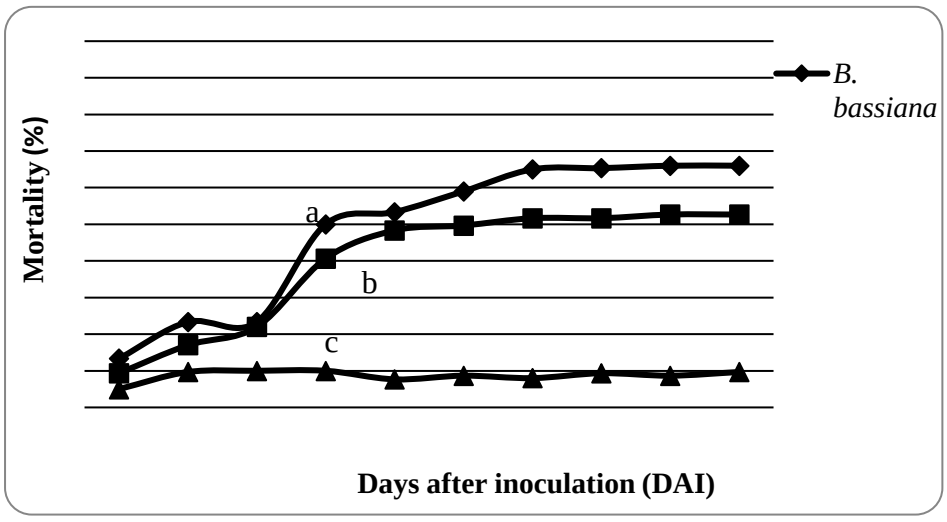

Figure 3. Mortality rate of BPH recorded in EEPF-treated rice seedling.

4. Conclusion

This study was first to report the establishment of *B. bassiana* and *M. anisopliae* as endophytic entomopathogenic fungi (EEPF) in rice. The rice-EEPF biotic association favors the increase in mortality rate of rice brown plant hopper, thus indicating its potential as micro-biological control against insect pests. It is necessary to continue the exploration on the rice-EEPF-brown planthopper interaction to fully understand its ecological impact in rice farming systems.

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Strategy for development of agroforestry system in agricultural land in Argosari Village Jabung District Malang Regency

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Abstract. Argosari Village is one of the villages in Malang Regency located on the slopes of the Bromo Tengger Semeru National Park area. The majority of people in Argosari Village have a livelihood as farmers. Therefore, agricultural activities need to be implemented in agriculture with agroforestry systems. Land management with agroforestry systems has benefits both ecologically, economically and socially for life so it is necessary to know the strategies for developing agroforestry systems. This study aims to find out strategies in the conservation of agroforestry systems. This research was carried out in Argosari Village, Jabung District, Malang Regency in November 2017. Data were analyzed using SWOT analysis. The results of the analysis show that the application of agriculture to agroforestry systems is very suitable. The implementation of the agroforestry system is felt by the community to be able to provide benefits in terms of economic, social and ecological. The results of the SWOT analysis show that the application of agroforestry systems is in Quadrant IV (Diversification) in Space G (Concentric Strategy). This means that the development strategy in this quadrant can be carried out simultaneously and in one department by one party. The main strategy in an effort to improve the sustainability of the agroforestry system in Argosari Village, Jabung District, Malang Regency is an increase in public awareness in the application of agroforestry systems, the establishment of institutions that regulate agroforestry activities ranging from cooperation with the government or related parties, regulating agricultural / forestry products, regulating. Collaboration with government / related parties for example in conducting training, facilitating in the form of assistance in the form of funding and seeds.

1. Introduction

Indonesia is one of the agricultural countries, Indonesia has a population that mostly works as farmers (1). In various regions in Indonesia, the community works as a farmer, one of them is in Malang, East Java. 45,888 Ha, in Jabung Subdistrict there are 1,225 Ha of agricultural land (2). Argosari Village is a village located on the slopes of the Bromo Tengger Semeru National Park (TNBTS). The majority of the population in Argosari Village have a livelihood as farmers. The community implements conventional farming systems, this system relies on a large number of results (3). The farming system in this way caused various problems in Argosari village, namely the occurrence of natural disasters such as floods and landslides in 2008. In addition, people also experienced drought for agricultural activities. This natural disaster occurred due to the lack of forest plants in Argosari Village.

Based on these problems, the community in Argosari Village began to implement agriculture with an agroforestry system. Communities combine agricultural crops and also forestry on the land they have (4). The implementation of agroforestry systems began in 2008 after a natural disaster occurred in Argosari Village. The application of agroforestry systems provides good benefits for the community, both in terms of economic, social and ecological (5). However, in its implementation

there are still some problems that cause the agroforestry system to not work properly. These problems start in terms of funding, the role of the government and people's understanding of agroforestry systems that are still low. Therefore, it is necessary to determine the strategies that can be done to further develop the agroforestry system in Argosari Village, Jabung District, Malang Regency.

2. Research Methods

The research was conducted in November 2017 in Argosari Village, Jabung District, Malang Regency. This research method using quantitative method. Data analysis used the SWOT (Straigh, Weakness, Opportunities, Treath).

3. Data Collection

Interviews using questionnaires were conducted purposively. Sample were chosen from argosari village. The samples was 84 people farmers.

4. Results And Discussion

The agroforestry system is an agricultural system by combining agricultural and forestry crops in one area both sequentially and concurrently. The application of agroforestry systems can provide benefits to the community both economically, ecologically and socially. Economic benefits are able to increase people's income. The ecological benefit is that the implementation of agroforestry systems can improve environmental sustainability, protect springs, prevent natural disasters and maintain soil fertility. While social benefits are able to increase cooperation between communities. Identification of internal or external factors in the formulation of sustainability strategies for agroforestry systems prepared by the SWOT method in addition to being based on the results of Rap analysis also directly in the field to key figures, experts or related parties. The results of the identification of internal factors are as follows:

Positive internal factors (Strengths / Strengths):

1. Availability of technology for making organic fertilizer (S-1)
2. Land fertility (S-2)
3. land productivity (S-3)
4. selling prices of agroforestry products (S-4)
5. the occurrence of conflict (S-5)
6. the existence of farmer groups (S-6)

Negative internal factor (Weakness):

1. Low level of education (W-1)
2. suitability of the application of agroforestry systems (W-2)
3. Use of inorganic fertilizer (W-3)
4. stability of the selling price (W-4)
5. sales results system (W-5)

Positive external factors (opportunities / opportunities):

1. community enthusiasm to maintain forest sustainability (O-1)
2. Commitment of farmer groups to further develop agroforestry systems (O-2)
3. Achievements obtained by farmer groups (O-3)
4. Increasing collaboration between communities (O-4)

Negative external factors (threats / threats):

1. Lack of seed assistance for the community (T-1)
2. Lack of counseling provided by the government / related parties (T-2)
3. lack of subsidies from the government both in funding / seed support (T-3)
4. Improving community skills from the government / related parties (T-4)5) Frequency of counseling (T-5)
5. Absence of laws regulating agroforestry systems (T-6)

4.1. Calculation of IFAS (Internal Strategic Factors Analysis Summary) and EFAS (External Strategic Factors Analysis Summary) values can be seen as follows:

Based on the results of identification of internal and external factors, then the analysis was carried out using IFAS and EFAS matrix. Making a matrix is done by calculating scores and weights, as well as the total number of multiplication scores and weights. Determination of the scale of the score scale, refers to the formula for determining the priority scale (SP) as the determination of the priority scale specified (6).

Calculation of IFAS and EFAS values is presented in Table. 1. While the results of external factor analysis are presented in Table 2.

Table 1. Analysis of IFAS preservation of agroforestry systems

No	Internal Factors Strengths (S) positive	Weight (a)	Rank (b)	Score (axb)
1	Availability of technology for making organic fertilizers	0.099099	3.6	0.356757
2	Soil fertility	0.099099	3.6	0.356757
3	Land productivity	0.099099	3.6	0.356757
4	Selling price of agroforestry products	0.099099	3.6	0.356757
5	Conflict	0.081081	3	0.243243
6	The existence of farmers group	0.09009	3	0.27027
	Total	**0.57**		**1.94**
	Weaknesses (W) negative			
1	Relative low education level	0.0901	3	0.2703
2	Suitability of the application of agroforestry systems	0.0811	3	0.2433
3	Used of organic fertilizers	0.0721	3.3	0.23793
4	Stable selling price	0.0991	3.3	0.32703
5	Sales systems products	0.0901	3.3	0.29733
	Total	**0.43**		**1.38**

Source: Research results, 2018

4.2. Quadrant IFAS and EFAS

Based on the results of the IFAS and EFAS matrix analysis, it can be seen the formulation of the steps for the sustainability strategy of agroforestry systems are as follows:

Based on the results of the IFAS and EFAS matrix analysis, it can be seen the formulation of the steps for the sustainability strategy of agroforestry systems are as follows:

X = strength + weakness
= 1.94 + (-1.38)
= 0.56 Y = opportunity + threat
= 1,406 + (-2.08)
= -0,674

So the axis position (X, Y) is at the point (0.56 and -0.674) and can be described as follows:

The results of the analysis of total weighting scores are carried out by the summing formula of the strength of 1.94 minus the value of the weakness of 1.38. The total weighting value obtained is 0.56. The results of the analysis of total weighting scores are carried out by the sum formula between the probability value of 1.406 minus the threat value of 2.08. The total value of weighting obtained is equal to -0.674.

Table 2. EFAS analysis of the conservation of agroforestry systems

No	External Factors Opportunities (O) positive	Weight (a)	Rank (b)	Score (axb)
1	Community spirit to preserve forests	0.11	4	0.44
2	Commitment of farmer groups to further develop agroforestry systems	0.1	3.6	0.36
3	Achievements obtained by farmers group in Arosari village	0.07	3	0,21
4	Enhance cooperation between communities	0.11	3.6	0.396
	Total	**0.39**		**1.406**
	Threats (T) negative			
1	Lack of seed assistance to the community	0.09	3.3	0.297
2	Lack of counseling provided by the goverment/related parties	0.10	3.6	0.36
3	Lack of goverment subsidies both in funding/seed assistance	0.10	3.3	0.33
4	Improvement of community skills from the goverment/related parties	0.11	3.6	0.396
5	Frequency of counseling	0.10	3	0.3
6	Absence of laws governing agroforestry systems	0.11	3.6	0.396
	Total	**0.61**		**2.08**

Source: Research results, 2018

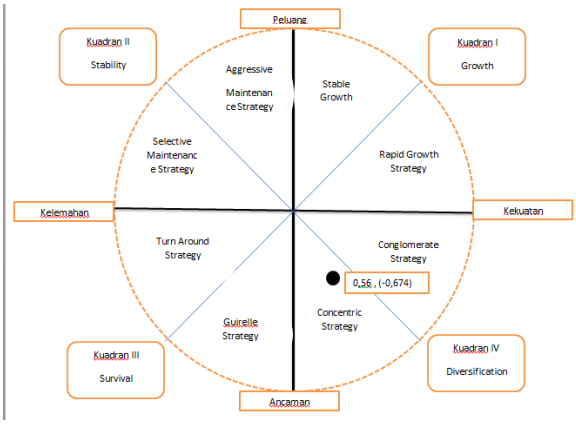

Figure 1. SWOT Quadrant Analysis (Source: Research results, 2018)

Based on the quadrant picture above, it can be seen that the application of agroforestry systems is in Quadrant IV (Diversification) in Space G (Concentric Strategy). This means that the development strategy in this quadrant can be carried out simultaneously and in one department by one party. This is in line with the quantitative SWOT analysis approach (7), the development of strategies in this quadrant position can be done by diversifying strategy (8). This means that the application of the agroforestry system in Argosari Village, Jabung District, Malang Regency is in a condition that can be accepted and run with the intended purpose, but in the implementation it also faces a number of threats.

4.3. Strategy Formulation

In formulating the most appropriate strategy for the implementation of agroforestry systems in Argosari Village, Jabung District, Malang Regency, it was carried out through a diversified strategy strategy, namely as follows:

1. Determine the Leading sector responsible for the development of the agroforestry system and determine the division of tasks and a clear role for the parties concerned. The division of tasks starts from the growth of public awareness, implementation, post-activity monitoring and evaluation and development stages.
2. Compile a Grand design or an integrated conceptual framework ranging from planning, implementation to monitoring and evaluation of each party concerned with the development of agroforestry systems.
3. Perform the implementation phase which includes:
   a. Planning phase
      Starting with the formation of a working group consisting of all parties related to the development of agroforestry systems.
      The working group formed has the task of developing a conceptual framework for the implementation of agroforestry system activities in accordance with their respective roles and tasks. Members of the working group can consist of community groups, the Forestry Service, the Agriculture Service and the Environment Agency.
   b. Implementation Phase
      At this stage it is divided into three aspects, namely the preparation of citizens, the application of agroforestry systems and the improvement of knowledge and technology.
      Community preparation
      Community preparations are carried out to emphasize efforts to foster awareness and awareness of the community, especially farmers about the dangers that will occur if environmental sustainability is not maintained properly.
      Infrastructure development
      To support the implementation of the agroforestry system so that it can run well is the need to build several facilities and infrastructures while still involving the community. The purpose of community involvement is so that people have more self-awareness and have more responsibility to look after it. Infrastructure facilities needed in the development of agroforestry systems, for example, the area for the manufacture of forestry plant seeds, the formation of institutions to sell farmers' products and the existence of institutions that are able to provide assistance or loans in the form of capital or seeds.
      Increased Knowledge and Technology
      In the development of agroforestry systems it is very important to improve skills for the community both from the relevant agencies and from those involved in the development of agroforestry systems. Whereas for the development of technology is also needed by the community, one of which is needed by the community is the development of technology in the manufacture of organic fertilizer. Improving public knowledge and improving technology can be done by counseling by the heads of farmer groups, related agencies or from those who are able to assist in improving the agroforestry system.
   c. Monitoring and evaluation phase
      This stage is monitoring the implementation of agroforestry systems that have been implemented by the community. As for evaluation used in policy making for further activities. With the evaluation can also be used to see whether the vision, mission and targets in the conservation of agroforestry systems have been achieved or not.
   d. Phase of the development of agroforestry systems
      The development of agroforestry systems is needed to provide added value both ecologically and economically for people who implement agroforestry systems or communities that have not implemented agroforestry systems.

From the SWOT analysis for the development of agroforestry systems it must be emphasized on all aspects ranging from ecology, economics, social and institutional. Because the implementation of agroforstri system is said to be successful if it is able to improve the environment, it can increase people's income and increase social relations between citizens. To achieve this goal, it must be supported by an institution that regulates the course of the agroforestry system.

The agroforestry system implemented in Argosari Village is ecologically successful. This was seen from the emergence of new springs in Argosari Village so that the drought that had been experienced by the community did not occur again. In addition, the community's dependence on forest products is reduced which is able to restore forest functions. But there is still a need to increase the number of forestry plantations on community farmland.

To support the success of the agroforestry system also needs to be strengthened in the economic sector. In the economic sector, for example, there is funding assistance or assistance from forestry plant seeds from the government or related parties. Whereas from the social situation is to increase cooperation between fellow communities or farmer groups for the continuation of the implementation of agroforestry systems.

Institutional factors have a very important role, it is expected that with the existence of institutions that regulate agroforestry activities are able to maintain the sustainability of the application of agroforestry systems. For example, the one that regulates the sale of both agricultural and forestry products so that people get a stable price every season. In addition, it is necessary to formulate policies that regulate the application of agroforestry systems.

5. Conclusion

The main strategy in an effort to improve the sustainability of the agroforestry system in Argosari Village, Jabung District, Malang Regency is an increase in public awareness in the application of agroforestry systems, the establishment of institutions that regulate agroforestry activities ranging from cooperation with the government or related parties, regulating agricultural / forestry products, regulating. Collaboration with government / related parties for example in conducting training, facilitating in the form of assistance in the form of funding and seeds.

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Potential banana-based agroindustry in Lampung Province-Indonesia

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Abstract. Banana is a potential agricultural commodity to be developed as economic value products for agroindustry. This study was aimed at determining the type of potential agroindustry of banana in Lampung Province and its added value. The research was conducted in Lampung province by using survey method. The type of potential agroindustry was determined by using the AHP method through expert choice software, and for the added value analysis was conducted by Hayami method. The results showed that the potential agroindustry of banana to be developed in Lampung province was banana chips with AHP value of 0.415. Processing of banana into chips products presented high added value (Rp3,281/kg) with an added ratio of 30.56%.

1. Introduction

Banana is a great fruit grown in Indonesia as this country is a top ten major world producers of banana and plantain [1]. Until 2015, total production of 7,299,275 tonnes of bananas were produced ranking it first place among the fruits, and Lampung Province was put first in the most considerable banana production following by East Java (1,629,437 tons) and West Java (1,306,288 tons) by contributing 1,937,349 tons or 26, 54% of total national banana production [2]. This data indicates the enormous potential of Lampung Province to become an ago-based area of banana processing in making highly demanded products. Most of the Indonesian exported agricultural commodities are raw materials with processing retention index of 71-75 %. Only 25-29 % of farm products are transported in the processed form [3]. Likewise, in Lampung province, the processing of bananas are generally still limited to traditional foods such as fried bananas and kolak [4]. The downstream of banana-based agroindustry is generally performed at home industry level. These indicate the need for a strategy to increase the value of bananas either in upstream or downstream processing. Some researchers [5], [6] and [7] argued that the development of agro-industry, a rural-based industry with business characteristics, and primarily engaged in the processing of agricultural products are the strategy to improve the welfare of the farming sector and attain overall economic growth.

The development of agro-industry is expected to be a right way for increasing banana 's value-added products as the processing would make bananas to be a more durable and high economic product, as well as providing more alternative processed products for marketing. Development of banana-based agroindustry should be done through a potential raw banana sources approach and the possible processed products approach. Source of bananas as a raw material is spread in 5 districts in Lampung province namely Lampung Selatan, Pesawaran, Lampung Tengah, Tanggamus and Lampung Timur. Meanwhile, the numerous potential processed products that have high demand in the Market are chips, "sale", puree, and banana flour [9]. The opportunity of banana processed in the form of puree was quite potential because this product is needed as a raw material in making baby food and juice. The increase in the world population especially a newborns baby (4 - 5 months) consuming banana area big potential for the banana processed products [10]. Processing banana to chip also a potential added value processed

products with a good market in the community [11]. Likewise, the processed products in the form of flour are possible as its utilization could substitute the wheat flour.

The assessment of the potential banana-based processing agroindustry needs a study on their sustainability to determine the viability of ideas that legally and technically feasible as well as economically justifiable. This research, therefore, was conducted with the aim to find the type of banana-based agroindustry that potential to be developed in Lampung Province by using Hierarchy Process Analysis (AHP). The ratio of added value was determined as well to support the data of selected potential banana-based agroindustry; According to Gittinger [8], the added value is the difference between gross output and temporary consumption value. In another word, this is the market price of goods or services produced which is reduced by price material goods or services and services purchased from other parties.

2. Methodology

The study was conducted in three stages. The first stage was a qualitative component of the study through interviews and a closed questionnaire survey with people related to banana agro-industry as well as with the experts from the local government. The experts consisted of 10 people in which five persons were coming from the Department of Industry, Department of Trade, the Office of Agriculture Food Crops and Horticulture, Food Security Agency, and Bappeda Lampung Selatan. While, others 5 were from the Department of agriculture food crops and horticulture, Department of Commerce, Department of industry, and Lampung province of Central Bureau of statistics. The objective of the interviews and questionnaire survey was to inquire into the perspectives of the banana production, business competitors, capital, and labor, process technology (machinery and equipment), products added-value, and market potential of banana-based agroindustry. The result of the questionnaire was then processed by using expert choice decision program (Hierarchy Process Analysis). The mindset of this analysis can be illustrated in Figure 1.

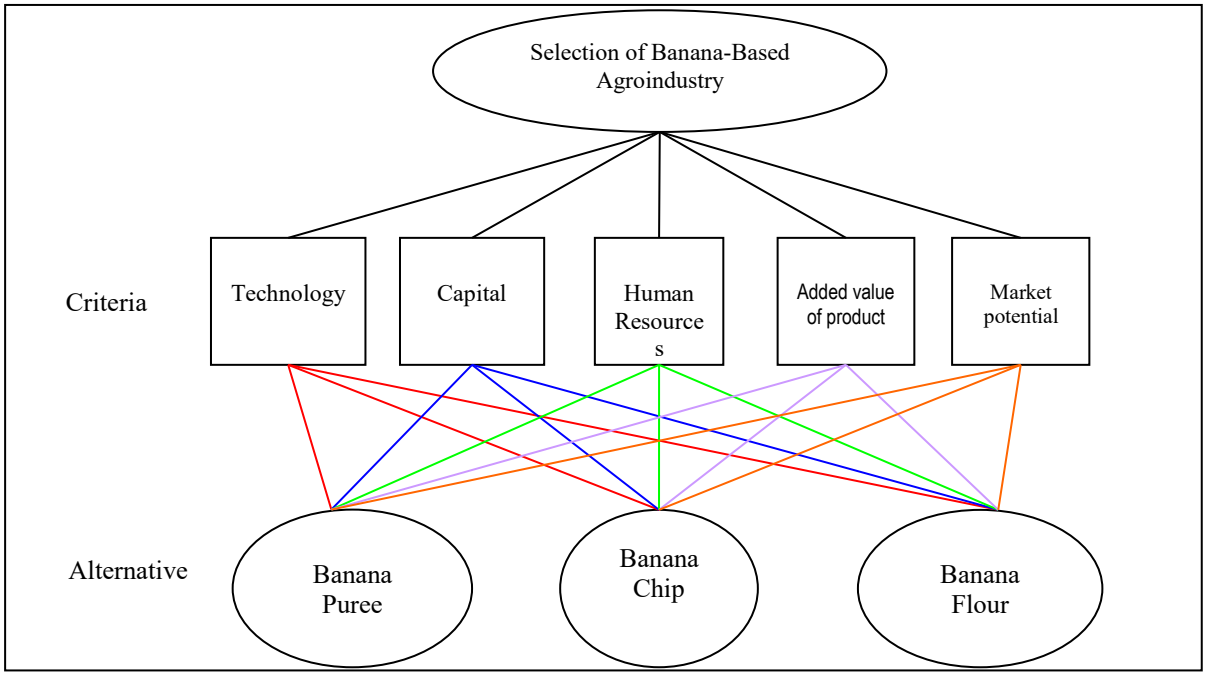

Figure 1. Product Alternative of Banana-Based Agroindustry.

The second stage was to determine the ratio of products added value, which was associated with the input-output, pricing, revenue and profit, and the retribution of the owners' production factors. The value-added analysis was conducted to find out the magnitude of the value added obtained from the processing of raw materials into a product. Procedure for the calculation of the value-added was a

method of Hayami. The final stage was to draw conclusions based on tools that were used for identifying potential banana-based agroindustry in Lampung province.

3. Resuts and Discussion

3.1. The Order of Priority Criteria

The results of the Hierarchy Process Analysis (AHP) test for the level criteria used as a determinant of the selection can be seen in Table 1. The most crucial rule was the market opportunity with an aggregate weight of 0.396 or 1.7 times more determinant than the Human Resources (HR), 2.5 times more decisive than the capital value, 3.6 times more critical than either technology or product value added. Market potential as the most critical factor is not surprising as this factor could indicate whether a production sector has good prospects or vice versa. In another hand, the market and marketing aspects are an essential determinant of the company.

Table 1. The order of priority criteria for determining an agro-industry selection results of the Hierarchy Process Analysis (AHP) test for the level criteria used as a determinant of the selection can be seen in Table 1. The most crucial rule was the market opportunity with an aggregate weight of 0.396 or 1.7 times more determinant than the Human Resources (HR), 2.5 times more decisive than the capital value, 3.6 times more critical than either technology or product value added. Market potential as the most critical factor is not surprising as this factor could indicate whether a production sector has good prospects or vice versa. In another hand, the market and marketing aspects are an essential determinant of the company.

Table 1. The order of priority criteria for determining agro-industry selection

Criteria	Description	Point	Order
Market Opportunities	Prospects of products to be developed both in domestic and international markets	0.396	1
Human Resources	Level of knowledge and technical ability and number of human resources in product development	0.237	2
Capital	Ability cost or all costs issued in the industrial implementation	0.155	3
Technology	The type of process technology used for producing and developing product.	0.110	4
Product Value-Added	The amount of profit to be gained if product developed	0.102	5

Human resources were the second rank (by 0.22) as a decisive criterion in the selection of agro-industries. The Human Resources play roles and responsibilities in leading the organization within the agricultural field [12]. As a determinant of the success of the project, human resources or labor must have suitable qualifications, skills, and expertise with the needs of the project [13]. Meanwhile, the capital factor was the third rank criteria that determine the establishment of agro-industry. The capital factor is essential as this is the primary factor of project production [14], capital is needed to start and develop the business either from internal or external sources [15]. Lack of capitals is one of the main people reasons to do not start a business yet. However, the capital will be no longer become an obstacle when the investors are interested in the attractiveness of people business model.

Technological factors and products value-added have similar level criteria in determining the priority sector of agro-industry types. Technical considerations are essential to make project keep in existing the trends and following the innovation opportunities. According to Kasmir and Jakfar [16], somethings to note in the selection of technology are the accuracy of technology with raw materials, technological success elsewhere, advanced technical considerations, the number of investment costs and maintenance costs, and possible development, as well as government considerations regarding labor.

The value-added products is another important factor for determining the priority criteria. This factor contributes a significant increase of the economic value of the banana'sperishable nature. Value-added is defined as an increase in the value of a product due to processing, transport or storage in a production process. The importance of value-added is apparently in the Hayami method, where the calculation of value-added products per kilogram of raw materials for one-time processing that produces a particular product will show a value-added processing of agricultural products.

3.2. Selected Product of Banana-Based Agroindustry

Among the priority banana-based agroindustry, the banana chip was the selected products with the highest cumulative aggregate value (41.5%) (Table 2 and Figure 2). Banana chips are fried banana slices that have characteristics with turned brown color and crunchy texture.

Table 2. Priority of Processed Banana Product.

Processed Banana Product	Criteria for selection					Aggregate	Percentage
	Technology	Capital	Human Resources	Product Added-Value	Market Potency		
Puree	0.091	0.650	0.559	0.393	0.111	0.346	34.6
Chips	0.677	0.103	0.151	0.351	0.698	0.415	41.5
Flour	0.232	0.246	0.290	0.256	0.192	0.239	23.9

CR = (*Consistency Ratio*) < 0.1 (10%)

Based on market potency, banana chips have the highest value, which was 0.698 compared to banana flour (0.192) and banana puree (0.111). Banana chips are popular as a snack food in many countries, consumed around the year by people of all age groups. The consumer acceptance of banana chips is based on quality attributes of the products influenced by processing [17]. Apriyani at al., [18] reported that banana chips are a potential product to be developed as these products are natural resources utilizing and are familiar with local human resources. Also, the processing of banana chips does not require much quantity and quality of high labor.

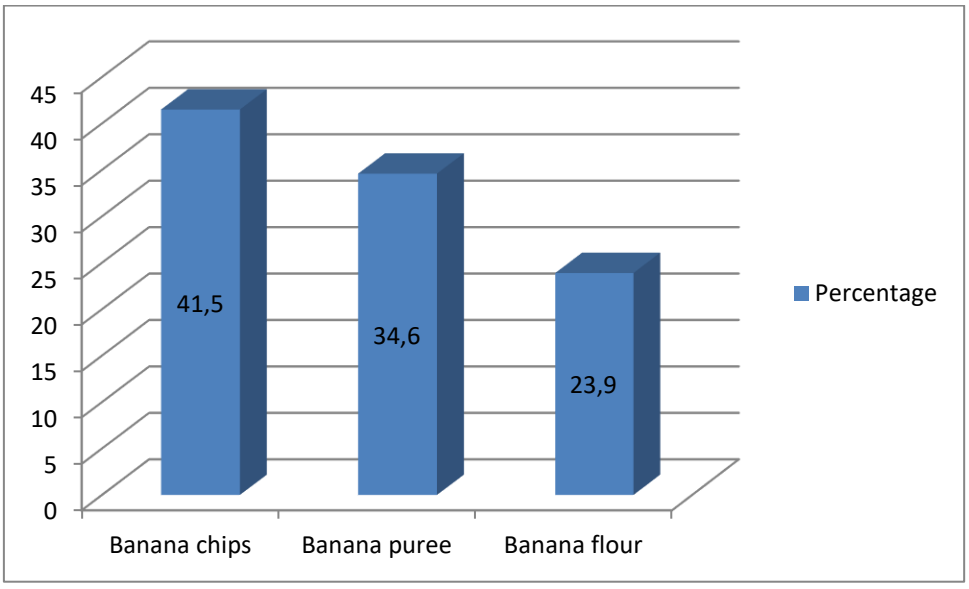

Figure 2. Priority of Processed Banana Product.

Based on technological factors, banana chips have the highest value compared to those of banana flour and puree. According to Siregar [19], the manufacture of banana chips is very simple and does not require significant venture capital. The technology of banana chips processing can utilize either the traditional or modern technology. However, production process using advanced technology will be faster and bigger capacity than those of conventional method. The use of the simple technique in the banana chips industry will be less capital required for operational implementation. According to [20], a capital factor is the most influential factor to the monthly net income generated by banana chips industry entrepreneurs. The higher the capital owned by the entrepreneur, the more significant quantities of banana chips can be produced, therefore it increased the net income of banana chips industry entrepreneurs.

Table 3. Calculation of value-added of banana chips agroindustry.

No	Variable	Calculation	
Output, Input and Price			
1	Output (Kg)	(1)	4,363
2	Raw Material (Kg)	(2)	14,223
3	DirectLabor (HOK)	(3)	45
4	Conversion Factor	(4) = (1) / (2)	0.31
5	Coefficientof DirectLabor (HOK/Kg)	(5) = (3) / (2)	0.0032
6	Price of Output (Rp/Kg)	(6)	35,000
7	DirectLaborCost (Rp/HOK)	(7)	50,000
Income and Value-Added			
8	Price of RawMaterials (Rp/Kg)	(8)	5,000
9	Price of Others Input (Rp/Kg)	(9)	2,456
10	Value of Output (Rp/Kg)	(10) = (4) x (6)	10,736
11	a. Value-Added (Rp/Kg)	(11a) = (10) – (8) – (9)	3,281
	b. Ratio of Value-Added (%)	(11b) = (11a) / (10) x 100	30.56
12	a. IncomefromD irect Labor	(12a) = (5) x (7)	158.19
	b. Pangsa of Direct Labor (%)	(12b) = (12a) / (11a) x 100	4.82
13	a. Margin (Rp/Kg)	(13a) = (11a) – (12a)	3,123
	b. Level of Margin (%)	(13b) = (13a) / (10) x 100	29.08
Reward for theOwner of Production			
14	Margin (Rp/Kg)	(14) = (10) – (8)	5,736
	a. Income of Direct Labor (%)	(14a) = (12a) / (14) x 100	2.76
	b. Contribution of AnotherInput (%)	(14b) = (9) / (14) x 100	42.81
	c. Profit of Company (%)	(14c) = (13a) / (14) x 100	54.44

3.3. Value-Added of Banana Chips

Value-added analysis and marketing margin of banana to processed banana", are needed to know the value-added given of banana chips on the banana raw material so it can be assessed whether the business efficiently runs and provide benefits or not [21]. According to Predita [21], value added is the increase in the value of a commodity because of the useful input imposed on the specialty concerned. The functional information is in the form of a form of utility, place utility, or time utility. Value-added

describes rewards for labor, capital, and management. The calculation of added value on banana chips agroindustry can be seen in Table 4 that is the value-added in 1 kg of bananas after being processed into banana chips.

4. Conclusion

The type of banana-based agroindustry potential developed in Lampung Province is banana chips with AHP value of 0.415, and a value-added ratio of 30.56.

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Purchasing behaviour and store atmosphere-the comparison of batik store and distro in Candi and Madura

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Abstract. This study investigates the effect of store atmosphere on purchasing behaviour under different products. Seventy five respondents participated in the study through house of Batik Tresna Art Madura plus ninety seven respondents participated in the study store atmosphere on revolution distro Candi Sidoarjo. Multiple regression analysis was utilized to determine the relationships effects amongst interested variables. The study shows that store atmosphere significantly affect purchasing behaviour. When store atmosphere is implemented, purchasing behaviour increase both on Batik Tresna Art and revolution distro Candi. Results suggest that purchasing behaviour is positively associated with store atmosphere and store atmosphere dimensions. Collectively, findings are consistent with the premise that store atmosphere shape purchasing behaviour at Tresna atr and Revolution Distro store. Partially, variable Exterior and General Interior, significantly influence purchasing behaviour however, Store Layout and Interior Display did not significantly influence purchasing behaviour. It was found that the relationship between store atmospheres on purchasing behaviour is stronger significantly. These findings provide further theoretical implications for marketing and consumer behaviour research, as well as practical guidelines for retailers who manage store atmosphere.

1. Introduction

The standard of living people are growing, the company must do ongoing research and development of his business, and how the company can meet the diverse needs and desires of consumers. Development of a business involves many aspects and one of them is improvement shop atmosphere.

In the modern economy like today, every company will face intense competition. Increasing competition intensity and number of competitors requires meet consumer needs, more satisfying than what its competitors do, so that an equal perception is needed in defining a product that has good quality. Products that have good quality are products that have excellent quality. Thus the company will be more seeing which business prospects will be undertaken and determining what strategies will be used to attract consumers' buying interest. This requires product differentiation from other companies.

The company can apply many business strategies and one of them is strategy of marketing be the very important in running a business. One example of part of marketing is atmospheric store that is an environmental design activities in-store purchases with determine the characteristics of the store through arrangement and selection of shop and physical facilities merchandise activities. According to Kotler [1, pp. 61] "Every store have a layout make it easier or more difficult for consumer to looking around inside"

Many phenomena in the business show that there is an influence on buying decision and it can be explained that store atmosphere have an important role in purchasing decisions. This also often occur in business in Bangkalan and Sidoarjo where the many business are restaurants, batik houses, clothing, food, and house's material shops.

Bangkalan and Sidoarjo is famous with culture, so many entrepreneurs establish business with cultural bonded like batik house. From many batik houses in Bangkalan, one of them is Rumah Batik Tresna Art, that makes everyone interested in visiting, cause has a difference with other shops, the atmosphere shop is unique and different from the other batik home atmosphere, and becomes its identity to attract consumers House of Tresna Art batik is one of the businesses model that shows the store atmosphere (store atmosphere) is able to influence consumers to make purchasing decisions on batik products. And we decide Revolution Distro in Sidoarjo because it has distinctiveness if we look compare with other home distro.

Based on study conducted by Widyanto et al [2] on his research entitled the influence of store atmosphere on purchasing decisions (surveys on consumers of the planet distro surf mall Olympic Garden in Malang) showed that store atmosphere is very influential on increasing product sales. There is also research from Dessyana [3] which explains that store atmosphere influences purchasing decisions. But there are also those who say that store atmosphere does not have a big effect on purchasing decisions. This happened in a study conducted by Mardhikasari [4] entitled the influence of store atmosphere, store location and product diversity on purchasing decisions.

Store atmosphere is one of the factors that influence a person's decision to make a purchase in a store, if the more convenient a store is, the more consumers are interested in shopping at the store. A good store design can also attract consumers' desire to know more about everything that the store has to offer [5]. Store atmosphere according to Berman and Evans [6] divides into several variables including: Exterior, General Interior, Store Layout and Interior Display. From background and introduction explanations above, this research will be conducted to examine whether the store atmosphere affect the consumers purchasing decisions both at Tresna Art and Revolution Distro.

2. Problem Formulation

Based on background is, problems can be formulated as follows, From the background above in accordance with the problem proposed then the problem statement can be detailed as follows:

1) Does the store atmosphere have a significant effect on buying decisions consumers of batik Tresna Art?
2) How does store atmosphere consist of exterior, general interior, store layout, interior display, and purchase decision at Revolution Distro in Sidoarjo?
3) Does the store atmosphere consisting of exterior, general interior, store layout, interior display have a positive effect on purchasing decisions in the Sidoarjo distribution revolution partially and simultaneously?
4) Which store atmosphere variable has a dominant influence on purchasing decisions?

3. Research purposes

Based on the formulation of the problems above, the objectives of this study are:To find out and examine the influence of store atmospheres on consumers' buying decisions at home batik Tresna Art, How does store atmosphere consist of exterior, general interior, store layout, interior display, and purchase decision at Revolution Distro in Sidoarjo?, Does the store atmosphere consisting of exterior, general interior, store layout, interior display have a positive effect on purchasing decisions in the Sidoarjo distribution revolution partially and simultaneously? And which store atmosphere variable has a dominant influence on purchasing decisions?

4. Literature Review

4.1 Definition of Marketing

Marketing is an activity between consumers and producers to exchange goods or services at prices was settled by doing interactions directly or indirectly to satisfy or to meet the consumers need. According to Kotler [1] "The definition of marketing is a social process whereby with the process, individuals and

groups get what they need and want by creating, offering, and freely exchanging valuable products and services with other parties". There are two factors that influence the company in marketing are:

1. External Environment of Marketing System.
2. Internal Variables of Marketing system.

4.2 Store atmosphere

The process creation of store atmosphere is an activity of designing an environment in a shop by determining the characteristics of the store through the arrangement and selection of physical facilities of the store and merchandise.

One of the factors that must be considered by the store owner is Atmosphere. From a marketer's perspective, the atmosphere of a store can have effects of consumers expectedly, thus increasing the likelihood of buying a product that might be ignored before. This can affect the amount of time and money spent while shopping. Utami [5] states that the atmosphere of the store is a combination of physical characteristics of the store such as architecture, layout, lighting, display, colour, temperature, music, and the overall aroma will create an image in the minds of consumers.

Whereas Store Atmosphere according to Sutisna [7] is the arrangement of internal spaces (in store) and outside space (out store) that can create customers comfortable.

The atmosphere relates to how managers can manipulate building design, interior space, floors, walls, aroma, colour, shape and music that customers experience which all aim to achieve a certain influence and buying decision finally. According to Berman and Evan [6] store atmosphere elements consist of four variables, namely Exterior, General Interior, Store Layout, and Interior Display.

According to Berman and Evans in Utami [5] elements of atmosphere divide into four parts, they are exterior shop, general interior, store layout and interior appearance. Explanation of the core elements are:

1) Store exterior (exterior of the store) has Influence on store image, therefore the outside of the store must be planned as best as possible. The shop exterior includes:

a. Storefront
b. Marquee
c. Entrance
d. Display windows
e. Parking facilities

2) General Interior

A good and successful store is a store that can attract consumers' attention and help consumers to easily observe, check and select goods, and ultimately make purchases when consumers enter the store. General interior can be created from:

a. Flooring
b. Colouring and lighting
c. Scent and sound
d. Alley of the room
e. Store personnel
f. Technology
g. Cleanliness

3) Store Layout

Store layouts must be planned in determining specific locations. Store layout will determine consumers will enter or exit the store. Store layouts that must be considered are:

a. Types of goods
b. Goods arrangement
c. Shop facilities
d. Store settings
e. Item group

4) Interior Displays
Interior display are the signs be used to provide information to consumers for affect the store environment, the aim of interior display to increasing store sales and profits. Interior displays such as posters, location signs, picture marks.

4.3 Buying decision
A specific process of purchasing consists of five stages, as follows: problem recognition, information search, alternative evaluation, purchase decisions, and post-purchase behaviour. "Being the task of marketers is that marketers must be able to understand the buyer behaviour of at each stage and what influence will react to these stages" [1, pp. 211]. Consumers through these stages in making purchasing decisions, but not all consumers who pass the five stages when making a purchase. The explanation of five stages:
1) Needs recognition
The purchase process starts when consumers recognize a problem or need.
2) Searching Information
Consumers who have been interested will encourage consumers to seek more information.
3) Alternative evaluation
Alternative evaluation is the process of evaluating products and brands that will be selected to meet the needs and consumers wants.
4) Purchase decision
There are two factors can influence purchase intentions and purchasing decisions, first is the attitude of other people and two is unexpected circumstances.
5) Post-purchase behaviour, Consumer satisfaction and dissatisfaction will influence next consumer behaviour.

4.4 Relationship of exterior with Purchase Decisions
Exterior Relationships with Shopping Decisions are Exterior having a positive influence on Shopping Decisions. Because with the Exterior, it will be able to attract customers to visit the shop and to shop. This is supported by research conducted by Prabowo and Rahardjo [8] who obtained the results that the Exterior variable significantly influences purchasing decisions.

4.5 Relationship of General Interior to Purchase Decisions
According to Berman and Evans [6] General Interior is a store display that makes visitors feel comfortable in the store. The General Interior of a store must be designed to maximize visual merchandising so it can attract buyers to come the store. But the most important thing to attract buyers after being in the store is in front of the display.

4.6 Relationship of Store Layout with Purchase Decisions
Planning of Store Layout includes structuring of space to fill available floor, classifying the products to be offered, setting in-store traffic, setting the required room width, mapping shop space, and arranging products offered individually.

Store layout will invite entry or cause customers to stay away from the store when consumers see inside through windows, storefronts or entrances. A good Store Layout will be able to invite consumers to be more comfortable traveling around and spending more money [6]. The results of research conducted by Dessyana [3] show that Store Layout has a significant effect on purchasing decisions.

4.7 Relationship of Interior Display with Purchase Decisions
According to Berman and Evans [6] each type of Interior Display provides information to customers to influence the situation. In other words, the Interior Display is a display of merchandise in the store.

Usually in terms of themes adapted to the event taking place updatable, the arrangement of shelves and storefronts, discount posters, so can attract visitors to shop.

4.8 Relationship of Store Atmosphere Variables with Purchase Decisions

Looking at the previous research that I used for the references, explained, that store atmosphere variables are very influential to increase sales in the store. The four store atmosphere variables, namely exterior, general interior, store layout, and interior display, make consumers more interesting and likely want to know more and ultimately tend to buy. A good store design can also attract consumers' desire to know more everything the store offer [5].

5. Research Methods

This research was conducted at the distro revolution located in the city of Sidoarjo, the village district of Ngampelsari village and Bangkalan too.

Population is the sum of all objects (units or individuals) whose characteristics are to be suspected. The population of this study is consumers or customers of the batik house Tresna Art.

A representative of the population called a sample that can be drawn conclusions later to generalize to the population. With the number of populations is infinite data which is not limited, in order to facilitate the research, the scope of the sample will be made smaller. For that the determination of the number of samples from the population is 25 times the number of independent research variables, the source of Roscoe in Ferdinand [9]. Then the minimum number of samples to be taken in this study is 50 respondents.

The sampling technique uses Purposive Sampling. "Purposive sampling is a technique determination of samples with certain considerations "[10, pp. 124]. This sample has specific criteria that are considered for research, while the criteria in sampling are consumers who have made purchases at the Tresna Art batik house. The number of samples to be used is 75 respondents. The amount of sample used in this study is 97 respondents.

Other side for study in revolution store Sidoarjo same method using purposive sampling technique. Purposive Sampling is a sample determination technique with certain considerations with the sample criteria in this study are visitors who have made a purchase or just visited the revolution store of the Sidoarjo Candi.

6. Results

Based on the results of this study some conclusions can be drawn as follows:

1. Store atmosphere from home batik Tresna Art has a significant effect in increasing the purchasing decisions made by its consumers.
2. For further researchers if doing research with the same object is expected to improve this research, with Adding other independent variables are examples of quality products on batik houses Tresna Art or prices offered by the house batik Tresna Art.
3. Based on the respondent's responses regarding the variable Store Atmosphere (X) which consists of Exterior (X1), General Interior (X2), Store Layout (X3), Interior Display (X4), and Purchase Decisions (Y) are in the good category. This can be seen from the assessment of some respondents who gave good scores on the variable Store Atmosphere and Purchase Decisions. If the Store Atmosphere variable is good, it can increase the Purchasing Decision on Revolution Distro, so that the product sales target of Revolution Distro can be achieved well. And assessments of Revolution Purchasing Decisions Distros are in the right category according to respondents' ratings. If the decision of the purchase made by the consumer is appropriate for the products sold, then it shows that the Store Atmosphere on Revolution Distro is good
4. Based on the partial test, it can be seen that the Exterior (X1) and General Interior (X2) variables have a significant influence on the purchasing decision variable (Y). While the Store Layout (X3) and Interior Display (X4) variables do not have a significant effect on the variable purchasing decision (Y) of the consumer at Revolution Distro Candi Sidoarjo. Based on F test, the significance

value was <0.05. Means from the four variables have a positive effect on the decision to buy Revolution Distro Candi Sidoarjo.

5. Based on the regression analysis can be seen that the General Interior variable (X2) has a dominant influence on the purchase decision (Y) Revolution Distro Candi Sidoarjo.

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Marketing and development strategy of siger rice from cassava in Way Kandis Village, District Tanjung Seneng-Bandar Lampung

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Abstract. The use of cassava is still dominated by large industries into tapioca and animal feed. Whereas the people in Way Kandis Village consume cassava is still limited to snacks. Utilization of cassava as an alternative staple food needs to change cassava into siger rice. This study aims to identify and determine the appropriate marketing and development strategies for siger rice in Way Kandis Village, Bandar Lampung. Analysis is carried out on internal aspects such as aspects of human resources, facilities and infrastructure, institutions, production, availability of raw materials, business locations and management and funding as well as external aspects such as technology and information, government policies, competitors, consumers and climate and weather. The results showed that siger rice produced in Way Kandis Village had white characteristics, the texture was rather sticky, the aroma was not typical of cassava, and was favored by panelists. The nutritional content of siger rice is water content (10.19%), ash (0.31%), fat (0.56%), protein (2.69%), crude fiber (4.50%), and carbohydrate (81.75%). The right marketing and development strategy for siger rice in Way Kandis Village is an aggressive growth strategy. Siger rice industry has a very favorable situation, has the power to overcome threats, and has the opportunity to overcome weaknesses so that it can expand the marketing area. As for the strategy for developing siger rice in Way Kandis, namely by conducting a diversification strategy, which means that the Siger rice industry is in a good position but still faces several challenges. Improved strategies can be achieved by increasing the amount of production offset by expanding the marketing network and improving the quality of products produced and increasing cooperation with the government to further increase production.

1. Introduction

Food security of a country said to be good if food needs the community has been fulfilled in terms of the amount and nutrition evenly and prices affordable. However, at the reality is now the community is in Indonesia generally and in the Province Lampung in particular has not been able to achieve conditions of food security due to still the size of community dependence consume rice. Dependency the community is due to being public perception that considers that rice is the only ingredient staples that contain carbohydrates the tallest.

One alternative to achieve food security is a program food diversification. Food diversification not just produce products which can reduce dependence the community will rice, but it is necessary product innovations that have more nutritional value to improve public health and reduce high degenerative disease. One form of diversification food is by using sweet potatoes

wood as an alternative to rice. Cassava can be used as an alternative food not only because of having womb good nutrition, but also have many availability in some regions, especially in Lampung Province. One food product from cassava which can be used as an alternative food is analog rice.

Analog rice is food functional derived from the cassava experience processing so that it is shaped granules like rice [1]. Meanwhile, according to Mishra et al. [2] analog rice is a processed product that can made from part or all of the material non-rice. Budijanto and Yuliyanti [3] declare analog rice shaped like rice grains can be made from all non-rice flour. One small rice industry analogues in Lampung Province that can produce rice analog with color white and or yellowish white is small analog rice industry in KWT Toga Sari Desa Way Kandis District Tanjung Seneng Municipality of Bandar Lampung. In the implementation of rice production activities analog, KWT Tirtaria is still experiencing obstacles. There are several obstacles happens both internal constraints comes from within the business and constraints external namely the obstacles that come from outside the scope of business that can affecting the development of rice business the analog.

Based on the background of the problem this research was conducted to collect information that can used as a basis for analysis for make decisions in damaging the right development strategy appropriate to be applied to the KWT Tirtaria.

The aim of this study to identify and determine appropriate development strategy KWT Tirtaria.

2. Materials and Methods

2.1. Place and time of research

This research was conducted at KWT Tirtaria Desa Way Kandis District Tanjung Seneng Municipality of Bandar Lampung in April-July 2017.

Research methods The research method used is a survey and interview method. Technique Data collection is done by method descriptive research conducted survey .. The analytical method used in this study is a skinative method and quantitative by analyzing good corporate environment environment internal and external environment. Results of analysis of external and internal factors this is then made as a matrix, namely the external strategy factor matrix (EFAS / External Factor Analysis Strategic) and strategy factor matrix internal (IFAS / Internal factor Analysis Strategic) The next stage is make use of all that information to formulate a development strategy and the right marketing strategy according to using matrix SWOT.

3. Results snd Discussion

3.1. Analysis of the Internal Environment of Siger Rice

Based on the survey results in the field with focus group and brainstorming methods it is known that the internal factors of siger rice in Way Kandis Bandar Lampung Village include aspects of product, price, place or distribution, promotion, human resources, management, and facilities and infrastructure. Furthermore, from each of these aspects detailed into points, determinants that can be the strength or weakness of siger rice in the village of Way Kandis.

3.1.1. Product Aspects

Based on the definition of the Kotler Philp product is everything that can be offered to the market to be considered, owned, used or consumed so that it can satisfy the desires or needs [4]. Meanwhile, according to the Indonesian Big Dictionary, the definition of products is goods or services that are made and added to their use or value in the production process and become the end result of the production process.

The products produced by KWT Tirtaria in Penawar Tama District have a much better quality compared to products produced by other producers. In terms of color, Siger rice produced by KWT Tirtaria has a white to yellowish white color. Siger rice is white and or yellowish white is siger rice with much better quality than other types of siger rice [5]. In terms of aroma, siger rice produced has a distinctive aroma of cassava. Because the raw material used is cassava without the addition of other ingredients. In addition, in terms of shape, the resulting siger rice has resembled the form of rice rice. So that psychologically people when consuming siger rice is the same as eating rice [1].

Packaging is a factor that is sufficient to support the quality of siger rice produced. Siger rice produced by KWT Tirtaria consists of 3 sizes, 250 g, 500 g and 1 kg. All products produced by KWT

Tirtaria use pouch packs flipped as primary packaging which is then sealed using a sealer. The type of packaging material used is very influential on the products produced, thus the function of packaging can be achieved, namely to protect products, facilitate distribution and attract consumers. This primary packaging is labeled as product information that contains the product name (brand), logo, composition, presentation suggestions, nutritional information, product benefits, net weight and production permits. However, there are still deficiencies in label, namely the lack of information on product halal, customer service, No. registration of the Ministry of Health of the Republic of Indonesia as well as information regarding product expiration. Whereas for secondary packaging, KWT Tirtaria uses cartons.

Based on the explanation above, it is obtained the strengths and weaknesses of the aspects of the products that exist in the Tirtaria KWT. The strength of the product aspect is that the quality of the siger rice product produced is good, namely yellowish white color similar to rice rice and has a nutritional content and good benefits for health, especially for diabetics. As for the weakness of KWT Tirtaria from the product aspect, that is still not complete information on the label.

3.1.2. Price Aspect

Price is an exchange rate of goods and services products expressed in monetary units. Price is one of the determinants of a company's success because the price determines how much profit the company will get from selling its products in the form of goods and services. Setting prices too high will cause sales to decrease, but if the price is too low it will reduce the profits earned [6]. Indonesian society in general still believes that product prices have a strong correlation with product quality. On the other hand consumers always want products with prices that are relatively cheap but have good quality. Therefore, KWT Tirtaria must be careful in determining product prices.

Siger rice produced by KWT Tirtaria is priced at Rp. 20,000 / kg while competitor products are priced at Rp. 17,000. The price offered is in accordance with the quality of the product produced in terms of color, aroma and shape better than other siger rice producers. Besides that, the form of siger rice produced also resembles rice rice while siger rice produced by other producers is still in the form of round granules like tiwul. In terms of packaging, siger rice produced using pouch packaging is more attractive than other siger rice packaging and the label is designed to be more attractive so that consumers are interested in reading and buying products.

At KWT Tirtaria, good and right market segmentation has not been implemented. One of them is price segmentation. This price segmentation is needed because one's economic strength must vary from one another. There are consumers who are able to buy products at high or high prices and there are also consumers who are able to buy at low prices or cheap. This price segmentation is needed so that KWT Tirtaria can reach all ranges of users or consumers of siger rice produced from the weak economy to strong economy. Thus, the benefits of consuming siger rice can be experienced by all consumers.

Based on the explanation above, it is obtained strength and weakness from the aspect of the prices that exist in the Tirtaria KWT. The strength of the price aspect is that the price offered is relatively affordable and in accordance with the quality of the product, packaging and label produced. While the weaknesses resulting from the price aspect is the absence of market segmentation, namely price segmentation.

3.1.3. Place or Distribution Aspects

A place or distribution is a company activity that makes a product available to the target customer [7]. Distribution can be interpreted as marketing activities that seek to expedite and facilitate the delivery of goods and services from producers to consumers, so that their use is in accordance with what is needed such as type, quantity, price, place and when needed [8]. Distribution is the activity of delivering products to the hands of consumers at the right time. Therefore, distribution policy is one of the integrated marketing policies which includes the determination of marketing channels and physical distribution. Both of these factors have a very close relationship in the success of the distribution and at the same time the success of product marketing. Distribution channels are needed to guarantee product availability in each channel chain [9].

The distribution system conducted by KWT Tirtaria is direct marketing channel. The resulting siger rice product is offered and sold directly through KWT Tirtaria's shop. KWT Tirtaria shop sells products in retail directly to consumers both in the size of 250 g, 500 g or 1 kg. The store is located in the production area so that the product transportation process can be carried out quickly and without the need for distribution costs. In addition to being distributed around the production site, Siger rice produced by KWT Tirtaria is also distributed to Bandar Lampung City through the Lampung Province Food Security Office. However, this distribution is not routine or unscheduled. This distribution is carried out if there is a request from the provincial Food Security Service.

KWT Tirtaria does not have cooperation with retailers who buy siger rice products for resale to consumers outside the production location. This causes the distribution area to be limited. However, in marketing its products, KWT Tirtaria strives to serve all market segments. This is done because KWT Tirtaria realizes that the products produced are functional food products that have health benefits to reduce risk and prevent diabetes and obesity. So that in creating its products, KWT Tirtaria does not carry out specializations aimed at certain market segments, namely for diabetics. But KWT Tirtaria continues to strive to provide products that have the best quality for consumers.

Based on the explanation above, it is obtained the strength and weakness of the aspect of the place or distribution in the Tirtaria KWT. The strength of the aspect of the place or distribution is a strategic place or distribution because it does not require distribution costs. While the weaknesses that result from the aspect of the place or distribution are the limited distribution of siger rice products.

3.1.4. Promotion Aspects

The promotion is an effort to increase the company's sales to carry out various things such as improving and expanding the distribution of its products and improving services to consumers [6]. Meanwhile, according to Suhendro [10], promotion is one of the variables of the marketing mix used by companies to communicate with the market. According to Ie et al. [11] Promotion is one of the marketing mix variables used by companies to communicate with the market.

In marketing siger rice products, KWT Tirtaria has not held a large-scale promotion. The promotional activities that have been carried out by KWT Tirtaria are participating in exhibitions and participating in bazaars organized by related agencies. KWT Tirtaria has not carried out the activity of distributing leaflets or brochures on the resulting siger rice products so that the siger brand or product produced by KWT Tirtaria has not been widely known by the public because of the promotion that has not been so intense. The existence of promotional assistance from the Food Security Service has a positive impact on KWT Tirtaria. Because the products produced at least began to be known by the public even though not yet extensive.

Based on the explanation above, it was obtained the strength and weakness of the aspect of promotion that existed at the Tirtaria KWT. The strength of the aspect of promotion is the promotion assistance from the Food Security Service. While the weaknesses resulting from the promotion aspect are the limited promotional activities carried out by KWT Tirtaria.

3.1.5. Human Resources Aspect

Human resources are one of the production resources, and are one of the factors of dynamics in long-term economic development. The availability of human resources in sufficient quantities, knowledge and skills and motivated to do work is a strength. It is because it will improve the performance of a business. But on the contrary, human resources that are weak both physically and mentally will become a point of weakness for a business or business.

Employees or workers are subject to factors of production that are very important in supporting the success of business ventures in various industrial activities. In fact, the success or failure of a business, whether or not a business is efficient, whether or not a business is effective is determined by human resources who participate in the business itself. Therefore, human resources must receive careful attention so that they can make optimum contributions in their work [12].

In essence, labor can be divided into three types, namely:

1. Trained workforce; usually the form of work that is occupied is not too need "theoretical skills"
2. Educated workforce; including the classification of workers who obtain theoretical education to a certain level and field / discipline. Can be divided into 2 types, namely experienced educated workforce and uneducated / uneducated workforce
3. Uneducated labor; including workers who did not obtain theoretical skills, so the main thing for them is "practical work KWT Tirtaria has been established since 2010 until now. The number of KWT Tirtaria employees is 25 people, consisting of 23 women and 2 male employees. The majority of KWT Tirtaria employees' final education is 16 junior high school graduates. Education is one of the factors that is sufficient to determine the level of skill possessed by the workforce in the Tirtaria Women's Farmer Group. So that the absorption of knowledge and information is much easier for the workforce to accept.

In addition to carrying out production activities, the workforce on this Siger rice also received training carried out by the relevant agencies. The training followed by KWT Tirtaria employees had a positive impact on the Siger rice. The benefits obtained are:

1. Working more efficiently, after participating in the training, of course the employees increase their knowledge, making it easier to complete a task.
2. Less supervision, after participating in training, the mistakes in working on the task can certainly be suppressed. If only a few mistakes are made, the level of supervision given is minimal.
3. Growing faster, employee development can indeed be left naturally in accordance with its capabilities. However, this development will be faster if employees attend training.
4. Stability of employees and a decrease in turn over, employees who have received training successfully so that they can grow certainly have a tendency to survive in the company.

Based on the description above, obtained strength and weakness from aspects of human resources on. Strengths from the aspect of human resources, namely the ability and skills that are quite good that have been owned by the workforce in the area and the training of related agencies. So that the ability and skills of KWT Tirtaria employees are growing. While the weaknesses of the aspect of human resources is the limited number of workers, which is only 25 workers. So that it is not yet possible to produce siger rice in larger quantities. For now, KWT Tirtaria is capable of producing 100-200 Kg / week of siger rice.

3.1.6. Management aspects

Management aspects are very vital aspects of a business. Because businesses that will or are being pioneered may fail if management in the business or organization is not going well. The management process itself also has rules so that businesses can run easily. The rules themselves can be clearly illustrated through the following management functions:

1. Planning, is a process to determine where and how a business will be run and started to achieve a goal that has been determined.
2. Organizing, is a process for grouping activities in certain units to be clear and orderly in accordance with the responsibilities and authority of the unit holder.
3. Actuating is a process where all planned things have been started by all units. Like a manager who directs all of his subordinates to start work in accordance with the tasks that have been assigned to him.
4. Controlling, is a process for measuring, evaluating and evaluating workers' results in order to remain in accordance with the initial plan and correcting various irregularities during the process of carrying out work [13].

In the Tirtaria KWT the management aspect has not been implemented optimally. Not yet optimal implementation of management at KWT Tirtaria because of the limitations of various parties and the number of workers at that. However, for organizing activities, implementation and supervision are sufficiently implemented. This is evidenced by the existence of direct directives during the

implementation of production activities, a mutual agreement in determining the timing of production activities, a clear coordination system either by telephone or direct coordination with the workforce, working together and directing the workforce in production activities for production steps that have not been understood and the existence of mutual agreement in providing production profits.

In addition, the entry and exit of costs and data on siger rice production at KWT Tirtaria have been regularly recorded. This is evidenced by the bookkeeping regarding the clear and written income and expenditure that is carried out in full by the head of the KWT as well as the business owner. Siger rice production data in the Tirtaria KWT can be seen in Table 7.

Based on the explanation above, the strengths and weaknesses of the management of Tirtaria KWT are obtained. Strengths in the management aspect are the implementation of clear and written books regarding income and expenditure and siger rice production data. While the weaknesses of the management aspect are that most management functions have not been implemented optimally this is due to the limitations of various parties and the limited number of employees.

3.1.7. Aspects of Facilities and Infrastructure

Facilities and infrastructure are the means of success of a business or organization. Because if these two things are not available then all activities carried out will not be able to achieve the expected results in accordance with the plan. The existence and availability of adequate facilities and infrastructure will greatly support the success of the business as well as the siger rice business in Way Kandis Village.

The Tirtaria Women Farmer Group has a number of processing equipment in the manufacture of siger rice which comes from the assistance of the related Dinas. Equipment obtained from related agencies is an extruder machine (siger rice forming machine). With the help of these machines, the siger rice production process becomes more effective and efficient. In addition, with the help of machinery from the relevant agency, KWT Tirtaria was able to produce anlog rice with a shape resembling rice rice. Other supporting equipment such as scales, press tools, basins and hands to dry the siger rice after leaving the extruder machine. KWT Tirtaria also has a large yard for drying siger rice.

Based on the description above, obtained the strengths and weaknesses of the aspects of facilities and infrastructure. Strengths in terms of facilities and infrastructure are ownership of adequate production facilities and infrastructure so that they can support production activities. While the weaknesses in the aspect of facilities and infrastructure are if the facilities and infrastructure owned cannot be utilized and used optimally.

The explanation of the seven internal aspects of Siger rice at the Tirtaria KWT at Way Kandis Bandar Lampung used in this study has produced some of these strengths and weaknesses. The strengths and weaknesses obtained are then determined and weighted by the rating which will produce an IFAS matrix before the development strategy is obtained.

Determination of the weight of each internal component to obtain strengths and weaknesses using the method of degree of relative importance. The determination of this weight involves one of the research respondents, namely the owner who is considered to be better understood and knows all production operational activities and knows the business constraints or obstacles. The internal factor matrix framework for strengths and weaknesses is presented in Tables 1 and 2.

3.2. Analysis of the External Environment of Siger Rice

The external environment analysis of siger rice is the identification of factors that are outside the KWT Tirtaria. These factors are opportunity and threat which can influence the existence and actions of both the direct and indirect performance of the KWT. Based on the survey results in the field, it is known that KWT Tirtaria's external factors include consumers, competitors, technology and natural resources. Furthermore, from this aspect it is detailed into a section that can be used as a determinant of opportunity or a threat to KWT Tirtaria.

Table 1. Framework for internal strategy factor matrix for strengths

Component	Strengths	Weight	Rating	Score	Rank
Product	The quality of the siger rice product produced is good	0.21	4	0.84	1
price	The price offered is relatively affordable and in accordance with the quality of the product	0.12	3	0.36	3
Place or distribution	Strategic place or distribution because it does not require distribution costs	0.17	2	0.34	2
Promotion	Promotional assistance from the Food Security Service	0.07	3	0.21	4
Human Resources	Good workforce skills and skills	0.12	3	0.36	5
Management	Clear and written bookkeeping regarding income, expenditure and data on siger rice production	0.17	3	0.34	7
Facilities and infrastructure	Ownership of adequate production facilities and infrastructure	0.14	3	0.42	6
Total				2.87	

Information on rating (strength): 4 (Strength that is very strong), 3 (: Strength that is strong), 2 (Strength that is low), 1 (Strength is very low).

Table 2. Matrix framework for internal strategy factors for weaknesses

Component	Weaknesses	weight	Rating	Score	Rank
Product	Still not complete label info	0.10	3	0.30	2
Price	The absence of price segmentation	0.10	2	0.20	1
Place or distribution	The limited distribution area of Siger rice	0.02	2	0.04	4
Promotion	Promotional activities are still limited	0.21	2	0.42	3
Human Resources	Limited number of workers so that production is limited	0.19	3	0.57	5
Management	Not yet optimal implementation of management functions	0.21	3	0.63	7
Facilities and infrastructure	Not optimal in utilizing facilities and infrastructure	0.17	3	0.51	6
Total				2.67	

Rating information (weakness): 4 (Weaknesses are very easy to solve), 3 (Weaknesses are easy to solve), 2 (Weaknesses are difficult to solve), 1 (Weaknesses are very difficult to solve).

3.2.1. Consumer Aspects

A consumer is someone who uses a product or service that is supplied. Consumers are divided into two, namely personal and organizational consumers. Personal consumers are individuals who buy goods or services for their own use, for use in the household, family members and friends. While the organizational consumer is a company, government agency or profit agency or other non-profit that buys goods or services and other necessary equipment used so that the organization can run well.

Many or no consumers of Siger rice can be influenced by consumer knowledge (product knowledge, purchasing knowledge and usage knowledge). Consumer knowledge of the product you want to buy is one of the important factors that can affect consumers. With complete information on the product, the consumer will be easier in determining which product to buy. Purchasing knowledge is one of the most important knowledge. Because with the knowledge of the purchase, the consumer can determine and decide to buy a product with the right, certain volume and frequency. In addition, knowledge of use is

also important for consumers. Knowledge of use is applied post-purchase, namely knowledge to use or use a product to meet needs. Every consumer who buys siger rice, has a different way of consumption, namely as a main food, support or companion, consumed with additional ingredients (side dishes) or only consumed without additional ingredients.

Siger rice consumers at KWT Tirtaria have knowledge and information about the products produced by it. In addition, KWT Tirtaria also has consumer trust and satisfaction on the quality of the Siger rice products produced. If the consumer has been satisfied with a particular product, if it stops production then the consumer who has been satisfied with the product will choose not to consume the same product with another brand.

This consumer purchase decision is also influenced by the price of the product to be purchased. Siger rice produced by KWT Tirtaria is priced at IDR 20,000 / kg while competitor products are priced at IDR 17,000. The price offered is in accordance with the quality of the product produced in terms of color, aroma and shape better than other siger rice producers. In addition, the form of siger rice produced also resembles rice rice while siger rice produced by other producers is still in the form of granules such as tiwul. However, prices are also very influential on the number of consumers even though the quality of the products produced is in accordance with the price of the products offered.

Based on the explanation above, then obtained opportunities and threats from the consumer aspect. Opportunities obtained from the consumer aspect are the knowledge and information that consumers already have about the siger rice products produced by KWT Tirtaria as well as consumer confidence in the siger rice products that have been consumed. While the threat obtained from the consumer aspect is that the price of siger rice is still high. This affects the number of siger rice consumers around.

3.2.2. Competitor Aspects

Competitors are companies or organizations that produce or sell goods and services that are the same or similar to the products offered. In the world of competition, the main task of entrepreneurs is to attract as many customers as possible both new customers and old customers and also how to turn off the pace of the development of competitors. Things that need to be known and continuously monitored are competitor products. Both in terms of quality, packaging, labels or other. Comparing the advantages of products owned by competitors and their weaknesses with their own products. In addition, producers must also be able to capture opportunities in the market before being captured by competitors.

Siger rice in the City of Bandar Lampung is only in the Penawartama Subdistrict, namely in the Way Kandis Village. This is an opportunity because there are no actors or similar business producers around the region. Thus, consumers who want to consume Siger rice can only obtain or buy from KWT Tirtaria.

In addition to competitors such as competitors from others can be a threat to Siger rice, namely rice rice business. The reason that underlies rice rice business as a competitor for the siger rice business is that the community is still dependent on rice. Besides that another reason is the selling price of Siger rice which is much higher than the selling price of rice rice with almost the same quality. The reason that underlies the community prefers to consume rice rice than siger rice. However, not a few people who want to buy Siger rice after knowing the benefits of Siger rice even with a high selling price.

3.2.3. Technology and Information Aspects

Technology is a scientific method for achieving practical goals or the overall means to provide goods needed for the survival and comfort of human life. The application of science and expertise is the core of the use of technology in the production process. The challenge now is how far the use of equipment as human power will increase productivity and quality. A product is not only affected by the quality of the raw material used but also influenced by the technology of the manufacturing process. This means that the machine to process the manufacture of raw materials into finished goods will affect the quality of the goods [12]. Generally, more sophisticated machine technology always produces better quality of goods.

Technological aspects include production equipment, production support infrastructure, means of mobility and information networks. The existence of technology in the Tirtaria KWT is reflected in the

presence of assistance in the form of production machines that facilitate the production of siger rice. However, in the information aspect of KWT Tirtaria still has not implemented an IT-based communication network and information system. By implementing an IT-based communication network and information system, it is expected to expand the marketing area of Siger rice. Marketing is not only done from mouth to mouth or through bazaars and exhibitions but can also be done with the help of the WEB. So that buyers can purchase Siger rice not only offline but also can purchase online.

Based on the explanation above, there are opportunities and threats from the technology and information aspects of the Siger rice. The opportunity is the existence of technological assistance in the form of production machines that can affect production time faster and produce higher quality siger rice. While the threat from the aspect of technology and information is not yet applied by other technologies other than production machines such as the application of information systems or technology in the form of computer operations that can help production operations.

3.2.4. Natural Resource Aspects

The availability of natural resources, either raw materials or supporting materials, greatly influences the sustainability of a business or organization. Raw materials are materials used for production purposes. Raw materials are tangible items that will be used in the production period. These items can be obtained from natural sources, purchased from suppliers, or made alone to be used in the next process [12]. Planning for raw material requirements is a process to ensure that raw materials are available when needed. When an effort to ascertain the demand for its products in the future, the time for new raw materials to arrive can be determined to reach the level of production that meets the predicted demand.

KWT Tirtaria gets cassava as raw material for making siger rice obtained from farmers, cassava traders or communities around Siger rice production houses. During the course of the KWT Tirtaria there was no difficulty in obtaining raw material for cassava only if a prolonged dry season the availability of cassava was limited and the price of cassava became higher. In addition to raw materials, the supporting materials used in the process of making siger rice are easy to obtain. Like water used for washing and helping the separation between starch and onggok obtained from around the production site. In addition, other supporting ingredients such as palm oil, salt, mono stearic glycerol and baking powder are easily available.

Table 3. The matrix framework for external strategy factors for opportunities

Component	Opportunities	Weight	Rating	Score	Rank
Consumer	Knowledge and information that consumers have	0.23	2	0.46	1
Competitor	The absence of actors or similar business producers around the region	0.20	2	0.40	2
Information Technology	Technology assistance in the form of production machines	0.27	3	0.81	4
Natural resources	The potential of large raw materials and continuity is guaranteed	0.30	3	0.90	3
Total				2.57	

Information on rating: 4 (Opportunities that are very easy to achieve), 3 (Opportunities that are easy to achieve), 2 (Opportunities that are hard to achieve), 1 (Opportunities that are very difficult to achieve)

Based on the explanation above, the potential of large raw materials and guaranteed continuity can be a reliable force for KWT Tirtaria. However, the availability of raw materials can be a weakness for a business if continuity is not guaranteed in the dry season. Business failure can occur due to the problem of continuity of raw materials that are not guaranteed. So that the existing siger rice stock is limited and results if at any time the consumer demand increases, siger rice cannot fulfill in full demand.

Explanation of some external aspects of Siger rice production at KWT Tirtaria in Way Kandis Village in Bandar Lampung shows several opportunities and threats. Opportunities and threats obtained are then

determined and rating weight that will produce an EFAS matrix before the development strategy is obtained. The matrix framework for external factors for opportunities and threats is presented in Tables 3 and 4.

Table 4. Framework for external strategic for threats

Component	Threats	Weight	Rating	Score	Ranking
Consumer	The price offered is still high retaif	0.23	3	0.69	3
Competitor	The selling price of siger rice is higher, affecting the number of consumers	0.30	2	0.60	2
Information Technology	Not yet implementing an IT-based information communication network	0.32	2	0.64	1
Natural resources	Continuity of raw material is not guaranteed during the dry season, which affects the stock of siger rice	0.15	3	0.45	4
Total				2.47	

Information on rating: 4 (threats that are very easy to overcome), 3 (threats that are easily overcome), 2 (threats that are difficult to overcome), 1 (threats that are very difficult to overcome).

Based on the description of the internal and external conditions of Siger rice production at KWT Tirtaria in Way Kandis Village, Bandar Lampung, the IFAS and EFAS metrics were obtained. Furthermore, the difference between the IFAS and EFAS metrics is presented in the SWOT analysis diagram. This SWOT analysis diagram will illustrate the condition of siger rice in what diagram. Weighting for SWOT diagram analysis of internal and external factors can be seen in Table 5.

Table 5. Weighting of SWOT diagrams of internal and external factors

Description	Internal		External	
	Strength	Weakness	opportunities	threats
Weight x rating	2.75	2.49	2.57	2.47
Difference	0.20		0.10	

From Table 5 above can be seen the difference between internal factors (strengths and weaknesses) and external factors (opportunities and threats). In the internal factor, the difference is +0.20 while the internal factor is the difference of +0.10. Furthermore, the selisisih value between internal and external factors of siger rice will be described on the x axis for internal factors and the y axis for external factors. After analyzing the external and internal environment, it can be formulated into a SWOT analysis that describes every strength, weakness, opportunity, and challenge that exists [14]. The meeting of the difference values of the two axes will produce a coordinate point on the SWOT diagram. The SWOT diagram of internal and external factors can be seen in Figure 1.

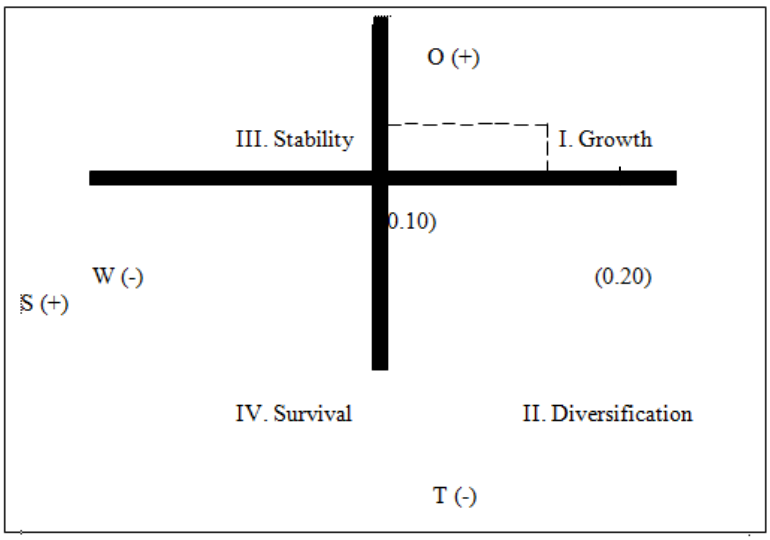

Figure 1. SWOT diagram of internal and external factors

Based on the SWOT diagram above, it can be seen that siger rice in Way Kandis Village, Bandar Lampung is in the first consciousness, namely aggressive growth. This means that siger rice has a very favorable situation, the company has the opportunity and strength so that it can take advantage of the opportunities that exist. The strategy that must be implemented in this condition is to support an aggressive growth policy (growth oriented strategy). A business or organization in this quadrant makes it possible to continue to expand, expand growth and achieve maximum progress.

KWT Tirtaria in Way Kandis Village, Bandar Lampung can use the existing opportunities and strengths such as the quality of the Siger rice products produced is good, the prices offered are relatively affordable and in accordance with the quality of the product, place or strategic distribution because it does not require distribution costs, there is promotion assistance from Food Security Service, the ability and skills of human resources that are quite good that have been owned by the workforce, clear and written bookkeeping regarding income and expenditure as well as data on siger rice production, ownership of adequate production facilities and infrastructure, knowledge and information already owned consumers, the absence of actors or similar business producers around the region, the existence of technological assistance in the form of production machinery, and the potential of large raw materials and guaranteed continuity. In addition, KWT Tirtaria continues to collaborate with local governments, universities, banks and entrepreneurs so that the production and quality of Siger rice can be guaranteed and a wider marketing network.

4. Conclusion

The siger rice produced by KWT Tirtaria has the characteristics of white rice such as rice grain, pulen texture, cassava aroma, panelists' preference, and contains water content (10.19%), ash (0.31%), fat (0, 56%), protein (2.69%), crude fiber (4.50%), carbohydrate (81.75%), and glycemic index 31. Siger rice quality assurance by applying SOP for making siger rice and clinical trials of rice siger in diabetic patients. Marketing strategies based on existing opportunities and strengths such as the quality of siger rice products that have been produced are good, the prices offered are relatively affordable and in accordance with the quality of products, places or strategic distribution because they do not require distribution costs, the promotion assistance from the Food Security Service, ability and skills quite good human resources that have been owned by the workers, clear and written bookkeeping regarding income and expenditure as well as siger rice production data, ownership of adequate production facilities and infrastructure, knowledge and information that have been owned by consumers, absence of actors or producers similar businesses around the region, the existence of technological assistance in the form of

production machines, as well as the potential of large raw materials and guaranteed continuity, as well as collaborating with local governments, universities, banks, and entrepreneurs so that the production and quality of siger rice can be guaranteed broader marketing style.

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Potential development of Madura corn products

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Abstract. Trunojoyo University has a focus on developing six sectors, including: the salt and tobacco sector, the food sector (corn, cassava, sugar cane, cattle and seafood), the energy sector (oil and gas and renewable energy), the education sector (formal, informal and non-formal), social sector, labor and women, tourism sector and creative economy. In 2016, Trunojoyo University successfully launched Madura Corn 1 and Madura Corn 2 with the Minister of Research, Technology and Higher Education. Therefore, it is necessary to do research on the potential development of Madura corn processed products. This research used a descriptive design, survey method, and Quality Function Deployment method. The populations in this study were Madura society. The primary data were obtained from a questionnaire filled by 100 Madura societies with random sampling techniques. The results of this study indicate that the content of Madura corn has high protein and fat content of 11.24% and 4.96%, so it is very good for consumption. The identification of consumer needs states that 72% of respondents expect the development of Madura corn processed products, one of the corn processed products that are in great demand by consumers is Madura corn rice products. Therefore the development of Madura corn rice products is highly expected in the diversification of Madura corn processed products and support in regional food preservation.

1. Introduction

Bangkalan is an area that has a tropical climate with dry soil types, so many farmers grow corn. In 2017 the potential of corn harvest area in Bangkalan was 58,850 Ha with a production of 142,329 tons [1]. This shows that the land area and productivity of corn in Bangkalan are very high. Corn is one type of grain crop, another name for this corn plant is Zea Mays L which is already popular throughout the world. Corn is one of the food crops that has a strategic role in agricultural development and the Indonesian economy because it has the potential in the needs of food, feed, industrial raw materials, and handicrafts [2]. Trunojoyo University has a focus on developing six sectors, including: the salt and tobacco sector, the food sector (corn, cassava, sugar cane, cattle and seafood), the energy sector (oil and gas and renewable energy), the education sector (formal, informal and non-formal), social sector, labor and women, tourism sector and creative economy. In 2016, Trunojoyo University successfully launched Madura Corn 1 and Madura Corn 2 with the Minister of Research, Technology and Higher Education. Therefore, it is necessary to do research on the potential development of Madura corn processed products.

2. Materials and Methods

This research used a descriptive design, survey method, and Quality Function Deployment (QFD) method. QFD method is very suitable for use in product development [3-6]. The populations in this study were Madura society. The primary data were obtained from a questionnaire filled by 100 Madura societies with random sampling techniques because it provides equal opportunities for each member of the population to become a sample, and this method is quite easy and fast in its implementation.

3. Results and Discussion

The content of Madura corn has high protein and fat content of 11.24% and 4.96%, so it is very good for consumption [7]. The development of corn-based products is one of the efforts in the implementation of food diversification by means of product manufacturing innovation. Fast food is a type of food that

is packaged, easily served, practical, or processed in a simple way. These foods are generally produced by high-tech food processing industries and provide various additives to preserve and give flavor to these products [8]. The identification of consumer needs states that 72% of respondents expect the development of Madura processed products. Some Madura corn processed products include corn chips (corn flakes), corn rice, corn sugar, corn oil, and corn flour.

Corn flakes are processed food products from starchy materials which are flattened into plates of a certain shape (usually round), dried, and fried crispy. This chips can be added to the spices according to taste, for example salty, spicy, savory, sweet, added sliced onion leaves, or added with other seasonings. The ingredients commonly used as chips are corn and cassava. The taste is crunchy, making corn flakes is a popular food process. Compared to other corn processed products, this corn flakes need more special and careful handling, especially because of its thin and easily destroyed physical form. Therefore, in processing corn flakes, the yield is only around 80%, around 20% of which is destroyed cannot be sold.

Instant corn rice is one of the technological developments that are expected to increase the consumption of corn rice. Instant corn rice has a protein content ranging from 10-11% [9]. Instant corn rice is a processed product in the form of ready-to-eat food textured like coarse ground flour and cooking process only by brewing it with hot water as desired. Corn chosen to be processed into instant corn rice is corn that is old so that when smoothing or milling is not soft. Corn rice is a typical Indonesian food made from corn as its basic ingredient. Corn rice is a food that contains nutrients needed by the body [10].

Corn sugar is a sugar extracted from corn plants. Corn sugar is said to be good for diabetics because it is included in the type of non-nutritive sweetener that has a fairly low-calorie level which is very good for controlling blood glucose levels. Corn sugar is included in the type of sugar from starch which is often referred to as High Fructose Syrup (HFS). HFS in liquid form is very beneficial for the use of the beverage industry. But now HFS is also widely used in alcoholic, animal food, candy, food, and pharmaceutical industries. The main content of corn sugar is glucose and fructose, the fructose content is between 42-90%. Corn sugar contains only a simple sugar substance called fructose, a type of sugar that is often found in fruits and has a sweeter taste than ordinary sugar. Corn sugar (fructose) is proven to have a lower number of calories compared to ordinary sugar (sucrose).

Corn oil is an oil that is rich in unsaturated fatty acids, namely linoleic and linolenic acids. Both of these fatty acids can lower blood cholesterol and reduce the risk of coronary heart attack. Corn oil is also rich in tocopherols (vitamin E) which function for stability to rancidity. In corn oil, there are dissolved vitamins which can be used as non-food ingredients, namely medicines. Corn oil can be used as an alternative for the prevention of coronary heart disease.

Corn flour is flour produced from dried corn by fine grinding corn endosperm containing 86-89% starch. Yellow corn flour with different brightness levels. Milling of corn seeds into flour is a skin separation process, endosperm, institute and tip stamp. Endosperm is part of corn kernels which are ground into flour and have high carbohydrate content. Skin that contains high fiber must be separated because it can make rough textured flour. In addition, the institution which is part of the highest fat content of corn seeds must also be separated so that the flour does not become rancid [11].

4. Conclusions

As many as 45% of consumers want Madura corn to be processed into Madura corn rice. There are seven consumer needs, among others, easy to cook, tasty, durable, soft texture, cheap, easy to store, and without BTP. Therefore the development of Madura corn rice products is highly expected in the diversification of Madura corn processed products and support in regional food preservation.

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Entrepreneurial skills and farming performance of organic farming

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Abstract. The purpose of this research were analyze the level of entrepreneurial skills of organic farmers and the farming performance of organic farming. The analysis is used quantitative analysis which using weighting of five variables of entrepreneurial skills (professional skills, management skills, opportunity skills, cooperative/networking skills, and strategy skills). This research was conducted in April-Mei 2018, with the number of respondents were 30 organic paddy farmers in the Komunitas Ngawi Organic Center (KNOC). The results of this research indicated professional skills 87.26 percent, management skills 83.78 percent, opportunity skills 80.20 percent, cooperation/networking skills 71.85 percent, and strategy skills 74.02 percent. Entrepreneurial skills of organic farmers are in the high level of entrepreneurial skills. Organic farming performance consists of productivity in the medium category (> 5-7) tons/ha/season with a percentage of 63.33 percent and profit IDR 9 102 000 ha/season is profitable category with R/C ratio 1.47.

1. Introduction

Entrepreneurship skills are concepts of relationships that refer to individuals and activities. On the one hand, describes individuals who know how to do things in business. On the other hand, it describes the tasks and activities that individuals need to know about how to do business. It must be emphasized that the concept of entrepreneurial skills explains about individuals [1]. In a study by [2] examined the entrepreneurial skills of farmers in three agricultural business divisions' namely conventional production, value added and non-food diversification. The results show that there are five entrepreneurial skills needed by farmers to be successful in business including: professional skills (plant production skills, technical skills); management skills (financial management and administration skills, human resource management skills, customer resource management skills, general planning skills); opportunity skills (recognising business opportunities, market and customer orientation, awareness of skills, risk management skills innovation skills); cooperation/networking (skills related to co-operating with other farmers and companies, networking skills, team working skills, leadership skills) and strategic skills (skills to receive and make use of feedback, reflection skills, monitoring and evaluation skills, conceptual skills, strategic skills, strategic decision making skills, goal setting skills).

The concept of entrepreneurial skills is derived from the element of entrepreneurial capacity. This concept is the result of studies from the ESoF (Entrepreneur Skill of Farmer) program European Commission 2006. The concept focuses on understanding entrepreneurial skills possessed by a farmer to become an entrepreneur [3]. The development of this theory is based on the results of studies conducted on the ESoF program "Developing the Entrepreneurial Skills of Farmers" at the European Union in 2005-2008. The concept of entrepreneurial skills based on the ESoF research project by [2] is explained in Figure 1.

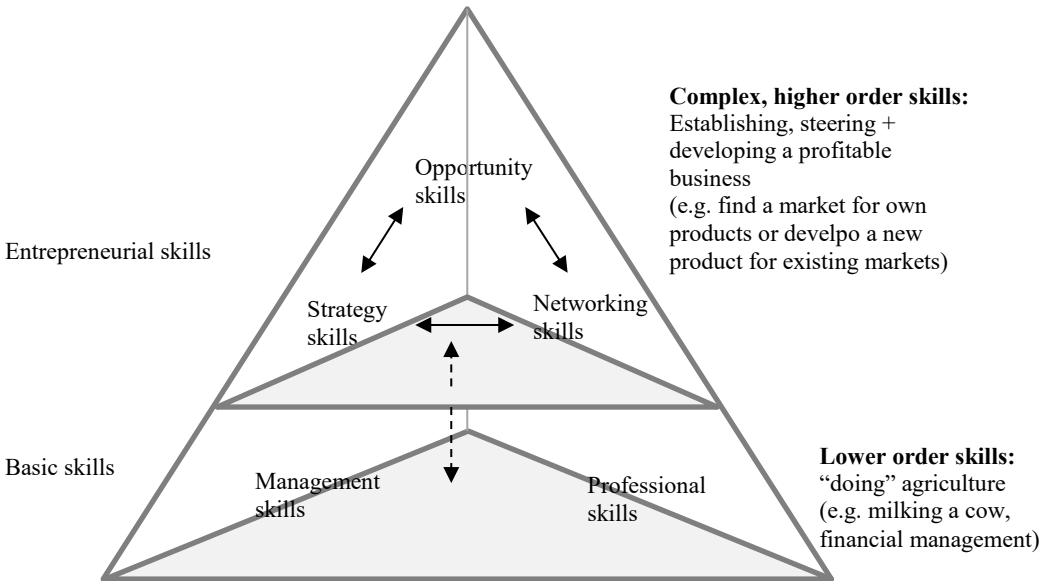

Figure 1. Five basic concepts of entrepreneurial skills of farmer

Entrepreneurial skills which are the basic skills according to [2] are categorized into (1) professional skills, and (2) management skills. As a synthesis of the research that professional and management skills are basic requirements for farmers. Skills as stimuli or motivators to arouse enthusiasm, improve the personal abilities of farmers, especially in terms of communication, management, innovation, developing business networks, stimulating creativity and ideas, increasing the ability to capture business opportunities and realize them. Whereas opportunity, networking, and strategy skills in entrepreneurial skills for farmers can be categorized as high / complex skill levels so that the three skills are worth mentioning as entrepreneurial skills.

These skills are seen as entrepreneurial skills which should be owned by farmers. Farmers who study entrepreneurial skills do not imply that other skills are considered irrelevant or unimportant. These skills are needed to find ways and strategies in developing business profits, realizing business opportunities and developing and enhancing businesses to stay sustainable. Efforts to compare the skills available in the research literature can be considered as part of the qualities possessed by an entrepreneur [4] and [5]. More clearly the categories of entrepreneurial farmer skills for farmers can be classified in Table 1.

Performance is a multidimensional concept that is formed from financial performance and non financial performance [8]. Similarly, [9] which describes the performance of a farm not only seen in terms of production and income, but also can be seen from the increase in assets owned by farmers and satisfaction of farmers doing their jobs. Each job has its own performance standards, so that a performance can be said to be good when it is able to meet or exceed the standards set previously. The standard in question is an innovation technology standard and business operational standard procedures. The application of good standards will provide good business performance. According to [10] that the application of agricultural innovation technology plays a role in increasing farm productivity. This is also in line with [11] who said that one way to improve the economy in rural areas is through technological innovation, especially agricultural technology. [12] who stated that the ability of entrepreneurs can improve business performance so that it can reduce unemployment in a region. [13] explain the relationship between business performance and income, where performance is a desire to grow reflected in income. Less optimal farmer performance in saprotan management, business management, capital, and marketing results resulted in an increase in income not being achieved.

Table 1. Category and indicator of entrepreneurial skills

No.	Agriculture entrepreneurial skills [6]	Category entrepreneurial skills [2]	Indicator underlying skills [2, 7]
1.	Learning skills, physical skills,	Professional skills	1)Plant or animal production skills 2)Technical skills
2.	Reading skills, Mathematic skills, Info communication, Technology Skills, Financial and Administration	Management skills	1)Financial management and administration skills 2)Human Resource management skills 3)Customer resource management skills 4)General planning skills
3.	Planning opportunity perception	Opportunity skills	1)Recognising business opportunities 2)Market and customer orientation 3)Awareness of skills 4)Risk management skills 5)Innovation skills
4.	Information collection, Communication skills and Foreign language skills,	Co-operation/ Networking skills	1)Skills related to co-operating with other farmers and companies 2)Networking skills 3)Team working skills 4)Leadership skill
5.	Creativity, Result orientation, Skills of logical thinking, Problem solving skills, Skill of analysis and feedback	Strategy skills	1)Skills to receive and make use of feedback 2)Reflection skills 3)Monitoring and evaluation skills 4)Conceptual skills 5)Strategic skills 6)Strategic decision making skills 7)Goal setting skills

The study conducted by [14] explained that the performance of a business can be measured from the level of sales, cost of sales, assets owned, brand image and fixed assets owned by the company. [15] added that business performance can be measured based on income, sales, output, productivity, costs, service acceptance, and customer reactions, continuity business and growth. Based on previous theoretical and empirical studies, business performance on organic farming which is considered important in this study are as follows:

1. Application of organic farming
   The application of organic farming is the extent to which organic farmers apply the rules of organic farming in every farming activity contained in organic farming standards and the Standard Operational Procedure (SOP).

2. Productivity
   Productivity is a term in production activities as a comparison between outputs (input) with input (input). Productivity can be used as a benchmark for the success of a business in producing a product. So that the higher the comparison, means the higher produced the product.
3. Profit
   Profit referred to in this study is that revenue is reduced by fixed costs and variable costs during one harvest. Profits are often also interpreted as farming income received by farmers.

At present, one of the farms that is of concern to consumers and even the world is organic farming. Organic farming arises from the impact of past agriculture that produces as much as possible for food fulfillment. This raises overall agricultural problems. The problem is no longer at the level of farming as a business unit, but has expanded to social issues, and farming culture itself. Organic farming provides incentives to preserve and build traditional / indigenous farmers' knowledge about agriculture and local ecosystems. The implementation of these innovative measures does not harm farmers, often because farmers must first invest in the learning process. With low innovation capacity, farmers must take the time to learn more diversity of practices [16]. The learning process is formed from the interaction of skills possessed by farmers. Skill interaction can support the development of entrepreneurial skills. Farmers develop their entrepreneurial skills primarily through the learning process while conducting farming activities because of the low level of formal education that farmers have. Change of perspective is very important to learn and that will happen when farmers change their perspective after being exposed to new ideas and various ways of doing things [1].

Organic farming systems are an innovative form of farming systems where different skills are needed from conventional business systems. Farmers must be able to apply entrepreneurial skills in every organic farming activity that complies with organic farming standards. The organic standard is a reference in organic farming activities and makes it easier for farmers to identify what entrepreneurial skills are needed in managing organic farming.

One of the problem in developing entrepreneurship is the weak quality of human resources related to entrepreneurial skills. Supposedly, the presence of organic farming can be an opportunity for potential entrepreneurial growth in the countryside. More than 35 million Indonesia national workers or 26.14 million households still depend on the agricultural sector [17]. Farmers' resources are available in very adequate quantities, however, the quantity of these farmers has not been matched with the quality of entrepreneurship they have. There are still many smallholder agricultural businesses whose motives for business are not yet in line with the paradigm of modern business, not a few farmers doing farming activities are still semi-commercially oriented, even some of them are subsistence. This shows that farmers still do not really view agriculture as a business. The process of transforming farmers from being initially oriented in business with traditional business motives (semi-commercial or subsistence) becomes the paradigm of modern (commercial) business or has an entrepreneurial spirit, requires awareness and volunteerism from the farmers themselves.

2. Material and Methods

The population of this study was all organic rice farmers in the Komunitas Ngawi Organik Center (KNOC) taken by census technique. Census technique is sampling technique if all populations are desirable as a sample [18]. The numbers of samples taken in this study were all KNOC organic rice farmers, amounting to 30 organic rice farmers. Location selection is purposive, with consideration that the area has implemented an organic farming system that has been certified organic and as an organic farming center in Ngawi Regency.

Entrepreneurial skills possessed by organic farmers are measured through five skills variables, namely: professional skills, management skills, opportunity skills, cooperative/networking skills, and strategy skills. The level of entrepreneurial skills of farmers in organic farming systems uses scores that have been processed as criteria for entrepreneurial skills of farmers based on the concept of de Wolf and Schoorlemmer 2007. The calculation of the percentage (%) level of entrepreneurial skills of each of the five indicators of entrepreneurial skills by all respondent farmers is as follows:

$$\text{Percentage of entrepreneurial skill level scores} = \frac{\text{Total of all respondents}}{\text{Maximum total score}} \text{ x } 100\%$$

After knowing the percentage of respondent's answers, then the calculation results are grouped according to the answer categories specified in Table 2. Calculation of the percentage of this score is used to facilitate in determining the answer categories of respondent's entrepreneurial skills. The number of indicators used by each different skill causes the highest difference in the number of scores on each entrepreneurial skill.

Table 2. Determination of the total score categories based on the percentage of respondent's answer categories

No	Percentage of Answer Categories (%)	Score category
1	0-25	Very Basic
2	26-50	Basic
3	51-75	High
4	76-100	Very High

To measure the performance of organic farming, it consists of three business performance, namely the application of organic farming, productivity and profit. First, the application of organic farming is measured using the [19]. Requirement regarding the principles of organic farming applied in Indonesia are in accordance with the Indonesian National Standards for Organic Food Systems, namely SNI 6729: 2016 (No 64 / Permentan / OT.140 / 5/2013). The method for calculating the percentage (%) of the level of application of organic farming is the same as measuring the level of entrepreneurial skills. Second, the productivity of organic farming is measured using the formula for the amount of organic rice production in ton divided by the area of land in ha as follows:

$$\text{Organic farming productivity} = \frac{\text{Total production (ton)}}{\text{Land area (ha)}}$$

Third, farming profits are the difference between total revenue and total costs [20] :

$$\pi = \text{TR-TC}$$

3. Result and Disscussion

3.1 Entrepreneurial Skills

The entrepreneurial skills of farmers are reflected by five variables, namely professional skills, management skills, opportunity skills, cooperation/networking skills, and strategy skills. Entrepreneurship skills are the highest stage in entrepreneurship. The results of the percentage scores on entrepreneurial skills of organic farmers are shown in Table 3.

Table 3. Level of entrepreneurial skills of organic farmers

Entrepreneurial Skills	Percentage (%)	Level of Entrepreneurial Skills
Professional skills	87.26	Very High
Management skills	83.78	Very High
Opportunity skills	80.20	Very High
Cooperation/networking skills	71.85	High
Strategy skills	74.02	High

In Table 3 shows that professional skills, management skills and opportunity skills are in a very high category. Whereas networking skills and strategy skills are in the high category. Judging from the results of the score showing all entrepreneurial skills possessed by organic farmers are in the category of high skill levels or entrepreneurial skills.

Entrepreneurial skills that have a very high percentage score are professional skills, management skills and opportunity skills. Indicators of professional skills are plant production skills and technical skills. Organic farmers can produce organic plants according to organic standards and are skilled in making organic input materials and used of modern technology. This skills are gained after joining the organic farming community, where all organic farmers receive regular organic farming training. The use of modern technology is widely used in the cultivation process while the use of information technology is only limited to call and send/receive messages without having internet access.

Entrepreneurial skills that have a very high percentage score are management skills. However, most organic farmers less attention to financial records and farm administration, they only keep in mind the real costs incurred in their farming. The amount of income obtained by farmers in one planting season, according to them, will get profit, but if a more detailed statement is made, the farmers will loss. Farmers do not take into account all costs, such as non-cash costs (labor costs in the family, depreciation of equipment, harvest labor, etc.). While the human resource management skills, customer resource management skills and general planning skills are good. The existence of a Komunitas Ngawi Organik Center is a community to improve the skills. So that, overall management skills in the category are very high. Entrepreneurial skills that also have a very high score are opportunity skills. Farmers are skilled at increasing profits by developing sustainable organic farming. Generally, organic farmers have gone through a period of transition/conversion in terms of doing cultivation and executing organic farming.

Networking skills and strategy skills have a high score percentage. Overall organic farmers are skilled in networking skills, but most of farmers are not expanding in establishing networks/relations to develop organic farming. In addition, organic farmers lack cooperation in groups. They still have a sense of distrust among their groups, so that suspicion arises in organic farming. For strategy skills, some organic farmers rarely reflect, rarely learn from previous experience, repeat mistakes again. Some farmers identify facts in the field, but do not find the right solution, leaving profits only to meet their daily lives. Another problem is that farmers generally understand the concept of organic farming but still depend on the organic farming community in providing farming inputs, learning from the process obtained, has a long-term plan but only wants.

3.2 Farming Performance of Organic Farming

The first performance is the application of organic farming assessed by measuring the level of application of organic farming based on the Indonesian National Standard (SNI) 6729: 2016 (No 64/Permentan/OT.140/5/2013). Calculation of scores from weighting will be used as a basis for measuring the application of organic farming. The score obtained is the result of a percentage of the score of the answer for each criterion of the indicator organic farming. The indicators of the application of organic farming based on SNI used in this study are conditioned by indicators in the field are fresh plants and plant products, crop production management, handling, transportation, storage and packaging, labeling and claims, traceability and recording documentation, source of organic products, other material requirements not contained in appendix, certifications and inspections. In Table 4 we can see the level of application of organic farming.

Table 4. Level of application of organic farming

No.	Application of organic farming	Weight of Application of Organic Farming		
		Total Score	Maximum Score	Percentage (%)
1.	Fresh Plant and Plant Products, plant production management:			
	– Conversion	96	120	80.00
	– Organic management maintenance	120	120	100
	– Parallel production and split production	47	120	39.16
	– Prevention of contamination	47	120	39.16
	– Land management, soil fertility and water	112	120	93.33
	– Plant selection and variety	120	120	100
	– Ecosystem management and diversity in plant production	95	120	79.17
	– Pest and Disease Management	114	120	95.00
2.	Handling, transportation, storage and packaging:			
	– Postharvest management	120	120	100
	– Packaging	44	120	36.67
	– Storage and transport	117	120	97.50
4.	Labeling and claims	87	120	72.50
5.	Traceability and recording documentation	66	120	55.00
6.	Source of organic products	109	120	90.83
7.	Requirements for other materials not included in the appendix	116	120	96.67
8.	Certification	120	120	100
9.	Inspection	120	120	100
	Total Score	1650	2040	80.88

The application of organic farming to organic farmers is not much different. This is because all organic farmers join the Komunitas Ngawi Organik Center (KNOC). Organic farmers easily get information and training related to the application of organic farming. In Table 4 shows that the level of implementation of organic farming as a whole in the category is very high with a percentage of 81.86 percent. Indicators of conversion, maintenance of organic management, land management, soil fertility and water, selection of plants and varieties, management of ecosystems and diversity, use of plant disturbing organisms, postharvest management, storage and transportation, labeling and claims, sources of organic products, material requirements others that are not included in the appendix, certification and inspection are in the very high category.

While the indicators of traceability and documentation of recordings are in the high category with a percentage of 72.50. Some farmers record or save documentation, but not complete and not saved for at least 5 years. Written data and documents that explain all types of goods, quantity and recipients / buyers

of goods sold must be saved. Written data or documentation must be kept so that it is possible for certification bodies and authorities to trace the origin, nature and quantity of all materials purchased, as well as the use of these materials. These problems can be minimized by joining organic farmers in the Komunitas Ngawi Organic Center (KNOC). The KNOC assists in the administration and documentation of organic farming.

However, in parallel production indicators and separate production, prevention of contamination and packaging are in the low category with percentages of 39.16 percent, 39.16 percent and 36.67 percent respectively. Parallel production indicators and split production, prevention of contamination and traceability and recording documentation are in the high category. Parallel production indicators and split production must pay attention to the limiting, handling, packaging, clear storage so that there is no mixing between organic and non-organic products. Parallel production are in a unit of land planted by similar plants (eg. rice), but not all blocks in the unit have organic status. Split production is in a unit of land planted by several types of plants (different), but not all types of plants are organic. Constraints that occur in the field are not all organic farming land planted organically. Conventional plants are close to organic plants, resulting in water and air contamination. To prevent water contamination, farmers carry out fertilization with husks which are inserted into sacks and stored in water sources.

While preventing air contamination, some farmers do not take preventing. Farmers only remind each other of conventional farmers in terms of spraying pesticides so that they do not lead to organic land. While in organic farming standards to prevent airborne contamination of annual crops is to plant buffer zones with a minimum width of 2 meters and managed organically. Buffer plants cannot be claimed as organic plants. Buffer plants must consist of different varieties so that they can be distinguished from the plants submitted for certification. Other forms of buffer zones can be trenches, roads, and the like as wide as a minimum of 3 meters. And make a barrier/barrier in the form of a living fence that is higher than the plants submitted for certification. Generally organic farmers prevent air contamination by planting buffer plants.

Indicator that got a low percentage is packaging, because most farmers do not packaging independently. They do not have modern packaging tools. Packaging is carried out by KNOC by using modern packaging equipment and packaging materials used that can be recycled. But there are some organic farmers doing packaging independently, packaging materials used can be recycled but packaging is still traditional. Problems in this application of organic agriculture can still be tolerated by organic certification agencies due to local environmental conditions. The organic farming activities that most violate the rules of organic certification institutions are providing pesticides to organic plants.

The second performance is productivity, productivity performance is assessed by measuring the amount of crop produced by organic farmers. Variation in the amount of production for each organic farmer varies depending on land area and soil fertility. Based on the [21] showed that the average productivity of organic rice in Tasikmala from 2005-2012 reached 7.68 ton per hectare. In Table 5 can be seen the productivity of organic rice (ton/ha) in one planting season.

Table 5. The level of productivity of organic rice farming per season

Productivity of organic rice (tons/ha)	Category	Number of Farmers	Percentage (%)
1-5	Low	3	10
>5-7	Medium	19	63.33
>7	High	8	26.67

The level of productivity of organic rice farmers in Table 5 shows> 5-7 ton/ha/season classified as medium with a percentage of 63.33 percent. This condition indicates that the organic elements used are not maximized in improving soil and nutrient structure. The facts in the field show that organic paddy fields are adjacent to conventional rice paddy fields so that they are contaminated through air and water. The research conducted [22] said that land that could be used as organic farming was a land that was free from contamination of agrochemicals from fertilizers and pesticides. Pollution-free land (air or water), both from industrial pollution and other agricultural areas that use chemical intake. If the soil

and nutrient structure is good and supported by an organic environment, it will increase crop productivity. Subsequent research by [23] regarding the opportunities for organic agriculture for sustainable agricultural development says that organic farming can increase the productivity of smallholder farming. The high productivity will affect the many benefits received by organic farmers.

The third performance is profit, which measured by looking at the level of net opinion obtained from organic farming as a whole. Profits are obtained from revenues less farm costs. Receipts are obtained from cash receipts, namely the value of money received by farmers from the amount of output, namely harvested dry grain multiplied by the selling price and non-cash receipts, namely the value of money from grain consumed by farmers. Farming costs are also divided into cash and non-cash costs. Cash costs are costs incurred by farmers in real and in the form of money. While non-cash costs are costs that are not spent in cash but are still taken into account. The component of cash costs on organic rice farming consists of fixed costs and variable costs. Fixed costs in organic rice farming consist of land rent, taxes, village fees, irrigation fees, tractor rental and harvesting machine rental. In addition there are variable costs, namely seeds, compost, fuel/electricity, profit sharing, wages for out-of-family labor, wages for transporting fertilizers, and harvesting transportation. Harvest transportation costs are calculated based on the distance of land location with grain storage location. For KNOC member farmers, GKP is directly stored in the KNOC barn. The components of non-cash costs on organic rice farming consist of MOL costs, biological agents, agricultural equipment depreciation, and wages in the family labor. Details of costs per hectare per season for organic rice farming can be seen in Table 6.

Table 6. Average production and revenue per hectare per season on organic rice farming in 2017-2018 (in thousands)

Cost component	Rupiah (Rp)	Percentage (%)
Cash Cost		
Fix Cost	3 093	16.07
Variabel Cost		
Seed	286	1.48
Compost	1 461	7.59
Fuel / Electricity	80	0.41
Wages of outside family labor	5 897	30.64
Profit Share	942	4.89
Fertilizer Transport Wages	494	2.56
Harvest transportation	235	1.22
Total Cash Cost	12 488	64.89
Non-Cash Cost		
Seed	117	0.60
Compost	1 414	7.34
Local micro organism	580	3.01
Biological agents	73	0.37
Depreciation of agricultural equipment	3 013	15.65
Wages of labor in the family	1 557	8.09
Total Non-Cash Cost	6 754	35.11
Total Cost	19 242	100.00

Based on Table 6 it is known that the average percentage of the largest costs in organic farming is found in the cost of outside family labor which is a cash cost. The percentage of out-of-family labor costs is high because activities on organic rice farming require more intensive work. Then the next

largest percentage of costs is fixed costs which are cash costs. Depreciation costs of equipment in non-cash costs also provide a high percentage. Most organic farmers have modern agricultural tools and machines. Outside family workers in non-cash costs also provide a high percentage.

Greater cash costs are incurred by organic farmers. Organic rice farmers devote more for out-of-family labor wages and fixed costs. Therefore, the proportion of cash needs is more issued by organic rice farmers. However, organic rice farmers need to consider the availability of inputs for fertilizers, local microorganism and organic pesticides. Also, the amount of fertilizers, local microorganisms and organic pesticides have never been experienced a limited supply. Organic farmers also consider the distance between paddy fields cultivated with KNOC locations. Relatively far distances will require more time and energy to distribute fertilizer, local microorganism and organic pesticides and yields.

The success of organic farming is measured using analysis of farm revenue and income. Acceptance of organic rice farming is obtained from the value of money revenue by farmers from the amount of production output, namely harvested dry grain multiplied by the selling price. The profit is used to measure how much value the farmer revenue from the costs incurred both in cash and non cash. The revenue and profit of organic rice farming can be seen in Table 7.

Table 7. Average production and revenue per hectare per season in organic rice farming in 2017-2018 (in thousands)

Information	Organic Rice
Total production (kg)	6 315
Price (Rp)	5 171
Cash revenue (Rp)	27 146
Non cash revenue (Rp)	1 197
Total revenue (Rp)	28 344
Cash cost (Rp)	12 488
Non cash cost (Rp)	6 754
Total cash (Rp)	19 242
Profit (Rp)	9 102
R/C ratio	1.47

Profit is one way to measure the success of a business. Every business that is run will always expect maximum profits. Based on Table 25 it can be seen that the average profit received is IDR 9 102 000/ha/season. The selling price of organic rice was determined by the KNOC, the average price of IDR 5,171. The high and low prices of organic rice were affected by the grain water content at harvest. Organic grain content of 19 percent to less than 21 percent will be purchased at a price of IDR 5,000 / kg for white rice grain and IDR 5,000 / kg for brown rice grain, while for black rice grain at IDR 6,000 / kg. If the water content of organic rice grain at harvest is more than 21 percent, the selling price will decrease according to the percentage increase in measured water content. Their tolerance limits accepted grain moisture content will affect the amount due when the milled rice yield.

When compared with conventional rice where the level of productivity and costs are the same, the price given for organic rice is a premium price. However, the price of conventional rice is around IDR 4,500 in the dry season and between IDR 3,200/kg – IDR 3,800/kg in the rainy season. The yield of organic rice is higher and more profitable. This has implications for the value of R/C ratio obtained 1.47 (R/C ratio > 1) then organic rice farming is profitable and feasible to cultivate. Cavigelli et al. [24] stated that the advantages of other methods of organic farming are, in the long run the yields increase and vice versa, production costs decrease. In general, some research results say that organic farming provides greater benefits and has a significant effect on farmers' income [25]. Organic farming provides positive results for farmers. In general, many studies evaluate the economic benefits of organic farming.

So that it can recommend its application as a more profitable production system [26]. Economic benefits are one of the main driving factors for converting certified organic farming [27].

4. Conclusion

Based on the results of entrepreneurial skills research and the performance of organic farmers' business can be concluded that entrepreneurial skills possessed by organic farmers in a high category are called entrepreneurial skills. The performance of organic farming business which consists of the application of organic farming is very high category, organic farming productivity in the medium category and organic farming profits is in the profitable category with R/C ratio 1.47.

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Feasibility of investment in oil palm plantations in Bengkulu Tengah

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Abstract. Palm oil is one of the prima donna of plantation crops which is one of the non-oil and gas foreign exchange earners for Indonesia. The bright prospect of palm oil commodities in the world vegetable oil trade encourages the Indonesian government to develop the palm oil industry in an integrated manner (agro-industry). The development of the palm oil industry is a process to increase added value for palm oil-based products, supported by government policies such as the plantation revitalization program 2006-2010 (Ministry of Agriculture, 2006) and subsistence investment for plantations (Ministry of Finance, 2006). Bengkulu Tengah Regency which is one of the potential areas for the development of the palm oil industry with an area of oil palm plantations has reached 7,918 ha and a total production of 35,793 tons (2017). The development of the palm oil industry, both land expansion and productivity improvements, has led to an increase in the total production of fresh fruit bunches (FFB), thus requiring an oil palm processing plant (PKS). The purpose of this study was to (1) Analyze the feasibility of investment in palm oil mill construction based on market, social and environmental aspects (2) Analyze the feasibility of palm oil mill investment based on social and financial environmental aspects, and (3) Analyze the sensitivity of the feasibility of palm oil mills to changes in production costs and decreased production capacity. The study was conducted in August-September 2018. The data used were primary and secondary data. The analysis is carried out qualitatively and quantitatively and is grouped into two scenarios, scenario I uses its own funds while scenario II uses bank credit loans. Qualitative analysis was carried out descriptively through observation and literature study while quantitative analysis was carried out using financial analysis methods based on NPV criteria, IRR, B / C Ratio, Payback Period and sensitivity analysis using an indicator of a 10 percent increase in production costs and a 10 percent reduction in production capacity. The results show that based on non-financial aspects consisting of market, social and environmental aspects there are no obstacles that can interfere with the operational process and the objectives to be achieved from the construction of the palm oil mill. Whereas from the financial aspect based on assumptions and criteria used for scenario I (own funds) it is feasible to carry out the NPV value of Rp. 106,698,657,000, IRR 22.34, B / C 2.30, PP 3 years 8 months. While scenario II (loan) is not feasible to be carried out financially according to the results of the NPV assessment (-Rp. 30,727,367,000, IRR 9.03, B / C 0.63, PP 6 years 4 months. Total investment needed for the construction of a coconut mill oil palm amounting to Rp.82,368,421,000.The results of sensitivity analysis with indicators of increasing production costs and decreasing production capacity, scenario I (own funds) is still possible to be implemented while in scenario II (loan) the construction of palm oil mills is not feasible.

1. Introduction

Agriculture is one of the important sectors in Indonesia which is an agricultural country. Agriculture contributes significantly to employment, sources of income, supply of food, industrial raw materials, feed, bioenergy, state foreign exchange sources, and capital formation. One of the agricultural sub-sectors that is very instrumental in the formation of the national economy is plantations. Indonesian plantations have several leading commodities both in food crops and non-food crops. Food crops that

are Indonesia's leading commodities include palm oil, rubber, coconut, cocoa, coffee, pepper, cashew, tea and sugar cane. Palm oil as a producer of palm oil (Crude palm oil) and the core of palm oil (Kernel Palm Oil) which is one of the prima donna of plantation crops that are a source of non-oil and gas foreign exchange for the country. Since 2005, Indonesia is the largest palm oil producer in the world with a total production of 36 million tons of palm oil or meeting 43.3% of the world's palm oil needs. Along with the progress of science and innovation on derivative products from palm oil that can be used as raw material for several other industrial sectors (downstream industry). The development of downstream industries, and the bright prospects of palm oil commodities in the world vegetable oil trade, encouraged the Indonesian government to develop the palm oil industry in an integrated manner (agro-industry). Integrated development of the palm oil industry by synergizing various existing potentials to create added value for products based on palm oil. In addition, the development of an integrated palm oil industry will encourage development growth, create new jobs, reduce unemployment and poverty and accelerate the process of technology transfer to the community (farmers). The development of the palm oil industry is also inseparable from the existence of government policies that provide a variety. The distribution and development plans of the palm oil industry (oil palm plantations) in Indonesia are mostly located in the regions of Sumatra, Kalimantan, Sulawesi and Papua.

Bengkulu Tengah is one of the regencies in Bengkulu Province which has enormous potential for oil palm development in Indonesia both in terms of area and production. In 2017 the area of oil palm plantations reached 7,918 ha and a total production of 35,793 tons. Based on the area of plantation and production, Bengkulu Tengah Regency has fulfilled the necessary conditions and sufficient conditions for the construction of a palm oil mill (PKS) capacity of 30 tons of FFB per hour, as recommended by the government related to the primary cooperative credit program package for member (KKPA) with an area of 6000 ha and above (PPKS, 2002). In addition, the continuity of the supply of FFB for palm oil mills is in accordance with the regulation of the palm oil mill construction permit (Minister of Agriculture Regulation No.26 / Permentan / OT.140 / 2/2007) which requires that the processing capacity is installed.

The development of palm oil mills (PKS) is an integral part of the development of the palm oil industry. Without a palm oil mill, the development of upstream industry (oil palm plantations) both land expansion and productivity improvements in regions, such as Bengkulu Tengah would be in vain because the nature of the large and perishable FFB products would be abandoned and rot around the collection point . causing a decline in the quality and selling price of FFB to be low, in addition to the transfer of regional revenue sources to other regions from the process of creating value added products that require rapid processing. So he embraced the presence of palm oil mills in the central regions so that it was very helpful for farmers to accommodate the production from the gardens they were working on. Investment in the development of FFB palm oil mills (PKS) in Bengkulu Tengah Regency in addition to providing benefits also creates costs and risks. This requires the need for proper and objective planning to analyze the benefits and risks of the investment activities. One analysis that is needed is an investment feasibility study. This analysis is carried out to see whether or not investment is feasible based on aspects that are reviewed so that it can provide an accurate picture to investors who are interested in making a decision to invest in Bengkulu Tengah Regency. With the construction of a palm oil mill, it will create a new economic area with the growth of formal and informal sectors such as schools, markets, health facilities, transportation and telecommunications. This of course will have a better impact on the socio-economic life of the community, local government, and other parties that are directly or indirectly related to economic activities in Bengkulu Tengah Regency. Based on the description of the conditions above, then the formulation of the problems that will be examined in this study is:

1. How is the investment feasibility seen from the social and financial aspects?
2. What is the sensitivity of investment in the development of palm oil mills to changes in costs and production capacity?

2. Research Methods

2.1 Research Location and Time

This research was carried out in Bengkulu Tengah Regency, Bengkulu Province. Site selection is purposively made because Bengkulu Tengah Regency is one of the potential areas in terms of area and total production for the development of the palm oil industry. Data collection time starts from August to September 2018.

2.2 Types and Data Sources

Data collected includes primary data and secondary data. Secondary data is obtained from existing information and data, searches through the internet, books, journals, research centers, government agencies, and literature related to research.

2.3 Analysis Methods

The analysis carried out in this study is quantitative and qualitative. Qualitative analysis was carried out to obtain an overview of the feasibility aspects of the development of palm oil mills (PKS) conducted in Bengkulu Tengah Regency which included market aspects, social environmental aspects and financial aspects. Quantitative data obtained is processed using Microsoft Excel Software then displayed in tabulation form to facilitate descriptive reading and interpretation. Quantitative analysis includes financial analysis of the development of palm oil mills (PKS) using investment feasibility criteria namely; Net Present Value (NPV), Internal Rate Return (IRR), Net Benefit Cost Ratio (Net B/C), Payback Period and sensitivity analysis.

3. Result and Discussion

3.1 Results of Environmental and Social Analysis

3.1.1 The positive impact of the construction of a palm oil mill

The palm oil mill construction project will have a positive impact on the opening of new jobs for people from various levels and types of expertise. The job creation process that occurs by palm oil mill construction projects will be even broader with the existence of a multiplier effect both backward and forward linkages from projects such as the emergence of employment in the trade, transportation and small and large industries. The opening of new jobs means additional income for the parties involved. Parties who directly gain an increase in income are farmers who sell FFB to palm oil mills (PKS) and residents around the project who are project employees. Other parties who obtain additional income are the regional and central government. Additional revenue for the government in the form of taxes consisting of PPh, PPn, PBB and PE. Furthermore, the sale of palm oil processing results adds to the export value of large companies, so that it will generate foreign exchange that can be used to finance national development.

3.1.2 Negative Impact of Palm Oil Plant Operational Activities

Possible impacts include: (a) the sound of factory noise in the area around the factory; (b) Activities of river water use and the emergence of waste disposal from factories; (c) The emergence of factory smoke. From these activities that can have a negative impact on the environment is item (b), while others are only local and have low intensity. The effect of the sound of the factory sound on the area around the plant will not interfere with the residential area and housing of the factory employees. While the effect of smoke emissions will not affect the air condition around the environment.

3.2 Investment Feasibility Criteria

Assessment of the feasibility of an investment in terms of financial aspects is done using several investment criteria. Each criterion used has advantages and disadvantages of each. The more criteria used, the more it provides a complete picture and better results. The criteria used in general to be analyzed in making investment assessment decisions are: Net Present Value (NPV), Internal Rate of

Return (IRR), Net Benefit Cost Ratio (Net B / C) and Payback period (PP). The following is a summary of the results of the investment criteria analysis for the two scenarios used (Table 1).

Table 1. Summary of Analysis of Criteria for Investment in Palm Oil Mills

Investment Criteria	Scenario I (Own Fund)	Scenario II (Loan)
NPV	106,698,657,000	- 30,727,367,000
IRR	22.34	9.03
B/C	2.30	0.63
PP	3 years, 8 months	6 years, 4 months

3.2.1 Net Present Value

Net present value is the difference between the net benefits obtained with the costs used in the project, calculated using a 7 percent discount rate for scenario I and 15 percent for scenario II. The discount rate is the cost of capital as an opportunity cost of an investment based on the scenario used. Use of discount rates this (7% and 15%) because the cost of capital invested in the project comes from different sources so that the costs incurred by each investment decision are not the same. The results of the analysis show a positive NPV at a discount rate of 7 percent for scenario I, amounting to IDR. 106,698,657,000 and scenario II at a discount rate of 15 percent with a negative value of IDR 30,727,367,000 for 15 years. The positive NPV value in scenario I is an indication that the investment plan for palm oil mill development is feasible because the results obtained are greater than zero. While the negative NPV value in scenario II indicates that the construction of the palm oil mill is not feasible to be carried out financially.

3.2.2 Internal Rate of Return (IRR)

Internal Analysis Rate of return with a discount rate of 7 percent and 15 percent is used to evaluate the ability of the project to generate profits that are associated with the time value of money. The IRR value reflects the amount of the discount rate which, if used to discount all cash inflows, will generate the same amount of cash as the amount of project investment. The results of the analysis show the IRR value of 22.34 in scenario I and 9.03 in scenario II. This shows that the plan to develop palm oil mills is able to produce an opportunity cost that is greater than the desired cost of capital in scenario I so that it is feasible to implement. Whereas in scenario II the IRR value is lower than the cost of capital that has been determined so that it is not feasible to be implemented in terms of financial aspects.

3.2.3 Net Benefit Cost Ratio (Net B / C)

Net benefit cost ratio is done to measure how much benefit can be received from each investment issued. The results of the analysis of the palm oil mill development plan resulted in a B / C ratio of 2.30 in scenario I and 0.63 in scenario II. This means that the profit generated from this project in scenario I is greater than the cost that must be spent so that it is feasible to carry out. Whereas in scenario II the resulting profit is smaller than the costs incurred, the construction of the palm oil mill is not feasible to be carried out financially in scenario II because the benefits generated are less than the costs invested.

3.2.4 Payback Period (PP)

Payback period analysis is carried out aimed to find out the payback period of the investment. The results of the analysis of the palm oil mill construction project will reach the point of return when the project is 3 years and 8 months in scenario I and 6 years 4 months in scenario II. When viewed from the age of the palm oil mill project which reaches 15 years, the construction of the plant is possible and feasible to carry out because the investment return time is smaller than the project life.

3.3 Sensitivity Analysis

Sensitivity analysis is used to see the sensitivity of the palm oil mill to changes in conditions beyond the range of assumptions that have been made at the time of planning. This analysis is carried out and directed at two indicators, namely if there is an increase in production costs and a decrease in production capacity by 10 percent. Determining a 10 percent increase in production costs refers to data on annual average inflation in Indonesia in the past decade that never exceeds 10 percent. Whereas a reduction in production capacity of 10 percent is a level of tolerance that is considered normal for a decrease in raw material supply caused by non-technical factors that might happen in the field.

3.3.1 Increase in Production Costs (10%)

In the indicator of rising production costs, sensitivity analysis is carried out assuming an increase in production costs by 10 percent. All variables of production costs are projected to increase except the cost of purchasing FFB and insurance costs. Exceptions are made because of the price of FFB has a correlation with the price of CPO and Kernel, because the rise and fall of FFB prices is influenced by the price of CPO and Kernel. While insurance costs are fixed so that it does not affect the smooth operation of production. The following is a summary of the results of the sensitivity analysis if there is an increase in production costs by 10 percent (Table 2).

Table 2. Summary of Results of Sensitivity Analysis on Increase in Production Cost Indicators 10%.

Investment Criteria	Scenario I (Own Fund)	Scenario II (Loan)
NPV	99,772,392,000	-35,189,724,.000
IRR	21.47	8.12
B/C	2.21	0.57
PP	4 years, 1 month	6 years, 8 months

Based on the results of the sensitivity analysis carried out if there is a 10 percent production cost increase, the palm oil mill construction in scenario I for all the investment criteria used, the construction of the palm oil mill allows and is feasible to be carried out. From the results of this analysis can mean that with a tolerance level of increasing production costs 10 percent of the operational activities of the plant is still able to provide benefits in scenario I. While scenario II is not appropriate to be carried out based on the results shown by negative NPV values, IRR is below the cost of capital and B / C ratio is small than one. Full details of the projected calculation of eligibility criteria if there is an increase in production costs of 10 percent.

3.3.2 Decreasing Production Capacity (10%)

Sensitivity analysis with an indicator of decreasing production capacity, is carried out with the assumption of a decrease in factory processing capacity by 10 percent. Decrease in capacity if it has implications for reducing the cost of procuring raw materials and the cost of supporting the production process. In addition, a decrease in capacity will result in a decrease in production volume that affects sales revenue or output. The following is a summary of the results of the sensitivity analysis if there is a decrease in production capacity of 10 percent in the following Table 3.

Table 3. Summary of Results of Sensitivity Analysis on Indicators of Declining Production Capacity 10%

Investment Criteria	Scenario I (Own Fund)	Scenario II (Loan)
NPV	84,671,172,000	-45,027,555,000
IRR	19.52	6.09
B/C	2.03	0.45
PP	4 years, 3 months	8 years, 1 month

From the results of the analysis carried out in the event of a 10 percent decline in production capacity (Table.3), the construction of a palm oil mill in scenario I is still feasible to be carried out based on the investment criteria used. This indicates that a decrease in production capacity at a 10 percent tolerance level is related to supply or availability the raw material in scenario I can still provide benefits and does not cause the palm oil mill operational activities to be disrupted. While in scenario II it is not feasible to carry out. Full details of the calculation projections caused by a decrease in production capacity.

4. Conclusion

From the results of the research that has been done, it can be concluded several things as follows:

1. Financially based on the assumptions used, scenario I (own funds) with a discount factor of 7%, the investment activity of the palm oil mill (PKS) capacity of 30 tons of FFB per hour is feasible in terms of all investment criteria used. NPV value is IDR 106,698,657,000; IRR of 22.34; Net B / C of 2.30; and Payback Period for 3 years and 8 months. While scenario II (loan) with a discount factor of 15%, palm oil mill investment activities are not feasible. The NPV value obtained is (-Rp. 30,727,367,000); IRR of 9.03; Net B / C is 0.63; and Payback Period for 6 years and 4 months. The total investment required is IDR 82,368,421,000.
2. The results of the sensitivity analysis of the palm oil mill (PKS) capacity of 30 tons of FFB per hour, on an indicator of a 10 percent increase in production costs and a 10 percent reduction in production capacity in scenario I are still feasible to be implemented while in scenario II it is not feasible to implement.

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Beef cattle farmers motivation towards the practice of profit sharing system of beef cattle business in Maiwa District, Enrekang Regency, South Sulawesi Province

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Abstract. The purpose of this research was to analyze the motivation of beef cattle farmers in practicing profit sharing of beef cattle business. Data was collected from January to February 2017 while the research was conducted in Maiwa Sub district, Enrekang Regency using survey research method. Total of 64 farmers were taken as respondents. The method used in the data analysis was SEM SmartPLS (Partial Least Square) 2.0. The results of the research was the variable of the farmers' motivation in practicing profit sharing system of beef cattle business was influenced by the variable characteristics of the farmers, the relationship with the stakeholders, and the desire to obtain progress.

1. Introduction

Maiwa Sub district in Enrekang Regency, South Sulawesi Province is one of the region where the farmers establish beef cattle business by implementing profit sharing system. The system is considered as a positive way to achieve welfare for farmers who practice the profit sharing system in Maiwa Sub district. The farmers who practice the profit sharing system are the ones who have 5-10 years' experience and have practiced this system throughout generations.

The implementation of profit sharing system in Maiwa Sub district is usually involved two parties which are the capital provider and the breeder. The party that acts as the provider of capital is the one who owns the cattle but usually does not have enough time to take care of those cattle. The provider then gives the cattle to a breeder to be taken care of according to the agreement they had previously. According to the breeder's statement, there is no written agreement made between the breeder and the capital provider. The agreement is built based on trust or kinship between two parties. Unfortunately, because of the said reason, sometimes it becomes less profitable for the breeder.

The farmers will be motivated to work if what they can meet their life necessities. Wahyjosumidjo [1] stated, that motivation is an internal force that encourages someone to take action. Hellriegel et al. [2] also added that there are three main groups of needs, namely: existence needs, relationships with stakeholders or related needs, and the need to have a progress or growth needs. This research focuses on those three factors which are expected to influence the motivation of farmers to practice the profit sharing system. The purpose of this research was to determine what factor that influence the motivation of farmers to practice the profit sharing system.

2. Research Method

This research used survey method. The research was conducted in Enrekang Regency, specifically in Maiwa Sub district from January to February 2017. The location of the research was in Maiwa Sub district, Enrekang Regency. The research location was determined purposively and total of 64 farmers

were taken (as the total samples). This research uses Partial Least Square (PLS) as the analysis tool and used four measurement methods, which were: Convergent Validity, R-Square, Significance Test, and SmartPLS model.

3. Result and Discussion

3.1. Overview of Research Location

Geographically, Maiwa Sub district is dominated by hills or mountains, which is about 84.96% of the total area of Enrekang Regency. Meanwhile the flat area only covers about 15.04% of the total area. The topography of Maiwa Sub district, Enrekang Regency includes such diverse features such as hills, mountains, valleys and rivers with a height of 47-3,293 m above sea level and does not have coastal area. Maiwa Sub district, Enrekang Regency, experiences two climate changes, namely rainy season and dry season. The rainy season occurs in November until July and the dry season occurs in August until October.

Table 1. Analysis Result of SmartPLS

No.	Variable	*Path Coefficient*	*Standard Error*	*T-Statistic*	Significant	R-Square	Explanation
1.	Breeder Characteristics => Performance	-0.214	0.088	2.433 > 1.96	0.074 (**)	0.7228	Accepted
2.	Necessity Fulfillment => Performance	0.076	0.077	0.985 < 1.96	0.162		Rejected
3.	Relationships with Stakeholder => Performance	0.485	0.103	4.706 > 1.96	0.000 (**)		Accepted
4.	Progress Obtained => Performance	0.500	0.090	5.530 > 1.96	0.000 (**)		Accepted

Source: Output SmartPLS

Based on Table 1, the decision of the test variables submitted in this research is obtained. Based on variable 1, the statement that the characteristics of farmers affect performance was proved by the Path Coefficients value by -0.214 and the Standard Error value by 0.088. It was also found that the T-Statistic value was more than T-Table (1.96) which means that the effect of the farmers' characteristics on the profit sharing system performance had significant effect with the trusting level of up to 99%. However, the results show in opposite direction. Which means: the higher the characteristics of the farmers, the performance of the profit sharing system would decrease. There are 5 valid indicators for characteristics of farmers: the number of livestock owned, non-farming income, profit sharing system income, agricultural land tenure and the number of calves produced. Meanwhile there are 6 invalid indicators which are: age, formal education, livestock farming experience, working time, numbers of family members, and the number of members involved in livestock farming maintenance. In accordance with the research conducted by Fauziyah [3], stated that the personal and psychological characteristics of

farmers influence technical competencies which subsequently affect the performance produced in beef cattle business.

Variable 2 shows that the necessity fulfilment affecting performance was not proven because the Path Coefficients value was 0.076 and the Standard Error value was 0.077. So, the T-Statistic value obtained was less than T-Table (1.96) which means the effect of the necessity fulfilment is not significant. It can be concluded that the need for existence is not the main factor driving livestock farmers to have a better performance in practicing the profit sharing system. In this case, farmers do not consider the necessity fulfilment. The reason was because most of the farmers who practice the profit sharing system in Maiwa Sub district, Enrekang Regency, work as farmers and consider it as a staple job. Livestock farming is only considered as a side job in helping the family's economical condition. This condition is contrasted with the opinion of Maryati [4] which states that needs or necessity can motivate everyone to carry any work in order to meet their needs. This fact also stands in contrast to Hartono's research [5] which explains that the business of raising beef cattle for farmers is a part to support the fulfillment of the farmers' needs. Needs will encourage human to push themselves to work harder in order to meet their needs or as a respond to the pressure they experience. However, this does not apply to livestock farmers who practice the profit sharing system in Maiwa Sub district, Enrekang Regency. Necessity fulfillment does not guarantee the high performance of beef cattle business with profit sharing system, because actually this system is not profitable but farmer feel respected by community by taking part in the system.

Variable 3 was about the statement that the relationship with stakeholders influencing performance was proven. With the Path Coefficients value of 0.485 which shows unidirectional results and the Standard Error value of 0.103, hence the T-Statistic value was more than the T-Table (1.96). This means that the relationship with stakeholders has a significant effect on performance where the level of trust reaches 99%. The higher the relationship with stakeholders, the higher the performance will be. When performance is affected by the relationships with stakeholders, it might also occur because livestock farmers who practice the profit sharing system was more concerned with good social relationships, such as relationships with capital providers, extension agents, government, surrounding communities, and other farmers. This fact is in accordance with Munandar's opinion [6] which states that humans have social needs. Humans are social beings who need each other and love to participate in any activity. Based on the research of Alfisyahrin et al. [7], they state that empowerment programs improve the living standard of farmers and the community's independence. Relationships with stakeholders have a positive impact where they can exchange experiences and information, especially information about livestock farming techniques and the adoption of better technology. In addition, the interaction between farmers and stakeholders is also expected to be well established so that there would be an impact where they would need each other, improve their skills and help managing beef cattle business.

Variable 4 was about the desire to obtain progress affecting performance was significantly proven because the Path Coefficients value was 0.500 which shows the same direction and the Standard Error value is 0.090, thus the T-Statistic value is more than T-Table (1.96). It means that the effect of the desire to obtain progress has a significant effect on performance with the level of trust reaches up to 99%. The higher the need to obtain more progress, the higher the performance will be. Performance that is affected by the desire to achieve progress because livestock farmers who practice the profit sharing system wish to develop their abilities; both in terms of knowledge, farming experience and to get an appreciation. This is in accordance with the opinion of Ardi [8] which states that achievement can

increase the motivation of farmers in partnering. In addition, this is also consistent with the opinion of Luanmase [9] which states that the higher the experience of farmers, the higher the motivation for raising livestock. On the contrary, the lower the level of experience of the farmer, the motivation for raising livestock will be lower. Cattle farmers consider that it is better to utilize their spare time making an income by doing a business with profit sharing system while fully working on their agricultural land, rather than wasting their spare time doing nothing it makes the desire the desire to obtain progress as one factor to have a real influence on the performance of farmers who practice the profit sharing system.

3.2. PLS Model

Firstly, several strategies to improve the welfare of farmers with profit sharing system are by trying to increase : 1) The number of livestock owned by farmers, 2) Non-farming income, 3) Revenue sharing system for farmers, 4)The area of agricultural land to farmers, 5) The number of calves born, and 6) Farmers' motivation through improving the relationships with stakeholders. The results of this research support the research by Alam [10] which states that the effect of social motivation has a significant effect on the activity of beef cattle cultivation. Secondly, farmers' motivation can be increased through the increase of the desire to obtain progress. The support of capital owners, extension agents, the government, the community, and other farmers was very much needed by livestock farmers who practice profit sharing systems in Maiwa Sub district, Enrekang Regency. By having stakeholders support, farmers will be motivated to establish beef cattle business.

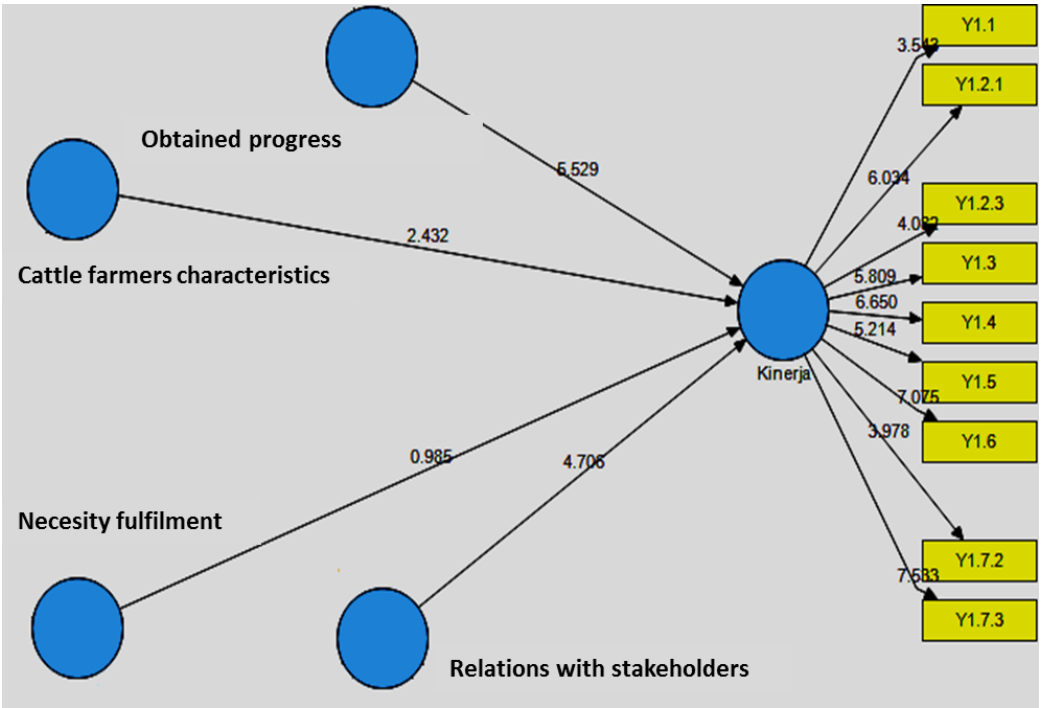

Figure 1. SmartPLS Final Model

Beef Cattle farmers in Maiwa Sub district, Enrekang Regency, will be motivated if they receive an appreciation and to obtain the knowledge and experience of livestock cattle farming from the profit sharing system. This would encourage farmer to be better in terms of beef cattle business with profit sharing system. Therefore, the alternative model for improving the welfare of farmers obtained is seen in Figure 2.

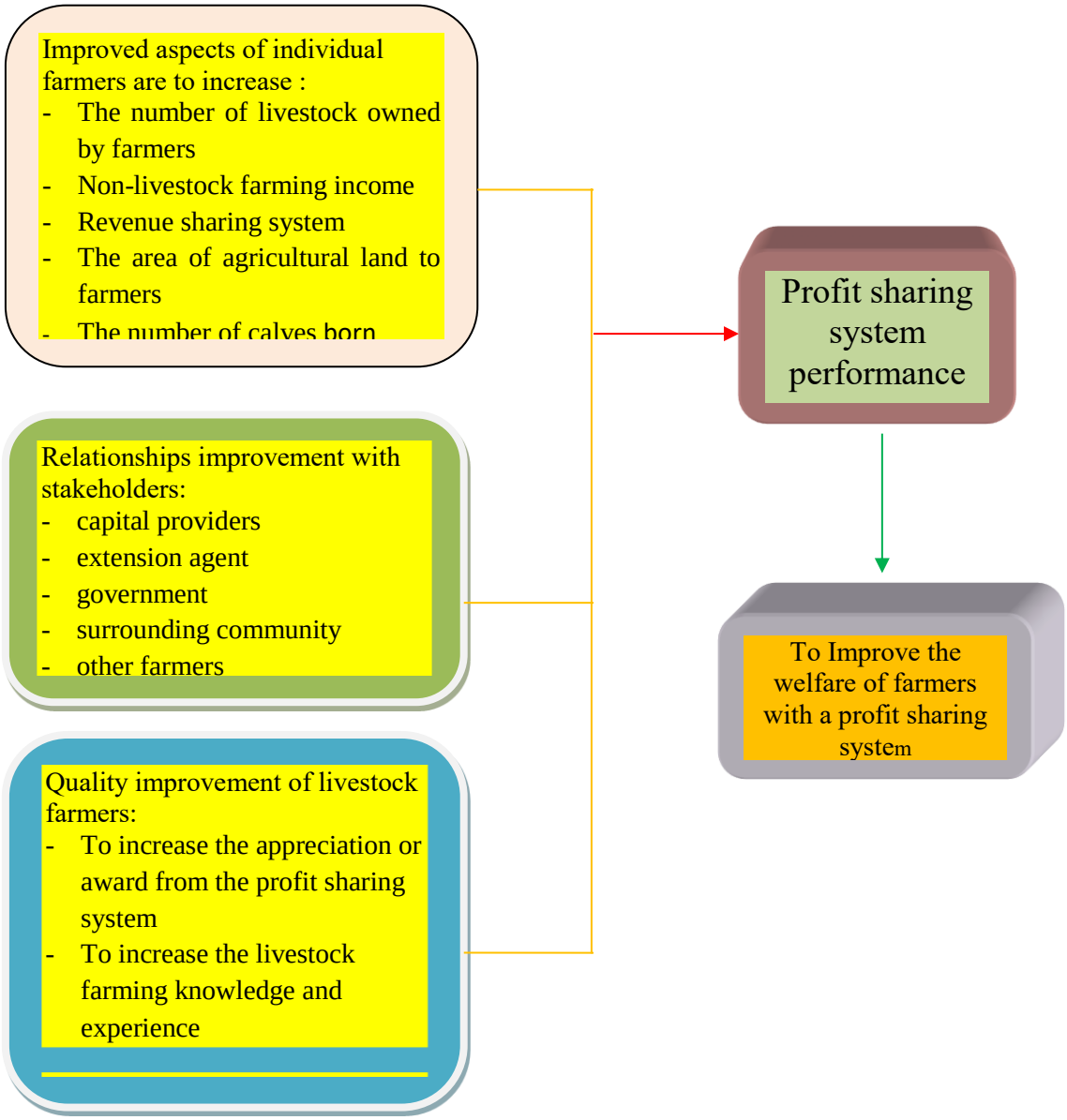

Figure 2. Alternative Model to Improving the Welfare of Livestock Farmer

4. Conclusion

The motivation of livestock farmers who practice profit sharing system for their beef cattle business in Maiwa Sub district, Enrekang Regency, is influenced by the characteristics of farmers, relationships with the stakeholders, and need to gain progress obtained.

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Profile of amino acid on *Pangasius djambal* fish finger processing

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Abstract. Amino acid is protein complier. Protein of an animal can retrieved from Jambal (*Pangasius djambal*). Jambal can be utilized as surimi and fish finger. The purpose of this study was to know the kind and comparison of amino acid content from Jambal from fresh meat, surimi and fish finger. This study used a descriptive method head for describe condition objectively, knowing profile of amino acid from fresh meat, surimi and fish finger. The parameters were moisture content, protein content and amino acid profile. The results showed that fresh meat, surimi and fish finger consist of 17 kinds of amino acid comprised 9 essential amino acids (valine, threonine, lysine, isoleusine, histidin, phenylalanine, arginine, leusine, and methionine) and 8 non-essential amino acids (serine, alanine, glutamate, tyrosine, proline, glysine, aspartate and cysteine). The processing can influence amino acid content from fresh meat, surimi and fish finger. Fresh meat with 32.21% of total amino acid content, surimi with 30.68% of total amino acid content and fish finger with 16.59% ot total amino acid content. Essential amino acid had highest content was lysine, fresh meat had 2.34% of lysine, surimi had 2.49% of lysine and fish finger had 1.21% of lysine, whereas non essential amino acid had highest content was glutamate, fresh meat had 5.14% of glutamate, surimi had 5.03% of glutamate and fish finger had 3.35% of glutamate.

1. Introduction

Amino acids are very usefull for our body, it's repairing the demage tissue after injured, protecting the liver from toxic agents, lowering blood pressure, regulating the cholesterol metabolism, leading the growth hormone secretion and reducing amonia level on blood [1]. According Wu [2], amino acids were classified based the capability of body to produce it, i.e essential amino acids (the body couldn't to produce, so that we must get from food) and non-essential amino acids (the body could to produce). Essential amino acids can be got from animal or vegetable protein, on food or drink.

Animal protein can be got from fiheries, that is Jambal (*Pangasius djambal*). Ismanto et al.[3] said that Jambal contains calorie and protein, the taste is delicious. Today, Jambal was the important and well-known fisheries comodity, because the market trend was rapidly. Before it, consumen rarely to know Jambal than the other seafood, such shrimp, salmon and tuna. One of the utilization of Jambal, is processing be surimi. Surimi is intermediated product, that is the aim of processing for longer the period storage than raw material [4]. The steps of processing surimi were cuting off the head and bone, pulverizing, washing, dehidration and adding cryoprotectant, then with or without freezing so that can make gel performing and binding water [5]. Furthermore, surimi can be turned into fish finger. Fish finger is food that made from milling meat and covered with breadcrumbs [6]. Addition from Jamshidi and Shabanpour [7], fish finger differently with nugget because it's using leek, the shape were longer and without steaming.

Frying were processing that use heat medium, it is cooking oil. Protein demage that be caused frying imply to amino acids conteins, i.e the nutrition product. The little information about amino acids content from fresh or processed Jambal (*fish finger*), this study was to know the kinds and comparison of amino acids content from fresh meat, surimi and fish finger Jambal.

2. Materials and Methods

2.1 Tools and Materials

The tools were used in the study consist of HPLC, sonifier, food processor, freezer and supporting tools like scales, stove, frying pan, and glassware. The materials were used in this study consist of Pangasius djambal, NaCl, HCl, NaOH, kjehdal tablets, H_2SO_4, H_3BO_3, methylene orange (Merck).

2.2 Methods

The method used in this study was deskriptif method with analyzed moisture content [8], amount of protein crude [8] and profiling amino acids by HPLC [9]. Fish finger making formulation with surimi and mince meat composting can be seen at Table 1.

Table 1. Formulation of making Jambal fish finger (in% of the amount of meat)

Materials	Amount
Mince meat	85 % (b/b)
Surimi	15 % (b/b)
Salt	1.5 % (b/b)
Garlic	2 % (b/b)
Sugar	0.3 % (b/b)
Pepper	0.4 % (b/b)
Cumin	0.5 % (b/b)
Wheat Flour	3 % (b/b)

3. Results and Discussion

3.1 Moisture Contents

Fresh Jambal meat has a high moisture content of 65.22 ± 1.46%. This shows catfish are food that is easily damaged. After the catfish meat is processed into surimi, the moisture content increases to 67.44 ± 1.04%. This increase is due to the fact that in the manufacture of surimi there are washing stages. According to Zhang et al. [10], the effect of washing causes the trapping of some of the washing water indoors or gaps that have been left behind by dissolved substances such as blood, pigments, proteins and mineral salts. The water content of surimi in this study is in accordance with SNI 2694 [11] which determines the maximum surimi water content of 80%.

In subsequent processing, fish finger shows that the water content is 47.08 ± 0.78% and meets [12] which sets a maximum limit of fish finger water content is 60%. Decrease in water content in fish finger because there are additional ingredients and frying pan. According to Elyasi et al. [8], decreased water content in fish finger processing due to the influence of additives such as flour. Bordin et al. [13] added, the frying process caused an increase in the temperature of foodstuffs so that water in foodstuffs evaporated. Water content in catfish meat, surimi and fish finger can be seen in Table 2.

Tabel 2. Moisture content of fresh meat, surimi, fish finger

Sample	Moisture (%)
Fresh meat	65.22 ± 1.46
Surimi	67.44 ± 1.04
Fish Finger	47.31± 0.78

3.2 Amount of Crude Protein

The protein content of the substance is known by protein content analysis. The contents of fresh meat, surimi and fish finger on a wet basis respectively were 18.75 ± 0.46%, 16.95 ± 0.55% and 10.71 ± 0.46%. According to Suryaningrum et al. [14] protein content of jambal fish was 13.13 ± 0.62%. The protein content of the fresh meat, surimi and fish finger is appropriate in Table 3.

Surimi has a protein content of 52.09 ± 2.01% (dry basis), lower than the levels of fresh meat protein. This is caused when the process of washing a portion of protein, especially protein, is water soluble, carried along with washing water. According to Mohan et al. [15], washing in the processing of surimi aims to increase the strength of the gel due to increased myofibril protein content and reduce sarcoplasmic protein and irritating components in the formation of gels such as blood, and digestive enzymes can be eliminated. SNI 2694 [11] determines the minimum protein content for surimi is 12%, so surimi in this study meets SNI.

Tabel 3. Amount of Protein Crude of fresh meat, surimi and fish finger

Sample	Protein (%)
Fresh meat	53.98 ± 3.12
Surimi	52.09 ± 2.01
Fish Finger	20.35 ± 0.98

Protein content in fish finger is 20.35 ± 0.98%. This value is in accordance with SNI 7758 [12] which sets a minimum fish finger protein level of 5%. Frying affects protein levels. According to Manning et al. [16], high temperatures in the frying pan (190 ° C) cause the peptide bond in the protein to break so that the protein content of fish finger drops.

3.3 Profile Amino Acid

The amino acid content of Jambal fresh meat, surimi and fish finger can be seen in Table 4.

Table 4. Analysis of amino acid from Jambal fresh meat, surimi and fish finger

No	Parameter	Daging (%)	Surimi (%)	*Fish Finger* (%)
1	Valin	0.73	0.70	0.38
2	Threonin	1.59	1.50	0.75
3	Lisin	2.34	2.49	1.21
4	Serin	2.27	2.15	1.09
5	Isoleusin	0.64	0.63	0.35
6	Alanin	1.76	1.68	0.93
7	Histidin	0.96	0.83	0.41
8	Phenilalanin	1.46	1.32	0.72
9	Glutamat	5.14	5.03	3.35
10	Tirosin	1.40	1.37	0.58
11	Prolin	1.85	1.42	0.92
12	Arginin	2.32	2.29	1.02
13	Glisin	2.79	2.67	1.29
14	Leusin	2.05	2.15	1.17
15	Aspartat	3.59	3.18	1.84
16	Metionin	1.20	1.18	0.52
17	Sistin	0.12	0.10	0.08
	Total	32.21	30.68	16.59

3.3.1 Essential Amino Acid

Histograms of essential amino acid levels in Jambal fresh meat, surimi and fish finger can be seen in Fig 1. Essential amino acids are amino acids that must be met from food because the body is unable to

form it (synthesis). Essential amino acids are important for maintaining body balance. The results of amino acid analysis using HPLC method obtained 9 types of essential amino acids namely valine, threonine, lysine, isoleucine, histidine, phenilalanin, arginine, leucine and methionine.

The results of the analysis showed that the essential amino acids which had the highest value were lysine, namely 2.34% in fresh meat, 2.49% in surimi and 1.21% in fish finger. According to Suryaningrum et al. [14], all types of Jambal have excess levels of lysine amino acids. Lysine plays a very important role in the body, such as blood antibody base ingredients, maintains the growth of normal cells and strengthens the circulatory system. Lysine with amino acids proline and vitamin C decreases blood triglyceride levels if in excessive amounts and will form collagen tissue. If the body lacks lysine it will cause reproductive abnormalities, anemia, stunted growth, hair loss, difficulty concentrating and fatigue.

The second highest amino acid in this study was arginine with a value of 2.32% for catfish meat, 2.29% for surimi and 1.02% for fish finger. According to Trisha et al. [17], humans do not need histidine and arginine amino acids for damaged cell replacement but these two amino acids are essential for growth. This is in accordance with Hammarqvist et al. [18] statement, that arginine is not essential for children and adults but is useful for infant growth, while histidine is not essential for adults but is needed by children.

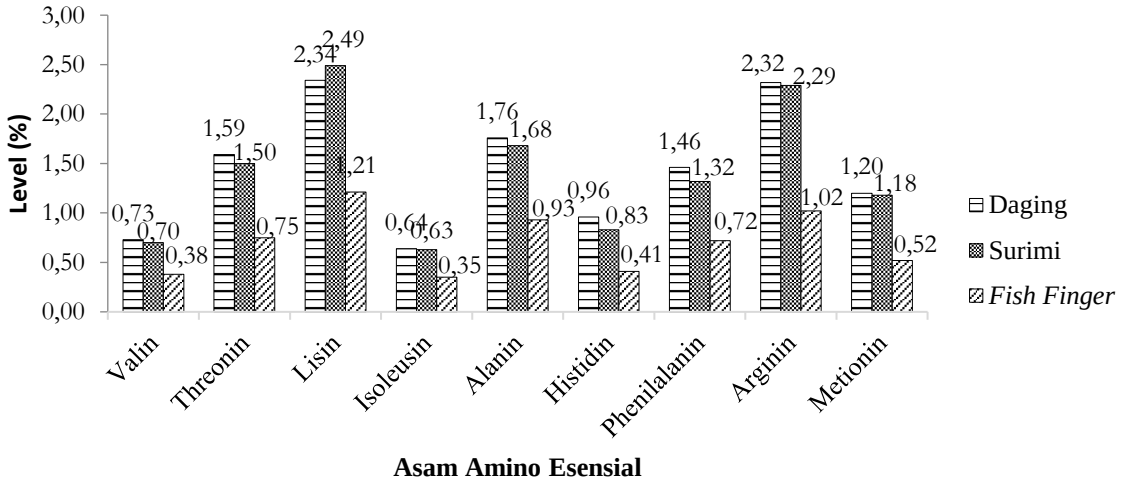

Figure 1. Concentration of esential amino acid

The lowest essential amino acid is isoleucine amino acid. Isoleucine amino acid levels are 0.64% for catfish meat, 0.63% for surimi and 0.35% for fish finger. According to Aristoy and Toldrá [19], isoleucine is an essential amino acid, is neutral, nonpolar and is an aliphatic amino acid.

3.3.2 Non-essential amino acid

Non-essential amino acids are amino acids available in the body because the body is able to produce them. Histograms of non-essential amino acids in Jambal fresh meat, surimi and fish finger can be seen in Figure 2. The results of the analysis contained 8 non-essential amino acids namely serine, alanine, glutamate, tyrosine, proline, glycine, aspartate and cystine. Figure 2 shows that non-essential amino acids with the highest value were glutamate with levels of 5.14% in catfish meat, 5.03% in surimi and 3.35% in fish finger. According to Mallick [20], free glutamic acid is present in organs and tissues and is naturally found in almost all foods such as milk, seafood, vegetables, meat, poultry, traditional soy sauce, fish sauce and other foods. Glutamic acid acts to provide savory or umami flavor to food. This causes Jambal to have a savory taste because the content of glutamic acid is quite high.

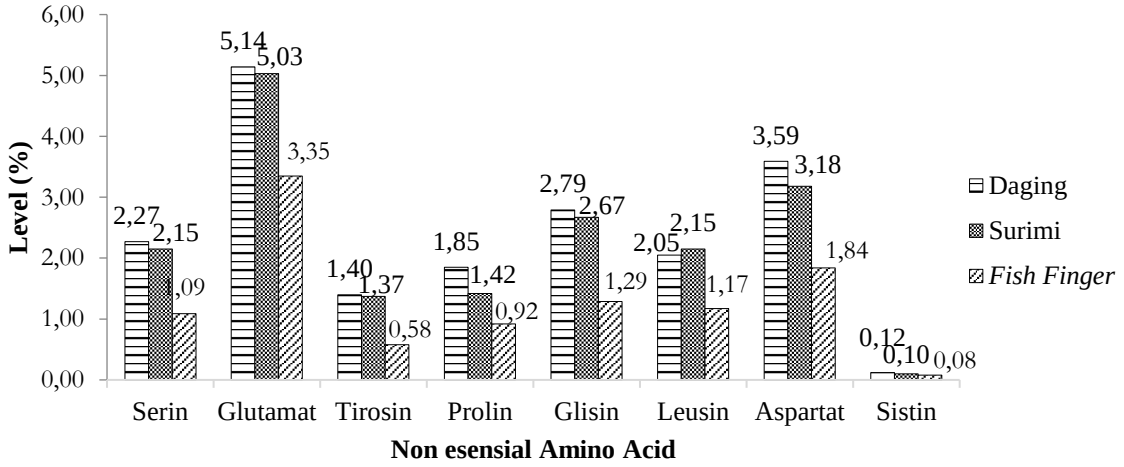

Figure 2. Concentration of non-esential amino acid

Glutamic acid is an amino acid that is able to provide a savory taste. Hydrogen groups on glutamic acid can be substituted with sodium to form monosodium glutamate (MSG) which has a strong savory taste that is widely used as a flavor enhancer. MSG is classified as a food additive which is added to food processing to strengthen taste [21]. Non-essential amino acids in this study found that cystine is the lowest non-essential amino acid. The level of amino acid cystine in catfish meat was 0.12%, in surimi as much as 0.10% and in fish finger as much as 0.08%. According to Abdulmumeen et al. [22], cystine functions as an antioxidant, helps fight the effects of free radicals on cells, and plays a role in protein stability.

4. Conclusion

The types of amino acids from Jambal meat (*Pangasius djambal*) in fresh form, surimi and fish finger are 17 amino acids consisting of 9 essential amino acids (valine, threonine, lysine, isoleucine, histidine, phenilalanin, arginine, leucine and methionine) and 8 non-essential amino acids (serine, alanine, glutamate, tyrosine, proline, glycine, aspartate and cystine). The total amino acid content of fresh meat was 32.21%, surimi was 30.68% and fish finger was 16.59%. Essential amino acids which have the highest levels are lysine in fresh meat of 2.34%, surimi of 2.49% and fish finger of 1.21%, while non-essential amino acids which have the highest levels are glutamate, in fresh meat 5.14%, surimi 5.03% and fish finger is 3.35%.

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Who produces vitamin B12 in Tempeh

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Abstract. Since 20 years ago people have known that vitamin B12 in tempeh is produced by activity of bacteria especially *Klepsiella* sp. and *Citrobacter freundii* during fungal fermentation. In this study, *Klebsiella* sp. and *Saccharomyces cerevisiae* were used as inoculums along with *Rhizopus oligosporus* in soybean fermentation for the making of tempeh (T). Each inoculum was inoculated separately along with *R. oligosporus* on to dehulled cooked soybeans as follows: soybeans + *R. oligosporus* + *Klebsiella* sp. (TRK), soybeans + *R.oligosporus* + *S.cerevisiae* (TRS), soybeans + *R.oligosporus* + *S.cerevisiae* + *Klebsiella* sp. (TRSK), and Soy + *R.oligosporus* (TR) and soybeans + *Klebsiella sp* (TK). Inoculated soybeans were then incubated at 30°C for 36 hours. Aspects were observed namely the growth of *Klebsiella* sp., *S. cerevisiae* and *R. oligosporus*, and vitamin B12 production. The results showed that the highest vitamin B12 was 3.15 mg/100 g in TSR, followed by 2.88 mg/100 g and 1.64 mg/100 g were in TSKR and TR, respectively. Meanwhile, vitamin B12 in TKR, 0.81 mg/100 g, was lower than in TK which was 0.96 mg/100 g. Nevertheless, the total number of bacteria in the tempeh was the highest (10^8 CFU / g) compared with the total number of *S. cerevisiae* (10^4 CFU / g) or the total number of *R. oligosporus* (10^3 CFU / g) in the tempeh. This suggests that the role of bacteria in producing vitamin B12 was less than that of *S. cerevisiae* or *R. oligosporus*. The conclusion was that *S. cerevisiae* has a significant contribution to the production of vitamin B12 in tempeh.

1. Introduction

It has been several decades ago, people known that vitamin B12 contained in tempe is synthesized by *Klebsiella pneumoniae* and *Citrobacter freundii* which are a contaminating bacteria during process of tempeh [1, 2] Co-inoculation of non-pathogenic *Klebsiella pneumoniae* was combined with *R. oligosporus*. *Rhizopus* is the main role of fermentation in the manufacture of tempeh but bacteria and yeast grow together and have an important contribution to the nutritional and functional properties of tempeh. Tempe is solid state fungal fermentation of cooked-dehulled soybeans by *Rhizopus oligposporus* activities at incubation temperature of 27-30°C for 36-40 h. During fermentation enzymatic activities of *R. oligosporus* leads to a significant increase in water-soluble nutrients, enhancing the biosynthesis of B vitamins and transformation of soy-isoflavones into antioxidant compounds [3]. Tempe is an extraordinary food because it contains vitamin B_{12} which is the only vitamin absent from plant-derived food sources unless contaminated or processed with with B12-synthesising microorganisms [4] Soybean itself contains low or undetectable of vitamin B_{12}, yet when soybeans are fermented to produce tempeh, the amount of vitamin B_{12} increases to 0.7 to 0.8 µg/100g. With these values of vitamin B_{12}, tempeh is a promising source of B_{12} for vitamin-derived foods such milk (0.3-0.4 µg/100g, meat (3µg/100g), and eggs (0.9-1.4 µg/100g) [2]. Industrial production of Vitamin B_{12} occurs through microbial fermentation, mostly use *Pseudomonas denitrificans, Propionibacterium shermanii*; however, this process has some drawbacks such as long fermentation cycle and expensive media requirements.

Saccharomyces cerevisiae is unicellular yeast and one of the most explored organisms in terms of industrial applications. It is used for bread making and various fermented food products and beverages

partly due to its contribution to flavour as well as vitamin B12 [5]. In addition to traditional alcoholic and fermented products, *Saccharomyces cerevisiae* has been used for diverse industrial purposes such as (i) lactose fermentation to ethanol, to produce lactose-free milk for people with lactose intolerance; (ii) the production of various alditols, such as glycerol or D-glucitol; (iii) protein production from alkanes and pulp-paper waste; (iv) providing enzymes, such as β-fructofuranosidase (invertase), α- and β-galactosidase and lipase; (v) production of compounds for research purposes, such as, novel carbon-carbon bonds and methyldiols of aldehydes and (vi) as biocontrol agents because they have antifungal activity, and (vii) cell biomass production (yeast food and feed), ingredient production, additives and as a processing aid for food processing, such as antioxidants, aroma, color, taste and vitamins, probiotic yeast, and yeast biocatalysts. *Saccharomyces cerevisiae* co-inoculated with Rhizopus oligosporus in fermentation of tempeh making, enhanced the aroma of tempeh by masking the beany aroma containing in tempeh [6]. Modification technique of tapioca by using *S. cerevisiae* is also beneficial in term of increasing of protein in modified tapioca [6]. This study was aimed to investigate the growth of *Saccharomyces cerevisiae, and Klebsiella sp* co-inoculated individually or together with *R.oligosporus* in fermentation of tempe making, and to evaluate the production of vitamin B12 in tempeh.

2. Materials and methods

2.1. Tempe making

Soybeans in this research were purchased in the Primkopti Bandar Lampung, and *Klebsiella* sp. was non-pathogenic bacteria isolated from the tempeh [7]. Tempe making was produced in the Microbiology laboratory according to the procedure done by Kustyawati et al. [8] with modifying in inoculation stages as follows, 300 g of soybeans were soaked in clean water overnight at room temperature, then manually removed the skin. Next, soybeans were boiled in clean water with a ratio of 1: 3 (soybean : water) for 30 minutes, drained, dried at room temperature, and ready to be inoculated with certain cultures. Inoculation was carried out as follows: 100 g of cooked dehulled soybeans were inoculated with 1ml of defined number of spore suspension of *R. oligosporus* and 1 ml of suspension cell of certain bacteria and yeast. Inoculated soybeans were packed in perforated plastic packaging and incubated at 32^{o}C for 40 hours. Five types of tempeh with the addition of different inoculated cultures produced on this study, namely (1) soybeans + *R. oligosporus* + *Klebsiella sp* (TRK), soybeans + *R. oligosporus* + *S. cerevisiae* (TRS), soybeans + *R. oligosporus* + *S. cerevisiae* + *Klebsiella* sp. (TRSK), and Soybean + *R. oligosporus* (TR) and soybeans + *Klebsiella* sp. (TK). Soybeans without inoculation as a control negative (Soybeans). Tempe making was made in duplicate.

2.2. Microbial count

Each of tempeh made was analyzed for its total number of bacteria, yeast and molds at the starting and the end of fermentation by growing culture on appropriate media. A total of 15 g tempeh was taken, mixed with 135 ml of 0.1% peptone water, homogenized with a stomacher for 5 minutes, and a series of dilutions from 10^{-1} to 10^{-8} was made in duplicate. Then one ml is taken from certain dilutions. Planting microorganisms was done by surface plate count method on the suitable solid media (BGBL agar, MEA, and PDA were for *Klebsiella* sp., *S. cerevisiae*, and *R. oligosporus*, respectively), and incubated at suitable temperature and time. The data obtained were analyzed descriptively and displayed in graphical form.

2.3. Vitamin B12 analysis

The analysis of vitamin B12 was done following the procedure run by *in house* method of LCIT Laboratory, University of Lampung. A total of 0.5 grams of tempe powder was weighed and put into 100 mL Erlenmeyer containing of 20 mL of milli-Q water. Each sample was verified using ultrasonic equipped with heater for 30 minutes. Sample volume was set for 25 mL, then centrifuged. Approximately of 2 mL of supernatant was pipetted and filtered using paper filter with 13 mm in diameter and 0.2 mm pore size. The filtrate was then placed in the vial bottle. The sample is ready to be injected into the HPLC (Shimadzu, CBM-20A controller, LC 20AD solvent delivering unit, CTO 10A

column oven, SPD M20-A photo diode array detector). HPLC running condition was using Agilent C-18 5 µm 125x4.6 mm column, column temperature of 35°C, mobile phase (water: acetonitrile: buffer phosphate 10 nM = 80:10:10), isocratic mobile phase method, with flow rate 1 mL/min, injection volume of 20 µL, wavelength detector 360 nm, and run time of 20 min.

3. Results and Discussion

Table 1 showed that the addition of *S. cerevisiae or Klebsiella* sp. together or separately did not affect the growth of *R. oligosporus*. *Rhizopus oligosporus* in tempeh is not contaminant microorganism instead it has to be deliberately added into the soybeans. *R. oligosporus* produces several enzymes during fermentation including lipase, protease, and phytase which hydrolyzes carbohydrate, lipid, and protein from the soybeans and produces fatty acids and amino acids which are then utilized by bacteria and yeast. Thus *R. oligosporus* plays a role in supporting the growth of other microorganism in the fermentation of tempeh. The low number of bacteria in this experiment can be caused by antibiotic produced by *R. oligosporus* [9]. *Saccharomyces cerevisiae* can live together with *R. oligosporus or Klebsiella* sp. during fermentation of tempeh making. They use carbon and nitrogen source from soybean as well as fatty acid and amino acid produced by *R. oligosporus*. On the other hand, the higher density of *Klebsiella* sp. during fermentation was caused by a contaminant microorganism. Therefore, it can be said that *R. oligosporus* serves as bacterial growth control while supporting microbial growth.

Table 1. Effect of variety culture inoculation of tempeh making on the growth of microorganism and vitamin B12 production

Type of the tempeh	Mold number (CFU/g)	Number of yeast (CFU/g)	Number of bacteria (CFU/g)	Vit B12 (mg/100g)
Soybeans + *R. oligosporus*	$3.35 \times 10^3 \pm 1.32$	5.9×10^2	$2.04 \times 10^9 \pm 2.03$	2.88± 0.01
Soybeans + *R. oligosporus* + *S. cerevisiae*	$4.5 \times 10^3 \pm 1.16$	$1.2 \times 10^7 \pm 1.66$	$1.09 \times 10^9 \pm 2.35$	3.15 ±0.01
Soybeans + *R. oligosporus* + *S. cerevisiae* + *Klebsiella* sp.	$5.6 \times 10^3 \pm 2.01$	$3.7 \times 10^7 \pm 2.11$	$2.54 \times 10^{10} \pm 1.85$	1.64± 0.0
Soybeans + *R. oligosporus* + *Klebsiella* sp.	$3.0 \times 10^2 \pm 1.44$	0	$1.86 \times 10^{10} \pm 2.41$	0.81± 0.0
Soybeans + *Klebsiella* sp.	0	0	$2.69 \times 10^{12} \pm 2.56$	0.96± 0.01

Note: The data in the table were average value of three replications.

Biosynthesis of vitamin B12 is limited to some bacteria and therefore the production depends on microbial fermentation. Fang et al. [10], that microbial de novo biosynthesis of vitamin B12 occurs through two alternative routes, they are aerobic and anaerobic pathways, in bacteria and archaea, respectively. Production of vitamin B12 during fermentation of tempeh making may be influenced by the inoculated cultures. Tempeh making with adding *R. oligosporus*, and *S.cerevisiae* contained the highest vitamin B12, but tempeh making added with *R. oligosporus* and *Klebsiella* contained the lowest vitamin B12. Even though the fermentation of soybeans with adding *Klebsiella* sp. did not produce tempeh, it produced vitamin B12. *Rhizopus oligosporus* could be better vitamin B12 producing than *Klebsiella* sp. in this experiment. Since soybean itself contains low or undetectable of vitamin B12, it indicated that *Klebsiella* was responsible on producing vitamin B12 in fermented soybean. Nevertheless, when *Klebsiella* sp. was co-inoculated with *R. oligosporus*, the amount of vitamin B12 in tempeh was low. This can be explained that either *R. oligosporus* or *S. cerevisiae* can produce antibiotic which may

inhibit the growth of bacteria including *Klebsiella* sp. On the other hand, *S. cerevisiae* can produce vitamin B12 and is rich of chromium [5, 11], and therefore addition of this can increase the vitamin B12 in tempeh. The production of vitamin B12 in tempeh inoculated with *S. cerevisiae* in this experiment (3.19 mg/100 g) was lower than that of done by Kustyawati et al. [8] which was 3.95 mg/100 g. Recently *Propionibacterium freundenreichii*, the food grade producer of vitamin B12, has been used to enrich vitamin B12 in tempeh making [4] and in lupin tempeh [12-14]. Even though, texture, taste and overall acceptance of lupin tempeh were no different from soy tempeh, this discovery may not be accessed by tempeh consumers in Indonesian who are familiar with the taste of tempeh. *Propionibacterium freundenreichii* was normally used in ripening of cheese making with its contribution to fatty compounds, important flavor in cheese [15].

4. Conclusion

Tempeh was a model in this experiment. The co-inoculation of *Klebsiella* sp. and *S. cerevisiae* with *R. oligosporus* was to reveal most of the responsibility for vitamin B12 production in tempeh making. *S. cerevisiae* was most contributor of vitamin B_{12} in tempeh and support the growth of *R. oligosporus*. Co-inoculation of *Klebsiella* sp. with either *R. oligosporus* or *S. cerevisiae* inhibited vitamin B12 production in tempeh, although it did not affect growth.

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Purification and characterization of celulase A7 cloned from *Orpinomyces sp*.Y102

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Abstract. Cellulose, a polymer of D-glucose, is the largest renewable resources and the most abundant carbon source. Its widely distributed in plant, fungi, even amoeba. Cellulose can not be easily degraded, because of the complex with hemicellulosa and lignin. Biodegradation is more attractive method to release glucose than chemical conversion. The complete degradation of cellulose requieres at least three types of cellulases, that is endocellulase, exocellulase and β-glucosidase. The rumen fungi have been proved to be a souce for active cellulases. A cellulase gene, designated cellulase A7 (celA7) was cloned from rumen fungus *Orpinomyces sp*.Y102 (NCBI under accesion number KM114221), that was predicted as exocellulase. The heterologous expression of celA7, was tried using maltose-binding protein as a fusion tag, and the biochemical properties was characterized. CelA7 was highly expressed in *E.coli* BL21(DE3) resulting in a 90kDa. The recombinant protein was purified 5.75 fold by DEAE Sepharose column chromatography, with specific activity 22.83 U/mg. The optimal reaction temperature pH are 50°C and pH 7.0. Its stable between pH 5.0-8.0 and 25-50°C. It was specific to glucose β-1,4-polymers. The activity of celA7 obviously higher than the commercial enzyme cellobihydrolase I (cloned from *Trichoderma reesei*).

1. Introduction

Cellulose, major component of plant cell wall and widely distributed in plant, some marine animals, fungi, bacteria, alvae, invertebrates and even amoeba [1,2]. A linear polymer of D-glucose linked by 1,4-β-D-glucosidic bond, is the largest renewable resource on the Earth. However, without appropriate treatment, agricultural waste have been accumulated and deposited, resulting in the risk of the enviromental pollution [3,4].Cellulose can not be easily degraded, because of the complex with hemicellulose and lignin, carbohydrate polimer are tightly bond to lignin, and hydrogen bond between cellulose chain [5,6]. Generally to hydrolysis cellulose into glucose use chemical conversion usually used sulfurid acid , but this process may produce produce furfural and 5-hydroxymethyl-2-furfural, acetic acid, phenol, heavy metals, levuliniv acid and formic acid that carry toxic inhibitory effect to the microbial growth [7,8]. Another method is biodegradation, is more attractive strategy to release glucose from cellulose chain, used enzyme originated from organism such as bacteria and fungi to digest cellulose. To convert biomass of cellulolytic wastes at least requiered three types of cellulase, that is endocellulase, exocellulase, and β-glucosidase.

Endoglucanase (endocellulase;endo-β-1,4-glucanase; EC 3.2.1.4) randomly cleaves the internal β-1,4 bond of cellulose, generating shorter chains of oligosaccharides [1]. Exocellulase (exoglucanase), enzymes that hydrolyze cellulose by releasing cellobiose successively from the reducing end (EC 3.2.1.176; Cellobiohydrolase I) or non reducing end (EC 3.2.1.91; Cellobiohydrolase II) of cellulose. Beta-glucosidase (EC 3.2.1.21) hydrolyzes cellobiose or oligoglucosaccharides into glucose [1].

Varieties of fungal and bacterial species produce cellulases and secret the enzymes to the environement. The cellulolytic enzymes of rumen fungi have been studied because of their

production of highly active cellulases [1,9,10,11],thus are regarded as a potential source for seeking highly efficient cellulases. Since cultivation of rumen fungi on a large scale is not easy due to their unique growth conditions, the number of cellulases cloned from rumen fungi is still limited [1,12]

Ion exchange chromatography, technique used in the separation of charged molecules acros a breadth of application and industries. Ion-exchange is a widely used techniwue in bioseparations since peptides, proteins, nucleic acids and related biopolymers have ionsable chemical moieties which render them susceptible to charge enhancement or reversion as a function of pH [13].

Previously, several genes which encode CMC-hydrolyzing enzyme were cloned from the fungus *Orpinomyces* sp.Y102 (NCBI accession number KM114221) isolated from the rumen of cattle (*Bostaurus*). One of them designated *celA7,* was predicted to encode an exocellulase belonging to the family 6 of glycoside hydrolase superfamily, using "Blast" program provided by NCBI (National Center for Biotechnology Information). In this study MBP was used as a fusion tag to assist the expression of *celA7* in *E.coli* in an attempt to characterize the biochemical properties of *celA7*.

2. Materials and Methods

2.1 Bacterial Strain

Escherichia coli BL21(DE3)-(Novagen, Madison, WI, USA) was used for protein expression and purification and *E.coli* DH5α (Yeastern Biotech, Taipei, Taiwan) was used for plasmid propagation and isolation.

2.2 Chemicals

Barley β-glucan, tryptone, amphicillin sodium salt, ammonium persulfate, N,N,N',N'-tetramethyle-ethylenediamine (TEMED), MOPS, 3,5-dinitrosalicylic-acid (DNS), bovine serum albumin, sodium chloride, pottasium sodium tartrate, sodium, sodium sulfite anhydrous, phenol, sodium dedocylsulphate(SDS), Tris-base, phenyl-methanesulfonylflouride(PMSF), cooma-sive briliant blue R-250, acetic acid, carboxymethylcellulose (CMC), D-(+)-Glucose, D-(+)-cellobiose, Pachman, *p*-nitro-phenyl-β-D-cellotriose(pNPC), *p*-nitro-phenyl-β-D-gluco-pyranose(pNPG), xylan (Oat spelt xylan).

2.3 Expression of cellulase A7

The vector containing the coding region of *celA7* was transformed into BL21(DE3) by heat shock. A colony in the resulting plate was picked and cultured in 5 ml LB/ampicillin medium at 37°C overnight. It was subsequently inoculated into LB/ampicillin medium (ratio 1:100), cultured at 37°C till OD_{600} of the culture reached 0.5-0.8. Then added IPTG (final concentration 0.1mM) and the culture was grown at 30°C for 4 hours. Cells were harvested by centrifugation for 20 minutes at 4°C. The pellet was resuspended into 50mM Tris-HCl buffer (pH 7.0) containing 1mM of phenyl-methanesulfonyl-flouoride(PMSF) and 1µg/ml leupeptin, and subjected to sonication on ice using a sonicator (S-400, Sunway Scientific corporation, Taipei, Taiwan), with program of four cycles of 30-s on and 30-s off at amplitude 40. After centrifugation for 20 minutes, the supernatan it was collected and filtered through a 0.45-µm microfilter (Millipore, Bedford, MA, USA) for purification, like previous study [1].

2.4 Purification of CelA7

Purification was carried out at 4°C. The filtered supernatant was loaded onto a-Diethylaminoethyl(DEAE)-Sepharose column (GE Healthcarem Piscataway, NJ,USA). Firstly, the DEAE-Sepharose ion-exchange resin was washed with ddH_2O at a flow rate of 1 ml/min. Followed by pre-equilibrated with Tris-HCl buffer (pH 7.0; equilibration buffer). Subsequently the crude extract of enzymes was loaded into the column, followed by wash with Tris HCl pH 7.0. Then the enzyme was eluted by 0.1-1 M NaCl gradient. Fraction of 3 ml were collected using a fraction collector (Amersham, Bioscience).

Selected fractions were measured for cellulase activity by 3,5-dinitrosalicic acid (DNS) method. Meanwhile, the concentrations of proteins were monitored by absorbance at 280 nm by UV-Vis

Spectrofotometer (UV-1700 (Pharma spec) Shimadzu, Japan). Selected fractions containing cellulase activity were subjected to SDS/PAGE to verify the protein content. The fractions containing a single band of 90 kDa were combined, concentrated by passage through a 10-kDa molecular weight cutoff membrane (Amicon ultra, Millipore). To reduce the salt concentration the sample was then diluted twice 10 fold with 50mM Tris-HCl pH 7.0 and concentrate again. The resulting enzyme was aliquoted and stored in -20°C

2.5 Activity assay and Protein Assay

The cellulase activity of enzyme was analyzed by measuring the production of reducing sugar from the hydrolysis of substrate using the (DNS) method. Assays were performed in 0.3-ml reaction mixture in Tris-HCl pH 7.0, substrate was barley beta-glucan. Reaction were incubated at optimal reaction condition (50°C), then proceeded for 10 min. Then added color stop to mixture then incubated at room temperature for 15 min after being added 40% sodium pottasium tartrate. After that absorbance at 575 nm, then measured by UV-Vis. A standard curve was established simultaneously using solution of various concentrations of glucose. One unit of enzyme activity was defined as the amount of enzymes requiered for releasing 1µmole of reducing sugar equivalent per minute under the specific reaction condition.

The protein concentration assay of all samples were measured by Bradford assay reagent (Bio-Rad, Hercules, CA, USA). Reaction was proceeeded for 10 min at room temperature and then absorbance at 595 nm was measured by UV-Vis. To determine protein concentration using a standard curve was established used 0.1 mg/ml bovine serum albumin (BSA). To measure total protein both of all samples were multiplying volume (L) of enzyme with protein content. Total protein content quoted in mg.

2.6 SDS-PAGE

Crude enzyme and each purified enzyme were added equal with 2x buffer sample. The sample was incubated at 90-95°C in dry incubator (Drybath Incubator, Ms (Major Science)) at 5 min and subjected to SDS-PAGE. Gel electrophoresis in 10% separating gel and 3% stacking gel were cast and run using (Bio-radpowerpac HC™). Gel electro-phoresis was performance 2 times using 40 volt for 40 min and 100 volt for 90 min. Molecular markers (Thermo scientific) were used as standard protein. Sample was stained with Coomasive briliant blue R-250, methanol and acetid acid for 20 min. After being stained, then it was destained with 10% of acetic acid and isopropanol overnight. Then gel was dried

2.7 Assays for optimum reaction temperature and pH

The optimum reaction temperature of CelA7 was measured by DNS method. 100 ng of CelA7 were added to reaction mixture containing the final of 2.5 mg/ml barley β-glucan in Tris-HCl pH 7.0. Reaction volume was 0.3 mL, the reaction mixture was incubated in temperature 30-65°C. Reaction was stopped at 10 min and the concentration of reducing sugar was measured by DNS method. The optimum activity was set as 100 %. Experiments were performed in triplicate. Data represent mean± SD of triplicates experiments.

The optimum reaction pH for CelA7 was determined in the specified buffers. The specified buffers consists of citrate buffer, phosphate buffer, MOPS, and Tris-HCl. Those buffers was designated in indicate pH. Citrate buffer was designed in pH 3.0 to pH 6.0, this buffers indicate less acidic environment. Phosphate buffers was designated in pH 5.0 - pH 8.0, thus indicate less acidin condition to less alkaline condition. MOPS buffer designated in pH 6.5 to pH 8.0. MOPS buffer designated as less acid condition to less alkaline condition with small range pH. And the last was Tris-HCl designated as less alkaline (neutral) to high alkaline condition, it was designed between pH 7.0 to pH 10.0. Then measured by DNS method.

Subsequently, 100 ng of CelA7 was added to reaction mixture. The reaction mixture consists of 2.5 mg/ml barley β-glucan and specified buffers. The reaction was processed for 10 min at optimal

temperature (50°C). The reducing sugar was measured by DNS method. The optimum reaction pH was set as 100%. Experiments were performed in triplicate.

2.8 Assays for themostability and pH tolerance

The thermostability of CelA7 was determined by incubating the enzyme in Tris-HCl buffer (pH 7.0) in (25-65°C) for 10 min. 100 ng of the enzyme was then withdrawn from the buffer, and added to a reaction mixture, and subjected to activity assay under the optimum reaction pH and temperature. The activity of un-treated enzyme (control) was set as 100%. Experiments were performed in triplicate.

To analyze the pH tolerance Cel A7, was incubated in specified buffers (MMOPS, Tris-HCl, citrate buffer, phosphate buffer) at the indicated pH in the room temperature for 30 min. Subsequently 100 ng CelA7 in the specified buffer was withdrawn and subjected to activity under the optimum reaction condition. The reducing sugar production was measured by DNS method. The activity of un-treated enzyme (control) was set as 100%. Experiments were performed in triplicate.

2.9 Assays for substrat specificity

To analyze the substrate specificity CelA7, was incubated in various substrate (soluble barley β-glucan, avicel, phosphorus-acid swollen cellulose (PASC), carboxymethylcellulose (CMC), pachyman, soluble xylan spelt oat at the Tris-HCl pH 7.0. Subsequently 100 ng CelA7 subjected to activity under the optimum reaction temperature and pH. The reducing sugar was measured by DNS-method. Experiments were performend in triplicate.

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3. Results

3.1 Overexpression of celA7

CelA7 was cloned in the vector pMAL-c5x to create a fusion protein in which the MBP is a fusion tag at the N-terminus.The predicted molecular weight of the fusion protein is 90 kDa. The CelA7 fusion protein was expressed in *E.coli* BL21(DE3). As shown in Figure 1, the recombinant *CelA7* was highly expressed in the supernatant of the crude *E.coli* lysate, after 4 h of IPTG induction (Lane 2), with a molecular weight of approximately 90 kDa as predicted.

3.2 Purification of CelA7

The crude extract was subjected to DEAE-Sepharose column chromatography. CelA7 was bound to the column, and eluted by NACl gradient solution. These fraction were bound to the column and show the highest activity of cellulase were subjected to SDS/PAGE to analysis the purity of these fraction. The recovery of the purification was 82.3%, resulting in a purification fold of 6.6 and the specific activity of CelA7 was 377.9 units/mg (Table 1).

Tabel 1. Summary of CelA7 purification

No	Purification step	Total volume (L)	Total protein (mg)	Total activity (Units[a])	Specific Activity (Units/mg)	Purifi-cation fold	Recovery (%)
1	Crude enzyme	0.05	481.9	27420.0	56.9	1	100
2	DEAE-Sepharose	0.05	59.7	22560.0	377.9	6.6	82.3

[a]One unit was defined as the amount of enzyme needed to catalyze the formation of 1µmole of reducing sugar equievalent per minute under the specified reaction condition.

3.3 Biochemical properties of CelA7

The optimal reaction temperature and pH for CelA7 was 50°C and pH 7.0 (Tris-HCl as buffer)(Figure 2). CelA7 was stable between 25-40°C(Figure 3.). Thus CelA7 is essentially not

tolerant to higher termpera-ture (>55°C). For pH tolerance of CelA7, was stable between pH 5.0-8.0 (Figure). CelA7 is not tolerant to < pH 5.0 and >pH 8.0.

3.3.1. Substrate specificity of CelA7
Substrate was analyzed as in Table 2

Table 2. Specific activity of CelA7 toward different substrates

Substrate (10 mg/ml)	Specific activity (units/mg)
Soluble barley β-glucan	377.9
Carboxymethylcellulose (CMC)	25.2
Avicel PH-101	ND*
Phosporus acid swollen cellulose (PASC)	12.2
Pachman	ND
Soluble oat spelt-xylan	ND

*ND: Not detectable

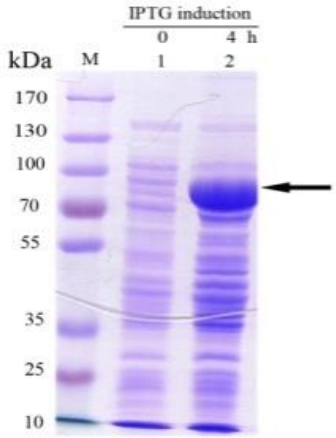

Figure 1. The SDS-Page analysis of the expressionof celA7; Lane 1, Control (0 hours induction); Lane 2, crude extract (4h induction IPTG)

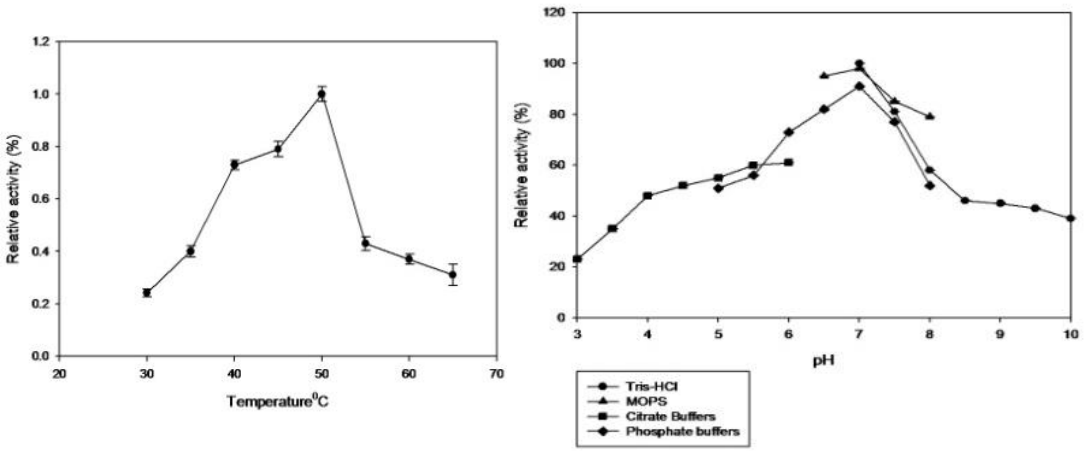

Figure 2. Analysis of the optimal reaction condition of CelA7. (A) Activity assay under various temperature, (B) Activity assay under various pH. The relative activities were calculates using the highest as 100%. Experiments were performed in tripicate. Data represent the mean ±SD

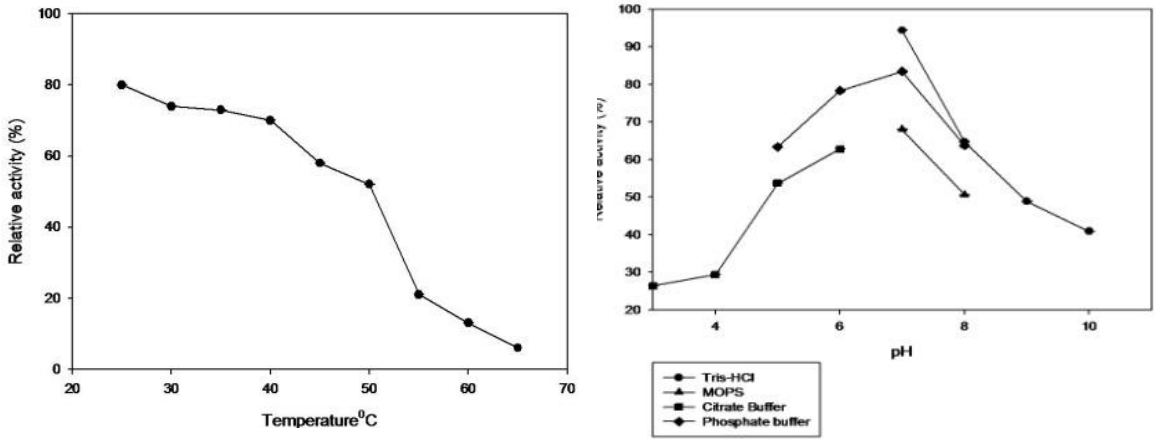

Figure 3. Analysis of the thermostability and pH tolerance of CelA7. (A) AEnzyme were incubated in indicated temperature for 10 min, withdrawn and assayed for activity under the optimal reaction condition. (B) Enzyme were incubated in the indicated pH for 30 min, withdrawn and assayed for activity. The relative activities were calculates using un-treated enzymes as 100%. Experiments were performed in tripicate. Data represent the mean ±SD

4. Discussion

Previously, A genes predicted to encode exocellulases were cloned from *Orpinomyces* sp.Y102, that is *celC7*, and *celA7*. CelC7 was predicted to encode an exocellulases. CelC7 has been expressed in *E.coli* as a fusion with a C- terminal 6x his tag, and has been purified and characterized as a cellobiohydrolase and cellotriohydrolase with a V_{max} of 5291.00 units/mg [1]. Thus, CelC7 was proved to be highly active among published exocellulases. In this study, CelA7 was succesfully expressed in a soluble form, using Maltose binding protein, indicating that MBP was helpful in increasing the solubility and/ or folding of the fusion partner [14].

Fussion proteins using MBP as a fusion tag can be purified using amylose column as the affinity chromatography [15,16] However, in this study, CelA7 was purified using one step purification by

DEAE-Sepharose column. Thus, the design of CelA7 renders the recombinant protein soluble and easily purified, facilitating it is large-scale production. The biochemical properties of CelA7 worked optimal at 50°C in Tris-HCl pH 7.0. CelA7 was stable over between pH 5.0-8.0 and 25-50°C. The biochemical properties CelA7 were similiar to celC7 (-17) [1]. The highest enzyme activities of CelA7 was obtained using barley beta-glucan, followed by CMC and PASC. Soluble barley β-glucan is a mixed- linkaged β-glucan, β-glucan a polysaccharide of D-glucose monomers linked by β-1,3 and β-1,4 glycosidic bonds in the structure [1,17]. CMC is a water-soluble long-chained with carboxymethyl substitutions. Its commonly used as a model substrate for detecting β-1,4-endoglucanase and it is known that CMC is a poor substrate for exocellulase [11]. Thus, if CelA7 is an exocellulase, it is reasonable that it specific to CMC is lower than β-glucan

5. Conclusion

Cellulase A7 cloned from rumen fungus *Orpinomyces sp*.Y102 can be highly expressed in *E.coli* BL21(DE3) using MBP as the fusion tag, and the fusion protein can be purified by just one step using DEAE-Sepharose column chromatography. CelA7 is a cellobiohydrolase specific to the polymer of glucose β-1,4 linkage which optimal rection condition being 50°C, pH 7.0. CelA7 were stable between pH 5.0-8.0, and stable between 25-50°C. The potential of CelA7 in biofuel production needs to be further evaluated.

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Microplastic in salt production areas of northern coast of east java

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Abstract. Increasing microplastic in marine environment, made the possibility of it entering human body through the most consumed marine product, such as salt. The purpose of this study is to identify the abundance and characteristics (types and color) of microplastic in salt that collected from three salt production areas at the Northern Coast of East Java i.e. Probolinggo, Surabaya, and Lamongan Cities. Salt particles were sampled from the crystallizing pond at the three salt production areas. Random sampling method were used to determine the sampling sites. Microscopy and FTIR methods were carried out to generate the abundance and polymer characteristics. The result showed that the abundance of microplastic were 303 particles/Kg. The dominant type of microplastic found in the salt particles were film and dominated by blue color. The polymer of microplastics were Polyprophylene, LDPE (Low Density Polyethylene), Polyethylene, Polystyrene, dan HDPE (High Density Polyethylene). Based on global salt consume (3.7 Kg/year), it will be about 1121 particles microplastics consumed by people of East Java per year. It will lead to high risk for human health. It is suggested that the government have to be concern in reducing any kind of plastic as the source of microplastic in the ocean.

1. Introduction

Plastic has been increase by year since mass production of plastic in 1950s[1], and become pollutant in worldwide environment.[2] Mount of plastic uses made new pollutant called microplastics (Nor and Obbard, 2014).[3] Microplastic are plastic particles < 5 mm size (Digka, 2018).[4] Small size of microplastic convenient to enter marine environment and consumed by some marine organism e.g zooplankton (vroom, 2017)[5], seabird (li, 2018)[6], fish (pedago, 2018; Digka, 2018)[4][7], Bivalve (Ding, 2018; Li, 2015). Whereas marine organism are product that will be consumed by humans. So that, made the possibility of microplastic to entering human body through marine product (yang, 2015).[8] Nevertheless microplastic also can entering to human body through other way, such as salt.

In the production of sea salt, saltwater is pumped into evaporation ponds, where it is concentrated by the action of sun and wind. Afer that, the salt condenses and crystallizes on the surface of the crystallizers.[9] There are needs consideration over the potential of sea water contaminant into sea salt after the evaporation and crystallization process. Salt is the common marine product that consumed by human, so that every human have a change to contamined by microplastic.

Microplastic can affect physical tissue or organ.[10] Other risk of ingested microplastic are additives and adsorbed chemicals can be released in the gastrointestinal fluids and potentially transfer to edible tissue. [11-13]Furthermore small plastic particles may enter the circulatory system, resulting in translocation and redistribution to most commonly consumed tissues.[10], [14]

The other study has been found microplastic in commercial salt. Yang et al. [8] found 7 – 680 particles/Kg of microplastic in different Chinese commercial salts. In other country, Karami et al.[1] was record the amount microplastic in commercial salt from different country 0-10 particles/Kg. Previous study in Spanish has been found 50 – 280 particles/Kg in sea salt sample[9], and 16 – 84 particles/Kg in commercial sea salt from Turkey[15].

Concern study of microplastic in Indonesia is rarely. Whereas Indonesia is the second country that producing plastic waste into marine environmental as many as 187.2 tons.[16] Need more reseach to cover microplastic pollutant. This study is the first concerning reseach microplastic inside salts in Indonesia.

2. Materials and methods

Three samples of salts particles were collected from salt production area in northern coast of east Java i.e. Probolinggo, Surabaya, and Lamongan Cities during August 2017. These salts represent three highest salt production in Northern Coast of East Java. A sample with a weight range from 250 g. Three replicate samples were used to compare among different site of the same location of salt production.

Approximately 250 g of salts from one sample of salts was directly placed into a 1 L erlenmayer. Approximately 100 mL of 30% H_2O_2 was added to each bottle to digest the organic matter. The bottles were covered and placed in a shaker at 65 ^oC at 80 rpm for 24 h and then at room temperature for 48 h. Then, a matter of 800 mL of filtered water was added to each bottle. A glass rod was used to stir the salts in the bottle until they were completely dissolved. The salt solution in one bottle of the four replicates was immediately transferred onto a piece of 5 µm pore size, 47 mm cellulose nitrate filter paper using a vacuum system. The filter paper was then placed into a clean petri dish with a cover and was dried at room temperature to observe the total number of particles. The filter papers were placed in clean petri dishes with covers and were dried at room temperature for further microplastic analysis.

The filters were observed under a Microscope Olympus CX21 and images were obtained with a smartphone. A visual assessment was performed to identify the types and colors of microplastics according to the physical characteristics of the particles. Some particles were randomly selected for verification using micro-Fourier Transform Infrared Spectroscopy (µ-FT-IR). The abundance of microplastics was calculated based on the microscopic observation and was confirmed with µ-FT-IR.

Particles of microplastic on the filter paper were randomly selected for µ-FT-IR spectroscopy.The spectrum range was set to 4000-400 cm^{-1} with a collection time of 3 s and with 4 co-scans for each measurement. The spectral resolution was 8 cm^{-1} for all samples, and the aperture size was 10 mm.

The spectrum analysis followed the method of Woodall et al.[17] Briefly, matches with a quality index ≥ 0.7 were accepted. Matches with a quality index < 0.7, but ≥ 0.6 were individually inspected and interpreted based on the proximity of their absorption frequencies to those of chemical bonds in the known polymers. Matches with a quality index < 0.6 were rejected[18].

3. Result and discussion

3.1. Abundance, type, and size of microplastic in salt

As a representative example, a photograph of one of the filters is shown in Fig. 1. The most common colors found were blue, white, and green. The number of microplastics was 303 particles/kg in salt particle. Different location showed significant differences in the abundance of microplastics in sea salts. Microplastic content was higher in Lamongan (540 particles/kg) than in Surabaya (244 particles/kg) and Probolinggo (124 particles/kg) (Figure 2A). The composition of microplastic types varied among different. However, fragments and fibers were the more prevalent types of microplastic particles in salt particles (Figure 2B). Foam and fiber accounted for less than 6% of the total number of microplastics in each from the three location. The sizes of the microplastic particles ranged from 45 µm to 4.3 mm in all of the salt particles.

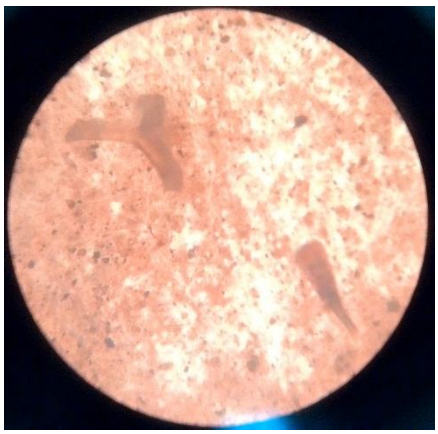

Figure 1. Optical microscope image of a flter afer the fltration of a salt sample

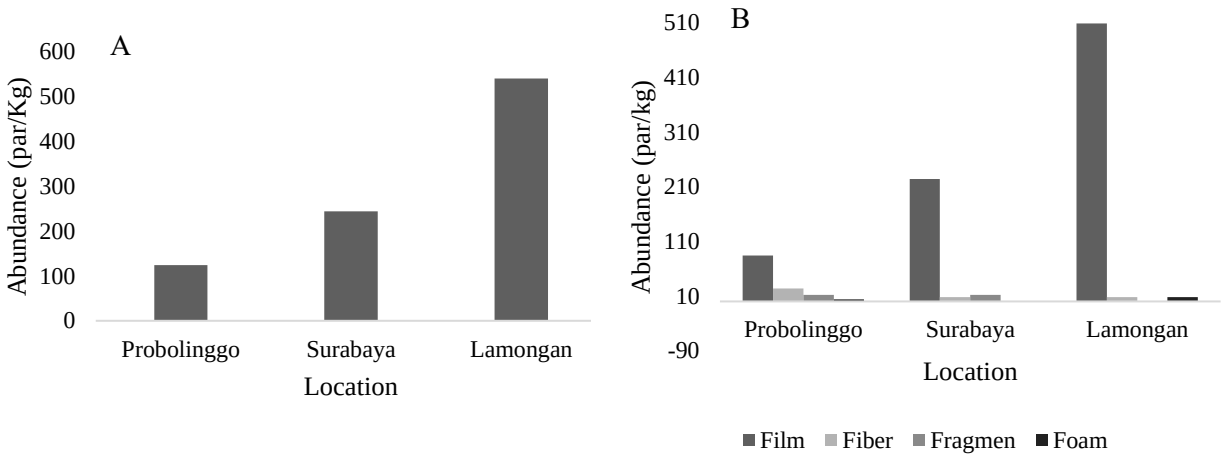

Figure 2. The abundance of each location (A), and abundence of microplastic types (B)

In this study, the amount of microplastic is caused by the location of salt production area close to garbage disposal. Factors like wind and rivers flowing to these marine environment might carry waste from nearby cities. Moreover, the process of production salt which use base of sack makes it possible to microplastic in salt particles. Wright and Kelly [13]report that agricultural plastics can contaminate water ecosystems through wind. In addition, neglect during the manufacturing process can cause transfer of microplastic from the production areas to the salt.[8] In fact, at this point, no matter how far these kinds of ecosystems are from cities, they can still contain high amounts of microplastic, as reported by various researchers.[19], [20] The high level of microplastic waste we have seen in lake salt in this study can be explained similarly. Since there are no studies that report the level of microplastic pollution in Northen coast of East Java, it was not possible for us to make any comparisons.

3.2. Type of microplastics in salts identified with μ-FTIR

The identification of the fibers found was done by Fourier Transform Infrared Spectroscopy (FT-IR), which is one of the most popular methods used to confrm the composition of microplastics[21]. Five particles were selected and identified using *μ*-FT-IR. For the 5 selected particles, film-blue; fragment; and fiber were identified as polyethylene (PE), film-white was identified as polypropylene (PP), and foam was polystyrene (PS) (Figure 3). Dominates chemical type of microplastic of all particles was

polyethylene. Polypropylene was the most common microplastic that found in salt particles from Surabaya.

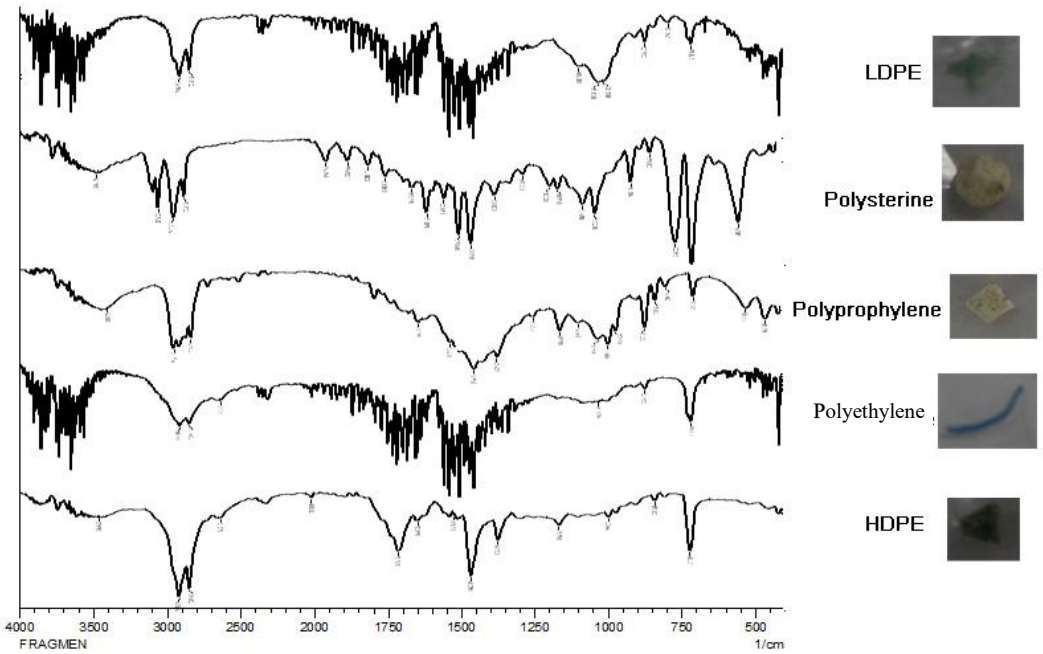

Figure 3. FTIR spectra of fbers found in the table salt samples

Iñiguez et al. was identified polyethylene terephthalate (PET), polyethylene (PE) and polypropylene (PP) in salt particles before grinding process. However, this result is not found in the present work, being the microplastics content very similar in salt. Eriksen *et al*.[22] report an estimate of the total number of plastic particles in the world's ocean, indicating a very high plastic pollution in north Pacifc compared to Mediterranean sea. Yang *et al*.[8] indicate that in China the sources of sea salts were from coastal waters in locations where the population density is very high, not being this true in the present study. In the present case, the data indicate that there is not a clear source of these micro-particles, but there is a background presence of the microplastics in the environment. In line with this microplastic pollution has also been detected in honey and sugar samples [23] and other [24], as well as in marine organisms [25], [17].

3.3. The risk of microplastics in salt to human health

Microplastics are a pervasive pollutant present in marine environments worldwide and tend to increase in concentration over time due to plastic fragmentation.[26] In 2010, the global daily sodium consumption was 3.95 g/day (equivalent to 9.88–10.2 g salt/day44) corresponding to 3.6 to 3.7 Kg salt per annum [27]. In this way, an average consumer of Indonesian salt would ingest a maximum of approximately 1024 plastic particles per annum. Humans will also ingest microplastics by consuming other sea products, such as mussels and fish, as well as other microplastic-contaminated food and water [28]. Although the amount of microplastics ingested through salt consumption is much less than that through bivalv consumption by the top European consumers (11,000 microplastic particles per year) [29], more individuals will be affected because table salts are required and consumed in our daily diet. Microplastics are a particular threat to organisms due to their small size and their capacity to absorb persistent organic pollutants [30]. The constituents of plastics, as well as the chemicals and metals they absorb, may ultimately be ingested by humans through the consumption of seafood. Due to the pollution of seawater, many contaminants have been found in sea salts, including plasticizers, such as di-(2ethylhexyl) adipate and benzyl butyl phthalate [31]. Plastic might be the direct sources of these

contaminants. However, plastics might absorb contaminants from the seawater and transfer them to the sea products. Therefore, the presence of marine microplastics in sea salts might pose a threat to food safety.

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Animal food demand inurban poor household in East Java: a quadratic almost ideal demand system approach

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Abstract. The number of food insecure households in Indonesia is 27% in urban areas, including East Java. This study aims to analyze the influence of socio-demographic variables, prices and income on the demand for animal food in urban poor households in East Java. Estimated demand system using Quadratic Almost Ideal Demand System (QUAIDS) with Poi procedure, using data from the Susenas 2016 as many as 1,639 households. All own-price elasticities are negative and expenditure elasticities positive. Increasing 1% in income will increase egg, chicken and milk demand by 0.57%, 1.59%, and 2.77%. Eggs are inelastic, while chicken and milk are elastic. Milk is a luxury and most sensitive to income changes. Beef is the substitute for eggs and chicken. Beef is the substitute with chicken and fish. The increase in income followed by falling prices for eggs, fish, and milk will increase consumption of chicken and beef.

1. Introduction

By 2016 the number of poor people in East Java, Indonesia is 4.7033 million people (12.05%). Among them, a total of 3.18451 million (10.01%) lives in rural areas, and 1.51879 million people (16.01 %) lives in urban areas. The poverty line (US $ / capita/month) in March 2016 amounted to IDR 321 761 in East Java. The line for the rural area is IDR 323 779 rural and for urban areas is IDR 319 662. The average monthly expenditure per capita (Rupiah) by province and urban rural classification, East Java has expenditure by 1,145,588 (urban) and 723,799 (rural). The monthly average expenditure per capita on type of food and non food by province and urban rural classification, the rural is greater than urban, that is rural as many as 57.94% for food and 42.06 for non food. While, urban as many as 46,45% for food and 53.55% for non food.

Monthly average expenditure per capita of food items by urban classification, in East Java, March 2017 for chicken eggs by 9.22 unit (IDR 10,775,-), duck eggs by 0.07 unit (IDR 127,-), broiler or local chichen meat by 0.5 kg (IDR 14,429), beef by 0.08 kg (IDR 8186), and fresh and shripm 1.13 kg (IDR 27,025) shrimp fresh fish. Total Consumption and these expenditures can be used to describe the pattern of consumption of animal food in urban poor households in East Java. Animal food consumption patterns can be used to look at the ability of households to meet protein needs. Protein supply is one of the indicators to describe the household poverty.

2.Material and Methods

2.1 The demand model: QUAIDS (Quadratic Almost Ideal Demand System)

The most commonly used method in demand analysis in the last two decades is the Deaton and Muellbauer [1] AIDS (Alamost Ideal Demand System) model. Indeed the AIDS model has a number of desirable demand properties such as allowing testing for symmetry and homogeneity through linier restriction among others. However, more recently Banks et al. [2] generalized the AIDS model by demonstrating that the appropriate form for some consumer preferences is of a quadratic nature contrary to the linier form in the basic AIDS. In addition, the QUAIDS model maintains the theory consistency and the desirable demand properties of the AIDS model.Formally, the share equations in the QUAIDS model [2] are:

$$w_i = \alpha_i + \sum_{j=1}^{n} \gamma_{ij} 1np_j + \beta_1 1n\left[\frac{m}{a(p)}\right] + \frac{\lambda_i}{b(p)}\left\{1n\left[\frac{m}{a(p)}\right]\right\}^2 + \varepsilon_i$$

Where w_i is a household's expenditure share for commodity i, defined as $w_i \equiv \frac{p_i q_i}{m} and \sum_{i=1}^{n} w_i = 1$
On the ither hand, the demand theory requires the following restrictions:
Adding-up: $\sum_{i=1}^{n} \alpha_1 = 1, \sum_{i=1}^{n} \beta_1 = 0, \sum_{i=1}^{n} y_{ij} = 0, \sum_{i=1}^{n} \lambda_i = 0,$
Homogeneity: $\sum_{i=1}^{n} y_{ji} = 0$
Slutsky symmetry: $\gamma_{ji} = \gamma_{ij}$

The QUAIDS model in this study was carried out accounting for socio-demographic efffects. Indeed, demographic factors can effect household behaviour in terms of demand and allocation of expenditure among goods [3-6]. Roy's (1983) 'demograpic scaling' method [7] was then used to take into account demographics in this study as in Poi [8]. In this approach, the effects of a change on the demographics are closed to the effect of a change in princes [4].

Considering z as a vector of S household characteristics z is a scalar representing the household size in the simplest case. Let $e^R(p,u)$ represent the expenditure function of a refference household with just single adult.For each household, Roy's method uses an expenditure function of household characteristics, without controlling for any changes in consumtion patterns. The second term control for a change in relative prices and actual goods consumed.

Following Roy [7], QUAIDS parameterized $\overline{m_o}(z)$ as $\overline{m_o}(z) = 1 + \rho z$
Where ρ is a vector of parameters to be estimated.

The expenditure share expenditure equation takes the following form:

$$w_i = \alpha_i + \sum_{j=1}^{K} \gamma_{ij} 1np_j + (\beta_i + \acute{\eta}_i z) 1n\left\{\frac{m}{\overline{m_o}(z)\alpha(p)}\right\} + \frac{\lambda_i}{b(p)c(p,z)}\left[1n\left\{\frac{m}{\overline{m_o}(z)\alpha(p)}\right\}\right]^2$$

Where $c(p,z) = \prod_{j=1}^{K} \rho_j^{n_{iz}}$
The adding-up condition requires that $\sum_{j=1}^{K} \eta_{rj} = 0$ for $r = 1, \ldots, s$.

The uncompensated price elasticity for the commodity group i with respect to changes in the price of commodity good j is:

$$\varepsilon_{ij} = -\delta_{ij} + \frac{1}{w_i}\left(\gamma_{ij}\left[\beta_i + \acute{\eta}_i z + \frac{2\lambda_i}{b(p)c(p,z)} 1n\left\{\frac{m}{\overline{m_o}(z)\alpha(p)}\right\}\right] * \left(\alpha_j + \sum_{1} \gamma_{ij} 1np_j\right) - \frac{(\beta_i + \acute{\eta}_i z)\lambda_i}{b(p)c(p,z)}\left[1n\left\{\frac{m}{\overline{m_o}(z)\alpha(p)}\right\}\right]^2\right)$$

The expenditure (income) elastcities for the good or commodity i is:

$$\mu_i = 1 + \frac{1}{w_i}\left[\beta_i + \acute{\eta}_i z + \frac{2\lambda_i}{b(p)c(p,z)} 1n\left\{\frac{m}{\overline{m_o}(z)\alpha(p)}\right\}\right]$$

The compesated price elasticities are derived from the slutsky equation:

$$\varepsilon_{ij}^c = \varepsilon_{ij} + \mu_i w_j$$

Note: all the lowercase greek letters other than α_0 are the parameters to be estimated. Two demographic variable were finally used in this study, namely area (urban and rural), and house hold size.

The parameters are estimated by interated feasible generalized non-linier least which are equivalent to the multivariate normal maximum likelihood estimator for this class of problem via Stata's 'nlsur'

command as sugested by Poi [8]. After the presentation of the demand model, it is worth discussing at least two major data issues, namely the price measure and the treatment of outliers and missing values.

2.2 Data

The data used in this research is secondary data in the form of Susenas data in March 2016. The data analyzed include socio-demographic data, namely household residence status, household member number, household income, household consumption, and expenditure. The animal foods in this study include eggs (chicken eggs, local chicken eggs, and duck eggs), chicken (local chicken meat and chicken meat), beef, fish (fresh fish and shrimp including fish, shrimp, squid, and shellfish) as well as milk (milk powder and infant milk). The sample size is 1.639 households. The unit of analysis in this study is household.

3. Result and Discussion

3.1 Parameter estimates

Domestic animal food demand can be approximated by calculating the amount of expenditure and consumption of animal food itself. Total consumption and expenditure depend on the price and household income. Through quadratic demand system functions , the coefficients of each domestic animal food can be obtained. The Results of QUAIDS analysis is shown in Table 1.

Tabel 1. Parameter estimates

Parameter (Coefficient and SEM)	Egg (1)	Chicken meat (2)	Beef (3)	Fish (4)	Milk (5)
Constant					
α	-0.39006 (0.14662)	0.13040 (0.19820)	0.64619 (0.07965)	-0.09568 (0.09260)	0.70915 (0.22897)
Income					
β	-0.09575 (0.04405)	-0.10662 (0.04934)	0.03230 (0.02338)	-0.02019 (0.02850)	0.19026 (0.04499)
Price					
γ_1	0.58442 (0.14005)	0.06828 (0.05612)	-0.04251 (0.03535)	-0.04759 (0.03215)	-0.56261 (0.12446)
γ_2	0.06828 (0.05612)	-0.58205 (0.07579)	0.14573 (0.02606)	0.08441 (0.02234)	0.28362 (0.10003)
γ_3	-0.04251 (0.03535)	0.14573 (0.02606)	-0.30603 (0.01918)	0.01639 (0.01070)	0.18642 (0.04268)
γ _4	-0.04759 (0.03215)	0.08441 (0.02234)	0.01639 (0.01070)	-0.09320 (0.01095)	0.03999 (0.04854)
γ_5	-0.56261 (0.12446)	0.28362 (0.10003)	0.18642 (0.04268)	0.03999 (0.04854)	0.05257 (0.15970)
Income Square					
λ	0.00962 (0.00126)	-0.01143 (0.00273)	0.00018 (0.00123)	-0.00219 (0.00150)	0.00383 (0.00259)
Demographic					
η_hhm_tot	-0.0064004 (0.0011852)	0.0043701 (0.0008855)	0.0005182 (0.0002881)	0.0008036 (0.0003623)	0.0007084 (0.0005138)
Demographic					
ρ_hhm_tot	-0.0831831 (0.0039288)	-0.0831831 (0.0039288)	-0.0831831 (0.0039288)	-0.0831831 (0.0039288)	-0.0831831 (0.0039288)

3.2 Price and expenditure elasticities

Table 2 shows the value of the income elasticity, price elasticity, and price elasticity Marshallian Hicksian. The income elasticity of 0.57 and a group of eggs is very significant. As for the chicken meat group significant at the alpha of 1.59 and 0.05. It means that the increase in household income of urban poor in East Java of 1% will increase the consumption of eggs and chicken meat by 0.57% and 2.77%. Eggs are normal goods and a staple of urban poor households in East Java. It indicated that the value of the income elasticity is less than 1. Chicken is a luxury item, determined by the amount of the income elasticity (more than 1).

Table 2. Expenditure elasticities andown-price elasticities

Animal Food	Income Elasticity	Price Elasticity		Total ART
		Marshallian	Hicksian	
Egg	0.56823	-1.19121	-0.79903	-0.0064004
	(0.01030)	(0.03778)	(0.03640)	(0.0011852)
Chicken meat	1.59053	-4.95303	-4.62894	0.0043701
	(0.03397)	(0.12913)	(0.13024)	(0.0008855)
Beef	3.44601	-26.52900	-26.48504	0.0005182
	(0.18598)	(1.10669)	(1.10603)	(0.0002881)
Fish	1.98376	-5.29697	-5.25032	0.0008036
	(0.13419)	(0.31181)	(0.31157)	(0.0003623)
Milk	2.76745	-7.05312	-6.86000	0.0007084
	(0.07688)	(0.28375)	(0.28353)	(0.0005138)

All Marshallian price elasticities and Hicksian price elasticity are negative. It is consistent with the economic theory that when the price increase, the demand for animal food will be going down. It fits well with studies in Nigeria [9], research in Ethiopia [10], research in Turkey [11], as well as research in Mexico [12]. Marshallian price elasticities have a higher value in absolute terms compared to the elasticity of the Hicksian. It can be explained by the theory that approach on the price elasticity Marshallian containing the effect of changes in income and the effect of price changes on the price elasticity while Hicksian only contains the price effect [13]. The amount of Marshallian price elasticities for a group of eggs of 1.19 and significant at an alpha of 0.05. It means that when the price of eggs rose by 1%, then the urban poor households in East Java will reduce the consumption of eggs by 1.19%.

Similarly, when it viewed from the Hicksian price elasticity, price elasticity that only Hicksian group of eggs (0.79) were significant at the 0.05 alpha. The price elasticity for the group of chicken, beef, fish, and milk was not significant. Both Marshallian and Hicksian, the price elasticity of a group of chicken, beef, fish, and dairy were not significant. It may be caused by the animal food price data obtained with the approach of the amount of consumption divided by the amount of spending that price variations are not sufficient to describe the actual price of animal food.

3.3 Demographic effects

Demographic factors in this study are reflected by the number of members in a household. The increase in the number of household members very significant effect on the demand for animal food (Table 2). In animal food eggs, The addition of 1 household members would reduce the consumption of eggs by 0.64%. As for chicken, beef, fish and dairy products, the increase of 1 household members will increase the consumption of 0.44%, 0.52%, 0.08% and 0.07%.

***3.4** Cross-price elasticities of animal food groups*

Table 3 shows that the uncompensated cross-price elasticities were mostly positive indicating substitution relationship of animal food groups whereas all of Hicksian elasticities also were positive. The estimates indicate that the cross-price elasticities of eggs, chicken, beef, fish and milk subtitution relationship. This implies that a unit change in the price of these food items has effect on the demand

for each other. Increasing in prices of beef by 1% will lead the poor households in Urban East Java to increase their demand for chicken by 0.511%.

Table 3. QUAIDS uncompensated and compensated price elasticities results

Marshallian Cross Price Ealasticity (Uncompensated Elasticity)					
Animal food	Egg	Chicken meat	Beef	Fish	Milk
Egg	-1.19121	0.51154	0.11764	0.00388	-0.01008
	(0.03778)	(0.03003)	(0.01227)	(0.01344)	(0.02241)
Chicken meat	1.01826	-4.95303	0.67740	0.21327	1.45357
	(0.10801)	(0.12913)	(0.04822)	(0.04786)	(0.08569)
Beef	4.39453	10.42311	-26.52900	1.12660	7.13874
	(0.67231)	(0.75928)	(1.10669)	(0.41932)	(0.67358)
Fish	-0.87811	1.76796	0.63189	-5.29697	1.79147
	(0.41109)	(0.41062)	(0.22758)	(0.31181)	(0.37175)
Milk	-1.58950	3.98253	1.31163	0.58101	-7.05312
	(0.22887)	(0.24736	(0.12329	(0.12516	(0.28375)
Hicksian Cross Price Ealasticity (Compensated Elasticity)					
Animal food group	Egg	Chicken meat	Beef	Fish	Milk
Egg	-0.79903	0.62732	0.12489	0.01724	0.02957
	(0.03640)	(0.03026)	(0.01226)	(0.01343)	(0.02240)
Chicken meat	2.11601	-4.62894	0.69769	0.25068	1.56456
	(0.10256)	(0.13024)	(0.04819)	(0.04782)	(0.08550)
Beef	6.77290	11.12529	-26.48504	1.20764	7.37921
	(0.66347)	(0.76898)	(1.10603)	(0.41935)	(0.67478)
Fish	0.49104	2.17218	0.65719	-5.25032	1.92991
	(0.39405)	(0.41387)	(0.22746)	(0.31157)	(0.37170)
Milk	0.32055	4.54644	1.34692	0.64609	-6.86000
	(0.22059)	(0.24890)	(0.12321)	(0.12507)	(0.28353)

4. Conclusion

As a source of protein, animal food is an essential food for human health, especially in reducing the problem of stunting. The estimation of the parameters used to obtain elasticity QUAIDS price and the expenditure of 5 groups of animal food. As the use of other microdata, the observed zero expenditure need to be accomodated to obstain consistent parameter and elasticity estimates. This issue of cencoring is especially challenging given the size of the demand system and level of disaggrgation considered in this study. The Poi's procedure is used to obtain the correct standard errors of parameter and elasticity eatimates needed for statistical inference.

All Marshallian price elasticities and Hicksian price elasticity are negative. The addition of 1 household members would reduce the consumption of eggs by 0.64%. As for chicken, beef, fish and dairy products, the increase of 1 household members will increase the consumption of 0.44%, 0.52%, 0.08% and 0.07%. The uncompensated cross-price elasticities were mostly positive indicating substitution relationship of animal food groups whereas all of Hicksian elasticities also were positive. The uncompensated cross-price elasticities were mostly positive indicating substitution relationship of animal food groups whereas all of Hicksian elasticities also were positive.

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Pesticides removal of fruit and vegetables by using ultrasound ozone

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Abstract. The facts show from WHO (World Health Organization) in 2015, there were about 2 million victims died each year due to unsafe food. Ultrasonication is a non thermal process that is used as a preservation method. Ultrasonic applications generally use vulnerable frequencies between 18-100 kHz and have an intensity between 10-1000W / cm^2. The application of ozone (O_3) in the handling of fruits and vegetables has good prospects because it is safe and effective, and ozone has high oxidation potential that can be used to kill pathogenic microbes. Based on these problems, the new innovation ideas which is sterilizer machines based on ultrasonic and ozone. The specifications of the machine are 80x70x14 cm, capacity of 1.5 kg, ozone concentration of 0.324 mg/l and ultrasound frequency of 40.32 kHz. The processing costs of Rp 107.58/kg with tool efficiency 94.32%. The ultrasonic treatment process on strawberries can reduce pesticide residues by 91.2%. The test kit results of ultrasound-ozone of strawberry, paprika, and mustard green showed free of pesticides.

1. Introduction

Food safety issues are indeed one of the most worrying and hot topics discussed in the world food forum.As a country with a large area, it is very important for Indonesia to be able to realize its consistency in handling the quality and safety of food products, especially in horticultural products such as vegetables and fruits. The development of the horticulture subsector, which includes vegetables and fruits, is known to have contributed to the value of food exports of around 12-17 percent [1]. If viewed in terms of benefits, fruits and vegetables are a source of provitamin A, vitamin C, protein, carbohydrates, and are rich in fiber and which are very beneficial for body health. However, on the other hand food security in Indonesia still does not meet the standards, namely the use of chemicals such as excessive pesticides by local farmers, with the aim of maintaining the quality of fruits and vegetables to maintain their quality when marketed. Basically, fruits and vegetables after being harvested will be easily damaged due to several influences such as physical, chemical, microbiological (viruses, bacteria, pathogenic microbes). For this reason, efforts are needed to handle harvests to improve the quality of vegetables and fruit. Application of ozone (O3) in handling fruits and vegetables has good prospects because it is safe and effective, and ozone has high oxidation potential that can be used to kill pathogenic microbes. Ultrasonic Ozone Treatment is a technology that can be used to reduce pesticide residues by lifting up to 91.2% [2]. This shows that Ultrasonic Ozone Cleaning is one of the effective technologies to reduce pesticide residues in post-harvest agricultural products. Ozone technology is considered as an effective technology to reduce pesticide residues and kill pathogens. This work designed and manufactured the sanitation preservation equipment based on ultrasonic ozone waves and edible coating which is able to maintain food security and extend shelf life

2. Method

Several stages were conducted in the production and implementation of the equipment developed in this work such as the design stage, equipment instrumentation installation, equipment testing and sample testing.

2.1 Materials and tools

In the manufacture and testing of fruit and vegetable preservation sanitation tools, the tools needed are, grinding, lathes and other supporting equipment such as cutting, welding and soldering tools. The materials needed are ozone generators, flow rate controllers, ozone analyzers, piezoelectric transducers, fan dryers, stainless steel slabs, nuts, bolts, edible coating chambers, water, plastics, and manufacturing materials

2.2 Instrumentation Stage

This tool has an instrumentation stage. The instrumentation stage can be seen in Figure 1

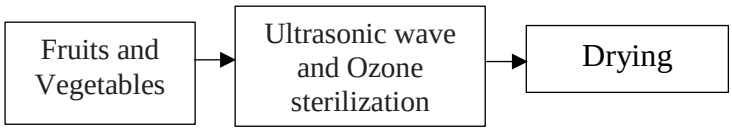


Figure 1. Tool Instrumentation System Stage

2.3 Tool Testing

The instrument tested ozone levels qualitatively by reacting ozone which was added with KI solution and quantitative test using titration method. Calculation of ozone levels, namely:

Ozone levels (mg / l): $\frac{Bm\ O3\ x\ V\ Na2S2O3\ x\ N\ Na2S2O3}{V\ sampel\ x\ e\ Na2S2O3}$ (1)

Besides testing ultrasonic frequency and calculating energy efficiency:

Energy Input = V input + I input (2)

Energy Output = Σ Power of each component (3)

Efficiency = $\frac{Energy\ out}{Energy\ in}$ x 100% (4)

2.4 Testing of Fruits and Vegetables

2.4.1 Microbial Testing

Microbial tests on samples were carried out to determine the performance of the equipment during the sanitation process. Samples were treated using the TPC method (Total Plate Count).

2.4.2 Pesticide Testing

Qualitative Pesticide Testing using a kit test.

3. Result and Discussion

3.1 Tool assembly

The assembly of sanitation and preservation equipment begins with the assembly of the ultrasonic ozone device, and the dryer is followed by making the tool frame. Ultrasonic-ozone device assembly uses ultrasonic components and ozone generators.

3.2 Tool Testing

This test is carried out with the aim to test the feasibility of the tools and products produced so that they can function properly and in accordance with what is expected. Based on the results of the equipment

feasibility testing, results were obtained that the fruit and vegetable sanitation preservation equipment had 440 watts of power.

3.3 Qualitative and Quantitative Ozone Level Testing

Qualitative testing of ozone levels is done by dissolving ozone into KI solution. Based on qualitative testing, the solution changes color from clear to yellow. This shows that the ozone generator in the appliance can function properly. While ozone testing quantitatively, the results of ozone concentration were 0.324 mg / L. Based on Kyu-Earn and Kang [3], ozone concentrations in the range of 0.1 ppm to 0.9 ppm are safe for fruits and vegetables and can be used to kill E. Coli, Vibrio, Salmonella, Yersinia, Pseudomonas, Staphylococcus and Listeria and can kill the virus. Another advantage of this technology is that it does not leave toxic residues on the fruit so it is safe to use [4].

3.4 Ultrasonic Frequency Testing

Based on the results of the test, the frequency of the ultrasonic device has met the standard with the frequency result of 40.32 kHz.

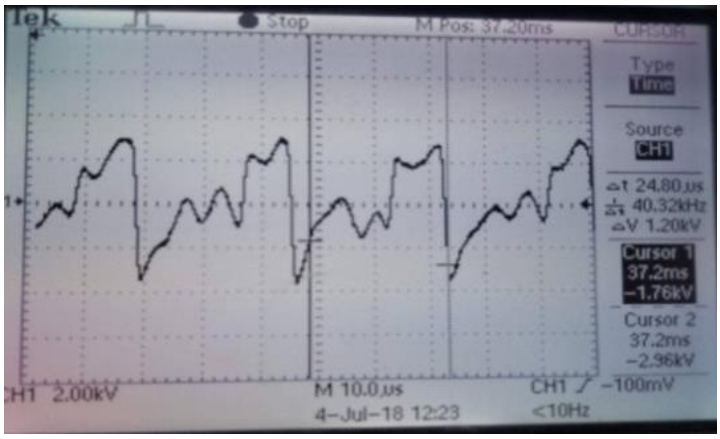


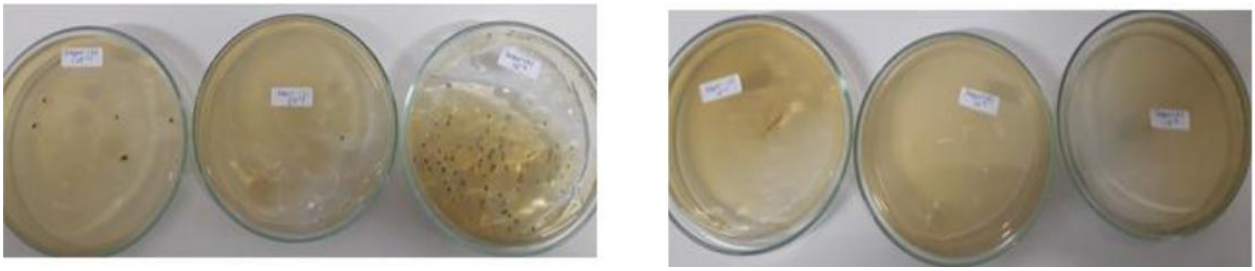
Figure 2. Ultrasonic Frequency Test Results

3.5 Efficiency

Efficiency testing of preservation sanitation tools is used to determine the balance of input and output energy used for the process of sanitation and preservation of fruits and vegetables. In this test, room temperature (25oC) is used for 15 minutes. Based on the treatment, the efficiency of the instrument was 94.32%.

3.6 Microbial Reduction Testing Tools

Microbial testing is carried out using the TPC (Total Plate Count) method to calculate the number of bacteria. The results can be seen in Figure 3. The tomato and strawberry fruit that were tested for control were positive for *Eschericia coli*, but with the treatment of sanitary preservation tools, the fruit of the vegetable fruit was reduced to 99.9% of *Eschericia coli* contaminants. This is consistent with previous research which states that ozone technology is a strong oxidizing agent and is used to remove pesticide residues and kill microbes [5], viral inactivation [6], and inactivation of bacterial spores [7].



(a) (b)

Figure 3. (a) Testing the TPC method for sample control, (b) treatment sample

Table 1. TPC Test Results for Strawberry and Sawi

Treatment	Number of microbes (cfu / gram)	
	Strawberry	Sawi
Control	$3.2\ x10^4$	$9\ x10^4$
Ultrasonic-Ozone	$2\ x10^3$	$1\ x10^2$

3.7 Pesticide Testing

Based on the results of testing in Table 2, the results obtained for strawberry, paprika and mustard positive control contain pesticides with a blackish blue color change. While the samples of strawberry, paprika and mustard greens that have been treated with this negative tool contain pesticides marked by the absence of discoloration. Ultrasonic treatment of coatings can help reduce particle size due to cavitation [8]. Qualitative Testing results can be seen in Table 2 below:

Table 2. Pesticide Test Results

Sample	Test Result		
Control	 Sawi (+)	 Strawberry (+)	 Paprika (+)
Treatment	 Sawi (-)	 Strawberry (-)	 Paprika (-)

4. Conclusion

This equipment was able to reduce microbial damage up to 99% indicated from the test kit results of ultrasound-ozone. The equipment processing costs was estimated as IDR 107.58/kg with the efficiency of 94.32%.

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Preparation technique in the production of Pantura fish fillet in Lamongan, East Java

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Abstract. Marine sector fisheries production in Lamongan Regency has increased every year. Sea fish that have low economic value are called trash fish. Trash fish has several disadvantages such as tight thorns, has little meat, and is high perishable. The purpose of this study is to get the right preparation technique so that the highest yield of fish fillets is produced and has good physical, chemical and functional characteristics. This research method is a completely randomized design, with the first factor is the type of fish consisting of Peperek, Juwi and Tembang fish. The second factor is the type of preparation technique which consists of mechanics, blanching, 1% acid immersion and 1% papain immersion. The data obtained were also analyzed descriptively from the preparation techniques in each observation parameter and presented in table form and then plotted in graphical form. Preparation techniques that produce the most yield and fastest preparation time are enzymatically with a concentration of 1%. Tembang, Juwi and Peperek fish possess good chemical content, namely: moisture content (77.46% - 80.13%), protein content (7.39% - 9.29%), fat content (8.01% - 9 , 49%) and ash content (1.55% - 2.83%). As well as functional properties, namely: froth power (17.68% - 61.87%), foam stability (50% - 57.14%), emulsion power (3.31% - 4.29%), emulsion stability (1, 91% - 3.37%), WHC (33.9% - 46.64%), and OHC (24.75% - 29.57%).

1. Introduction

Indonesia's abundant natural resources in the marine and fisheries sector. Marine sector fisheries production in Lamongan Regency has increased every year. Production in 2014, 2015 and 2016 was 71,553 tons, 72,346 tons and 73,142 tons [1]. Sea catching fish in Indonesia have very diverse types. In general, fishing operations have high economic value because of their high nutritional content, but there are several types of fish that have low economic value. Fish that are not included in the main catching destination are called bycatch or side capture results. The fishermen in some areas also call this fish the term trash fish. Trash fish is usually used as animal feed or at least processed into salted fish and sometimes just thrown away, resulting in a foul odor during the harvest season. There are three types of trash fish that have the potential to be developed, Juwi fish, Peperek fish, and Tembang fish [2] .

Nutrient content of trash fish is not much different from other types of fish, so it can be processed into raw materials for processed fish products. In processing, of course, a fish preparation technique is needed to produce high yields of fish meat. Physical fish preparation techniques are by slicing fresh fish directly or mechanically [3], chemically [4], and enzymatic [5]. Treatment in preparation techniques to increase their economic value. Trash fish has different morphology so that in the

preparation technique it will certainly use different methods. The purpose of this study is to obtain the best preparation technique for trash fish so that the resulting fillet has a high yield and can facilitate the subsequent process of processing food products.

2. Materials and Methods

2.1. Materials

Some fresh fish there were Chacunda gizzard-shad ((*Anodontostoma chacunda*), Orangefin ponyfish (*Leiognathus bindus*), and trash fish with the average weight of 50-100 g/fish were purchased from a fish central market in Lamongan district, East Java Province, Indonesia. Fishes were kept in cold storage immediately. Fishes were kept in an icebox and transported to the Department of Agro- industry Technology, Faculty of Agricultural Technology, University of Jember within 5 h. Upon the arrival, fishes were immediately kept in a freezer. Before analysis, fishes were immediately thawed at room temperature, washed, filleted, and minced to uniformity by using a chopper. Gelling agents (agar and *i*-carrageenan) were purchased from chemical shop in Hiroshima, Japan.

All chemical were used were analytical grade. There were NaCl, phosphate buffer 0,1 M pH 7, HCl, sodium hydroxide, Lowry reagent, H_2SO_4, selenium, boric acid 4 %,methanol, SDS 0,1 % Tris- HCl buffer pH 6,5, sucrose, urea, 2 % SDS, 2 % 2-mercaptoethanol, 50 % glycerol, CBB, staining solution, Na_2CO_3, $CuSO_4$, sodium potassium tartrate, phenolic solution, glutaraldehyde, ethanol.

The ingredients used are Juwi, Peperek and Tembang fish obtained from the north coast of Lamongan. lime, enzyme papain and vinegar. While the analysis material used is TCA, SDS, oil, selenium, concentrated H2SO4, 10% NaOH, 3% boric acid and petrollium benzene.

The tools used are measuring cups, ovens, analytic balance sheets, desiccators, filter paper, vortex, porcelain exchange rates, ignition furnaces, excicators, kjeldahl flasks, distillation flasks, color readers.

2.2. Preparation of fish fillets

The three types of marine fish are prepared in four ways, namely mechanical, blanching, chemical and enzymatic. Preparation techniques carried out on fish are given the same time limit of 5 minutes for blanching, and 30 minutes for vinegar and enzymes. Mechanical preparation techniques for fish are done by filling the fillet directly with fresh fish. In this process, the three types of fish are pre-washed using running water, while the other fish are temporarily accommodated in the frezeer. The fish that has been washed is discarded with scales and stomach contents. Then the fillet process is carried out from the end of the tail, splitting the back to the end of the head. Fish that are clean and detached from thorns are weighed and their weight is recorded and then stored in a frezeer. In the blanching technique, the three types of fish are cleaned alternately using running water and fish waiting for the washing process to be stored in the freezer. After washing, the fish is boiled at 100oC for 5 minutes. After boiling, the fish is removed and cooled in the open air. After cold, the three types of fish are thorn removal and fillet process is carried out. Fish that are clean and detached from thorns are weighed and weighed and then stored in a freezer. Chemical and enzymatic preparation techniques are carried out by means of soaking fish. Chemically, fish is soaked with 1% vinegar acid solution while enzymatically, fish is soaked with 1% protease enzyme solution. This soaking is done for 30 minutes. After that the fillet process is carried out then the results are weighed and analyzed physically, chemically and functionally. The process can be seen in Figure 1.

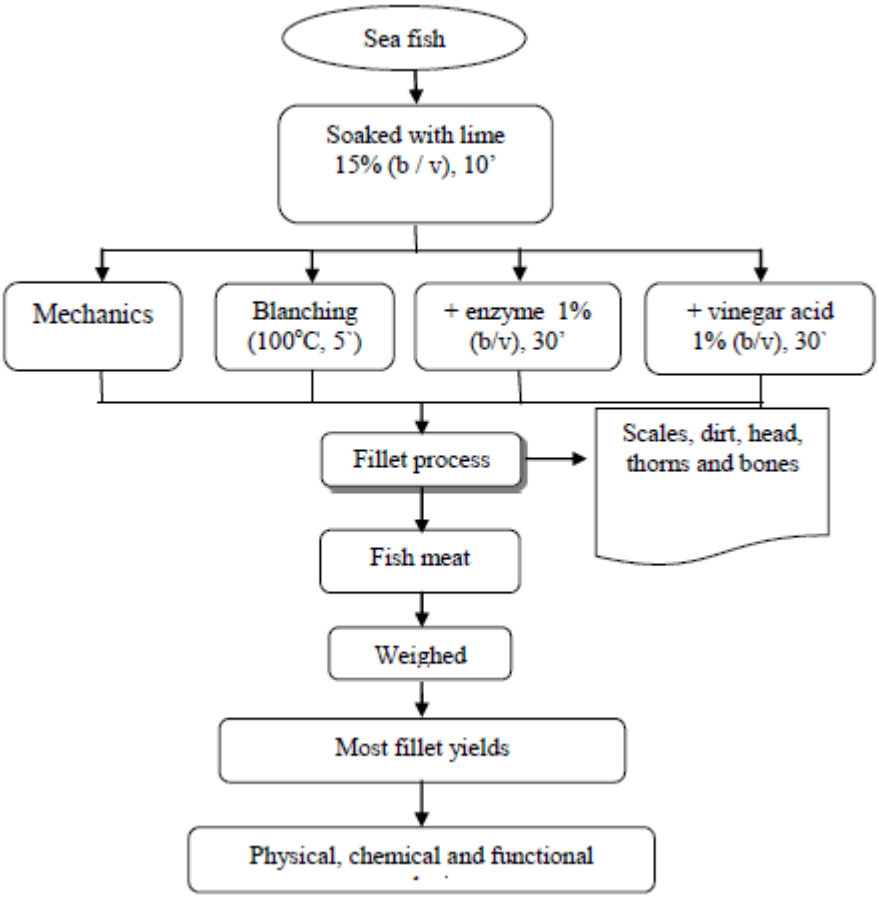

Figure 1. Flow chart of trash fish preparation techniques

2.3. Data analysis

The research design used in this research is Experimental Laboratory. Observation data from all testing parameters will be calculated using Microsoft Excel and presented in table form and then plotted in graphical form, then analyzed by ANOVA - One Way test (SPSS 16) and continued with Duncan test (p 5 0.05) if there are significant differences.

2.4. Analysis procedure

Fillet yield, lightness [6], moisture content [7], fat content [7], ash content [7], protein content [7], water holding capacity [8] oil holding capacity [9].

3. Results and Discussion

3.1. Physical Properties

3.1.1. Yield

The average mechanical fillet yield in Juwi, Peperek and Tembang fish was 51.97% (67.3%; 33.4% and 55.2%); blanching treatment was 53.47% (64.4%; 44.8% and 51.2%); the treatment of soaking vinegar acid at a concentration of 1% was 54.7% (57.9%; 46.1% and 60.1%); and the mmersion treatment of papain enzyme solution at a concentration of 1% was 55.7% (56.4%; 47.5% and 63.2%). Fish fillet yield can be seen in Figure 2.

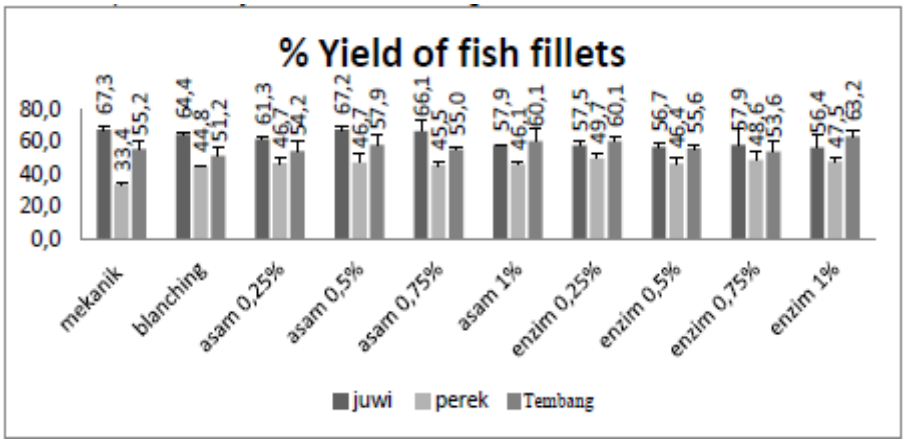

Figure 2. Yield of fish fillets

The results of analysis of variance at the test level (α) were 5%, indicating that the preparation technique had an effect on the yield of trash fish fillets. Figure 2, shows that the treatment of fish fillets with mechanical treatment has the largest yield for Juwi fish species. This is because morphologically the Juwi fish is fusiform in shape, flat rather elongated with thorns on the bottom of the body so that in the preparation process it is much easier when compared to Peperek fish which has a much thinner and smaller morphology. From these data it is proven that papain enzymes can function as meat collectors so that the fillet process runs easily and produces high yields. The mechanism of action of enzymes in crushing meat is the occurrence of a peptide bond termination reaction so that the protein chain is cut into pieces to form a shorter chain. Termination of this bond will cause the connective tissue and flesh fibers to be cut off and the binding strength becomes weak so the meat becomes soft [10].

3.1.2. Lightness

Brightness of fish fillets ranged from 18.83-25.01 and tended to approach black or gray. The results of fish lightness fillet measurements are shown in Figure 3.

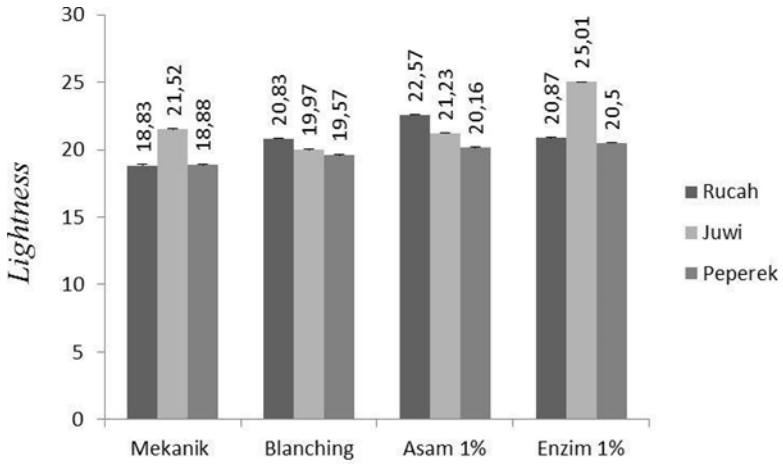

Figure 3. Lightness value of Fish Fillets

The results of analysis of variance at the test level (α) of 5%, showed that the preparation technique had a significant effect on the brightness of trash fish fillets. According to [11], soaking fish with vinegar can maintain fish quality, because vinegar that seeps into fish meat can inhibit microbial growth and the activity of histidine-breaking enzymes into histamine. This acid can also reduce pH so that bacterial activity will be inhibited. Figure 2, shows that the highest brightness fish fillets in Juwi fish with 1% enzyme immersion treatment is equal to 25.01 and the lowest brightness value in trash fish (Tembang) with a mechanical treatment that is equal to 18.83. The low brightness value in mechanical treatment is caused by the absence of pretreatment before filleting techniques are carried out, so that the fish will be overgrown with decaying microorganisms and hemoglobin oxidation becomes methemoglobin which changes the color of the remaining blood from bright to darker [12].

3.2. Chemical Properties

3.2.1. Water content

The water content contained in fish fillets of Tembang, Juwi and Peperek is between 76.57% to 80.13%. The highest average water content is the treatment of 1% enzyme immersion which is 78.35% and the lowest in blanching treatment is 74.90%.

C)

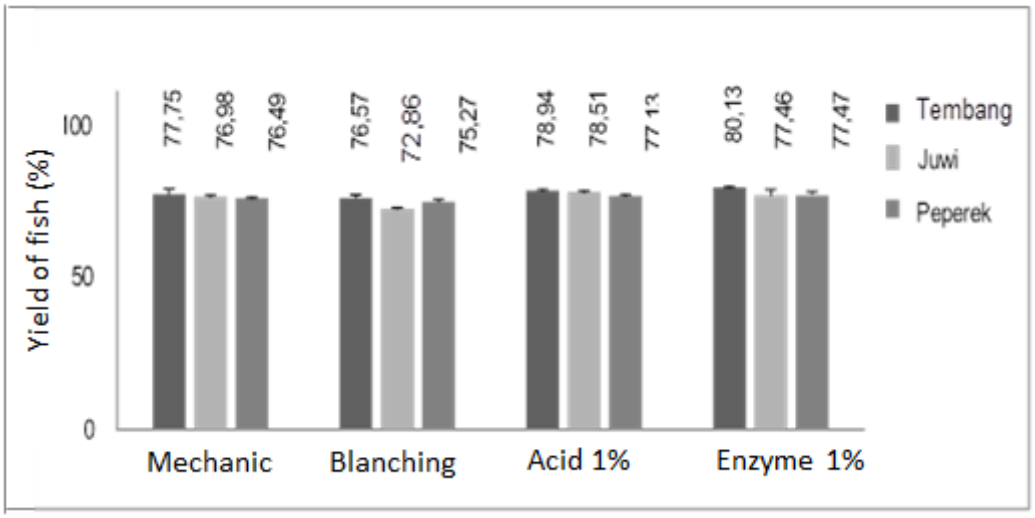

Figure 4. Fish Fillet Water Content

The results of analysis of variance at the test level (α) of 5%, showed that the preparation technique had a significant effect on the water content of trash fish fillets and produced different chemical characteristics. Figure 3, shows that the water content of fish fillets ranges from 70-80%, and is quite good when compared with the water content of Mackerel fish which ranges between 63- 82.1%. In general, the degree of freshness of fish food has a relationship with the water it contains. Water content is also very influential on the durability of foodstuffs [13].

3.2.2. Protein levels

The average protein content contained in fish fillets of Tembang, Juwi and Peperek fish with mechanical treatment was 9.39%; the blanching treatment is 9.55%; with treatment of immersion of 1% vinegar acid solution is 8.56%; and with the treatment of immersion of papain 1% enzyme solution is 8.30% (Figure 4). The highest protein content of fish fillets was found in blanching treatment. However, when viewed as a whole, each fish with different treatments also has different protein content. One factor that causes differences in protein levels is the consumption of different fish feeds. The diversity of fish protein composition is caused by several factors including food,

species, sex and age of fish. The protein content of fish fillets as a whole is slightly lower when compared to fish protein levels in general, which is 18% [14].

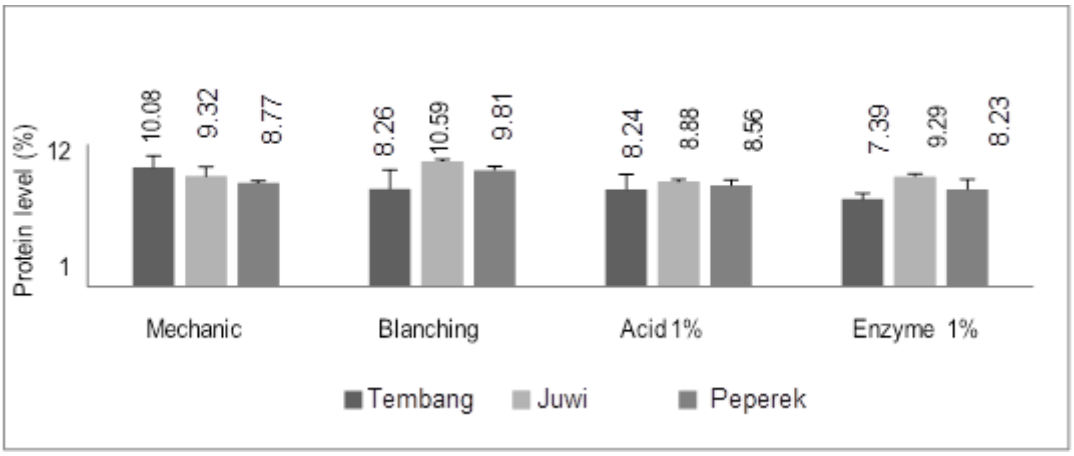

Figure 5. Fish Fillet Protein Levels

3.2.3. Ash Content

The average ash content contained in fish fillets in Tembang, Juwi fish and Peperek fish with mechanical treatment was 2.17%; and the blanching treatment was 2.97%. While the average ash content with treatment of 1% vinegar acid soaking was 2.44% and the treatment of 1% papain enzyme soaking solution was 2.14%. Tembang fish, Juwi fish and Peperek fish have high ash content. However, the ash content is lower when compared to skipjack fish which has ash content of 5%, and is higher when compared to anchovy which has ash content of 0.97% [14]. The content of ash and its composition depends on the type of fish and how it is treated. Besides that ash is an element of minerals or inorganic substances contained in food and is a residual element that remains after the material is burned to be free of carbon. Ash also includes non-volatile components and remains in combustion and annealing of organic compounds [15].

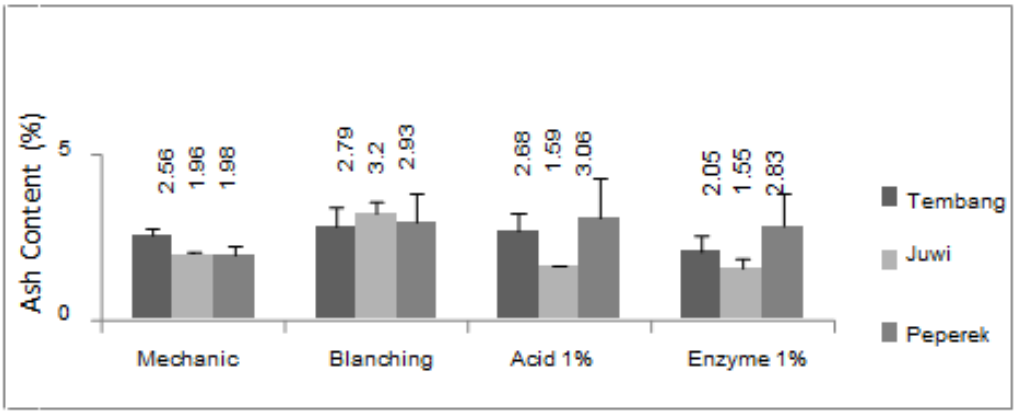

Figure 6. Fish fillet ash content

3.2.4. Fat level

Fats circulating in the body are obtained from two sources, namely food and liver products which can be stored in fat cells as energy reserves [16]. Fat content contained in fish fillets in Tembang, Juwi fish and Peperek fish with mechanical treatment were 6.63% respectively; 8.54% and 8.69%. Fat content in blanching treatment was 6.58%; 5.87% and 7.58%. Fat content in the treatment of

1% vinegar acid soaking was 8.19% respectively; 7.42% and 8.64%. While the fat content in the treatment of immersion of papain enzyme solution 1% was 8.01%; 8.69% and 9.49%.

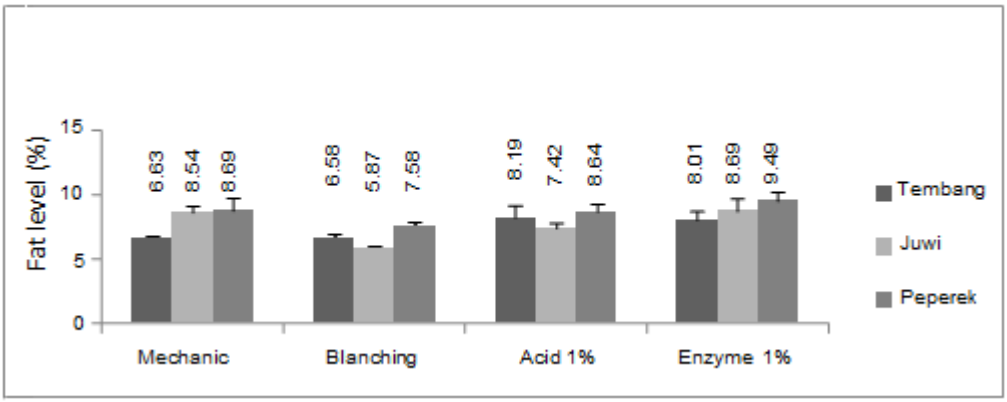

Figure 7. Fat content of fish fillets

The results of the analysis of variance at the test level (α) were 5%, indicating that the preparation technique had a significant effect on the fat content of trash fish fillets. Fat content of the three types of fish above is still lower when compared with fish Mackerel 14.4%. Fish with the lowest fat content is found in blanching treatment. The blanching process causes some of the chemical content to dissolve, so that the chemical content in fish has a lower percentage. Fat content in each type of fish is different, this is caused by an increase in water content. Increased moisture content of the ingredients causes the proportion of fat to decrease [17].

3.3. Functional Properties

3.3.1. Water Holding Capacity (WHC)

WHC is the ability of a food ingredient to hold water. WHC values for fish fillets of Tembang, Juwi fish and Peperek fish in mechanical treatment were 42.72% respectively; 34,697% and 27.64%. WHC value for blanching treatment was 78.9%; 112.39% and 43.24%. WHC value in the treatment of immersion of 1% acid solution was 35.96% respectively; 17.98% and 44.44%. WHC value in the treatment of immersion of 1% enzyme solution is 33.9%; 43.11% and 46.64%.

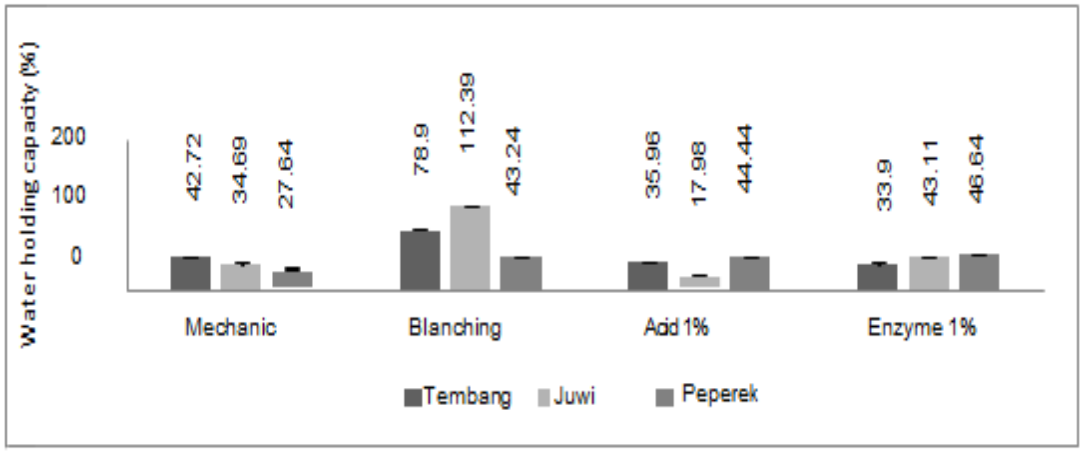

Figure 8. Value of Fish Fillet WHC

The highest WHC value is in fish fillets with blanching treatment. This can be caused by the

bond in the fish fillet tissue that has changed due to the influence of high temperatures, making it easy to absorb water. Besides that the WHC value is influenced by the protein content of meat. The three types of fish have good protein content so they can hold water in fish meat. According to [18], the ability of meat to bind water depends on the amount of reactive protein meat. The increasing levels of meat protein, meat WHC will increase because of the ability of proteins to bind water chemically and decrease in meat fat content.

3.3.2. Oil Holding Capacity (OHC)

Protein in food has the ability to hold and absorb oil. This ability is a functional characteristic that is very important in protein applications in food processing products. OHC values for fish fillets in Tembang, Juwi fish and Peperek fish in mechanical treatment were 34.55% respectively; 23.25% and 13.33%. OHC value in blanching treatment was 59.14% respectively; 71.52% and 55.63%. The OHC value in the treatment of immersion of 1% acid solution was 8.64%; 21.94% and 12.58%. OHC value in the treatment of immersion of 1% enzyme solution was 29.57%; 24.75% and 26.49%.

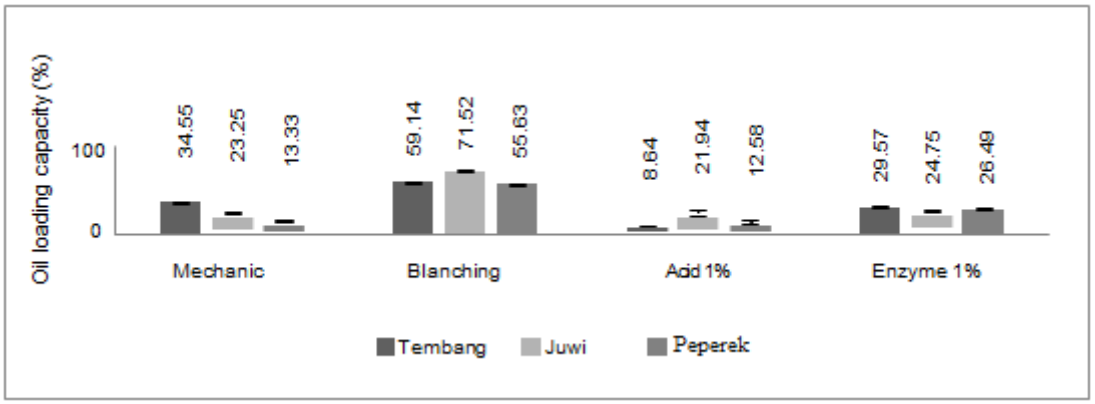

Figure 9. Value of Fish OHC Fillets

Based on the data above it can be seen that the largest OHC value is found in fish fillets with blanching treatment. This can be caused by the bonding of fish fillet tissue that has changed due to the influence of high temperatures, making it easy to absorb oil. In addition, [19] states that oil absorption is not only due to oil being trapped physically in protein but also the presence of non-covalent bonds such as hydrophobic, electrostatic and hydrogen bonds in the interaction of protein fat.

4. Conclusion

Preparation techniques that produce the most yield and fastest preparation time are enzymatic preparation techniques with a concentration of 1%. The best preparation techniques are 1% enzyme, 1% acid, blanching and mechanics. Tembang fish, Juwi fish and Peperek fish possess good chemical content, namely: moisture content (77.46% - 80.13%), protein content (7.39% - 9.29%), fat content (8, 01% - 9.49%) and ash content (1.55% - 2.83%). As well as functional properties, namely: froth power (17.68% - 61.87%), foam stability (50% - 57.14%), emulsion power (3.31% - 4.29%), emulsion stability (1.91% - 3.37%), WHC (33.9% - 46.64%), and OHC (24.75% - 29.57%).

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Economic impacts of agrarian reform on rice farms in the Philippines

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Abstract. Agrarian reform has been, and continues to be, one of the biggest issues in the Philippines' agriculture sector. The most recent and comprehensive policy that aims to improve farmer livelihood is the Comprehensive Agrarian Reform Program (CARP) passed in 1988. Using secondary data of beneficiary farmers provided by the Department of Agrarian Reform (DAR) and through interviewing non-beneficiary farmers, randomly selected through random sampling, the impact of the support services on income, productivity, farm technical efficiency, and farmer welfare were analyzed. Income data showed that agrarian reform beneficiaries were able to better maximize their profits, earning higher incomes, through lower costs provided by the support services. However, production data showed that average farm yields for both beneficiary and non-beneficiary were roughly the same, yet the technical efficiency of beneficiaries were higher. Overall, there was a positive welfare change for beneficiaries

1. Introduction

Land reform is a key aspect of growth in a nation. According to Aghion et al. [1] inequality in land ownership and distribution plays a huge role in the lack of economic growth in developing nations. It is then implied that, through appropriate land reform measures, poverty in a country can be greatly reduced since land is an essential source of income and by providing greater access to land, farmers will have a greater income and, by extension, greater social welfare.

The importance of agrarian reform in the development of the Philippines has always been highlighted by the continuous change in the policies implemented in the country. There have been numerous attempts at establishing a solid agrarian reform program in the Philippines dating back all the way to the Spanish occupation era up until the most recent program, RA 6657 [2] or the Comprehensive Agrarian Reform Program (CARP). However, the implementation of CARP in 1988 added a different aspect to land reform by incorporating the importance of social welfare in its policies. This meant that CARP not only redistributed land, but also included support services such as credit availability, infrastructure support, marketing assistance, and many others which aim to improve the livelihood of farmers by reducing costs and increasing farmer productivity, according to Reyes [3].

Due to the close association between economic growth and the agricultural sector, the Philippines' population is highly dependent on the access to land as a source of income, livelihood and general welfare. Therefore, to increase social welfare within the country's poorer rural populace, the Philippine government undertook agricultural development to spur growth and productivity. However, despite more than two decades under the program, GIFT [4] states that farm productivity in the Philippines remains low compared to other countries in the South East Asian region and there is still a huge gap between the incomes of the rural and urban sectors of society. There is still an income gap between the large landowners prior to the implementation of CARP and Agrarian Reform Beneficiaries (ARBs) today. After more than 20 years of CARP, there is still the question as to by how much the current agrarian reform program increased farm income – if at all - and whether it has improved farmer welfare.

2. Materials and Methods

Data was gathered from 60 respondents, 30 of which represented the Agrarian Reform Beneficiaries (ARB) while the remaining 30 respondents represented the non-Agrarian Reform Beneficiaries (NARB) of *barangay* Dila. Farm productivity, production costs and income were collected through for both groups through primary and secondary data.

2.1. Cost and Returns Analysis

In order to determine the impact of support services on the income and profitability, the net farm income must be determined on a 'per hectare' basis. This is computed by:

$$NFI = TR - TC \quad (1)$$

Where: NFI is the net farm income per hectare (in Pesos), TR is the total revenue per hectare (in Pesos), and TC is the total cost per hectare (in Pesos). The net farm income used in the study shows, not only the investment of the farmer in production, but also the inputs used in farm operations to show provide a picture of farm level income.

2.2. Farm Productivity Analysis

To analyze the effects of CARP on farm productivity, farm yield was computed using the following formula:

$$Y_t = \frac{TP_t}{A_t} \quad (2)$$

Where Y is the average farm yield (cavans/ha), TP is the total production (cavans), A is the area planted to crop per hectare (ha), and t is the time or period.

2.3. Farm Efficiency Analysis

Farm level efficiency is important in order to find out whether farmers are maximizing their production and, in turn, their profit. A production function is estimated using the linear Cobb-Douglas form:

$$lnq_j = \beta_0 + \sum_{i=1}^{5} \beta_i lnx_{ij} + \varepsilon_j \quad (3)$$

Where *q is the* quantity produced in kgs, β is the parameter for estimation, x_1 is the quantity of Land (in hectares), x_2 is the quantity of Fertilizer (in bags), x_3 is the quantity of Chemicals (in Pesos), x_4 is the quantity of Seeds (in kg), x_5 is the quantity of Labor (in man days), *i* is the input to be measured, *j* is the farmer, and ε is the error term. Using the statistical analysis tool STATA12, estimation models were used on 5 different inputs, namely: Land in hectares, fertilizer in bags (50 kg per bag), chemicals in Pesos, seeds in kg and labor in man days, all of which are on a per farm basis, and the values of these inputs turned into natural logarithm to get the log-linear frontier model.

2.4. Economic Surplus Model

In order to analyze the effect of the Comprehensive Agrarian Reform Program on the welfare of ARBs, an economic surplus model was used to find out whether there are welfare gains or losses [5]. An adaptation of the economic surplus model used by Quilloy [5] and as developed by Alston et al. [6] was used. This model places the welfare upon the changes in the price elasticities of the demand and supply curve as well as the change in the cost of rice, assuming a closed economy.

Demand: $Q_d = e_d P_t + e_1 Yt + E_{1t}$ (4)

Supply: $Q_s = e_s P_t + e_2 Q_{t-1} + E_{2t}$ (5)

Where Q_d is the volume of rice demanded, Q_s is the volume of rice supplied, e_d is the elasticity of demand, e_s is the elasticity of supply, Y_t is the average annual price of rice, Q_{t-1} is the lagged value of rice supplied, and E_{1t},E_{2t} are error terms. The elasticity of supply and demand was used to see the changes in consumer surplus after the implementation of CARP. The elasticity can be computed using the computations used by Quilloy [5]:

Percentage change in the volume of rice due to RA 6657	$E(Y) = \left(\frac{(Q_1 - Q_0)}{Q_0}\right)$	(6)
Change in cost of rice due to RA 6657	$k = \left(\frac{E(Y)}{\varepsilon}\right)$	(7)
Relative change in price of rice RA 6657	$Z = kx\left(\frac{\varepsilon}{(\varepsilon + \omega)}\right)$	(8)
Change in Total Surplus	$k \cdot P \cdot Q_0 \cdot [1 + (0.5 \cdot k \cdot \omega)]$	(9)
Change in Producer Surplus	$Z \cdot P \cdot Q_0 \cdot [1 + (0.5 \cdot k \cdot \omega)]$	(10)
Change in Consumer Surplus	$(k - Z) \cdot P \cdot Q_0 \cdot [1 + (0.5 \cdot k \cdot \omega)]$	(11)
Net Welfare Change	$k \cdot P \cdot Q_0 \cdot [1 + (0.5 \cdot k \cdot \omega)] - P \cdot Z \cdot Q_1$	(12)

Where P is the farmgate price of rice per kg, Q_0 is the quantity of rice in kg, $\overline{P}$ and $\overline{Q}$ are the mean values of price and quantity, ε is the elasticity of demand, and ω is the elasticity of supply.

3. Results and Discussion

3.1 Cost and Returns Analysis

Profitability of both ARBs and NARBs were tested according to their beneficiary status and are shown in Table 1. The table shows that, in terms of gross income, NARBs earn PhP14,224.50 more than ARBs. However, it is in the costs that ARBs have an advantage over those not under the program.

However, in terms of cost, one of the largest costs incurred by NARBs comes from the land rent that they pay to the landowner which amounts to an average of PhP24,816.80 per annum. This is in comparison to ARBs who own their land and pay no rent. Furthermore, since ARBs can borrow machinery from their cooperatives, they pay far less on fuel costs.

Overall, Net Revenue is higher for ARBs by PhP9,022.80, as likewise found by previous studies such as Reyes [3] despite the lower gross income. The lack of rend payment significantly lowers the overall cost for ARBs and they are able to spend more on other inputs.

3.2 Productivity Analysis

Comparing the averages of the two different farmer groups, we can clearly see a difference between ARBs and NARBs, as shown in Table 3. ARBs averaged 9,789.43 kg of *palay* per hectare on their farms while NARBs only managed 9,506.14kgs per hectare. We must also note that per cropping yield of the two farms are far higher than national averages, which is 3,700kg/ha according to GIFT (2013), with NARBs and ARBs producing approximately 1,000kg/ha/cropping and 1,100kg/ha/cropping more than the national average *palay* yield, respectively. The figures shown in Table 4 even surpass global rice production per hectare.

However, given the number of support services provided for beneficiaries, the difference is not as large as expected. This implies that CARP has not had much of an impact on the productivity of the farms. This is perhaps because most of the economically focused support services under the program focus on the postharvest processing and marketing support such as farm-to-market roads and storage facilities rather than the actual production process.

Table 1. Average annual costs and returns (PhP) per hectare of ARB and NARB farms according to beneficiary status, Dila, Bay, Laguna, 2013

Item	Beneficiary Status		
	NARB	ARB	Difference
RETURNS			
Cash Returns			
Sales	141,197	126,972.50	14,224.50
Total Cash Return	141,197	126,972.50	14,225.50
Non-Cash Returns	-	-	-
Total Non-Cash Returns	0.00	0.00	0.00
Total Returns*	141,197	126,972.50	14,224.5
COSTS			
Cash Costs			
Inputs			
Land Rent	24,816.80	0.00	24,816.80
Fertilizer	12,503.38	10,990.51	1,512.90
Chemicals	3,979.15	6,942.15	(2,963.00)
Seeds	4,151.80	4,711.86	(560.10)
Fuel	1,347.65	160.18	1,187.50
Irrigation	704.318	2,565.10	(1,860.80)
Production Costs			
Land preparation	14,702.63	18,649.77	(3,947.10)
Planting	5392.96	4007.54	1,385.40
Fertilizer App.	751.30	876.61	(125.30)
Chemical App.	1,141.12	665.71	475.40
Weeding	816.67	5306.09	(4,489.40)
Harvesting	13,638.87	11,476.45	2,162.40
Threshing	14,044.87	11,476.45	2,568.40
Hauling/Transport.	4,151.80	3,124.46	1,027.30
Other Costs	2520.00	0	2,520.00
Total Cash Costs[1]	104,201.12	80,952.87	23,248.30
Total Costs	104,201.12	80,952.87	23,248.30
Net Cash Revenue	36,995.88	46,018.63	(9,022.80)

Table 2. Proportions of annual *palay* yield per hectare of ARBs and NARBs, Dila, Bay, Laguna, 2013.

	Beneficiary Status	
Characteristic	NARB	ARB
Average annual yield (kgs/ha)*	9,506.14	9,789.43
Average yield (kgs/ha/cropping)*	4,753.07	4,894.72

3.3 Technical Efficiency

For NARBs, there were 4 different inputs that were highly significant. The inputs Land, Chemicals, Seeds and Labor were all significant at a confidence level of 1%. The input with the highest coefficient out of these significant inputs was Land with a coefficient of 0.6941 as seen in Table 3. Fertilizer on the other hand, is not significant in affecting the technical efficiency of Non-ARB farms. The high significance of these inputs suggests that mismanagement of one input could lead to high inefficiency which is most likely why NARB farms were far less efficient when compared to ARBs. The huge effect on the technical efficiency of different inputs could also be due to the lack of credit support and other forms of services brought about by CARP.

Following the discussion of Quilloy [7], there is a big difference in the variables that influence the technical efficiency of the farms of ARBs. As shown in Table 3, only Land and Fertilizer are seen to have a significant effect on the technical efficiency of the ARB farms, only one of which is deemed to be highly significant, which is Fertilizer which is seen to be significant at a confidence level of 1%. Chemicals is significant with a P-value of 0.0150 –. According to the coefficients, Land has the highest impact on the technical efficiency of the ARB farms at 0.4667.

Table 3. Result of OLS regression for NARBs and ARBs in Dila, Bay, Laguna, 2013.

Variable	Beneficiary Status	
	NARB	ARB
	Coefficient (Standard Error)	
Constant	7.1014***	5.8237***
	(0.1896)	(1.0687)
lnLand	0.6941	0.4667**
	(0.0250)	(0.1889)
lnFertilizer	0.1111***	0.2991***
	(0.1270)	(0.0510)
lnChemicals	0.0912***	0.0019
	(0.0017)	(0.0092)
lnSeeds	0.1112***	0.1549
	(0.0020)	(0.2074)
lnLabor	0.2110***	0.2255
	(0.0557)	(0.1628)
Sigma-squared	0.2156	0.0399
	(0.0557)	(0.0105)
Log-Likelihood	1.2400	5.7526
F-Value	16.74	27.74
R-squared	0.77	0.85

*significant at a 10% confidence level, ** significant at a 5% confidence level, ***significant at a 1% confidence level

Table 4 breaks down the different efficiencies and it shows that NARBs have an even spread among the different efficiency ranges. Most farmers that are not under CARP are shown to be between 70-80% or 90-100 % technically efficient in their input use. However, there is still a large proportion of farmers (20%) who are largely inefficient and fall below the 60% range of technical efficiency. Looking at the ARBs, however, show that 100% of farmers under CARP and who receive the programs support services are all within the 90-100% efficiency range which implies that ARBs are better trained and more knowledgeable on farm input usage as compared to NARBs.

The computed efficiencies of the two groups showed a large difference in the average percentages between NARBs and ARBs. NARB farms were found to be only 73.76% efficient while ARB farms were almost totally efficient at 99.57%. That despite the small difference in average yields per hectare; Agrarian Reform Beneficiaries are far more efficient in use of these inputs for production than non-ARBs. The higher efficiency implies the ARBs are able to use fewer inputs still produce roughly the same amount as NARBs, which, in turn, helps ARBs cut down on production costs.

Table 4. Ranges of technical efficiency between ARBs and NARBs, Dila, Bay, Laguna, 2013

Efficiency Range	Beneficiary Status			
	NARB		ARB	
	No. of respondents	%	No. of Respondents	%
90-100	7	23.33%	30	100.00%
80-90	4	13.33%	0	0.00%
70-80	7	23.33%	0	0.00%
60-70	6	20.00%	0	0.00%
< 60	6	20.00%	0	0.00%
Total	30	100.00%	30	100.00%
Technical Efficiency	75.18%		99.72%	

3.4. Welfare analysis.

To test the welfare effects of the CARP on the beneficiaries, an economic surplus model was estimated. The estimation of the economic surplus model is shown in Table 5.

The model showed that there is a 0.03% increase in the volume of rice, as shown by the variable *E(Y)*, produced due to the implementation of CARP. This can be attributed to the higher technical

efficiency and better use of inputs as shown earlier in the study. We can also see that the cost of rice production (k) fell by 0.09% for the ARBs. Various infrastructure improvements have made certain post-harvest handling and transportation cheaper and this lower cost of production can be largely attributed to the lack of 'land tax' or land rent that the non-land owners such as NARBs have to pay. Lastly, there is also a relative decrease in the in the price of rice by 0.17%.

Table 5. Surplus model estimation on the welfare effects of CARP in Dila, Bay, Laguna, 2013

Welfare Characteristic	Change in Surplus Model
Elasticity of demand (ε)	-0.50
Elasticity of supply (ω)	0.33
Price of rice/kg *(P)* -	10.51
Quantity of rice produced prior to CARP (Q_0) – (kgs/ha)	9506.41
Percent increase in the volume of rice ($E(Y)$) - (%)	0.03
Relative decrease in the cost of rice production *(k)* - (%)	0.09
Relative decrease in the price of rice *(Z)*- (%)	0.17
Change in total surplus	8,822.36
Change in producer surplus	16,674.55
Change in consumer surplus	7,852.20
Net Welfare Change	8,749.48

Computing for the economic surplus model, the change in the total surplus amounts to PhP8,822.36. The largest change in the surplus model is in the rightward shift of the supply curve which is depicted by the large change in producer surplus of Php16,674.55, indicating and increase in farmer welfare under CARP. This is coupled with an increase in consumer surplus of PhP7,852.20 and the welfare change is quantified by the net welfare change of PhP8,749.48.

4 Conclusion

The Comprehensive Agrarian Reform Program provides its beneficiaries with much needed support services to help aide in farming necessities such as credit support, cooperatives and trainings. Since the extension of the program ends in the year 2014, the study aims to evaluate the extent to which the program has helped the lives of farmers. The study showed that despite lower production quantity as a whole, farmers under the program improved yield by 100kg/ha/cropping over NARBS. Income for ARBs also increased, earning roughly PhP 10,000 more per year than NARBs.All these improvements can be attributed to support services which help with the way farmers utilize the inputs they use. The true objective of the study, and perhaps most significant, is to show whether there has been an improvement in the welfare of the farmers under CARP. The study showed a significant increase in the net surplus. The study has proven that in the Dila ARC of Bay, Laguna, there has been an increase in the welfare of farmers by Php8,749.48 after the implementation of CARP implying that farmer-beneficiaries are better off and have a better standard of living than their non-ARB counterparts.

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The risks of parasitic helminths and protozoan infection in Philippine native swine: Its implication to environmental health and food safety

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Abstract. The study was conducted in selected backyard native swine farms in five municipalities in Quezon Province, Philippines. Farmers are involved with backyard farming of native swine to earn small income. The study aimed to determine the presence of parasites in native swine and the extent of contamination in soil and water in backyard farms. Also, it aimed to asses certain factors in the farming management that could lead to their abundance and prevalence. The results revealed 16 parasite taxa, of which 14 were recovered in feces, 11 in soil and three in water samples. These parasites were *Ascaris*, *Metastrongylus*, *Oesophagostomum*, *Strongyloides*, *Trichuris*, *Balantidium*, *Blastocystis*, *Cryptosporidium*, *Eimeria*, *Entamoeba*, *Giardia* and *Isospora*. *Toxocara* sp. and *Hymenolepis diminuta* were also observed in soil samples. *Strongyloides ransomi* (83.33%) showed the highest prevalence in swine feces, *Ascaris* sp. (55.56%) in soil, and *Crysptosporidium* sp. (11.11%) in water samples. Moreover, the study revealed that the mean density of soil parasites recovered in depth 1 (0-5cm) was much higher (93 eggs/ cyst/ oocyst per gram) compared to depth 2 (9 eggs/ cyst/ oocyst per gram). The difference was shown to be statistically significant at p=0.004. Study also revealed significant association between parasite contamination rates with some farming practices. This would entail that native swine is susceptible to a wide range of parasite infections posing animal and human health threats thus also suggest that proper animal manure disposal should be adopted by the farmers for effective strategies for food safety and public health concern.

1. Introduction

Native swine in the Philippines is considered one of the important animal genetic resources that provides livelihood in the countryside. It has important adaptation traits to unfavorable environment and capable to thrive under low input type management. These desirable qualities are essential for achieving sustainable swine farming. However, mismanagement of livestock wastes can lead to risk to zoonotic diseases [1, 2]. Wastes such as animal feces is a source of pathogenic organisms, mainly bacteria, viruses, parasites and fungi. Depending on prevailing environmental conditions, developmental stages of zoonotic parasites may remain viable in the environment for several months to years until ingestion by definitive hosts [3- 5]. The environmental route of transmission is important for many protozoan and helminth parasites with water, soil and food being particularly significant. Both the potential for producing large numbers of transmissive stages and their environmental robustness (with the ability to survive in moist microclimates for prolonged periods of time) pose persistent threats to public and veterinary health [6, 7]. The increasing demand on agricultural products increase the likelihood of encountering contaminated environments with parasites [8].

The local farmers and residents in the province of Quezon are currently involved in raising organic native swine in their backyards. These organically-produced swine are bound for the production of export quality, the roasted whole swine meat or locally known as *"lechon"*. This economic activity has been openly supported by their respective local government units. However, along with the promotion of this agricultural activity is the lack of baseline knowledge and information on the different parasites present in the organically-produced swine. This increases the exposure of the farmers, the households and consumers to risks pertaining to health and nutrition.

This study aimed to contribute to the knowledge on how we can reduce the risk of parasite contamination in local farms for animal and food safety concerns. In general, the study aimed to determine the extent of parasite contamination in selected backyard native swine farms in Quezon Province, Philippines, and the risk factors associated with knowledge, attitudes and practices in native swine farming.

2. Methodology

2.1 Study Area

The study was conducted in selected backyard farms of native swine in Quezon province (Figure 1.0). The province shared the highest number of farms in the region. And it has estimated human population of about 1,987,030 as of 2010 census. The province topography is characterized by rolling, steep to very steep terrain with few plains, valleys and swamps. Climate of the province is characterized by the absence of dry season. Average annual rainfall is about 111.56 inches [9].

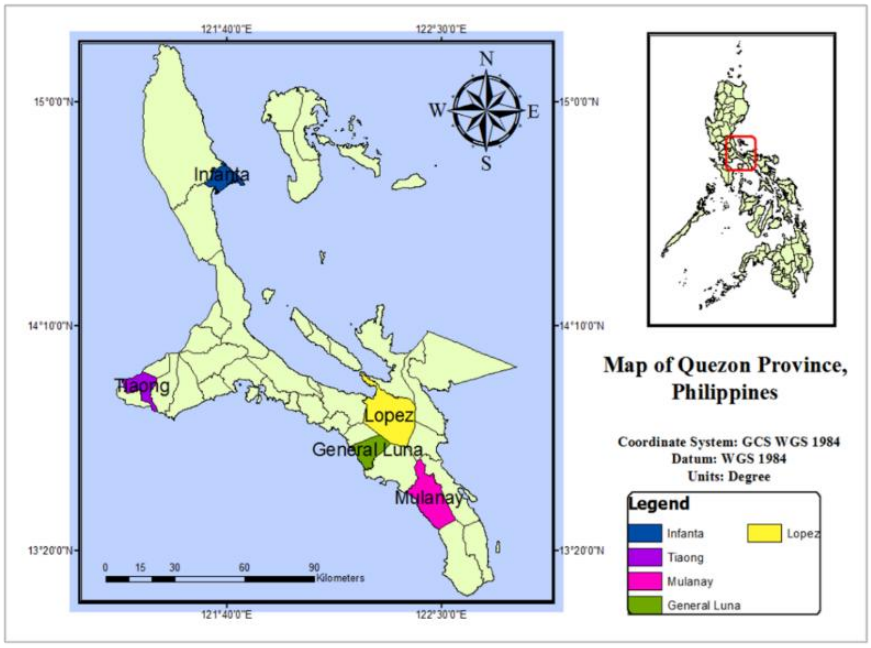

Figure 1.0. Map of Quezon province showing the study area.

2.2 Collection of Samples

Fecal samples were collected from 36 native swine which did not undergo deworming procedure for past six months to avoid bias results. Prior to fecal collection, ethics clearance was secured from the Institutional Animal Care and Use Committee (IACUC) with protocol number 2015-004. Upon collection, about five grams from freshly passed out feces from native swine was stored in a container with 10% formalin prior to laboratory examinations.

A total of 90 soil samples were also collected randomly. In order to improve the precision of the resulting soil-transmitted helminth (STH) estimates, three replicates of soil samples was established in

each farm, and each of this replicate has 1-5 *cm* (layer 1) and 6-10 *cm* (layer 2) depths. Soil sample of 200 grams was collected per layer then air-dried overnight and kept in the laboratory at room temperature.

For water samples, the collection was done via available sources (faucet, drums and deep well) for native swine consumption. A total of 135 water samples were collected, of which three replicates per water source were being sampled in each farm. Subsequently, each sample with 10 liter volume was directly poured into container and transported within 24 hours in the laboratory for filtration of suspected parasites.

2.3 Processing of Environmental Samples

Formalin Ethyl Acetate Concentration Technique (FEACT), modified Sucrose Floatation Technique (SFT), and Acid-fast staining (AFS) and Immunofluorescence Assay (IFA) were used to isolate parasites from feces, soil and water samples, respectively. Briefly, in FEACT, 7 ml of a suspension formalinized feces was strained and added with 3 ml ethyl acetate then centrifuge for about 1800 rpm for 5 minutes. After centrifugation, decantation follows leaving the pellets at the bottom of the tube intact for examination of parasites. Meanwhile in SFT, 2 grams of strained and completely air-dried soil was processed. Briefly, 2 grams of soil per sample was washed with distilled water, mixed and then centrifuged at 1800 rpm for 10 minutes. Followed by decantation, the sediment was added with sucrose solutions with specific gravity of 1.2, then in 1.3.

Furthermore, AFS and IFA were used to recover *Cryptosporidium* oocysts and *Giardia* cysts protozoans from water, feces and soil samples. Samples were filtered using modified filtration device adopted from Komatsu et al., (2014). After filtration, the filter membrane was removed and washed with appropriate amount of distilled water. The washings were collected into the test tubes and centrifuge for about 10 minutes in 1100 rpm. Followed by decantation, the precipitate was kept for protozoan analysis. IFA test was done to confirm further the presence of *Cryptospordium* and *Giardia*. Procedure was followed according to manufacturer's instruction. This test allows binding of the fluorescein-labelled mouse monoclonal antibody reagent to a specific oocyct/cyst. Observation of cyst and oocyst were done using 200x magnification, then 400x, and 1000x for confirmation. *Cryptosporidium* and *Giardia* fluoresced bright apple green. The IFA test is positive if one or more oocysts or cysts are present.

2.4 Knowledge, Attitudes and Practices (KAPs) on Native Swine Farming

A key informant interview was conducted to record KAPs in each farms. Prior to data collection, informed consent was obtained from all participants. The survey questions was modified from several previous studies related to animal and public health, sanitation and hygiene, environmental sustainability, to food security and biosafety [10-14]. The questionnaires were translated with their local dialect and was approved by ethics committee. To reduce information bias, the questionnaire was pretested.

2.5 Data Analyses

The contamination rate (%) and mean density of parasites were obtained for feces, soil and water samples. Data were analyzed through IBM SPSS Statistics version 20. Spearman rank's correlation analysis was used to associate the parasite intensity and the age and body weight of the native swine. Chi-square Test was also used to associate the knowledge, attitudes and practices (KAP) in native swine farming to the parasite intensity in feces, then parasite density in soil. In addition, Point-biserial correlation analysis was used to associate the contamination rate of parasites recovered in water and the KAP results. Furthermore, comparison of mean density of parasites between two soil depths was analyzed using Independent sample's T-test. Descriptive analysis was also employed for the qualitative data generated from the coded KAP survey.

3. Results and Discussion

3.1 Prevalence and intensity of parasites in native swine feces

The results of the study revealed that out of 36 native swine sampled, 35 (97%) were infected with two or more parasite species. As shown in Table 1 a total of 14 parasite taxa were recovered from fecal samples and it showed that *Strongyloides ransomi* has the highest prevalence (83.33%). Association between the helminth parasite intensity showed no correlation with native swine weight and age. However, *Trichuris suis* showed significant positive correlation with sex (r=0.343). On the other hand, parasitic protozoans, *Balantidim coli* showed significant moderate correlation with host's weight (r=0.494) and age (r=0.453). While *Giadia* spp. showed significant negative moderate correlation with weight (r=-0.423) and age (r=-0.441). *Isospora suis* also showed negative moderate correlation with sex (r= -0.368). And lastly, *Eimeria* sp. also showed significant negative moderate correlation with sex (r=-0.368) and age (r=-0.391).

Strongyloides ransomi, commonly known as swine threadworm is considered ubiquitous and common nematode parasite infecting swine and reported pathogenic in young suckling swine while adult swine were immune to threadworm infection due to well-developed immune system [15]. However, this contradicts the results of this study which shows that *Strongyloides ransomi* infection showed no association with swine age and body size. *S. ransomi* for instance has complex life cycle and undergo series of transformations which involves development in important medium, the soil environment [16, 3]. This nematode is unique among helminths because all stages are considered parasitic, the female worm inside the intestine of its hosts, and the free-living generations, the males and females outside its host which are both parasitic [17]. The said life cycle might be part of the caused why *S. ransomi* showed the highest infection rate among helminths infecting native swine in the study area. Consequently, this parasite is already reported in tropical countries however, infection among native swine have generally received little attention from veterinary parasitology, despite of worldwide prevalence reported [18]. On the other hand, among the parasitic helminths only *Trichuris suis* showed significant moderate positive correlation with swine sex and body weight; all other helminths showed no association. Similar finding showed that males are more often susceptible to parasite infection because of their competitive ability and behaviour [19].

Moreover in protozoans, *Balantidium coli* was already reported for being zoonotic and causes diarrhoea in a wide range of mammals all around the world especially domesticated swine serving as their reservoir host [20, 21]. Recent study on prevalence and sustainable control of *B. coli* in swine was studied and showed that infected swine were being controlled effectively using synthetic medicines [22]. In addition, *Giardia* sp. showed moderate significant association in the native swine weight and age. *Isospora suis* also showed moderate significant association in sex of native swine. Moreover, *Eimeria* sp. also showed moderate significant association in sex and age of the native swine. According to some previous studies, *Giardia, Isospora and Eimeria* are the common coccidians infecting swine [23-26].

Table 1. Prevalence and mean intensity of parasites recovered from native swine feces in selected backyard farms in Quezon Province, Philippines.

Parasite Taxa	No. of Infected Swine (n=36)	Prevalence (%)	Mean Intensity (egg/ cyst/ oocyst per gram)
Helminth Eggs			
Ascaris suum	8	22.22	167
Metastrongylus sp.	4	11.11	16
Oesophagostomum sp.	15	41.67	42
Strongyloides ransomi	30	83.33	427
Trichuris suis	8	22.22	48
Protozoan Cyst/ Oocyst			
Balantidium coli	6	16.67	35
Blastocystis spp.	8	22.22	164
Cryptosporidium sp.	11	30.56	3
Eimeria sp.	4	11.11	10
Endolimax nana	4	11.11	45
Entamoeba spp.	9	25.00	132
Giardia spp.	3	8.33	24
Iodamoeba butschlii	17	47.22	248
Isospora suis	7	19.44	5
Total	**35**	**97.22**	**1063**

In general, sex, age and weight of the native swine were among the morphological factors in which protozoan infection is prevalent in the study area. The young swine when compared to adult swine is more susceptible to *Balantidium coli, Giardia, Isospora* and *Eimeria* infection [22]. However, indicating that there are some of the swine morphological features showed no association with the recovered parasites, hence some swine are reported to develop resistance to parasite infection [27]. The respective diseases which these parasites cause are reported to be zoonotic. These parasites are among the major biological constraints contributing to the low productivity of swine and hampered the economic benefits. Moreover, outcomes of these diseases to mortality among animals and humans have shared significant impact on the water industry, hence affordable or effective water treatment strategies must be addressed [28].

Furthermore, there are various trends in parasite infection in native swine in relation to age and body size. This could be also due to other factors such as farming practices and management systems. Some of the confounding factors might as well contribute to the parasite transmission dynamics such as immunocompromised native swine, inadequate waste disposal systems in the farm, availability of possible reservoir hosts in the area such as the wild rodents, availability of septic tanks, or in general could be due to the farmers that lacks awareness for proper and standard farming management and practices [29, 30].

3.2 Contamination rate and density of parasites from soil and water samples

Out of 90 total soil samples examined, 79 (87.78%) were contaminated with parasites. Contamination rate was higher with helminths (52%) compared to the protozoans (48%). Soil samples recovered a total parasite count of 1153 with mean density of 15 eggs/cysts/oocysts per gram. Among the parasites recovered from the soil, *Ascaris* sp. has the highest prevalence (55.56%). Furthermore, soil contamination with parasites between depth 1 (0-5 cm) and depth 2 (6-10 cm) also revealed significant difference between the soil depths in terms of intensity of parasites ($F(1,10)=13.67$, $p<0.004$) and proved that depth 1 (0-5cm) was higher ($M=2.40$, $SD=0.373$) compared to depth 2 ($M=1.21$, $SD=0.696$). Consequently, the parasites recovered between different soils depths varied in composition. In depth 1 (0-5cm), *Cryptosporodium* sp. showed the highest mean density, followed by *Giardia* sp., *Ascaris* sp., *Isospora* sp., *Strongyloides* sp., *Trichuris* sp., *Toxocara* sp., *Oesophagostomum* sp., *Eimeria* sp.,

Metastrongylus sp., and then *Hymenolepis diminuta*. On the other hand, in depth 2 (6-10cm), *Metastrongylus* sp., *Oesophagostomum* sp., and *Eimeria* sp. were not recovered.

Meanwhile, water samples were detected contaminated with protozoans, namely; *Isospora* sp. (43%), *Cryptosporidium* sp. (36%) and *Giardia* sp. (21%). Highest mean density was observed in *Cryptosporidium* (11 oocysts per gram), followed by *Isospora* sp. (4 oocysts per gram), then *Giardia* sp. (3 cysts per gram). However, there was no significant difference among the mean density of the three protozoans (p=0.361). These protozoans were isolated from water sources such as deep well and water drums from 10 farms. Also, it is important to note that no helminth eggs was detected in the water samples.

3.3 Knowledge, Attitudes and Practices (KAP) in Backyard Native Swine Farming

Among the recorded KAPs in the survey, the kind of feeds (r=-0.550), herd size (r=-0.566) and the kind of roughage (r=-0.584) showed significant relationship with the parasites intensity in native swine feces. The association between the KAP and mean density of parasites recovered from soil showed high association to the level of confinement, floor type of pen, farmers perform pest/ rodent control, farmer's awareness on proper composting of manure, and willingness to attend training courses on livestock farming. Moreover, contamination in water with protozoans showed significant association to the floor type of pen, farming experienced, routinely used of disinfectants, source of water for pigs, kind of dewormer, farming experience and attendance to training courses about livestock farming. Consequently, in order to maintain an effective preventive measures on parasitosis, public health awareness must be addressed among people especially those in agricultural remote areas with poor medical facilities [7]. Moreover, epidemiological surveys on soil-transmitted helminths infection must be addressed especially on analyzing wide range of possible route of infection because soil is an effective substitute for fecal examination as well [32].

4. Conclusion

Assessment of parasites in livestock farming shows importance in establishing good quality agricultural products. Those parasites recovered were considered as zoonotic or the infection can be transmitted from animal to humans. Many in the rural areas in developing countries have little access to health services and this pose another challenge to public health. Thus, good agricultural practices should be adopted based on science-based policy recommendation through capacity building of various stakeholders.

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Agriculture extension technique as an accelerator of adoption innovation

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Abstract. Agriculture is an important industry. Most European countries that indutrialized are known to have been based primarily agricultural communities. Agriculture in Indonesia specially rice was mostly personal production (not indutrialization) which mean every farmers can manage their own farm freely. The government facilitating farmers development with agriculture extension that help farmers overcome their problems in fields and giving information about new technique or ways to gain a better results. The role of agriculture extension can be accelerator of agriculture development in Indonesia because they can be the bridge between the researcher and farmers. They can help farmers to adopt new technique that can be applied in their fields to improve their production One of the most intensive training to do a success agriculture training was on fields training. This method of learning can help farmers to conduct a better way of planting and growing rice that can produce a better harvest. The purpose of this research was to introduce an agriculture extension intensive training to help accelerate adoption of innovation for better farming activity on farm. The result showed that intensive agriculture training was an effective way to accelerate farmers decision to adopt new innovation.

1. Introduction

Every year there are a lot of research and publication generated by the researcher about better way to achieve greater harvest and green agriculture. The researcher always give a lot of effort and funding to conduct a research. It was not only require time and work but also spent money. All of this research results propose a better way in conduct farming on farm that will make farmers have a better knowledge in how to manage their farming business. Unfortunately not all the farmers in Indonesia are proactive to learn new method of farming. They usuallly conduct their farming business on how their ancestor teach them.

Indonesia's citizens are depended heavyly on agriculture for livelihoods. The numbers of farmers household was a lot and they depended their livelihood solely from the harvest. It was important to introduce a new and effective method of agriculture to them. The effectiveness and efficiency of agricultural technology transfer and its advisory service play an important role in agricultural development and can improve the welfare of the farmers who lived in rural areas.

Indonesia's farmers facing an constraining structure such as a unwillingness of farmers to adopt new innovation, old farmers with a low education level, and ineffective agriculture extension method. Based on Giddens [1] social systems are composed of patterns relationships between actors or collectives reproduced across time and space. Giddens also states that structures can be analysed as rules and resources. We want farmers to be able to adopt new innovation quicker, hence we can try to remove constraining structure and replaced it into enabling structure. One way to achieve that by conduct on fields training for farmers.

On the other hand there is also agency that can be enabling or constraining factor. Agency based on Kinseng [2] capacity of an actor to think and act as independent person, free and autonomous. This agency in this case was farmers. They can make their own decisions. They can be enabling or constraining factors on adoption innovation process. We need to conduct proper way to convey new

innovation that will help them made the best decision for their farm. The purpose of the study was to explain the impact on fields training to improving farmers decisions in adopting innovation in their farm.

2. Methodology

A qualitative research method using a case study approach was used for this research. The case study, as describe by Yin [3] and case-based reasoning are appropriate methods given the context and restrictions of the inquiry. This case was used as sampling of certain area that already doing fields training in Indonesia. Semi structured questions was used to get the data for this research. The main criterion of informant selection was that they must have had close links with the fields training that happen in that area. He/she must have insightful knowledge on fields training and able to describe the activity that happen in that area.

Semi-structured, probing questions were framed from the contextual setting to increase clarity and completeness. We also conduct observation in the area that doing fields training to get better understanding on the transfer knowledge process that happen in fields. Observations and documentations complemented data collection to triangulate results for trustworthiness.

As per data analysis, comparative method was used to compare information from the informant and used it against each other for emergent themes and consistency was done to ascertain the credibility of the information obtained.

3. Results and Discussion

Agricultural extension officer that handling Gotong Royong 2 farmers group in Klasemen village, Probolinggo district, were asked question pertaining to their ability to transfer appropriate agricultural technology to small-scale farmers. The question was regarding their understanding of the new planting technique that will be adopt by the farmers group member. The question was to make sure that the agricultural extension officers was having appropriate informations on the correct way to applied the innovation.

The famers also given the question not only about the new technique but also on their impression of the agriculture extension officers and the fields training. We probe more about their understanding on the innovation and compare to the answer of the agricultural extension officers and the results was the same. This means that the farmers get better understanding on how to applied the innovation in their fields.

The fields training is start from the stage of preparing seeds, until harvest. But the most critical process is on the planting because the agriculture extension officer is trying to introduce new planting technique that will generate more harvest. The agriculture extension officer was welcome by the farmers because they trust her. She use local languange to the farmers so they can understand better. Not only using local languange but she also giving guidance to farmers and their laborer about how the proper way to applied the planting technique on fields. In this way, farmers who never know or heard about the innovation able to practice the innovation in a correct way.

The results after adopting innovation from the researched that conduct by Putri [2018] was increased of productivity from 6,4 ton/Ha to 7,1 ton/Ha. This increasement is the results of applying an innovation in correct way. Structures change from constraining to enabling trough on fileds training that have a good impact on raising farmer’s harvest. When convey or introducing new technique or innovation to farmers was tricky because if the farmers didn’t see obvious results after applied a new innovation it will be hard for them to trust agriculture extension again next time. Hence we need to be sure that farmers applied the technique/innovation in correct ways that similar with the information from scientist.

4. Conclusion

Agriculture extension was playing an important role as the bridge between researcher and farmers. They can convey the innovation that created by researcher to farmers. This activity was better by doing fields training. In fields training farmers not only get the visual information but they also get to know the information from the example that given by the agriculture extension officer, he or she also able to give

guidance to the labor that works in fields, with this close monitoring the information of new innovation will transfer better.

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Physical and anthropogenic characteristics-based landslide spatial pattern analysis in agricultural catchment

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Abstract. Landslides are naturally occurring events when the soil material move downhill on it's slip plane. This phenomenon is often occured in hilly areas in Java Island, Indonesia, especially in Karangkobar Catchment. Even though it occures naturally, landslide events can increase dramatically due to interference from anthropogenic activity. This study aims to understand the pattern of occurrence of landslides based on anthropogenic and physical characteristics of landslides in agricultural catchment of Karangkobar. Spatial analysis using a geographic information system was carried out on anthropogenic and physical parameters that were overlaid at landslide events to obtain landslide occurrence patterns. The anthropogenic parameters are described by land use while the physical parameters are described by the elevation, slope, aspect, distance to the river, and physical properties of the soil. Furthermore, cross tabulation analysis was carried out between the physical and anthropogenic characteristics of landslide events. Landslides in the Karangkobar watershed mostly occur on slopes facing north and southwest with a moderately steep slope (15-45%) located between 1000 and 1100 msl. Many landslide event are mostly occured in moderate permeability loam soil. Intensive agriculture on farm land present the highest landslide event compared to other types of land use.

11. Introduction

Landslides are naturally occurring events when the soil material move downhill on it's slip plane. This phenomenon is often occured in hilly areas in Java Island, Indonesia, especially in Karangkobar Catchment. Even though it occures naturally, landslide events can increase dramatically due to interference from anthropogenic activity. Intensive agriculture in farm land represent the anthropogenic activity in the Karangkobar Catchment area. This activity is not only carried out in flat areas but also in hilly areas. Mountain farming which practiced in Karangkobar is widely practiced in various places in the world [1-4] as well. This study aims to understand the pattern of occurrence of landslides based on anthropogenic and physical characteristics of landslides in agricultural catchment of Karangkobar.

Figure 4. Landslides in Karangkobar Catchment

12. Methods

12.1. Research Location

Karangkobar Catchment (7°16'00.00"S 109°43'39.02"E) is located in Karangkobar Sub-District, Banjarnegara District Central Java, Indonesia. Karangkobar Catchment is a 4th Strahler's river order catchment and encompass 1,047 hectares area. Agriculture is the main activity of the catchment dwellers.

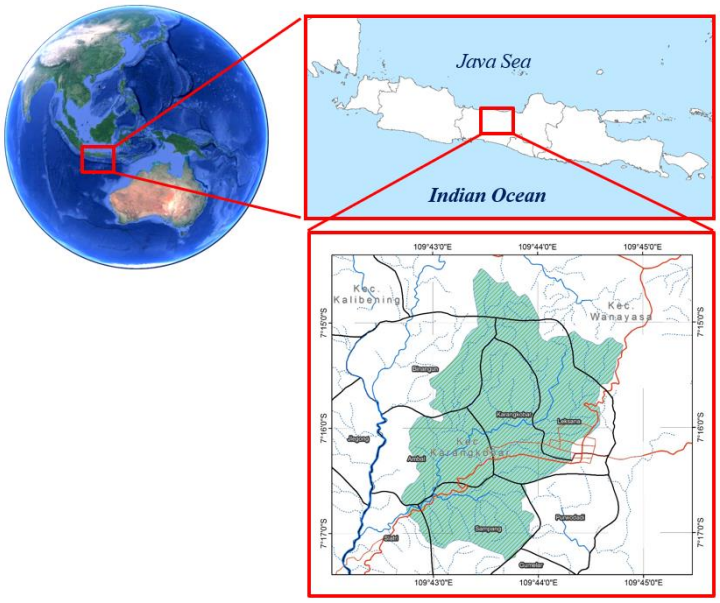


Figure 5. Karangkobar Catchment Location

12.2. Landslide Spatial Pattern Analysis

Analysis of the spatial pattern of landslides is done by overlaying between landslide events maps with physical and anthropogenic characteristics. Overlaying both maps is done on geographic information system software. The characteristics used in this analysis are physical aspects and anthropogenic aspects. The physical aspects used are elevation, aspect, slope, distance from the river, toposequen, and soil physical properties (texture and permeability). The anthropogenic aspects used include land use which represents an anthropogenic characteristic of a land.

12.3. Most Occurrence Analysis of Landslide Events

Analysis of landslide events that occur most frequently in a parameter is analyzed so that the most commonly encountered landslide events occur in each parameter. Most occurrence analysis is carried out and represented using bar chart. In addition to analyzing each parameter singly, multi parameter analysis is carried out using cross tabulation.

13. Result &andDiscussion

3.1. Landslide Event Spatial Distribution

Karangkobar Catchment is a landslides prone catchment. Census of landslides carried out in August 2018 found a total of 45 landslide event with spatial distribution as shown in Figure 3. The middle stream area of Karangkobar Catchment has the most landslide events, which is 30 events (67%). The upstream area has 8 landslide events (18%) and downstream has 7 incidents. The difference in the number of occurrences between the downstream, middle stream and upstream which is quite striking is presumably influenced by several parameters used in this study both physical and anthropogenic parameters. In some locations there were clustering of landslide events, at least 3 clusters of landslides were found. The upstream area was discovered by a landslide cluster which contained 8 landslide events.

middlestream areas are found in two clusters, namely the northern cluster and the southern cluster, each cluster has 15 landslide events.

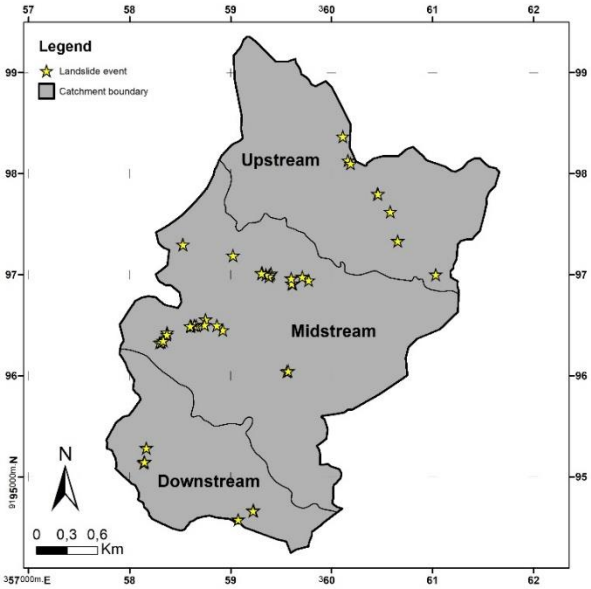


Figure 6. Karangkobar Catchment Location

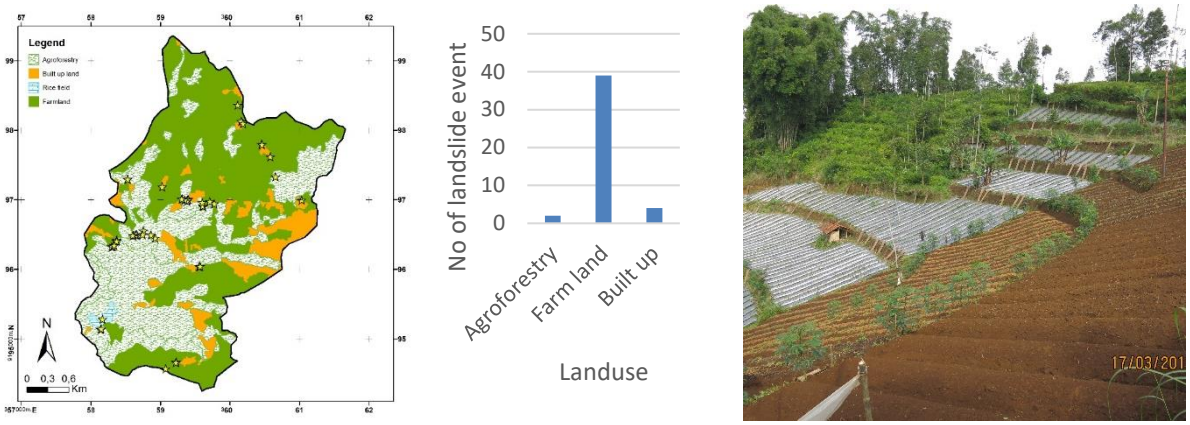
The spatial distribution of landslides found in the Karangkobar Catchment analyzed the pattern using several parameters both anthropogenic characteristics and physical characteristics of the land. Anthropogenic characteristics are indicated by land use conditions. The physical characteristics of the land include elevation, slope, aspect, and soil physical properties (soil texture and soil permeability).

3.2. Anthropogenic Characteristics of Landslide Event

Anthropogenic properties of land can be represented properly using land use. Land use is a form of activity carried out by humans. The type of land use that can be found in the Karangkobar Catchment consists of 4 types of land use, namely agroforestry (39%), built up land (10%), rice field (1%), and farmland (50%) as presented in Figure 4. As the most extensive type of land use, farmland has the highest number of landslide events, namely 39 landslides. The second highest event occurred in built up land, namely 4 events and in agroforestry 2 events.

Farmland is intensive agriculture with agricultural commodities in the form of crops (cabbage, potatoes, chili, spring onion, cauliflower). The pattern of farmland cultivation is mainly on land with sloping slopes mostly perpendicular to the slope. Moreover, on farmland land there are no agroforestry patterns or no / very few tree stands. The implication is that there is no canopy cover in addition to seasonal crops, there is no deep root that serves to grip the ground given the roots of annual plants are only a maximum of 30 cm. In addition, the land on the farmland land is intensively processed by means of planting, this activity can increase infiltration so that more water enters the soil and increases the potential for soil derailment in the sloping area so that landslides occur.

Agroforestry is a land cultivation system or landuse where forestry, agriculture and livestock activities are combined together [5]. In the research area, agroforestry is mix planting between annual crops and tree stands. This type of land use is more beneficial both ecologically and economically. Ecologically, agroforestry land conditions have a better hydrological ability, namely by means of standing trees able to intercept some of the excess water so that not all of them fall to the surface of the soil and which is infiltrated so as to reduce the potential for derailment. In addition, the roots of deeper trees can make the soil have stronger aggregates.



(a) **(b)** **(c)**

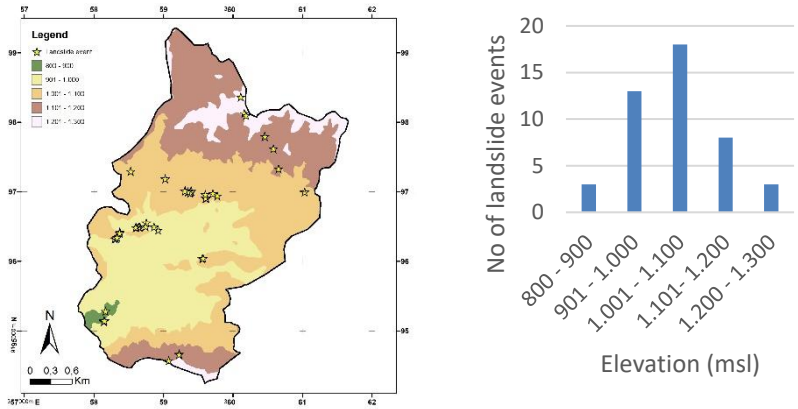
Figure 7. Anthropogenic Land Properties and Landslide Events,
(a) spatial distribution, (b) most occurences analysis, (c) farmland in research area

3.3. Physical Characteristic of Landslide Event

a. Elevation

The elevation characteristics of the Karangkobar catchment are illustrated in Figure 4a. Karangkobar's catchment elevation has variations ranging from 800 to 1,300 msl. The highest elevation lies in the northern region which is also the upper catchment area and the southern part of the catchment. The lowest elevation is located in the downstream catchment area exactly adjacent to the Karangkobar Catchment outlet. The pattern of landslide events based on elevation forms a parabolic curve along with the increase in elevation. At low elevations, landslide events are relatively low, along with increasing elevation landslide events increase until the peak occurs at an elevation of 1,001-1,100 msl. Then the landslide event falls again along with the increase in elevation.

Crosstabulation between landuse and elevation shows that the highest landslide occurrence occurs in elevation conditions between 1,001-1,1000 msl in the type of farm land use (Table 1). The second most landslide occurred at an elevation of 901-1,000 msl which also occurred in the type of farm land use. This confirms the analysis in the previous section. At this elevation landslides occur in many types of farmland land use. This indicates that farm land use is carried out intensively in the elevation range. In addition, landslide events on farm land also occur even at the lowest elevation class of 800-900 msl. In contrast, agroforestry land use types have the least landslide events, namely 2 landslide events. Both landslide events only occur at an elevation of 1,001-1,200 msl.



(a) (b)

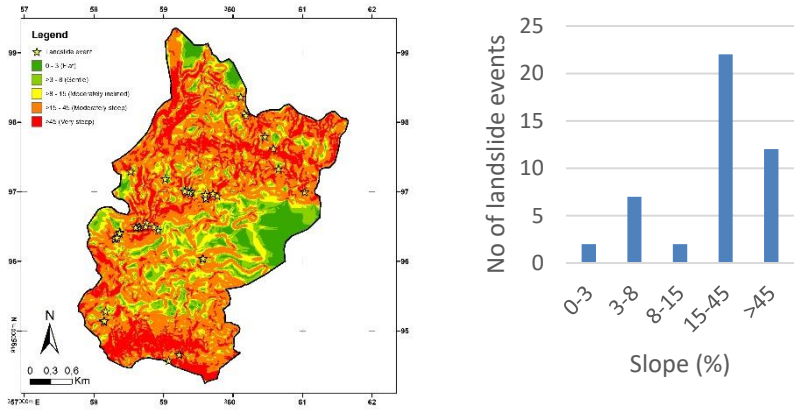
Figure 8. Land Elevation and Landslide Events,
(a) Elevation map, (b) Number of landslide event per elevation class

Table 4. Landslide Event by Land Use and Elevation Crosstabulation

Landuse	Elevation (msl)				
	800 - 900	901 - 1.000	1.001 - 1.100	1.101- 1.200	1.200 - 1.300
Agroforestry			1	1	
Farm land	3	9	17	7	3
Built up land		4			

b. Slope

The slope in the study area is divided into five slope classes (Figure 4b). These classes are from flat (0-3%) to very steep (> 45%). Very steep slope classes can be found in almost all regions, both upstream, middle and downstream catchments. The regions that are classified as flat (0-3%) to gentle slope (3-8%) are located in the middle of the right part. The region is the center of economic activity in the Karangkobar District. The pattern of landslide events on the slopes in the Karangkobar Catchment also has the same tendency, which is parabolic. Landslide events increase along with the increase in slope and the peak is at a slope of 15-45% (slightly steep) then falls again on the slope> 45% (steep).



(a) (b)

Figure 9. Slope and Landslide Events,
(a) Slope map, (b) Number of landslide event per slope class

The results of crosstabulation analysis showed that the land with the type of farm land on the slopes between 15-45% was the area that had the most landslide events, namely 21 events (Table 2). Not only occur on slopes with high grade only, landslides also occur on low slope slopes, even in flat slope classes (0-3%). In contrast, the smallest occurrence occurred in agroforestry land, namely 2 events. Landslide events in agroforestry areas only occur on steep slopes (15-45%) to very steep (> 45).

Table 5. Landslide Event by Land Use, Slope and Aspect Crosstabulation

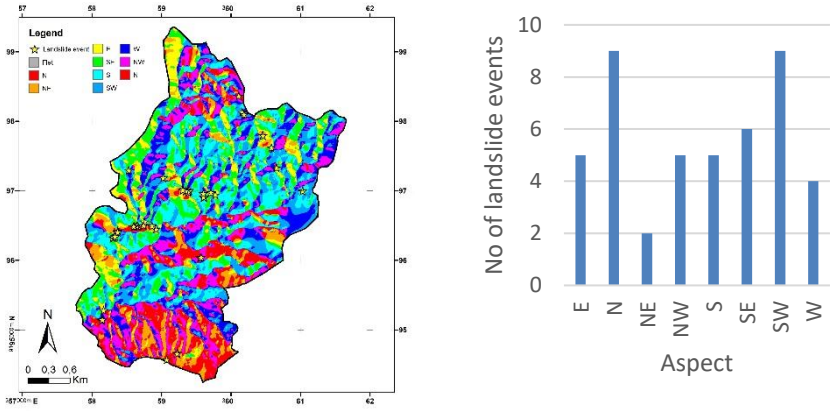
Landuse	Slope (%)					Aspect							
	0-3	3-8	8-15	15-45	>45	E	N	NE	NW	S	SE	SW	W
Agroforestry				1	1	1						1	
Farm land	2	7	2	21	7	4	5	2	5	5	6	8	4
Built up land					4		4						

c. Aspect

Aspect is divided into 8 directions. Aspect has a close relationship with sun exposure both duration and intensity. In the tropics, the variation of the moon's irradiation time is not too different because the distance of the sun is not too large. Even so, the direction of the sun's radiation always occurs as much as 4 times when the sun moves north to the tropic of cancer and when the sun moves south to the tropic

of capricorn. The study area is located at coordinates 7 ° 16'00.00 "S 109 ° 43'39.02" E which indicates that this region is located in the southern hemisphere. The distance of the sun is closest to the study area if the sun is at the tropic line of capricorn, at that time the intensity of the sun's radiation is higher than when the sun is in the tropic of cancer. As a result, the intensity of the irradiation of the group of slopes facing south is higher than the slope group located in the north.

Data processing proves this. The slope group facing south (S, SE, SW) has a higher number of landslide events (20 events) than the north-facing slope group (N, NE, NW) which has 16 events. Furthermore, when viewed from the western and eastern slope groups, the west side slope group (W, SW, NW) had a higher incidence of landslides (18 events) than the east-facing slope group (E, SE, NE) which had 13 events. This shows that the solar radiation intensity on the morning to afternoon is lower than solar radiation in the afternoon to evening. This results in more intensive physical weathering, especially on land that does not have vegetation cover. This result is strengthened by crosstabulation analysis (Table 2) which shows that on land that does not have a cover (farmland) has the highest landslide incidence compared to other types of land use.

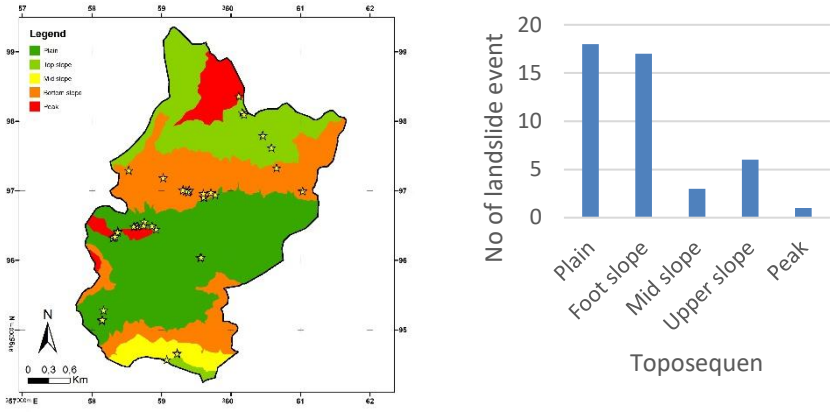


(a) (b)

Figure 10. Aspect and Landslide Events,
(a) Aspect map, (b) Number of landslide event per aspect class

d. Toposequen

Toposequen is a sequence of topography that represents the elevation and slope of the slope. Toposequen has a close relationship with the geomorphological process. This study uses 5 toposequents, namely peak, upper slope, middle slope, lower slope, and plain. Toposequen peak can be found in the upstream and central regions, toposequen slopes that are directly below the peak toposequen can be found in the northern and southern regions of the catchment. The rest are topose lands. Logically, the terrain topose has the chance of the lowest landslide, the slope has a higher chance, and the peak topose has the highest chance of a landslide. Even so, landslide events found in the study area show the opposite (Figure 8). Landslide events found in the Karangkobar Catchment showed that in the toposequen the plains were found at most landslide events, the more towards the peak of the landslide, the fewer were found. At the top of the slope, the lower slope is the top spot that has the most landslide events compared to the middle slope and upper slope.



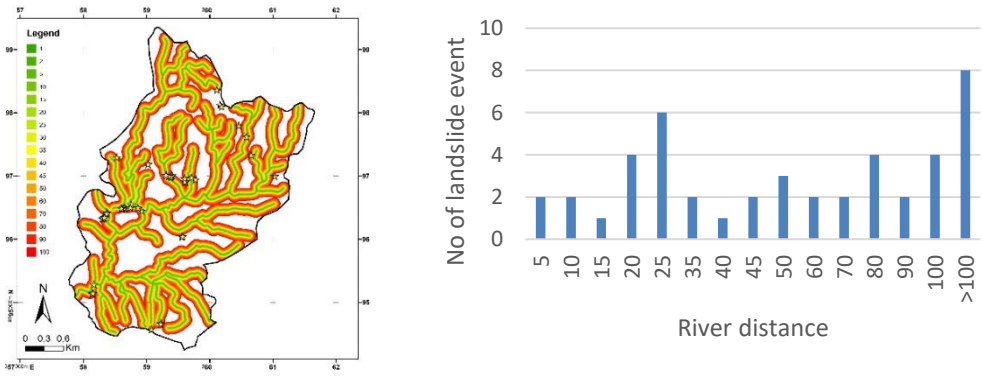
(a) (b)

Figure 11. Toposequen and Landslide Events,
(a) Toposequen map, (b) Number of landslide event per toposequen class

Crosstabulation analysis in Table 3 clarifies the previous analysis. In the lower plains and slopes, landslide events were found in farmland land use. Even landslide events found in the middle to peak slope toposides are also found on farmland land. From this analysis it can be interpreted that the farmland is carried out in all study areas from the plains to the peak areas. Nevertheless, the most intensive farmland activity occurs in the plains to the lower slopes, considering that access is much easier than the middle slope to the top.

Table 6. Landslide Event by Land Use, Texture, and Permeability Crosstabulation

Landuse	Toposequen				
	Plain	Foot slope	Mid slope	Upper slope	Peak
Agroforestry			2		
Farm land	14	15	3	6	1
Built up land	4				



(a) (b)

Figure 12. Distance from river and Landslide Events,
(a) River distance map, (b) Number of landslide event per river distance class

e. Distance from River

The distance to the river is thought to affect landslide events given the frequent occurrence of lateral erosion and intensive vertical erosion on riverbanks, causing landslides in several locations. Based on the results of data processing, it turns out that the indications were not found in the study area. Areas that are very close to the river (5-10 meters) do not indicate a large number of landslides, only found 4

landslide events in this catchment area. The frequency of finding the highest landslide occurrence is more than 100 meters from the river. Seeing this pattern means that in the area of distance study of the river does not have a close enough relationship with landslide events.

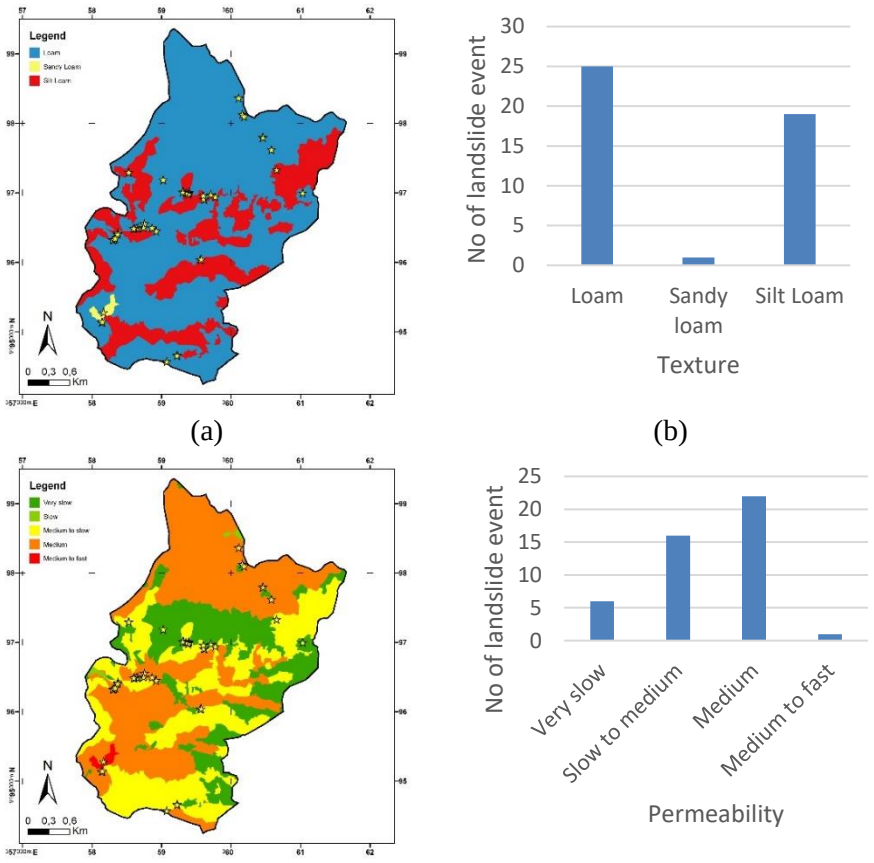


Figure 13. Physical Soil Properties and Landslide Events
(a) soil texture map, (b) Number of landslide event per soil texture class,
(c) soil permeabillity map, (d) Number of landslide event per soil permeability class

f. Physical Soil Properties

The physical properties of the soil used in this study are texture and permeability. Soil texture is a comparison of large fractions of grains of sand, dust and clay. This parameter is closely related to landslides, which is related to the ability to store water, pass water, and the potential for formation of skid fields due to eluviation / iluviation processes in the soil layer. The study area has 3 soil texture classes, namely loam, sandy loam, and silt loam. The three soil texture distributions are presented in Figure 10a. The soil texture is a type of soil texture that dominates the study area, no wonder most landslides occur on land that has a type of soil texture that is 25 events (Figure 10b). Silt loam is a type of texture that has a difference in landslide events that are not too far from the loam texture even though the extent of the area is quite different.

Permeability is the ability of the soil to deliver water. Permeability found in the study area consists of 4 classes, starting from very slow to medium to fast. The spatial distribution of soil permeability in the Karangkobar Catchment is presented in Figure 10c. The pattern of landslide events in the permeability class found in the study area is in the form of parabolic which increases with the speed of permeability and reaches the peak in the medium permeability class and then decreases again in the medium to fast permeability class. The faster the permeability, the higher the potential for landslides

because rainwater that falls to the surface of the soil will be able to be delivered to the slip field quickly and in large quantities. The medium to fast class has only a few landslides because the area is very narrow.

The results of crosstabulation between landuse and soil texture and soil permeability showed that the highest number of landslides occurred in the type of farmland land use which had loam soil texture and medium permebility (Table 4). Loam soil texture is a very good texture for agricultural activities because it is easy to cultivate, therefore this area is in great demand by local residents to be processed as agricultural land, especially vegetables. Permeability that is owned by the land in the farmland tends to be large considering that the agricultural land is periodically loosened by means of hatching with the aim that the air circulation in the soil is smooth. So that it appears that on land farmland with medium and slow to medium permeability has a high landslide incidence.

Table 7. Landslide Event by Land Use, Texture, and Permeability Crosstabulation

Landuse	Texture			Permeability			
	Loam	Sandy loam	Silt Loam	Very slow	Slow to Med	Medium	Med to Fast
Agroforestry	1		1	1	1		
Farm land	20	1	18	5	15	18	1
Built up land	4					4	

14. Future Research

Further research is needed on the physical and antrophogenic conditions that affect landslides. These parameters are further analyzed using a statistical approach, especially for parameters that are known to be able to describe landslide patterns. The parameters referred to include elevation, aspect, slope, land use, physical properties of the soil, and toposequen.

15. Conclusion

Farm land shows the highest landslide events. All physical conditions of land in the farm land type were found to have landslide events even at low elevation and slope. In contrast, agroforestry land shows a very minimal number of landslide events. Landslides only occur on the physical characteristics of the land that are quite extreme such as at altitudes above 1,000 msl and steep slope (15-45%) up to steep (> 45). This can clearly illustrate that anthropogenic characteristics have a major role in influencing landslide events in the Karangkobar Catchment. Physical characteristics are determinants of landslide susceptibility, while anthropogenic characteristics are a trigger for landslides. Thus, the control of landslide events in Karangkobar Catchment needs to be emphasized more on human activities in land processing.

Acknowledgments

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Antibacterial ability of *Streptomyces* sp. E404 in different media formula and incubation time and molecular characterization of antibacterial compound encoding gene

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Abstract. *Streptomyces* sp. E404 was isolated from mangrove rhizosphere in Segara Anakan Cilacap, Indonesia, and observed to produce antibacterial compounds that inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*. The synthesis of antibacterial compounds is influenced by production medium formula and the incubation time. The study aimed to determine the effect of different formula of medium (carbon sources: starch and oatmeal, nitrogen sources: sodium nitrate and yeast extract) and incubation time (7, 14, 21 days) on the inhibitory ability of antibacterial compounds produced, molecular characterization of 16S rRNA and Non Ribosomal Peptide Synthetase (NRPS) genes. The study was conducted experimentally using a completely randomized design (CRD) with factorial patterns for antibacterial study and survey method for molecular characterization. The results showed that oatmeal and sodium nitrate with 21 days incubation time resulted in the highest inhibition of *E.coli* with inhibition zone diameter was 14.5 mm. Meanwhile, the same formula with 14 days incubation time resulted in the highest inhibition of *S. aureus* with inhibition zone diameter was 23.5 mm. Based on 16SrRNA gene analysis, *Streptomyces* sp. E404 has 80% similarity with *Streptomyces* sp. strain 2438 (access number EU864310.1) and E. value 8e-145. Isolates have a 706 bp NRPS gene and 82% homology to NRPS gene of *Pseudomonas orientalis* strain F9 and *Pseudomonas fluorescens* strain L321 that were homolog to *Streptomycin biosynthetic gene cluster* dan *Syringomycin biosynthetic gene cluster.*

1. Introduction

Isolate actinomycetes E404, isolated from the rhizosphere of *Avicenia marina* mangrove plants in Segara Anakan, Cilacap, Indonesia, produced antibacterial compounds that inhibit the growth of *E. coli* and *S. aureus*. Conventional characterization showed that isolate actinomycetes E404 performed filamentous morphological properties, powdery colony surface, spiral mycelium type, responded positively to casein, catalase positive, oxidase positive, were able to use various carbon sources (monosaccharides, oligosaccharides and polysaccharides), positively result in indole, methyl red, O/F , and nitrate reduction assays. Based on Holt et al. [1], isolate actinomycetes E404 was determined as *Streptomyces* sp. E404 [2]. The conventional identification is not enough to ensure the organism to the species level. Identification to species level requires 16SrRNA sequence gene analysis.

The growth of *E. coli* and *S.aureus* were inhibited by filtrate of *Streptomyces* sp. E404 cultured in Starch Casein Nitrate Broth (SCNB) medium. The diameter of inhibition zone were 12.5 mm and 13 mm on *E. coli* and *S.aureus* lawns. The older the culture filtrate produced higher inhibition. Inhibitory

ability was also seen in the MIC and bioautography tests [2]. The antibacterial compounds synthesis can be influenced by the formula of the production medium and the incubation time. Optimization of cultural conditions is very important to obtain high metabolic results [3]. The variations of carbon and nitrogen sources have been known to influence antibiotic biosynthesis in actinomycetes [4]. The formulation of fermentation medium is very important in the production of secondary metabolites [5]. Production medium can be formulated by regulating carbon sources and nitrogen sources.

Starch is one of the carbohydrate biopolymers and is often used as a carbon source for growing and producing metabolites in *Streptomyces*. Starch consists of 20 to 25% amylose (linear) and 75 to 80% amylopectin (branched) by weight. Granule surfaces may contain small amounts of proteins and lipids which may have a significant effect on the physical properties of starch [6]. Oatmeal is an alternative complex carbon source for *Streptomyces* growth. The oat contains 12% carbohydrates, including 2% dietary fiber, and 2% each of protein and fat. Bundale et al. [7] showed that the effects of using various carbon sources such as glucose, galactose, fructose, sucrose, maltose, mannitol, starch, manose, xylose, and arabinose influenced on the antibacterial compounds production in *Streptomyces* sp isolates against *Bacillus cereus* growth which is seen from the diameter of the inhibitory zone formed.

The sources of nitrogen, both organic and inorganic nitrogen sources, play an important role in the production of secondary metabolites results in increased production of antibacterial compounds. Organic nitrogen is a source of nitrogen contained in living biomass such as proteins and amino acids while inorganic nitrogen is not [8]. Yeast extract as a source of organic nitrogen, not only contains a nitrogen source but there is also a carbon source in it. Examples of inorganic nitrogen compounds are $NaNO_3$ and KNO_3 in either solution or solid phase. Sources of organic nitrogen such as yeast extract are the best substrates that can also function as carbon sources [9]. According to Pandey et al. [10], the effect of using yeast extract on the antibacterial compounds production of *S. kanamycetius* M27 produced 6.93 units / mL, higher than that of $NaNO_3$ inorganic nitrogen, which was 4.33 units / mL. Incubation time affects *Streptomyces* in producing antibacterial compounds because the longer the incubation, *Streptomyces* will be more active, the more the cells number the greater ability to break down the substrate [11]. Biosynthesis of antibacterial compounds begins at the end of the logarithmic phase until the end of the stationary phase, after the cell division and multiplication process quits [12]. Decreasing and increasing the inhibitory ability of antibacterial compounds can be caused by the optimal time of secondary metabolites production that varies among isolates. The similar treatment may give different results. Isolates that do not produce inhibitory zones are possible or have not passed the optimal time, while isolates that form an inhibitory zone are possible to undergo a production phase of secondary metabolites [13]. According to Usha et al. [14], several other parameters in cultivation such as the medium pH and incubation temperature also play a major role in the production of bioactive metabolites.

The ability of *Streptomyces* spp. in inhibiting the production of antibacterial compounds due to the presence of gene encoding Nonribosomal Peptide Synthetase or NRPS synthesis [15]. The NRPS gene fragment belongs to a structural gene that codes for enzymes involved in the biosynthesis of secondary metabolites. NRPS is known as part of the type of secondary metabolites produced by microorganisms, including antitumor agents and antibiotics that are very medically valuable [16]. NRPS gene characterization can provide gene sequence information by sequencing and can provide information on gene cluster homology for the analysis of secondary metabolites produced by bacteria [17].

This study aimed to ensure the species of isolate *Streptomyces* sp. E404 based on the 16SrRNA gene sequence, the effect of different types of medium and incubation time on the antibacterial ability of *Streptomyces* sp. E404, and the characterization its NRPS encoding gene.

2. Materials and Methods

2.1. Research design

The investigation of the 16S rRNA gene and NRPS gene were carried out in a survey method with paramater the index similarity of 16S rRNA and NRPS genes. The study on the effect of medium and incubation time was carried out experimentally using completely randomized design (CRD) with

factorial pattern. The first factor was a culture medium consisting of four levels, namely: medium A (g / Ll: basal medium; 1% starch; 0.2% yeast extract), B (g / L: basal medium; 1% starch; 0.2% sodium nitrate), C (g / L: basal medium; 1% oatmeal; 0.2% yeast extract), and D (g / L: basal medium; 1% oatmeal; 0.2% sodium nitrate). The second factor was the incubation time which consisted of three levels: 7, 14, and 21 days. The treatment was repeated three times. The main parameter was the diameter of the inhibitory zone produced by the crude extract of *Streptomyces* sp. E404 against coli *E. coli* (Φ IZE.EC) and *S. aureus* (ΦIZE.SA), while as supporting parameters were the weight of crude extract (WE), weight of mycelium biomass (WM), pH value of filtrate, substrate reducing sugar concentration (RSC).

2.2. The analysis of 16S rRNA gene and NRPS gene

Initially investigation was genomic DNA isolation. DNA quality was visualized with 1% agarose gel electrophoresis using a UV transiluminator. Quantification of DNA by looking at the DNA concentration value using the Qubit 2.0 Fluorometer machine.

The amplification of 16S rRNA gene using forward pA primer: AGAGTTTGATCCTGG CTCAG (8-28), and reverse pH primer: AAGGAGGTGATCCAGCCGCA [18]. The composition of the ingredients used in PCR: master mix 25 µL, primer F 2 µL (23.2 nmol / 0.14 mg), primer R 2 µL (26 nmol / 0.16 mg), 19 µL Free Water Nuclease, 2 µL sample DNA (0.1 µg / mL). DNA was amplified using a PCR machine with predenaturation conditions at 98 oC for 2 minutes, followed by 40 reaction cycles] consisting of denaturation at 98 °C for 10 seconds, annealing stage at 52 °C for 15 seconds, primary elongation at temperature of 68 °C for 30 seconds, final extension at 68 °C for 1 minute. Ended with holding at 8 °C. Amplification of 16S rRNA gene with a total volume of 50 µL had a final concentration of 0.1 µM. Composition for PCR was 19µL nuclease free water was filled in PCR tubes, followed by 2µL primary forward, 2µL reverse primer, 25µL master mix PCR, and 2µL sample DNA.

Amplicon sequencing was done at 1st Base Pte Ltd. Singapore. The sequence of DNA sequences was analysed through the Basic Local Alignment Search Too (BLAST) program (www.ncbi.nlm.nih.gov/BLAST/), compared to the DNA sequence database contained in Genbank NCBI. Alignment sequences were carried out with the Bioedit program. Phylogenetic tree was analyzed by Neighbor joining method, genetic relationship analysis using MEGA version 7 program, bootstrap value 1,000 times, the species used as comparison was taken from Genbank data.

NRPS gene amplification: Predenaturation of DNA template at 98 °C for 2 minutes, followed by 40 reaction cycles consisting of denaturation at 98 °C for 10 seconds, primary annealing at 55 °C for 15 seconds, primary elongation at 68 °C for 1 minute, followed by a final extension at 68 °C for 1 minute.

Primers used were A3-F GCS TAC SYS ATS TAC ACS TCS GG and A7-R STA CCG SAC SGG BGA CST S with a size of 700 bp. The standard volume used for one PCR reaction was 50 µl consisting of water 19 µl, master mix 25 µl, Primers (0.5 µM) 2 µl x 2, DNA (50 ng) 2 µl. PCR results were run on electrophoresis chamber using 1% agarose for 60 minutes at 70 Volts. The PCR product is visualized on a UV Transiluminator device.

Sequencing was carried out at First Base Malaysia Laboratory. Sequence alignment is carried out with the BioEdit program. Subsequent analysis used the BLAST nucleotide (BLASTn) program on the NCBI website (blast.ncbi.nlm.nih.gov) for verification of sequences studied. The access numbers obtained were used for analysis with the AntiSmash program (https://antismash.secondarymetabolites.org) to predict the types of metabolites produced [17]. The AntiSmash program analyzes the NRPS gene sequence so that information on the description of the homologous gene cluster appears. Information on gene clusters showed secondary metabolite produced.

2.3. Subculture of isolate and preparation of inoculum

Isolate *Streptomyces* sp. E404 was subcultured on Starch Casein Nitrate Agar (SCNA) slant medium [19] and cultivated on SCNA plate medium, incubated for 7 days at room temperature [20]. The growing culture was then formed some plugs that function as an inoculum.

The bacteria tested *E. coli* and *S. aureus* were subcultured on Nutrient Agar (NA) medium, incubated at 37 °C for 24 hours.

2.4. Production of Antibacterial Compounds [21]
Production media was prepared with starch and oatmeal as carbon sources, yeast extract and $NaNO_3$ as nitrogen sources, so that there is a combination of media A (containing starch and yeast extract), B (containing starch and $NaNO_3$), C (containing oatmeal and yeast extract) and D (contains oatmeal and $NaNO_3$) with a volume of 250 mL each.

A total of 13 inoculum plugs of *Streptomyces* sp. E404 was inoculated, culture was incubated at 30 ^{o}C for 7, 14 and 21 days.

At the end of the incubation period, the broth was filtered to obtain the weight of mycelium biomass (WM). The filtrate was measured its pH, reducing sugar concentration (RSC), inhibitory zone diameter resulted by filtrate against *E. coli* (ΦIZ.F.EC) and *S. aureus* (ΦIZ.F.SA), and then extracted to obtain a crude extract of antibacterial compounds (WE).

2.5. Filtrate inhibition assay with diffusion method [20, 22]
A 1.5 mL filtrate was transferred to a microcentrifuge tube, then centrifuged for 5 minutes at 3,000 rpm. The 20 µL supernatant was filtered with Millipore (membrane pore diameter 0.22 µm), dripped on disc paper (6 mm diameter) and placed on NA medium which had been inoculated with 1 mL of *E. coli* and *S. aureus* broth culture. Then, incubated at 37 °C for 24 hours. The inhibition zone formed was measured in diameter, which was the average of vertical diameter and horizontal diameter.

2.6. Weighing the mycelium biomass of Streptomyces sp. E404 [23]
Separation of mycelium biomass and filtrate using Whatman No. 1 filter paper that previously weighed (X1). Biomass was dried in an oven at 70 ºC for 2 hours to get the dry weight of biomass. Dry biomass is weighed as gross weight (X2). Dry weight of mycelium biomass (WM) was gross weight minus the weight of filter paper (X2-X1).

2.7. Determination of reducing sugar concentration using Nelson-Somogyi Method [24, 25]
1 mL of medium A, B, C, D with incubation time of 7, 14 and 21 days were prepared in a test tube. Then each tube was added with 1 mL of Nelson reagent and heated in water bath for 20 minutes. All the tubes were then cooled immediately until the tube temperature reaches 25 ºC and then 1 mL of arsenomolybdat reagent was added, shaken until all the Cu_2O dissolve and added 7 mL of distilled water, shaken until homogeneous. Optical density (OD) of each solution was measured using a spectrophotometer at a wavelength of 540 nm. The concentration of reducing sugar was determined using the regression equation of the standard curve of glucose concentration that was $y = 0.0615x + 0.0897$.

2.8.Extraction of antibacterial compound and measurement of antibacterial compounds weight
The filtrate was extracted twice with ethyl acetate (1: 1 v / v) in a separating funnel for 20 minutes. The extract obtained was evaporated to obtain a moist extract and stored in a vial bottle that had previously been weighed (Xv1). Then the crude extract obtained was weighed as gross weight (Xv2). Gross extract weight was gross weight minus vial bottle weight (Xv2-Xv1) [26].

2.9. Extract inhibition assay with diffusion method against E. coli and S. aureus
E.coli and *S.aureus* cultures were taken as much as one ose then inoculated into Nutrient Broth (NB) medium and incubated for 10 hours for *E.coli* and 8 hours for *S.aureus*. As much as 1 mL of *E.coli* and *S.aureus* cultures were inoculated by pour plate method on NA medium. The disc paper (diameter 6 mm) was then placed on the NA medium and dripped with 20 µL of crude extract, incubated at 37 °C for 24-48 hours. The inhibition zone (mm) formed was measured in diameter, which was the average of vertical diameter and horizontal diameter.

3. Results and Discussion

3.1. 16S rRNA gene analysis and NRPS gene

DNA isolation results showed DNA quantification values of 0.0377 µg/mL or 37.7 ng/mL. According to Latifah et al. [27], genomic DNA concentration values ranging from 0.016 to 0.85 µg / mL or equivalent to 16.3 to 854.5 ng / µL can still be used in the amplification process to produce a good product or amplicon.

Based on the results of 16SrRNA gene analysis, *Streptomyces* sp. E404 has 80% similarity to *Streptomyces* sp. strain 2438 (access number EU864310.1) and value E. value 8e-145.

Streptomyces sp. E404 NRPS gene was successfully amplified using A3F and A7R primers, in accordance to Ansari et al. [28], it produced a visualized band that was very clear (bright). *Streptomyces* sp. E404 has a NRPS gene sequence with a band size of 706 bp, has 82% homology with the NRPS gene of *Pseudomonas orientalis* strain F9 and *Pseudomonas fluorescens* strain L321.

In general the NRPS gene cluster in *Streptomyces* sp. E404 was homologous with the Streptomycin biosynthetic gene cluster and the Syringomycin biosynthetic gene cluster. Secondary metabolites generated in the AntiSmash data base refer to the name of the product produced. This analysis showed that the prediction of secondary metabolites produced including Streptomycin and Syringomycin.

3.2 Effect of medium type and incubation time on the inhibitory ability of antibacterial compounds against E. coli and S. aureus

The results showed that antibacterial compounds extract of *Streptomyces* sp. E404 grown in different mediums and incubation time was able to inhibit the growth of *E. coli* and *S. aureus* (Figure 1). The actualy results showed: inhibition of *S. aureus* was higher than that of *E. coli*, a higher inhibition was produced by crude extracts of D medium containing oatmeal and nitrate, a high inhibition of *E. coli* produced by 7 days culture crude extract while against *S. aureus* by 14 days culture crude extract. The treatments interaction D21 was the highest inhibited *E. coli* (ΦIZ.E.EC : 14.5 mm) and against *S. aureus* was D14 treatment (ΦIZ.E.SA : 23.5 mm) (Figure 2). According to Devi and Mulyani [29], the antibacterial inhibitory strength is categorized as strong (10-20 mm inhibitory diameter) and very strong (> 20 mm inhibitory diameter). According to Qin et al. [30], the antibacterial activity of ethyl acetate extract from broth culture, in addition to being influenced by culture conditions such as media composition, aeration and the number of inoculums, is also influenced by the extraction process, including the solvent used and the duration of mixed shaking.

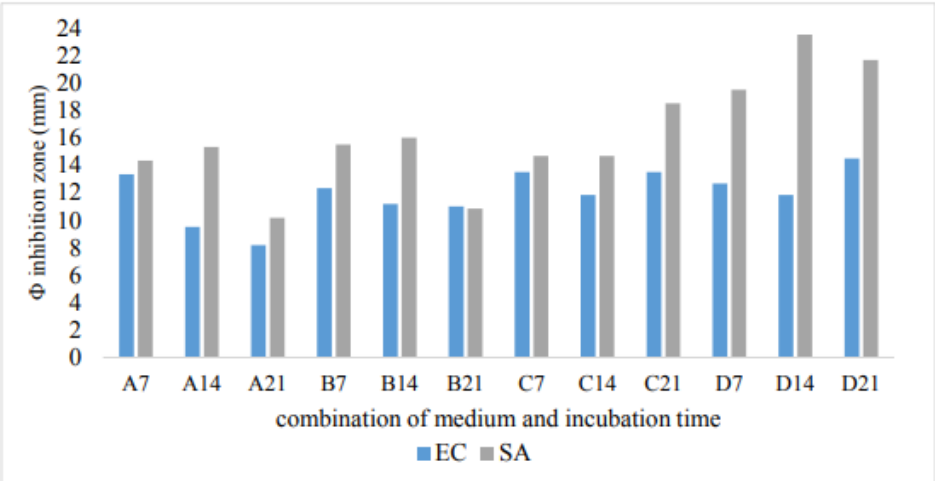

Figure 1. Histogram of extract inhibitory zone diameter of *Streptomyces* sp. E404 against *E.coli* (EC) and *S.aureus* (SA) influenced by different types of medium (A: medium contain starch and yeast extract,

B: medium contain starch and $NaNO_3$, C: medium contain oatmeal and yeast extract, D: medium contain oatmeal and $NaNO_3$) and incubation times (7: 7 days, 14: 14 days, 21: 21 days).

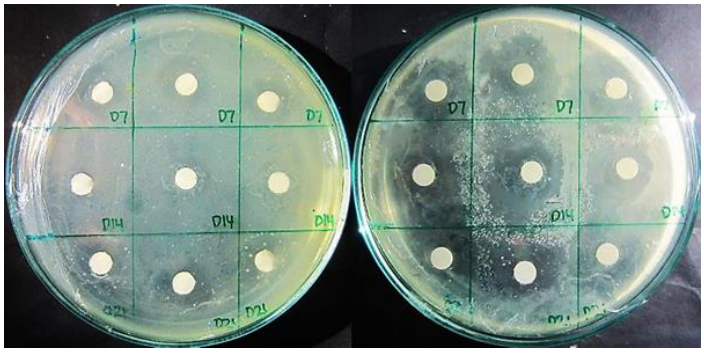

Figure 2. Inhibitory zone of *E.coli* (A) and *S.aureus* (B) resulted by crude extracts of *Streptomyces* sp. E404 from D medium

The analysis of variance (ANOVA) of inhibitory assay of *Streptomyces* sp. E404 crude extract against *E.coli* showed that the type of medium and incubation time significantly affected the bacterial growth ($p < 0.05$). The 5% LSD test showed that the most influential medium in inhibiting *E.coli* was the type of medium contains oatmeal and sodium nitrate (D) and the most influential incubation was 7 days. While the interaction between the type of medium and the incubation time on the inhibitory ability of *Streptomyces* sp. E404 antibacterial compounds significantly affected *E. coli* and the most influential treatment was D21. The inhibitory zone produced by extracts from culture on oatmeal and nitrate medium with an incubation period of 21 days exhibited the largest inhibitory zone, which was 14.5 mm in diameter. The analysis of variance (ANOVA) of inhibitory test of *Streptomyces* sp. E404 against *S. aureus* showed that the type of medium had a significant effect ($p < 0.05$) and the incubation time was not ($p > 0.05$). The 5% LSD test showed that the most influential medium type in inhibiting *S.aureus* growth was the type of medium with oatmeal and sodium nitrate (D). Meanwhile, the most influential interaction between the type of medium and the incubation time for *S. aureus* growth inhibition was D14, which resulted in the highest inhibition zone diameter, which was 23.5 mm.

The type of medium and the length of incubation affect the isolate in synthesizing antibacterial metabolites. The effect of medium type and incubation time on antibacterial compound synthesis can also be seen in supporting parameters of mycelium biomass weight, crude extract weight, reducing sugar concentration on substrate, inhibitory zone diameter produced by filtrate and medium pH value (Figure 3). Supporting parameters confirmed the production of antibacterial compounds by *Streptomyces* sp. E404 which was influenced by the type of medium and the incubation time.

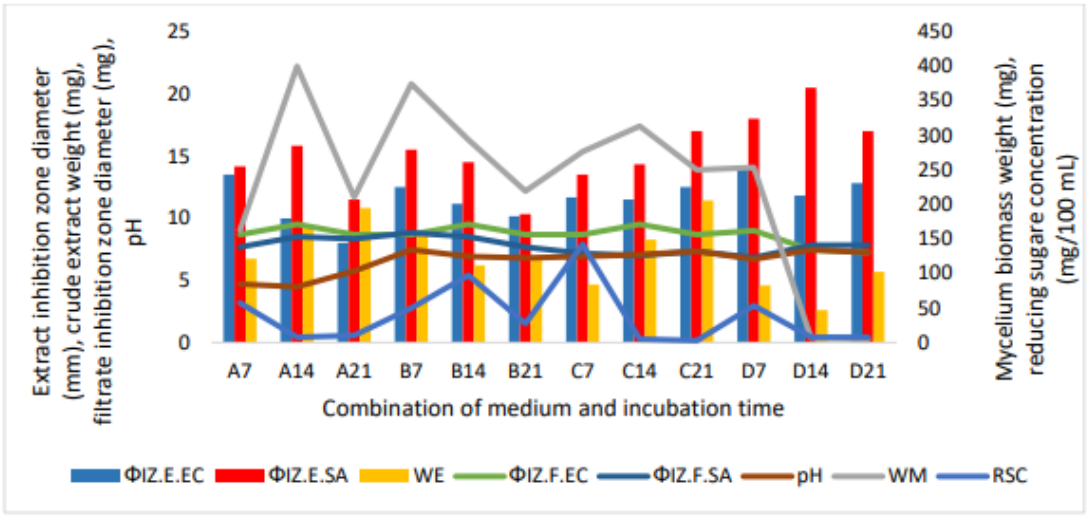

Figure 3. Histogram of extract inhibitory zone diameter on *E. coli* (ΦIZ.E.EC, mm), diameter of *S. aureus* inhibitory zone (ΦIZ.E.SA, mm), crude extract weight (WE, mg / 100 mL), and mycelium biomass weight curve (WM , mg /100 mL), the inhibition zone diameter produced by filtrate against *E. coli* (ΦIZ.F.EC, mm) and *S. aureus* (ΦIZ.F.SA, mm), the concentration of reducing sugar (RSC, mg/100 mL) and the pH value produced by the isolate *Streptomyces* sp. E404 on four media formulas (A: medium contain starch and yeast extract, B: medium contain starch and $NaNO_3$, C: medium contain oatmeal and yeast extract, D: medium contain oatmeal and $NaNO_3$) and incubation times (7: 7 days, 14: 14 days, 21: 21 days)

Oatmeal and sodium nitrate increased the production of antibacterial compounds with high concentrations resulting in the highest inhibition zone against *E. coli* and *S. aureus*. The results of the study were in accordance with Al-Zahrani [31], the production medium of antibacterial compounds of *Streptomyces* sp. J12 containing starch produced a inhibition zone of 26 ± 0.6 mm against the growth of *S.aureus* and did not inhibit the growth of *E. coli*. The production medium containing oatmeal produced a higher inhibitory zone, which was 29 ± 1.5 mm against the growth of *S. aureus* and inhibitory zone of 20 ± 1.5 mm against the growth of *E. coli*.

According to Bundale et al. [7], the production of secondary metabolites is stimulated by complex carbon sources which are slowly assimilated. Optimal production is achieved by growing microorganisms in a medium containing a nutrient source that is slowly utilized. In addition, the growth of isolates is also influenced by type of nitrogen sources added in the media. Organic nitrogen sources induce relatively higher biomass yield for *Streptomyces* sp. E404 compared to inorganic nitrogen sources. Based on the results of Bundale et al. [7], the effect of using various kinds of nitrogen sources has an effect on the production of antibacterial compounds of *Streptomyces* spp. towards the growth of *B. cereus*. Organic nitrogen sources produce higher cell biomass compared to inorganic nitrogen sources.

Other factors that also affect the results and quality of actinomycetes metabolites are the incubation time, which was correlated to the bacterial growth phase and bacterial death phase. The longer the incubation period correlates with increased production and concentration of inhibitor enzymes, fluctuations in pH and biomass values and the production of secondary metabolites that limit the growth of other bacterial cells so that the clear zone produced tends to be greater. According to Song et al. [32], increasing the incubation time can produce more secondary metabolites but, it can also make microorganisms produce more toxic compounds to inhibit the production of antimicrobial metabolites. There are 3 things that influence the cessation of antibiotic biosynthesis, ie the irreversible destruction of several enzymes of the antibiotic biosynthetic pathway, the feedback effect due to the accumulation of antibiotics produced and the reduction of intermediate precursors in antibiotic biosynthesis [33].

Different responses from *S. aureus* and *E. coli* to antibacterial compounds were due to their differences in sensitivity. The wider inhibitory zone of *S. aureus* indicates that these microorganisms are more sensitive to test compounds. This is possible because the cell wall of *S.aureus* has a single layer (monolayer) with low fat content (1-4%) so that antibacterial compounds are likely to be easily absorbed. While the *E.coli* cell wall has three layers with high fat content (11-22%), causing antibacterial compounds difficult to absorb. Similar results were obtained from research conducted by Agoramoorthy et al. [34] which showed that Gram-positive bacteria were more susceptible than Gram-negative bacteria. Likewise, the results of the study by Ozcelik et al. [35] which states that there are differences in sensitivity between Gram-positive and Gram-negative bacteria.

Production culture with a 14-day incubation time resulted in a higher inhibitory ability against *S. aureus*. Whereas for *E. coli*, a higher inhibition was produced by a production culture with an incubation time of 7 days, although the 7-day mycelium weight was lower than the weight of the mycelium at other incubation ages. Based on the relationship of these parameters, it is possible that compounds that play a role in inhibiting the growth of *E. coli* and *S. aureus* are different compounds.

Extract weight did not affect the inhibitory zone formed, because the inhibitory ability was influenced by the high and low concentration of antibacterial compounds. Crude extract weights only show antibacterial compounds have been produced and secreted into the media. However, culture on medium containing yeast extract, the longer the incubation period, the higher the yield of crude extract weight. According to Bundale et al. [7], the growth of isolates was also influenced by the nature and type of nitrogen sources added in the media. Organic nitrogen sources produce higher cell biomass compared to inorganic nitrogen sources. Al-Zahrani's research [31] also showed the effect of using yeast extract to increase the production of antibacterial compounds of *Streptomyces* sp. J12 compared to $NaNO_3$.

The results also showed that the low crude extract weight produced a high inhibitory diameter. The highest weight in C21 treatment, which was 11.42 grams and produced a high inhibitory zone (18.5 mm), while the lowest weight in the D7 treatment was 4.60 grams. However, the D7 treatment produced a high inhibitory effect of 19.5 mm. Oatmeal is a natural substance that contains starch and its derivative components and protein in low concentrations. It is suspected that complex natural components give effect to growth and synthesis of metabolites better than other medium. Nitrates function as cofactors in enzyme activity, thus increasing the synthesis of bioactive compounds.

The results of the reduction sugar test showed that the average high concentration during the incubation period was 7 days and decreased in the longer incubation period. These results indicate the breakdown of starch and oatmeal at the beginning of incubation and high utilization at the end of the incubation period. The longer the incubation, the lower the concentration of glucose in the medium. The results of measurement of reducing sugar were in line with observations of biomass weight, crude extract weight and inhibition zone diameter.

The capability of isolate *Streptomyces* sp. E404 uses starch and oatmeal due to amylolytic enzymatic properties. Hydrolysis of starch by the amylase enzyme causes starch to break down into smaller molecules, namely dextrin (intermediate yield) and maltose. The use of maltose substrate requires the enzyme maltose- glucoamylase which breaks down maltose into glucose, and the enzyme maltose-phosphorylase which breaks down maltose into glucose-1-phosphate. According to Hoque et al. [36], several *Streptomyces* isolates isolated from the soil were able to produce maltase enzymes. Then glucose-1-phosphate is isomerized to glucose-6-phosphate, so that the pathway becomes the same as glucose [37]. Glucose is a monosaccharide compound which is generally the most easily metabolized by microorganisms compared to other sugars, so that it is referred to as a primary substrate [38]. The glucose metabolic pathway mostly follows the Embden-Meyerhof trajectory. Glucose is converted to glucose-6- phosphate, which in turn is converted into pyruvic acid. This compound is a carbon source and the main energy for most microorganisms and is the starting point for most of the metabolic pathways of microorganisms.

Observation of the pH value of the medium can be used to determine the presence of cell growth activity. During the growth period, organic acids are produced as a product of metabolic processes. The hydrolysis of sugar converted into organic acids causes the medium to become acidic. In the production

culture of antibacterial compounds by Streptomyces sp. E404 is known that there is a small change in the pH of the medium, even though when the high biomass is produced, the pH value of the medium is lower and vice versa. The increase in pH in the medium is caused by the occurrence of protein deamination which can cause the culture to become more alkaline. According to Wang et al. [38], the use of organic nitrogen sources tends to trigger an increase in pH of the medium caused by the occurrence of amino acid deamination. Cell lysis or damage to some cells in the medium can also increase the pH of the fermentation medium. Cells are prepared by several organic proteins, in the event of cell damage, amino acid deamination occurs which results in an increase in the pH of the medium. According to Bundale et al. [7], medium pH is one of the most important environmental factors because it gives a real effect on the activity of several enzymes that catalyze metabolic reactions, and gives a significant influence on membrane permeability and cell morphology. Initial pH changes affect many cellular processes such as regulation and biosynthesis of secondary metabolites.

Culture filtrate of Streptomyces sp. E404 is able to inhibit the growth of pathogenic bacteria that prove the presence of antibacterial compounds secreted into the media. The inhibitory test with the same filtrate volume as the extract of the test bacteria showed that the filtrate had a lower inhibitory ability than the crude antibacterial compound extract (Figure 3). The difference can be caused by the dilution effect of the antibacterial compound concentration in the liquid medium. Susilowati et al. [39] stated that the more antibacterial compounds secreted into the media, the greater the diameter of the inhibitory zone. The test results also showed that the filtrate treatment incubation period of 14 days on average resulted in a higher inhibition zone diameter than the other incubation period filtrate. This result is in accordance with mycelium biomass, ie the average mycelium weight is higher during the 14-day incubation period. High mycelium biomass is a source of high synthesis of antibacterial compounds.

The diameter of the inhibitory zone produced by Streptomyces sp. E404 for each treatment varies. The size of the inhibitory zone shows the ability to inhibit an isolate against the test bacteria. The inhibitory ability can be influenced by various factors. According to Susilowati et al. [39, the zone of inhibition of bacteria is largely determined by bacterial growth and sensitivity and the speed of diffusion of antibiotic compounds on agar medium. Antibacterial compounds produced by *Streptomyces* sp. E404 is found in the filtrate supernatant. The active compound found in the supernatant shows that the antibacterial active compounds produced are extracellular [40]. Culture filtrate of *Streptomyces* sp. E404 also has inhibitory power against Gram positive and Gram negative test bacteria, so that active compounds are thought to be broad spectrum antibacterials.

4. Conclusion

Based on the analysis of 16S rRNA sequences of *Streptomyces* sp. E404 has 80% similarity with *Streptomyces* sp. strain 2438 (access number EU864310.1) and value E. value 8e-145. Isolate *Streptomyces* sp. E404 has a NRPS gene with a size of 706 bp and has a homology of 82% with the NRPS gene *Pseudomonas orientalis* strain F9 and *Pseudomonas fluorescens* strain L321. The type of medium containing oatmeal and nitrate with an incubation time of 21 days resulted in the highest inhibition of *E.coli* with a inhibition zone diameter of 14.5 mm. Meanwhile, the same formula with an incubation time of 14 days resulted in the highest inhibition of *S. aureus* with a inhibition zone diameter of 23.5 mm.

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Sweet potato greens 'neglected vegetables rich in bioactive compounds' (part I): radical scavenging activity, inhibitory effect on α- amylase, total phenolic and flavonoid contents of local sweet potato (*Ipomoea batatas*) leaves

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Abstract. Sweet potato greens or leaves are commonly consumed by rural people in some part of Indonesia, Malaysia, The Philippines, and other part of the world. In addition to their nutritive value, sweet potato leaves have been reported to content an appreciable amount of bioactive compounds such as polyphenol including flavonoids beneficial for health. Over the years, phenolic including flavonoids are intensely studied for their physiological health benefits as functional foods. However information on bioactive contents of sweet potato greens grown locally in Lampung are inexistent, therefore this paper reports some components believed to be potential for human health. The leaves of locally grown three sweet potato genotypes used in this study were harvested six weeks after planting. The results showed methanol extract of sweet potato leaves had 63-79% DPPH radical scavenging activity 55-59 % inhibitory effect on α amylase , 0.81-1.75 g GAE/100 g dry weight of total phenolic content, and 7.57-10.51mg QE/100 g dry weight of flavonoids.

1. Introduction

Sweet potatoes are cultivated widely around the world primarily for their underground tubers utilized as a raw material for starch production, staple and snack foods . They can be harvested up to three times a year. In addition to be regarded as biomass waste after harvesting the tubers, sweet potato leaves are regularly harvested and consumed in some Asian countries mainly because it has a high content of protein, mineral substances, vitamins and bioactive compounds as well as soluble and non-soluble dietary fiber. Sweet potato leaves contain up to 15-27% protein on dry matter basis depending on the varieties [1] , and are a valuable source of iron, vitamins E, beta carotene lutein and polyphenol [2]. These compounds have been reported to have antioxidant properties, inhibit α glucosidase and α amylase activities [3] which is very potential to manage blood glucose level in diabetic patients.

Bioactive components, namely polyphenol, of sweet potato leaves have been intensively studied because of their ability to protect against free radicals. The over production of free radicals and nitrogen species could result in oxidative stress that increases the onset of chronic non-communicated diseases (NCDs) such as some types of cancers, neurogical problem, and cardiovascular[4,5]. The main polyphenol contents of sweet potato leaves are caffeoylquinic acid (CQA) derivatives[6]. The amount of polyphenol in leaves is higher than those of in the tuber flesh and the peel[7]. Eugenio et al.[8] reported sweet potato leaves contain high amount of phenolic compounds mainly quercetin, chlorogrenic acid, and rosmarinic .

In Lampung areas, sweet potatoes are grown widely among small growers. However, despite of their valuable content of protein and bioactive compounds , sweet potato leaves have no economic value, they are either fed to cattle or utilized as compost. Therefore it is important to provide information on various health-promoting biological activities of sweet potato leaves to encourage people to utilize sweet potato leaves as part of their healthy diet. This study revealed the antioxidant property, α amylase inhibitor, flavonoid and total phenolic contents of three sweet potato genotypes namely LPG-01, LPG-06, and LPG-07 grown at Politeknik Negeri Lampung Experimental Field. These sweet potato genotypes are still on-going experiment and have not been grown commercially.

2. Material and Methods

2.1 Materials

Three sweet potato genotypes namely LPG-01, LPG-06, and LPG-07 grown at Politeknik Negeri Lampung Experimental Field were used in this study. Sweet potato leaf tips used in this study had the desirable attribute for human consumption. They had small stems and petioles they were glabrous, tender and showed some vein purple color. The 15 cm long tips were harvested 6 weeks after transplanting. For each genotype, harvested fifteen sweet potato tip stems were rinsed with tap water, then steam blanched for 3 min, and cooled at room temperature. They were then cut into small pieces, put into plastic bags, and frozen at -15°C until analysis was performed. The samples were evaluated for their DPPH radical scavenging activity , α amlylase inhibition, total phenolic, and total flavonoid contents. The chemicals such as Folin-Ciocalteau reagent, DPPH, methanol were purchased form Sigma-Aldrich via local supplier.

2.2 Sweet Potato Leaf Extraction

Samples of frozen blanched sweet potato leaves (50g) were macerated in 100 ml of 60% methanol in a closed container for 24 h at 4°C in the dark room, then filtered through Whatman No. 42 filter paper. The remaining residues were washed with 100 ml of methanol, both filtrates were collected as the crude extract filtrate. The methanol in the crude extract was evaporated by the rotary evaporator (0.1 MPa, 40°C) until the volume was 50 mL, kept in a dark brown bottle and stored at -20°C until further analysis.

2.3 Scavenging Radical Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity of leaf extract was determined using method described by Chung et al. [9] with slight modification. Two µL of the methanol extracted leaves (as described in above procedure) were mixed with 2.0 mL of 2×10-4 M DPPH in methanol. The mixture was shaken vigorously and left in the dark at 25°C for 30 min. The absorbance of mixture was read immediately at 517 nm using a GENESYS 10S S UV-Visible spectrophotometer (A_{sample}). The mixture of 95% methanol (2 mL) and sample (2 mL) serve as blank (A_{blank}). The control solution was prepared by mixing methanol (2 mL) and DPPH radical solution (2 mL) ($A_{control}$). The ability of sweet potato leaf extract to scavenge DPPH radical or antioxidant activity was calculated by the following equation:

$$\text{Scavenging Radical Activity} = \left[100 - \frac{A_{sample} - A_{blank}}{A_{control}}\right] \times 100\ \%$$

2.4 Invitro α-amylase Inhibition Study

The α-amylase inhibitory activity was determine using method described by Zengin[10] with modifications. Briefly, 250 µl of leaf extracts with varying concentrations (125–500 µg/ml) and 250 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing α-amylase (from Aspergillus oryzae , Merck) solution (0.5 mg/ml) were incubated for 10 min at 25°C. After preincubation, 250 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube at 5 s intervals. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 500 µl dinitrosalicylic acid (Sigma, St. Louis, USA) color reagent. The tubes were then incubated in a boiling water bath for 5 min and cooled to room

temperature. The reaction mixture was then diluted by adding 5 ml of distilled water, and absorbance was read at 540 nm using a GENESYS 10S S UV-Visible spectrophotometer. the α-amylase inhibitory activity was calculated as percentage inhibition, using the formula.

$$\% \text{ Inhibition} = ([Abs_{control} - Abs_{samples}]/ Abs_{control}) \times 100$$

2.5 Total Phenolic

Total phenolic content in sweet potato leaf extract was determined by colorimetry method using Folin-Ciocalteu reagent assay as modified from Singleton and Rossi [11]. SP leaf extract (1ml) was mixed with 1ml of Folin-ciocalteu's phenol reagent and allowed to react for 3 minutes. Then, 0.8ml of 7.5% (w/v) sodium carbonate was added. The mixture was agitated and allowed to stand for further 30 minutes in the dark. The absorbance of leaf extracts and a prepared blank were read at 765 nm using GENESYS UV-Visible spectrophotometer. Gallic acid with the concentration range from 10 µg/mL to 100 µg/mL, was used for the standard curve and the total phenolic content was expressed as mg gallic acid equivalents per gram of dried sample (g GAEs/100g dry weight).

2.6 Total Flavonoids

Total flavonoid content was determined using the method described by Vuong et al. [12] with slight modification. Extract (0.5 mL) was added 2 mL of deionized (DI) water and 0.15 mL of 5% (w/v) $NaNO_2$, kept at 25°C for 6 min. Then, 0.15 mL of 10% (w/v) of $AlCl_3$ was added and allowed to stand for 6 min, followed by the addition of 2 mL of 4% (w/v) NaOH and 0.7 mL of DI water. The mixture was mixed thoroughly and left at 25°C for 15 min. The absorbance was read at 380 nm using GENESYS UV-Visible spectrophotometer. Quercetin with the concentration range from 10 µg/mL to 100 µg/mL was used as a standard and the total flavonoid content was expressed as mg quercetin equivalents per gram of dried sample (mg QEs/100g dry weight).

2.7 Statistical Analysis

All the analysis were conducted in triplicates for each genotype, and the data were reported as means ± standard deviation

3. Results and Discussion

The results of scavenging activity, enzyme inhibition, total phenolic and total flavonoid contents are summarised in Table 1.

3.1 Scavenging Radical Activity

The DPPH radical scavenging activity of the three sweet potato genotypes can be ranked as LPG-7 > LPG-6 > LPG-2. The difference in total antioxidant activity among sweet potato varieties grown under similar condition might be influenced by the plants tolerance against viruses and fungi [13]. DPPH is a free radical generating compound used to measure the scavenging ability of sweet potato leaf extract. The radical scavenger of the leaf extract will decolorize the color of DPPH in methanol solution (purple) to yellow color because of reduction of the stable DPPH radicals to diphenyl-pricrylhydrazine in the presence of H-donating antioxidant . In this study, the antioxidant property of the sweet potato leaf extract was determined using only single method (DPPH assay). The decision was based on its availability and simplicity, furthermore Steed and Truong [14] reported that the antioxidant activities assayed by DPPH and ORAC (oxygen radical absorbance capacity) showed a significant correlation.

3.2 α-Amylase Inhibition

The inhibitory effect on α-amylase of three genotypes sweet potato leaf extracts were between 55 to 60%. LPG-7 leaf extract showed the strongest inhibitory effect , followed by LPG-2 and LPG-6. The variation of inhibitory effect on α-amylase could be due to variation in the phenolic content. It was claimed that the higher levels of chlorogenic acid (one type of phenolic) may contribute to α-amylase and α-glucosidase inhibitory effect [15].

3.3 Total Phenolic

The total phenolic contents among leaf extract from three genotypes sweet potato were between 0.82 and 1.75g GAEs/100 g extract. Phenolic compounds contribute to the ability to scavenge the reactive oxygen species (ROS)[12], and therefore are important to keep healthy body. Phenolics also has important role in modulating the carbohydrate absorption [16], which may be used to manage blood glucose in diabetic patients.

3.4 Total Flavonoids

The flavonoids contents were between 7.57 – 10.51 mg QEs/100g dry weight. The highest was found in LPG-6 leaf extract, followed by those of LPG-2 and LPG-7. Flavonoids have been intensely studied mainly because of their function as antioxidant . The mechanism of flavonoids as antioxidant or in removing free radicals can be divided in to two, which is scavenging and chelating [17].

Table 1. Antioxidant ability, enzyme inhibition, total phenolic and total flavonoid of sweet potato leaf extracts

Sampel	DPPH Scavenging radical activity (%)	α-amylase inhibition (%)	Total Phenolic (g GAEs/100g dry weight)	Total Flavonoid (mg QEs/100g dry weight)
LPG-2	79.68±0.13	57.95±005	1.75±0.27	8.23±0.51
LPG-6	75.72±0.24	54.92±0.03	0.81±0.09	10.51±0.72
LPG-7	63.39±0.36	59.84±0.15	1.49±0.05	7.57± 0.18

4. Conclusion

The methanol extract of sweet potato leaves from 3 genotypes have shown to contain considerable level of antioxidant properties, enzyme inhibition, total phenolic and total flavonoid. Based on these findings, sweet potato leaves are very potential to be promoted as promising natural source of antioxidant, and α-amylase inhibition that could be used to overcome hyperglycemia problem.

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A temperature distribution study in a heated bed component mode from butyl rubber for dust mite allergy patients

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Abstract. Asthma and allergy symptoms affect life quality of their patients since the patients have to cope and deal with the symptoms directly and side effects of them. Allergies can be stimulated by many substances while the allergy caused by dust mites is often found outdoor and indoor because dust mites eat dead skin layers, dandruff and scurf falling from human and living things as their food to survive. Dust mite related allergy does not cause by dust mites themselves but their feces is the main substance to trick the allergy symptom in the dust-mite allergy patients. The allergy symptoms can be indicated by sneezing, rash or, even, swallowed organ tissues. Many ways to reduce a number of dust-mite related allergy are to get rid of dust mites; the dust mites cannot survive in environments with temperature above 60 Celsius longer than 45 minutes, and to have dust-mite-free environments. One place where there are always dust-mite food is a bed; bedding components such as mattress and pillow can be home of million dust mites. This work focused on investigating temperature distributions on a heated bed component made from butyl rubber, numerically and experimentally, to evaluate possibility to fabricate dust-mite-free bedding components made from butyl rubber. The component was designed by computer aided design (CAD) based on a pillowcase shape to be fed with hot water to heat the component up to unacceptable dust-mite living conditions. The design component was simulated for temperature distributions of the component while it was also fabricated locally by using recycle butyl rubber sheets and fed with hot water to experimentally investigate the practical temperature distributions on the fabricated component. From simulation results of hot water flow into the component at an inlet water flow rate of 2.25×10^{-5} m^3/s during observed 45 minutes, temperature distributions on the component surface were above 60 Celsius. There were limited conditions in fabricating the butyl rubber sheets locally, the fabricated component was smaller than the size of the simulated component. Experimental results of hot water flow into the component at an inlet water flow rate of 2.25×10^{-5} m^3/s during observed 45 minutes showed that temperature distributions on the component surface were also above 60 Celsius. Therefore, these results showed potentials of the design to be developed as dust-mite-freebedding components to help creating dust-mite-free environments for dust-mite allergy patients.

1. Introduction

Allergy affects many patients to start asthma and to have lower quality of life. There was a report stating that 70% of Thai people were affected by allergy [1]. Allergy is caused by many stimulants depending on patients such as dust, dust mites, food, etc. When the patients take their stimulants into their bodies, the bodies will react to these stimulants faster and worse than normal people. There are numerous body reactions to these stimulants; itchiness, tearing, running nose, sneezing and swollen tissue. The worst swollen tissues around ears, noses, throats and mouths can block air ways causing lower oxygen level in patient system and other severe organ failures. One of stimulants; which can be found in natural

environments and in households, is a dust mite, it can survive by consuming dandruffs, scurf and dead skin. Dust-mite-allergy patients are not stimulated by dust mites but they are stimulated by dust-mite feces. Dust-mite-allergy can be detected by allergy medical specialists who perform allergy tests; for an example is a skin test. The specialists always recommend their patients to avoid contacting with stimulants of patient allergy and to control their environments to be stimulant-free environments. To get rid of dust-mite feces, one may get rid of dust mites and the feces. The dust-mite feces can be eliminated their stimulant affects by coldness while the dust mite, themselves, cannot survive in environments with temperature above 45 Celsius longer than 40 minutes [2].

Wadsö and Svennberg [2] introduced their important information about dust mites. They experimentally investigated the activity of House Dust Mites (HDM) as a function of humidity and temperature by applying a laboratory culture of Dermatophagoides farina (Df or the scientific name of the dust mites) and the measurement of the heat produced by the mite respiration. They found that the HDM activity generally decreased after about one month, they suspected that the food supply which did not last longer than one month was the cause of lower HDM activity. They delivered important information from their studies with different humidity levels that the Dermatophagoides farina died after 40 minutes exposure to 45°C environment.

Many commercial vacuum cleaners were claimed to be able to take dust mites out of beds such as MARTEX trade mark [3], BOSCH trade mark [4] and Phillips trade mark [5]. The MARTEX vacuum cleaner was cited that water and High Efficiency Particulate Filters (HEPA) were used as dust-mite traps, the operating time was 15 – 20 minutes per one matter. The BOSCH vacuum cleaner was described as the HEPA and Bionic Filter Vacuum Cleaners.

2. Materials and methods

Our team designed heated bed components by using the computer aided program as shown in Fig 1. Then, we designed and fabricated heating systems for pillows. There were two types of the heating systems, one was a direct heating system by a plate heater and another one was an indirect heating system with hot water. The indirect system combined of two types of bed components, one was the available commercial rubber bag and another was the in-house-made butyl rubber bag. The total of three bed components were fabricated according to the size of the bed component which was designed by the computer aided design program. All three fabricated bed components were experimentally studied for their potential as the heated pillows as shown in Fig 2.

Figure 1. A designed bed component.

(a) a bed component with a plate heater (b) a commercial rubber bag (c) an in-house butyl rubber bag

Figure 2. Three bed components which were used to investigate for the workable heating system.

While the bed components and the heating systems were processed, the designed bed component from Fig 1 was numerically investigated as it was heated by 100 Celsius hot water by using the commercial Computer Fluid Dynamic (CFD) program, Fluent. The governing equations in the numerical simulation were as following

Conservation of Mass

$$\frac{\partial U_i}{\partial x_i} = 0 \tag{1}$$

Conservation of Momentum

$$\frac{\partial U_i U_j}{\partial x_i} + \frac{1}{\rho}\frac{\partial}{\partial x_j}\left\{\mu\left\{\frac{\partial U_j}{\partial x_i} + \frac{\partial U_i}{\partial x_j}\right\}\right\} + \frac{1}{\rho}\frac{\partial P}{\partial x_i} = 0 \tag{2}$$

Conservation of Energy

$$\rho\frac{D\left(C_p T\right)}{D_t} = \nabla.\left(k\nabla T\right)$$
$$+\mu\left\{2\left[\left(\frac{\partial u}{\partial x}\right)^2 + \left(\frac{\partial v}{\partial y}\right)^2 + \left(\frac{\partial w}{\partial z}\right)^2\right] + \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x}\right)^2 + \left(\frac{\partial u}{\partial z} + \frac{\partial w}{\partial x}\right)^2 + \left(\frac{\partial v}{\partial z} + \frac{\partial w}{\partial y}\right)^2\right\} + S_i \tag{3}$$

3. Results and Discussion

The size of the CAD bed component was 150 mm. in width, 800 mm. in length and 80 mm. in height, the fabricated bed component was also in the same dimension. The CAD component was simulated for its surface temperature distributions by using Fluent, the commercial Computational Fluid Dynamics (CFD), with operation conditions as shown in Table 1.

Table 1. Working conditions for the simulated bed component in the CFD program.

Conditions	
Inlet water temperature	373.15 k or 100°C
Inlet water velocity	2.25×10^{-5} m^3/s
Component surface temperature	302.15 k or 29°C
Ambient temperature surrounding the component	298.15 k or 25°C

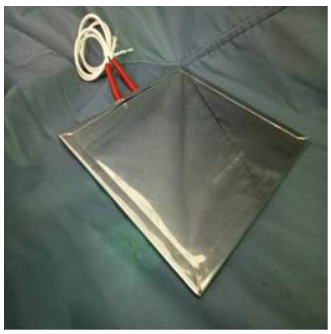
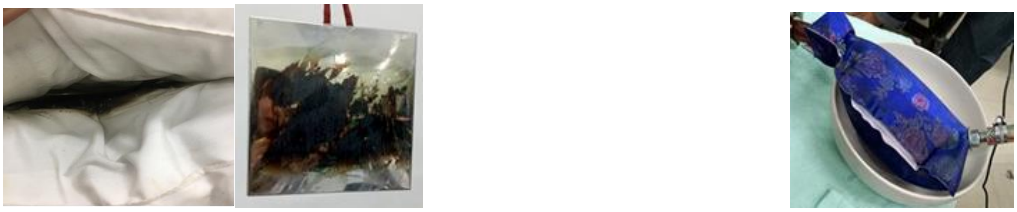


Figure 3. A plate heater

Among all three fabricated bed components, the 900 Watt plate heater (Figure 3) was chosen to heat the first fabricated bed component. The commercial rubber bag and in-house made butyl rubber bag was attached with connectors to connect with a hot water hose from a hot water tank. An infrared thermometer trademarked FLIR E40 was used to investigate temperature distributions and average

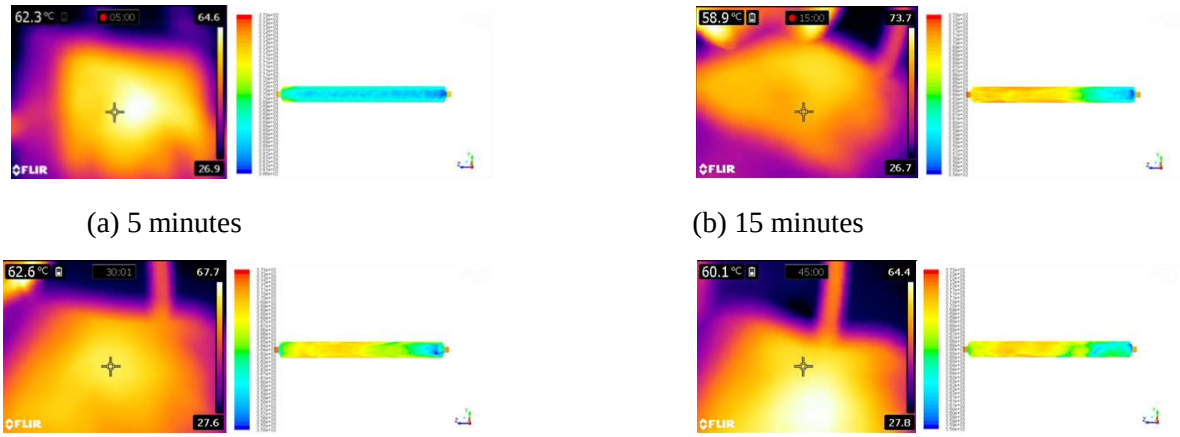
temperatures of the bed component surface. All temperatures were measured and recorded real-time since the heating system started and during the experimental investigations, the experimental conditions were set as same as indicated in Table 2. [6]

From the simulation result, the temperature of the CAD component surface reached 60 Celsius in 20 Milliseconds 60 seconds. The fabricated component which was heated by the plate heater was burned (Figure 4a) within 1 seconds after the heater stared working while the commercial rubber bag was damaged (Figure 4b) by heat from the fed hot water after the hot water was fed within 8 seconds after we started supplying hot water into the bag. Since the in-house butyl rubber bed component was the only workable component practically, the temperature distributions and average temperatures of the components obtained from the simulation and experiment were compared as shown in Figure 5 at 5, 15, 30 and 45 minutes after both surface temperatures were over 60 Celsius. From (Figure 5a), the beginning period of the heating time, the average temperature of the simulated component was around 95 Celsius. and the temperature distribution was not uniform while the average temperature of the tested component was around 54.4 Celsius. and the temperature distribution was not uniform. At 15, 30 and 45 minutes, the average temperature and the temperature distribution of the simulated and experimental bed component were getting closer than the beginning of the heating period. The dimensionless average component surface temperatures (or the average component surface temperature divided by the inlet water temperature) of both components at different periods of times were compared in Figure 6, the percentages of the different temperatures at different times; 5, 15, 30 and 45 minutes, were 49.3%, 52.8%, 45.4%, 49.8% respectively.



(a) the burned component by a heater (b) the damaged commercial rubber bag.

Figure 4. Experimental bed components; (a) the burned component by a heater and (b) the damaged commercial rubber bag.



(a) 5 minutes (b) 15 minutes

(c) 30 minutes (d) 45 minutes

Figure 5. The average temperature and temperature distribution of the simulated and fabricated butyl rubber bed components at 5,15,30 and 45 minutes respectively.

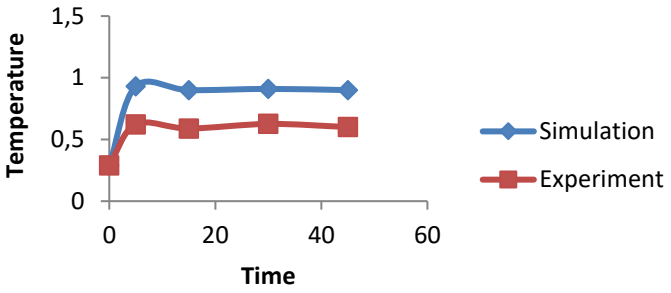


Figure 6. The dimensionless average temperatures of the simulated and fabricated butyl rubber bed components.

4. Conclusion

The objectives of this work were to design, fabricate and tests the heating bed components to find potentials of the design component to be developed as the dust-mite-free bed component for dust-mite allergy patients. The quality of the patient life could be improve by improving environmental conditions around the patient since bed components touched the patient skin in everyday life and the bed components were the suitable places for dust mites to live. Dust mites could be get rid of by maintaining their environment above 60 Celsius at least 45 minutes. The design component was drawn by using the CAD program and simulated to find its temperature distributions and average temperatures by using Fluent. Three fabricated components were experimentally investigated. The experimental results of the three bed components revealed that the the direct heating system damaged the bed component, the bed component surface near the heater was burned and the experimental investigation was stopped immediately. The commercial rubber bag was fed with 100 Celsius water, the edges of the commercial bag was damaged, the commercial rubber bag was not workable. The last investigation was focus on the in-house-made butyl rubber bag, the only fabricated bed component with no damage occurred during the experimental investigation. The average temperature differences between the simulated and experimental bed components were in the acceptable range. Therefore, the butyl rubber with the in-house-made fabrication showed its potential to be improved as components of the dust-mite-free bed for the dust-mite allergy patients to improve their quality of life.

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Quality of hygiene, sanitation and identification of *Eschericia coli* O157: H7 in Sate Languan related with traveler's diarrhea in Bali

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Abstract. Bacterial contamination in food is still a health problem that can cause food poisoning. Currently there are many kinds of food traders in the environment around us, including in the area of Bali with one of the traditional foods is Sate Languan. Sate Languan is not only favored by Balinese people, but also by tourists. Supervision of Satay Languan needs to be done in accordance with food quality standards that can prevent the occurrence of traveler's diarrhea cases. This study aims to determine the microbiological quality and hygiene of Sate Languan traders in the tourism area of the Pantai Lebih, Gianyar Regency, Bali. This study uses mixed method with sequential explanatory design, which combines two forms of research, namely quantitative and qualitative, where in the first stage takes quantitative data and then qualitative data. Quantitative approaches are also carried out with direct questionnaires and observations by researchers. The number of samples of all satay traders at Pantai Pantai and its surroundings were 19 restaurants and 19 samples of Satay Languan. The examination began with the identification of Eschericia coli O157: H7 with culture on eosin methylene blue agar (EMBA) media, identification of E. coli O157: H7 followed by growth of bacterial isolates on sorbitol MacConkey agar (SMAC) selective media followed by confirmation test with O157 latex. The results of this study indicate that including the category of unfavorable as many as 17 respondents (89.47%), while the respondents included in the good category as many as 2 respondents (10.53%). The results of observations on the habit of hand washing after mixing raw materials mostly (70.83%) have been carried out by respondents. In addition, almost all the guides have short and clean nails (79.17%), and usually clean the place after they finish selling (87.50%). However, most food handlers (83.33%) did not wear aprons and (100%) did not wear headgear while working. Of the 19 samples examined coliform and E. coli, 17 stalls contaminated with E. coli were found on average 5 x 106 cfu / gram, only 2 stalls E. coli were still within safe limits. Further test results revealed that the Eschericia coli negative O157: H7. Statistically related variables are food handlers' hygiene.

1. Introduction

Satay languan is one of the best known culinary products of Balinese food. The main ingredients are fish chopped using tools and ground and then roasted. The satay is very popular in the Pantai Lebih area, Gianyar. Often local and foreign tourists come to restaurants around the beach to enjoy this special meal.

Traditional Balinese food is still vulnerable to microbiological contamination [1][2]. Research on Lawar food in Gianyar also found high microbiological contamination [3]. Research in the Kuta tourism area also shows the same results. The vulnerability of traditional Balinese food to bacterial contamination needs to get serious attention from all parties because it is related to the health of tourists. Many tourists come to enjoy culinary tours, especially traditional Balinese food. So it is necessary to know the causes and efforts to solve the problem of food contamination.

Eschericia coli O157: H7 belongs to the E. coli (EHEC) enterenterohaemorrhagic group which is a pathogen that produces shiga like toxin which is harmful to humans[4]. Examination of E. Coli O157 content is necessary to determine the level of pathogens from these foods. Research in South Africa has detected E. coli O157: H7 contamination in pork, beef, human waste and animal waste[5]. The presence of E. coli O157 can harm food products that are marketed.

Sate Languan is a typical food on the Pantai Gianyar, but the quality of hygiene and microbiological food needs to be studied so that it can do the right guidance. Identification of pathogenic bacteria is needed to determine the level of contamination. That is why this research is to measure the level of merchant hygiene and sanitation as well as microbiological examination of food in the Pantai Pantai, Gianyar.

2. Material and Methods

This research uses mixed method with sequential explanatory design research design that combines two forms of research namely quantitative and qualitative where in the first stage retrieves quantitative data and then qualitative data. Quantitative approaches are also carried out with direct questionnaires and observations by researchers. Then the data obtained is analyzed to determine the effect of variables with each other. A qualitative approach was carried out to find out the causes of E. coli contamination in food, traders' perceptions of hygiene, perceptions of the owner of the satay tools by in-depth interviews with 7 respondents consisting of 5 traders, 2 owners of satay looms. Traders usually bring meat to the soldier to be chopped into minced meat called melt. The yield is then processed by traders into satay and meatballs sold at the beachside. The results of the interview became the skin data which became the transcript of the interview and then analyzed the contents according to the related theme.

The place of this research was carried out on Pantai Gianyar, Bali. Samples were taken to all merchants in the coastal area of more than 19 stalls. The data collected in this study are primary data in the form of food handler's characteristics, level of knowledge, and attitude of the handler through question and answer using questionnaires, while the practice of handlers, availability of sanitation facilities, and environmental conditions based on observations at the research location using the check list form. The satay sample is taken directly from the Sate Languan Trader and stored in an ice flask and taken to the laboratory.

2.1. Isolation and identification of Eschericia coli

Identification of Escherichia coli is done by this dilution method using a series of dilutions from the sample which is then planted on the medium. After incubation, the growing colonies can be calculated with the assumption that one colony that grows comes from one cell. Diluted samples were taken as much as 0.1 ml and inoculated on agar media prepared for E. coli using Eosin Methylene Blue Agar (EMBA) media. Next the sample is spread with a bent glass rod until it is evenly spread over the surface of the agar. Then the petri dish was incubated in an incubator (inverted position) at 37^0C for 24 hours. After incubating the growing colonies and metallic green were counted as E. coli colonies.

The positive colony of E. coli from EMBA media planted on nutrient to slant is then inoculated on sorbitol MacConkey Agar (SMAC) media. After incubating at 37^0C for 20-24 hours, serotype E. coli O157: H7 was detected from the appearance of clear colonies and was considered to be sorbitol negative [6]. For a more convincing confirmation that the colony was an E. coli O157 serotype, the test was continued using E. coli O157 Latex Agglutination Test (Oxoid DR620 M). Positive reaction is indicated by the formation of precipitation on the latex paper according to the control.

3. Results and Discussion

After taking the data through interviews through questionnaire instruments, observation with a check list form, and microbiological examination in the laboratory, the characteristics of the respondents (food handlers) are obtained.

Table 1. Distribution of Respondents (Food Handlers) according to Gender, Age, Education, Length of Work at Pantai Lebih, Bali

characteristics of respondents	Frequency	Percentage (%)
Gender		
Male	1	5,3
Female	18	94,7
Age		
Teenagers	2	10,5
Adult	15	78,9
Elderly	2	10,5
Education		
No school	1	5,26
Elementary school	4	21
Middle school	4	21
High school	10	52,6
Length of work		
< 10 years	10	52,6
10–20 years	3	15,7
20-30 years	2	10,5
> 30 years	4	21
Personal hygiene		
good	4	
not good	15	
availability of sanitation facilities		
available	9	47
not available	10	53
cleanliness		
clean	10	53
not clean	9	47
ownership of the tool		
yes	4	
no	15	

The youngest age of the respondent is 18 years old and the oldest is 65 years. The age of the respondents is grouped into 3 groups, including youth groups (12-20 years), adults (20-30 years), and elderly (more than 60 years). While the working period is grouped into 4 categories, including for less than 10 years, 10-20 years, 20-30 years, and over 30 years. Based on the table above, it can be seen from 19 respondents there were 18 respondents (94.7%) of female sex while the male sex was only 1 respondent (5.3%). Most respondents included the adult age group as many as 15 respondents (78.9%), the elderly age group as many as 2 respondents (10.5%), and the teen age group only 2 respondents (10.5%). The lowest education owned by respondents was no schooling as many as 4 respondents (21%). The highest level of education of respondents was 10 respondents (52.6%). In addition, the length of work of respondents with working groups less than 10 years is at most 10 respondents (52.6%), while the working group for 10-20 years is 3 respondents (15.7%), working groups for 20-30 years as much as 2 respondents (10.5%), and working groups for more than 30 years as many as 4 respondents (21%).

Personal hygiene practice variables are grouped into good personal hygiene practice groups and poor personal hygiene practices. There are more proportions of unfavorable practices compared to good practices. Can be seen in Table 1. that of the 19 respondents included in the unfavorable category as many as 17 respondents (89.47%), while the respondents included in the good category were 2 respondents (10.53%).

The results of observations on the habit of hand washing after mixing raw materials mostly (70.83%) have been carried out by respondents. In addition, almost all the guides have short and clean nails (79.17%), and usually clean the place after they finish selling (87.50%). However, most food handlers (83.33%) did not wear aprons and (100%) did not wear headgear while working.

The availability of sanitation facilities is categorized as adequate sanitation facilities and inadequate sanitation facilities. There is a proportion of inadequate sanitation facilities compared to adequate sanitation facilities. In this variable, which included 9 categories of inadequate sanitation facilities (47%), while 10 sanitation facilities were adequate (53%). The results of the observation on the respondent's selling place, most (79.17%) already had washing facilities and cooking utensils, and (91.67%) using dish soap. However, there are still many sate restaurants (75.00%) which have no toilets for consumers or traders. Almost all places do not have closed bins (95.83%), because most of them use open bins or only use plastic.

The cleanliness of the surrounding environment where the respondent sells is categorized into a clean environment and a less clean environment. There is a proportion of less clean environmental conditions compared to a clean environment. It can be seen in Table 1 that respondents who belong to the less clean environment category are 9 respondents (47%), while those included in the net environmental category are 10 respondents (53%). As a result of the observations made, most (75.00%) of the places where the respondents sold were not scattered. However, there were 66.67% of the selling places whose floors were not clean and 83.33% of the places to sell were insects such as flies, mosquitoes, cockroaches, and so on around the selling place. There are only 4 traders who do have their own tools as long as the rest are still wearing it to the yielding place.

Based on the results of laboratory examinations, it is known that according to the 2014 CFS standard there are 4 food stalls that meet the standard amount of germ contamination. The high germ contamination in making satay is not separated from the manufacturing process starting from the selection of raw materials, the process of grinding the fish into sate, equipment used, contaminating food handlers and storage at standard temperatures. Based on the results of the bivariate analysis in Table 2 it is known that related to the presence of E. coli in the Satay Lang, namely food handler hygiene. While other variables have no significant relationship. Traders' habit of washing their hands when making and processing sate is very influential on the contamination of germs.

Table 2. Results of bivariate analysis of variables of hygiene, sanitation, facilities, length of work and education.

Variable	*E. coli*		P value
	Positive (%)	Negative (%)	
Hygiene food handlers			
Not good	17 (89,5%)	0	0.06
good	0	2 (10,5%)	
Sanitation facility			
Not good	10	0	0.21
Good	7	2	
Cleanliness			
Not good	9 (47,5)	0	0,474
Good	8 (42%)	2 (10,5%)	
Education	9 (47,5%)	0	0,474
Low	8 (42%)	2 (10,5%)	
High			
Length of working			
Less than 10 years	8 (42%)	2 (10,5%)	0,474
More than 10 years	9 (47,5%)	0	

Based on the results of field observations and interviews it was found that the quality of the fish before processing played a major role in becoming a source of microbiological contamination. Fish storage that does not meet temperature standards. This can cause food contamination. Sate-making equipment is very susceptible to contamination because it is not washed with disinfectants and has long been used. Most traders take the yield material from one source, which turns out that the equipment contaminates the material, only 4 traders have their own tools. The quality of the tool used has a major role in the occurrence of E. Coli contamination. Tools that are not cleaned with disinfectants after use are susceptible to contamination.

Figure 4. Use of the hand if it is not cleaned properly is susceptible to contamination, the yield tool that is rarely cleaned is also susceptible to contamination.

The hygiene behavior of food handlers in the process of making satay is enough to play a role. The habit of washing hands with soap before taking the sate is still low. Traders must be orderly in cleaning their hands before taking food. The quality of the existing clean water is in accordance with the standard because it is obtained from the PDAM. The satay storage place before roasting is less standard because most of it is placed in a thermos where the temperature cannot be adjusted. This condition is prone to cause contamination and accelerate bacterial growth. The tools used also need to be routinely washed at a minimum after use.

Perceptions of hygiene

Some traders admit that they don't use tools like aprons, wash their hands before taking food. They feel more complicated to prepare protective equipment. Though this is important to avoid the risk of contamination in food.

"I am lazy to use tools like apron cloth, it is rather complicated, like that, you don't have to use it like that" (PD2)

"... he ... he ... (laughs), don't use any hand slop, I don't want to be a bit troublesome. Washing my hands but not always having to do it, cook every time I take hand-washing food (PD5).

"... if I don't smell my money, I don't wash my hands, just prepare the food ... the workforce is also less, especially having to wash everything. (PD3).

Meat storage

The remaining meat is stored in a cooling container or freezer. Traders store the meat in a cooling container so they will remove it if someone buys. each trader has a freezer to store satay and fish ingredients.

"I take the meat from the freezer if I want to make the satay or meatballs we remove, I keep the fish so that it lasts" (PD4)

"... I keep it in the freezer in the refrigerator if there are still leftovers. The marinade has been separated (PD3)

Perceptions about the cleanliness of the tool

According to the service provider to make the melted or minced meat tools that are used there are routinely cleaned ones that only cleans after finishing use. They also consider the cleanliness of the tool will also attract customers to come buy their services. A clean and healthy place will make their customers happy.

"I use the tool first when it's finished using the newly washed" (PD1)

"I pay attention to the cleanliness of the tool because it is related to the pleasure of the customer as well, usually after someone brings the meat here to make it melt, I immediately clean it. This is so that customers are also happy, so it's clean, healthy too. If they are dirty, there are lots of flies, they are also not happy ”(PD2)

Most traders do not have the tools to melt, so they usually bring the meat to be chopped to melt to the nearest meat processing plant.

"If I don't have a tool, I will bring it to the yielding place there ... I have subscribed (PD4).

"I have been subscribed for a long time there, I usually don't have a problem ... well the same person is still my brother" (PD6)

"I also consider the cleanliness of the appliance well, so if it is clean it is good means" (PD7).

"I already have my own tools so I have cleaned them regularly. This is important, especially if you want to sell to people so this is our good name" (PD3)

The results of this study indicate that the microbiological quality of the Satay Languan in the Pantai Lebih, Gianyar is not good, this is also related to the hygiene and sanitation of the traders. As one of the attractions, many domestic and international tourists enjoy the culinary. Efforts to improve the quality of microbiology are needed to be able to become a decent culinary tourism area for foreign tourists.

Other research results on the contamination of microbiology of Balinese lawar foods in Kuta Region also found 46.55 positive E. coli [2]. Research in Pontianak also found that contamination of seafood in traditional markets[5]. The existence of E. coli findings in food can cause food poisoning cases [6].

Research in several countries has also found the same thing that food handlers can have good knowledge and attitudes but their behavior is still poor [7][18][19][20]. There are many cases of food poisoning caused by low hygiene from food handlers. Other studies also show food handlers who have poor personal hygiene can transmit the gastrointestinal infection[8].

This study also shows the use of personal hygiene from traders such as using masks, aprons, food pickers is still low. Even the tendency to wash your hands with soap every time you take contaminated items is also low. This behavior actually causes contamination from hand to food, from the equipment used if not diligently cleaned is also a source of transmission.

Hand hygiene is very important for everyone, especially for food handlers. Hand washing habits are very helpful in preventing transmission of bacteria from hand to food. This is in line with research[9] which shows that there is a very significant relationship between behavioral category variables and germ numbers. The results of the examination of E. coli that did not meet the most requirements in the behavior in the low category (66.7%), and who met the most requirements in the respondents in the medium category (88.5%). Statistical test results show that there is a significant relationship between behavioral variables with E. coli. There is a greater proportion of personal hygiene practices than good personal hygiene practices.

Research in Depok [10] also found that the presence of E. coli in ketoprak and gado-gado foods was caused by contamination from the handler itself. It was concluded that there was a significant relationship between the nails of food handlers and food contamination. Besides that, the habit of not washing hands before serving the buyer and after going to the toilet, is a source of contaminants that are quite influential on the cleanliness of food ingredients. Smoking habits that are often seen when they wait for buyers are other contaminant factors.

Based on the results of observations in this study it is known that most food handlers do not use aprons, in accordance with the results of research in Palembang [11] which presented the results that no

street food merchants were found wearing aprons while touching food at the location of trading in the elementary school environment. Observations were also made on the use of head covers on food handlers. Of the 23 respondents found only 60.9% of respondents used a head covering, even the majority (86.9%) of respondents did not wash their hands when they wanted to touch food. All sellers have trash bins, but there are still some that have no cover. Trader hygiene practices affect the quality of food handled, poor hygiene practices can cause microbiological contamination in food because food handlers are the main and potential source of food contamination and the movement of microorganisms [12].

Based on the results of the study in Semarang [12] out of 13 rujak samples, 9 samples (69.2%) contained E. coli and as many as 30.8% did not contain E. coli. The presence of E. coli in the rujak is caused by lack of knowledge about individual hygiene practices. Chances are, most traders do not get counseling about the application of hygiene practices in handling food. Increasing knowledge of food handlers through the provision of training and courses can reduce morbidity and mortality due to food [13][17]. To prevent the transmission of diseases caused by food and beverage handlers, it is necessary to have good supervision and guidance. Food handlers are obliged to apply the 6 principles of food sanitation, namely; material selection, material storage, processing, storage of cooked food, transportation, and presentation. Although it is a must for every handler to maintain health and hygiene, there must still be supervision to ensure that a food handler is in good health while working [14].

Based on observations in this study found all traders do not have a trash can with a cover because they use open bins or only use plastic, as well as observations from research in Depok [10] which showed that some traders did not provide a garbage can complete with a lid. Jasaboga Hygiene Sanitation Requirements [15, 16] states that trash cans such as plastic bags or paper, covered trash bins must be available in sufficient quantities and placed as close as possible to the source of waste production, but can avoid the possibility of contamination of food by garbage. The person in charge of the jacket must maintain all buildings and facilities or equipment properly to avoid possible contamination of food, accumulation of dust or microorganisms, increased temperature, accumulation of waste, breeding of insects, mice and puddles of water.

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Antioxidant, α-glucosidase and nitric oxide inhibitory activities of *Phyllanthus acidus.*

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Abstract. *Phyllanthus acidus* (Euphorbiaceae) is commonly known as star gooseberry or cermai. This plant has been traditionally used to reduce pains, inflammatory and oxidative stress related disorders. Despite its traditional uses, the phytochemical constituents and bioactivities have inconsistency results. Therefore, the aims of this study were to evaluate the bioactivities of different parts (leaf and fruit) of *P. acidus* extracted with different ethanol ratios. The inhibition of α-glucosidase and nitric oxide (NO), and the antioxidant activities of two parts (leaf and fruit) of *P. acidus* extracted with various ethanol ratios (0, 50 and 100%) were studied. The results showed that extraction of the leaves with 50% ethanol gave the most active extract with the lowest IC_{50} value for α-glucosidase at 1.53 µg/mL, moderate NO scavenging and inhibitory activities (IC_{50} = 158.17 and 180.06 µg/mL, respectively), and the highest total phenolic content with 33.20 mg GAE/g extract. The 50% ethanol extract of the fruit showed the highest total phenolic content, DPPH free radical scavenging, NO scavenging, α-glucosidase and NO inhibitory activities with values of 9.42 mg GAE/g extract, 48%, 49%, 2.44 µg/mL and 43%, respectively. Hence, this is an important step in establishing the validity of the traditional claims as anti-inflammation and set the preliminary step towards developing this plant into high claim products for phytomedicinal preparations.

1. Introduction

Intake of plant phytochemicals might have beneficial effects in protecting the body from various diseases [1]. The leaf is the site of photosynthesis and biosynthesis of the phenolic compounds that migrate from the leaf to the other parts, including the stem, fruit and root [2].

Phyllanthus acidus has several common names, including star gooseberry, "Otaheiti" gooseberry or "Mayom" (Thailand) and in Malaysia, it is known locally as "Chermai", kemangul or chermala [3]. *P. acidus* can be grown in Indonesia, Vietnam, Malaysia and India [3][4]. It has long been used in numerous medical treatments. The fruits are usually pickled or processed into juice, jam or jelly [4][5]. Traditionally, *P. acidus* is used in the management of several disorders associated with pain, inflammation and oxidative stress, including rheumatism, bronchitis, asthma, respiratory disorders, hepatic disease, and gonorrhoea [6][4]. This plant is known in folk medicine, where every part of it can be used to treat several diseases. The leaves are mostly used for the treatment of hypertension, liver disease and itchy skin [7][8]. The fruits are used as a liver tonic and blood purifier, as well as for the management of several pathological conditions, such as bronchitis, constipation, vomiting and diabetes [6][9]. Previously, 4-hydroxybenzoic acid, hypogallic acid, kaempferol and caffeic acid were isolated from n-butanol leaf extracts, while gallic acid, dihydroquercetin, quercetin and myricetin were identified in the fruit juice. Many health promoting effects have been ascribed to these compounds [10, 11].

Solvent extraction is the most commonly used method for the preparation of plant extracts, but the efficiency of the extraction depends on the polarity of the analyte in the sample matrix and the polarity of solvents, as well as the time, temperature, and techniques used [11]. In this study, the leaf and fruit samples were extracted with various ethanol ratios. Ethanol at various concentrations was used due to

its ability to extract hydrophilic and lipophilic compounds and the fact that it is nontoxic and less expensive than other solvents [4]. Thus, the present study aimed to evaluate the total phenolic content, DPPH, nitric oxide scavenging, α-glucosidase and nitric oxide inhibitory activities of extracts from the leaf and fruit of *P. acidus* using various ethanol ratios.

2. Materials and Methods

2.1. Chemical reagents

Absolute ethanol, dimethyl sulfoxide (DMSO), and sodium phosphate buffer were supplied by Merck (Darmstadt, Germany), while formic acid, LCMS grade methanol and purified water were supplied by Fisher Scientific (Geel, Belgium). The other chemicals including gallic acid, α-glucosidase enzyme, glycine, 2,2-diphenyl-1-picrylhydrazyl (DPPH), *p*-nitrophenyl-α-D-glucopyranose (PNPG), lipopolysaccharide (LPS), sodium phosphate buffered saline (PBS), and recombinant murine IFN-γ were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Plant materials

The fresh leaves and fruits of *P. acidus* were collected in March 2016 at the Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor and authenticated by Dr. Shamsul Khamis, an in-house botanist and the voucher specimen (SK 2990/16) was deposited in the herbarium of the Institute.

2.3. Plant extraction

The fruits and leaves were dried at 45°C in a forced-air convection for 5 days. The dried samples were ground using a laboratory blender and stored at -20°C for a maximum of 4 wk. About 5 g of each powder sample was mixed with 100 mL of various ethanol ratios with deionized water (0, 50 and 100% v/v) and extracted using an ultrasonic bath sonicator at 40°C for 1 hr with a power setting of 90 W at a frequency of 53 KHz. Each extract was filtered through a Whatman filter paper no 1, then dried using a BUCHI rotary vacuum evaporator and lyophilized using a Scanvac freeze drier. All extracts were kept at 4°C until further analysis for a maximum of 4 wk.

2.4. Total phenolic content (TPC)

Total phenolic content was done using the Folin-Ciocalteu method as previously reported [2].

2.5. DPPH free radicals scavenging assay

The assay was used to determine the scavenging activity of the sample according to the previous method [2].

2.6. Nitric oxide (NO) scavenging assay

The NO scavenging assay was done using the previous method [12].

2.7. α- Glucosidase inhibitory assay

The α-glucosidase inhibitory assay was done using the previous method [2]. The percentage inhibition of the extracts was calculated as: % inhibition of sample = [(An-As)/ An] x 100%, where A_n and A_s are the absorbance values of the negative control and test samples, respectively.

2.8. Nitric oxide inhibitory activity

Nitric oxide (NO) production by RAW 264.7 macrophages (ATCC) was done using the method previously [13, 14].

2.9. Statistical analysis

The bioassay results were given as mean ± standard deviation of 6 replicates. Analysis of variance (ANOVA) and Tukey's honest significant difference (Tukey-HSD) multiple-comparison tests were done to show the significant difference among the results at a confidence level of 95%.

3. Results and discussion

3.1. Effect of various ethanol ratios on the biological activities of P. acidus extracts

The total phenolic contents in the *P. acidus* samples extracted with the various ethanol ratios are shown in Table 1. For both *P. acidus* leaf and fruit extracts, the 50% ethanol ratio resulted in the highest amounts of phenolics ($p<0.05$). Furthermore, the result showed leaf extracts have higher TPC ($p<0.05$) compared to the fruit. Therefore, the leaf of *P. acidus* extracted with 50% ethanol had the highest TPC. A previous study showed that different ethanol concentrations differed in their ability to solubilize compounds, which thus affected the extraction of the phenolic compounds [15]. The leaves have been found to contain the maximum amount of phenolic compounds especially when maturity is reached [2]. In addition, previous study reported that the leaf had higher amounts of phenolic compounds and flavonoids as compared to other parts that are synthesized using the shikimic acid pathway [16].

The free radical scavenging activity of the extracts using the DPPH test are shown in Table 1. For the leaf extracts, the 100% ethanol showed the highest percentage inhibition ($p<0.05$). There was a significant difference ($p<0.05$) between leaf and fruit extracts for the 100 and 50% ethanol ratios, whereas there was no significant difference between the 0% ethanol extracts. However, the IC_{50} values for fruit extracts were not determined because the percentage inhibition was <50%. The 100% ethanol leaf extract was the most active in the DPPH assay. However, the highest TPC was seen in the 50% ethanol extract. Therefore, it could be proposed that the polar phenolic compounds in 50% ethanol extracts of *P. acidus* may not be contributing directly to antioxidant activity, whereas other phytochemical compounds apart from the phenolics, were responsible for the activity showed by the 100% ethanol leaf extract. Furthermore, research has been done on the optimization of extraction of the phenolic compounds in *Centella asiatica* as a function of ethanol concentration, where the results showed the optimized condition was achieved with a 40% ethanol concentration [17]. This result was in agreement with the work previously [17], who found that the different polarity solvents varied in antioxidant activities. Thus, the study concluded that the ethanol concentration used for the extraction had a significant effect on the phenolic contents and antioxidant compounds in the extracts.

Based on Table 1, the evaluation of NO scavenging activity of *P. acidus* extracts showed significant differences ($p<0.05$) among samples. The results showed similar trend with free radical scavenging activity. The highest NO scavenging activity among the samples was observed for the 100% ethanol leaf extract while for the fruit extracts, the greatest activity was observed for the 50% ethanol extract. The leaves extracts showed the highest percentage inhibition indicating the accumulation of more antioxidant compounds in the leaves, which are known to be a site of secondary metabolites production [3]. The phenolic and flavonoid compounds that are present as secondary metabolites in plants have been reported to act as scavengers of singlet oxygen and free radicals and might also be responsible for the nitric oxide scavenging activity [18]. Furthermore, a previous study showed that the anti-inflammatory activities in *P. acidus* extracts based on NO scavenging and NO inhibition in the presence of natural antioxidants, such as kaempferol and quercetin, contributed to the anti-inflammatory activities of the *P. acidus* extracts [19].

Table 1 shows the percentage inhibition of α-glucosidase activity and the statistical analysis showed there were significant differences ($p<0.05$) among the different extracts. The 50% ethanol extracts of the leaves and fruits of *P. acidus* showed the highest α-glucosidase inhibitory activity and the lowest IC_{50} values. Therefore, 50% ethanol was the most suitable solvent system to extract *P. acidus* compounds that have anti-diabetic properties. The results for fruit extracts were in agreement with a previous study in which the fruit juice of *P. acidus* was shown to have the highest percentage of α-glucosidase inhibitory activity among various tropical fruits juices [8]. Although the leaves have traditionally been used for the treatment of oxidative stress-associated diseases, such as diabetes [7]

there is still not sufficient scientific evidence to support this claim. The current work showed that both parts of *P. acidus* showed high percentages of α-glucosidase inhibitory activity, which may support the traditional claims.

The NO inhibitory activity of the *P. acidus* showed that the 50% ethanol extract of the leaf had a greater percentage inhibition followed by 100 and 0%. However, there was no significant difference between the IC_{50} values for the 0 and 50% ethanol extracts. These findings were further supported in previous studied [19]. The leaf extracts of *P. acidus* suppressed the production of NO due to the content of caffeic acid, kaempferol and quercetin based on their HPLC analysis. The evaluation of NO inhibition was carried out on the basis of the traditional uses of this plant for treating several oxidative stress-associated diseases, such as skin disorders, rheumatism, bronchitis, diabetes, and psoriasis [19].

4. Conclusions

The 50% ethanol extract of the leaves of *P. acidus* showed antioxidant activity, a high total phenolic content, and substantial inhibition of α-glucosidase and NO, which strengthened the traditional claim of its value for treating oxidative stress related diseases. The present study may help to ascertain the potency of *P. acidus* as a potential source of bioactive compounds for oxidative stress associated diseases, such as diabetes and inflammation. However, extensive studies need to be carried out to show the potential use of this plant as a natural therapeutic agent and to be incorporated as an element of functional foods or nutraceutical products.

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Table 1 TPC, DPPH scavenging, NO scavenging, α-glucosidase and NO inhibitory activities of *P. acidus* extracts

Part	Ethanol ratio	Total phenolic content, mg GAE/g extract	DPPH radical scavenging activity		Nitric oxide scavenging activity		α-Glucosidase inhibition		Nitric oxide inhibitory activity		MTT
			Percentage inhibition, % (500 µg/mL)	IC_{50}, µg/mL	Percentage inhibition, % (500 µg/mL)	IC_{50}, µg/mL	Percentage inhibition, % (500 µg/mL)	IC_{50}, µg/mL	Percentage inhibition, % (500 µg/mL)	IC_{50}, µg/mL	Percentage inhibition, % (500 µg/mL)
Leaf	100	$10.95^{Aa} \pm 0.53$	$57.34^{Aa} \pm 2.05$	$221.25^{a} \pm 18.68$	$80.02^{Aa} \pm 3.63$	$113.83^{a} \pm 9.60$	$32.44^{Aa} \pm 3.46$	ND	$85.46^{Aa} \pm 2.75$	$208.94^{a} \pm 16.30$	$105.28^{Aa} \pm 6.94$
	50	$33.20^{Ab} \pm 1.55$	$40.45^{Ab} \pm 3.80$	ND	$76.24^{Aa} \pm 1.95$	$158.17^{b} \pm 11.88$	$99.52^{Ab} \pm 0.24$	$1.53^{Aa} \pm 0.23$	$96.74^{Ab} \pm 1.35$	$180.06^{ab} \pm 17.67$	$71.59^{Ab} \pm 2.43$
	0	$20.70^{Ac} \pm 1.02$	$33.27^{Ac} \pm 2.51$	ND	$44.86^{Ab} \pm 2.84$	ND	$92.06^{Ac} \pm 2.54$	$6.29^{b} \pm 0.98$	$84.22^{Aa} \pm 1.58$	$164.45^{b} \pm 15.83$	$96.35^{AC} \pm 2.07$
Fruit	100	$1.56^{Ba} \pm 0.07$	$23.89^{Ba} \pm 2.74$	ND	$41.11^{Ba} \pm 1.74$	ND	$24.31^{Ba} \pm 3.55$	ND	$35.03^{Ba} \pm 8.97$	ND	$89.57^{Bab} \pm 4.17$
	50	$9.42^{Bb} \pm 0.44$	$48.41^{Bb} \pm 1.51$	ND	$49.39^{Bb} \pm 0.89$	ND	$98.34^{Bb} \pm 0.78$	$2.44^{A} \pm 0.77$	$43.30^{Ba} \pm 5.78$	ND	$92.80^{Ba} \pm 6.53$
	0	$6.98^{Bc} \pm 0.43$	$35.20^{Ac} \pm 3.02$	ND	$45.25^{Ac} \pm 1.10$	ND	$37.78^{Bc} \pm 2.48$	ND	$37.85^{Ba} \pm 6.34$	ND	$82.28^{Bb} \pm 3.52$
Quercetin				$2.58^{b} \pm 0.34$		$7.43^{c} \pm 0.71$		$0.82^{c} \pm 0.16$			
Curcumin										$10.94^{c} \pm 0.43$	

Data expressed as mean± standard deviation.
Different capital letters indicate significant differences ($p<0.05$) between plant parts while different lowercase indicate significant differences ($p<0.05$) among ethanol ratios.
ND: Not determined

Protein derived from plant pest (*Erionata thrax*) for growth medium of antagonistic bacteria

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AbstractPropagation of bacteria biological agents usually use medium ECG (Potato Extract Sugar) which has low protein content. A compatible medium, NB (Nutrient Broth) is very expensive. Some pests are found to be a good protein source for nutritional purposes such as caterpillars. This study was carried out to find the feasibility of using pest as substitution medium to grow bacteria biological agens. Research methods include substitution test medium for the propagation of *B.subtilis* with various concentrations (pour plate) and antagonis activity test (clear zone). Substitution medium of caterpillars can replace Nutrient Broth. Final density of *B.subtilis* (after 48 hours shaker) is 10^{18} CFU/ml (increase 10^{8} CFU/ml) for caterpillars medium. Protein content test results of substitution liquid medium about 5.4 to 5.8% for in all treatment concentrations, equivalent with protein content of Nutrient Broth which is about 5.88%. Generation time (g) of *B. subtilis* is 26.34-26.57 minutes and 1.6 hour^{-1} for growth rate constant (k). Biological agents *B. subtilis* still antagonistic to *Erwinia carotovora* with the clear zone 0.65 cm as well as nutrient broth medium, 0,65 cm.

1. Introduction

Erwinia carotovora is one of the causes potato tuber soft rot disease, both in the field and in the storage which damage between 15-100% [1]. Javandira [2] suggested that *Bacillus subtilis* is potentially to be controlled the pathogen *Erwinia carotovora* by producing antibiotics. *Bacillus subtilis* are produced siderophores and antibiotic that can be inhibited the growth of pathogens [3]. Antagonist of *Bacillus subtilis* is used as a biological agent that is started has been developed.

Propagation of bacteria biological agents usually use medium ECG (Potato Extract Sugar) which has a low protein content, whereas the bacteria need a lot of protein to grow. One compatible medium is NB (Nutrient Broth). Nutrient Broth is a common medium used to grow bacteria in laboratories. This is a basic medium composed of a simple peptone and a beef extract. As the readily available culture media are expensive, there is a need to find alternative media or substitute medium. There are many sources of protein in the nature, especially kind of pests such as snails, caterpillars and grasshoppers. Groups of insects (including caterpillars or larvae) about 40-60% [4]. Potential proteins of that pest are needed to be studied to be used as a substitution growth medium of bacteria biological agents. The present research is aimed at replacing the nutrient source by a protein formulation from pests and also antagonistic activity of *B. subtilis* in controlling *E.cartovora*.

2. Material and Methods

2.1. Medium formulation

The samples were finely powdered separately using electric blender and sieved. The powder was stored separately in sterile containers until its use. Four different solid media were prepared as follows, 8 g per liter distilled water from each protein source was taken and mixed with 20 g of agar (as substitution of Nutrient Agar). Substitution medium treatment were used liquid medium with various concentration medium, they are P1 = 4 g/L, P2 = 6 g/L, P3 = 8 g/L, P4 = 10 g/L, P5 = 12 g/. After

boiling the substitution medium, it was incubated at 2-5^{0}C for 24 hours at least and then sterilized on autoclave at 121^{0}C and 2 atm for 20 minutes. The design used was completely randomized design (CRD) with Duncan further test (DMRT) at 5% significance level.

2.2. Substitution medium test for the propagation of bacteria biological agents b.subtilis

The test used liquid medium. Observations were made every 6 hours for 48 hours to calculate the density of bacteria using methods puor plate through previous dilution. Each treatment using a sample of 10 ml of liquid medium plus 100 mL *B. subtilis* 10^{10} cfu / mL and repeated 3 times. The treatments are type dan concentration of flour g/L distilled water. They are: P0: 8g Nutreint broth (NB)/L BP1: 4g caterpillar flour (*Erionata Thrax*)/L, BP2: 6g caterpillar flour/L, BP3: 8g caterpillar flour/L, BP4: 10g caterpillar flour/L, BP5: 12g caterpillar flour/L.

Variable observtions of this test are population of bacteria and bacterial growth rate. It also show the generation time that calculated by the formula [5]:

$$g = \frac{t}{3.3 \log (b/B)}$$

where g is the generation time, t is the time difference measurement, B is the initial population, b is the population after time t and 3.3 is the conversion factor log2 be log10. The rate of growth of biological agents B. subtilis is calculated by formula [6]:

$$k = 0.693/g$$

where k is the growth rate (h-1); g is the generation time (minutes); and 0.693 is the number of statutes.

2.3. Antagonis Activity Test of Biological Agents B. Subtilis Against Erwinia Carotovora

Antagonistis activity test of *B. subtilis* is performed in vitro on a petri dish with the overlay method, or better known as the clear zone by Wakimoto *et al* [7] and Hara and Ono [8]. Tests were conducted to determine the antagonistic activity of biological agents against E. carotovora B. subtilis after propagated by substitution medium. B. subtilis to be tested taken from the shaker for 7 treatments are**:** P0: 8g Nutreint broth (NB)/L (control), BP1: 4g caterpillar flour (*Erionata Thrax*)/L, BP3: 8g caterpillar flour/L, BP5: 12g caterpillar flour/L. Variable quantity observations seen through the clear zone index calculation formula [8]:

$$I = \frac{B - A}{B}$$

Where I is clear zone index (cm), A is colony diameter of biological agens (cm) and B is clear zone diameter (cm).

2.4 Protein Test

The method of analysis used to test protein is a semi micro Kjeldhal, Soxhlet method for analysis of fat, alkaline acid digestion method for fiber analysis, a method for the analysis of the ash furnace, oven method for the analysis of water, and the method of calculation based on nutrient (BETN).

3. Result and Discussion

3.1 Substitution Test Medium for The Propagation of Bacteria Biological Agents B.subtilis

The data is presented in four forms: growth curve, generation time, rate of growth, and growth analysis every 6 hours. It showed that substitution medium from source pest protein can grow *B. subtilis* as well as nutrient broth (as liquid medium) and nutrient agar (as solid medium).

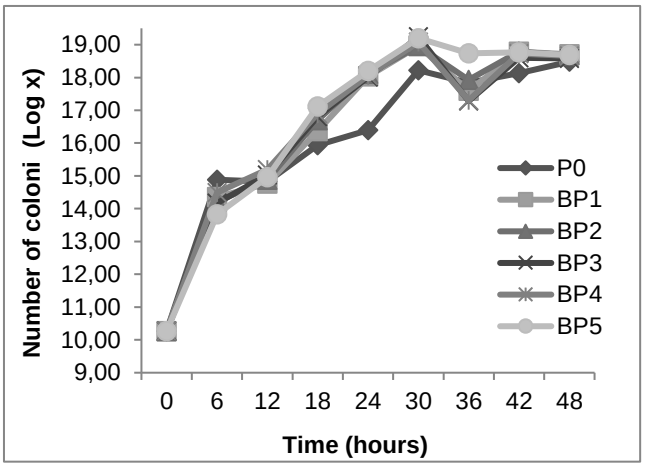

Figure 1. Growth curves of *B. subtilis* on Substitution Medium of Pests. P0: Nutrient Broth (8gr / L); (a) Substitution Medium of snails. BP1: Caterpillar flour (4g/L); BP2: Caterpillar flour (6g/L); BP3: Caterpillar flour (8g/L); BP4: Caterpillar flour (10g/L); BP5: Caterpillar flour (12g/L).

Figure 1 showed an exponential and stationary phases. There are four phases of bacterial growth when grown in batch culture lag phase, exponential phase, stationary phase and death phase [9]. Lag phase is not clearly visible because the time of observations were took every 6 hours so it could to be seen the growth during the interval of 6 hours. Volk and Wheeler [10] suggests that the lag phase (grace) lasted for one hour to several days depending on the type of bacteria, the age of the culture and the nutrients contained in the medium. An increase in the production of enzymes by engineering the medium concentration, pH of the medium, temperature, carbon and nitrogen sources indicate that Bacillus sp. TPT-20 adaptation during the lag phase + 5 hours [11]. Phase of death has not been 5 seen because observations only 48 hours. The rate of death is accelerating became exponentially dependent on the species, all cells died within a few days or a few months [6].

Table 1. Average generation time and growth rate constant of *B. subtilis*

Treatments	value	
	g (minutes)	k (hours^{-1})
P0B (Kontrol)	26.34	1.58
BP1 Caterpillars Flour (4 gr/L)	26.57	1.57
BP2 Caterpillars Flour (6 gr/L)	26.48	1.57
BP3 Caterpillars Flour (8 gr/L)	26.33	1.58
BP4 Caterpillars Flour (10 gr/L)	26.44	1.57
BP5 Caterpillars Flour (12 gr/L)	26.47	1.57

Generation time and growth rate constant of *B. subtilis* for each treatment has ranged from 26.33 to 26.57 minutes and 1.6 to 1.7 h^{-1} after propagated using substitution medium, as well as Nutrient

browth (Table 1). According Maryanti et al. [12], *Bacillus subtilis* generation time is 33.43 minutes and the growth rate constant (k) is 1.15 h^{-1}. The other research found that generation time (g) and growth rate constant (k) for *Bacillus subtilis* is 45.04 minutes and 0.92 h-1 [13]. The difference in value is based on the ability of each bacterial metabolism [13].

The ability of substitution medium also showed high density after shaker for 48 hours. First density of *B. subtilis* is known 1.87 x 1010 cfu/mL for POB (Table 2). Average density for substitution medium from snails in all treatments is known 10^{10} cfu/mL (Table 2). This means that the population of *B. subtilis* increases as much as 10^{8} cfu/mL for 48 hours shaking, as well as control (Table 2). Substitution medium ability was analised. Growth analysis of biological agents B. *subtilis* every 6 hours inter treatment in snails medium showed that the observation of 12 hours and 42 hours affects the growth of B. subtilis. Growth analysis in caterpillars medium showed that there are effects of inter medium treatments every 6 hours. At the 36-hour observation, BP5 has the best ability in growing *B. subtilis*. There are significant ecffects between treatments from different source medium per 6 hours observation. In general, the data showed that BP (caterpillars medium) has better ability in growing *B. subtilis* than control and other medium source at the 18, 24 and 30 hours observation. In the end of observation (42 and 48 hours), the ability of caterpillars medium as good as grasshopper medium and control (POB).

Table 2. Average population of *B. subtilis* end results propagation in substitution medium

Treatments	Density (cfu/mL)
P0B (control)	4.01×10^{18}
BP1 (4 gr/L)	5.78×10^{18}
BP2 (6 gr/L)	5.05×10^{18}
BP3 (8 gr/L)	3.95×10^{18}
BP4 (10 gr/L)	4.70×10^{18}
BP5 (12 gr/L)	4.97×10^{18}

Description: P0B: using Nutrient broth (8 g / L). BP1: Caterpillars Flour (4 g/L); BP2: Caterpillars Flour (6 g/L); BP3: Caterpillars Flour (8 g/L); BP4: Caterpillars Flour (10 g/L); BP5: Caterpillars Flour (12 g/L).

The high ability of substitution medium pest source are depend on nutrients contained in the medium. Bacteria need a source of energy, carbon and some nutrients to grow. Bacteria require a small amount of certain organic compounds for growth as an important substance that is not able to be synthesized from the nutrients available, it is referred to as a growth factor (need in small amounts by cells to play a role in the biosynthesis). Needs are managed on metabolic pathways in the cell. According to Todar [14], growth factors are divided into three, namely, (1) a purine and pyrimidine for the synthesis of nucleic asan (DNA or RNA); (2) amino acids for protein synthesis; and (3) vitamins as coenzyme.

Table 3. Chemical content of pest flour as medium substitution *Erionata thrax* (per 100gr)

Content	Sample of flour (%)
protein	57.759
fat	4.627
carbohydrate	14.657
fiber	12.758
ash	10.169
water	7.211

Nutrient broth has 3 g of beef extract composition and 5 g of peptone per liter of distilled water. Beef extract serves as a source of vitamins and other growth factors while peptone serves as a source of amino acids, N, S, and P. In the substitution medium, beef extract requirements are met with a fat

content, peptone replaced by protein content. Klimov et al. [15] suggested that carbohydrate is in the medium can lead to an increase in the growth of microorganisms, but if it is in large numbers to have a negative effect on enzyme production. Substitution medium has high protein source Table 3 show that caterpilar flour have high protein content, 57,759%. Protein test for substitution medium <5% in all average treatments for caterpillars (Table 4).

Table 4. Average protein and pH of liquid substitution medium in all treatments

sample	Protein (%)	pH value
P0 (control)	5.88	6.68
BP1 (4 gr/L)	5.60	6.43
BP2 (6 gr/L)	5.73	6.53
BP3 (8 gr/L)	5.82	6.42
BP4 (10 gr/L)	5.71	6.21
BP5 (12 gr/L)	5.84	6.48

Description: P0: Nutrient broth (8 g / L). BP1: Caterpillars Flour (4 g/L); BP2: Caterpillars Flour (6 g/L); BP3: Caterpillars Flour (8 g/L); BP4: Caterpillars Flour (10 g/L); BP5: Caterpillars Flour (12 g/L).

In addition, the bacteria also require appropriate physical conditions in the growth media such as O2 concentration, temperature and pH [14]. The results showed that, all treatments of snails medium (AP) had an average pH of pH 7-8, while the medium caterpillars (BP) and grasshoppersmedium (CP) having a pH of 6-7. Some of the main factors that effect the growth of microorganisms involve the supply of nutrients, time, temperature, water, pH and oxygen availability. According to Volk and Wheeler [10], some bacteria grow at pH 6, are not rare organisms that grow well at pH 4 or 5 and very rarely an organism can survive well at pH 4.

Different pH values on a medium showed changes in the concentration of hydrogen ions (H +) in the medium [14]. According to American Public Health Association (APHA) [16], setting the standard pH value of Nutrient Broth is around 6.8 as same as the result in this study, around 6.86. It has similarities with pH value of caterpillars and grasshoppers medium, 6.21-6.78 that be measured in this study. While the snails medium have a higher pH, between 6.72-8.03. Biological agents' *B. subtilis* can grow at pH 6-11, and optimum at pH 7 [17].

3.2 Antagonis Activity Test of Biological Agents B. subtilis against Erwinia carotovora

Antagonist test results showed that *B. subtilis* still have antagonistic activity against *Erwinia carotovora* (disease causing soft rot of potato tubers) after propagated by substitution medium. *B. subtilis* have antagonistic mechanism that is more inclined to the ability to produce antibiotics [18]. *B. subtilis* can produce several peptides that act as an antibiotic and antifungal, such as: subtiline, aterimin, bacitracin, subtilosin, mikobasillin, subsporin, ituirin, serexin, surfaktin, basillomicin, basillisin, 10 acid cyanide, fengimisin, and basillisosin [19]. Based on research Javandira [2], *B. subtilis* showed potential inhibiting the growth of pathogenic *E. carotovora* the clear zone, which is about 0.35 cm - 0.45 cm. Index produced a clear zone of biological agents B. subtilis using substitution medium range between 0,5- 0,7 cm (Table 5). The difference in value of the index clear zone thought to be caused by differences in the concentration of *B. subtilis* was used in the study.

Table 5. Average test antagonists biological agents propagation medium b. subtilis results against *Erwinia carotovora* substitution

Treatments	Clear zone indeks (cm)
P0 *Nutrient Broth* (8 gr/L)	0.64
BP1 caterpillar flour (4 gr/L)	0.65
BP3 caterpillar flour (8 gr/L)	0.63
BP5 caterpillar flour (12 gr/L)	0.63

4. Conclusion

From this present research, it can be concluded that substitution medium of caterpillars can replace Nutrient Broth. *B. subtilis* generally grows well in all the substitution medium treatment for each protein formulations and still antagonistic to *E. carotovora*. It recommended to use this substitution medium with formulation 4g/L distelled water the liquid medium and 8g/L plus 20g of agar for solid medium. Further research should be carried out with various types of bacteria and its antibiosis activity.

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Isolation and characterization of cellulolytic bacteria from forest land and waste disposal places in Bali

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Abstract. This study aimed to obtain a new strain of cellulolytic bacteria that could degrade lignocellulose waste as one of the abundant biomass into simple sugars such as glucose before being converted into bioethanol. The result of this research with cellulolytic media obtained 20 isolates that were able to grow and utilize cellulose as a carbon source. But among them only 6 isolates produced a clear zone with Gram's Iodine test ranging from 11.03 to 36.9. Four isolates having the largest clear zone would be further treated by using the filter paper or filter paper degradation test. KJ03 bacteria isolate had the highest ability to lower cellulose on paper Whatman no. 1 whose percentage was 82.08%, followed by isolates TS04, TS06 and BG03, the percentage of which was 74.94%, 39.43%, and 35.24%, respectively. DNA sequencing analysis of the 4 best isolates was most likely the genus of *Klebsiella*, although it might still be possible from other genera such as *Pseudomonas* or *Shigella*. KJ03 was identified as *Klebsiella quasipneumoniae*, TS04 was identified as *Klebsiella* sp., TS 06 was identified as *Klebsiella quasipneumoniae* and BG03 was identified as *Klebsiella pneumoniae*.

1. Introduction

The actualization of a circular economy through the use of lignocellulosic wastes as renewable resources can lead to reduce the dependence from fossil-based resources and contribute to a sustainable waste management. The integrated bio-refineries, exploiting the overall lignocellulosic waste components to generate fuels, energy, and chemicals, are the pillar of the circular economy [1]. Every year, millions of tons of lignocellulosic waste are formed by plant cell walls [2]. Lignocellulose is an organic component consisting of three polymers namely cellulose (35-50%), hemicellulose (20-35%), and lignin (10-25%) [3]. Cellulose is the largest component of plant cell wall constituents and becomes organic waste that is degraded for a long time [4]. Cellulose $(C_6H_{10}O_5)_n$ is a linear homopolysaccharide composed of 100-4000 units of monosaccharides β-glucose via β-1,4-glycosidic bonds [5]. Cellulose is shaped like a fiber, clay, insoluble in water, and is found in the walls of plant protective cells, especially on stems, branches, and all woody parts of plant tissues. Conventionally, this biomass is handled by burning so that it can cause air and environmental pollution. Whereas the biomass residue of this plant, with the help of cellulolytic microbes that secrete cellulase enzymes, can be hydrolyzed by cutting the β-1,4-glycosidic bond on the long chain of cellulose into products that have added value, such as glucose, biofuels, organic chemicals and sources quality nutrition, good for animal feed or food sources [6].

Cellulolytic bacteria are bacteria that can produce cellulase enzymes and have the ability to break down cellulose into glucose monomers and make it a carbon source or energy source [3]. Some genus of cellulolytic bacteria are *Clostridium, Cellulomonas, Bacillus, Thermomonospora, Ruminococcus,*

Bacteroides, *Acetivibrio*, *Misrobispora*, and *Streptomyces* which can produce cellulase enzymes effectively [7]. Each cellulolytic bacteria produces a different cellulase enzyme complex, depending on the genes and carbon sources used [8]. The use of microbial as an enzyme producer is chosen because it has several advantages, including cheap production costs, can be produced in a short time, has a high growth speed, easy to control [9].

Many studies have been carried out to obtain cellulose degrading bacteria which are capable of producing cellulase enzymes that are more effective and efficient from various sources such as soil, decaying plant material, sources of hot springs, organic matter, ruminants and compost [2, 10]. Shaikh *et al*. [11], cellulolytic bacteria from several different environments (paper industry waste, municipal waste, sugar cane plantations, parks and wood furniture) and obtain 11 isolates are able to degrade cellulose with specific activities cellulolytic is 8.4 U/mg. Zin *et al*. [12], isolate cellulolytic bacteria from cow manure and soil and obtain 6 isolates that can degrade cellulose with the highest cellulolytic activity is 1.702 U/mL. The soil is one of the habitats of cellulolytic bacteria [13]. The characteristics of soils that have a lot of cellulolytic bacteria are soil that contains litter or parts of plants that have died (stems, twigs, branches, leaves, skin, etc.) and organic waste.

The cellulolytic bacteria as the cellulase enzyme-producing microbes offer great potential in the field of handling cellulose waste and bioconversion of cellulose into glucose as an intermediate product that can be used as an industrial raw materials, one of which is for bioethanol production [14]. Therefore, it is necessary to conduct isolation, test the ability of cellulolytic bacteria in order to potentially degrade cellulose and identify them with 16S rDNA amplification and sequencing.

2. Research methods

2.1. Materials and cellulolytic medium preparation

Soil samples were collected from the forest of Batu Village, Gianyar Regency, Kutuh Village, Badung Regency, and the Final Waste Disposal Site (TPAS) of Suwung Village, Badung Regency, Bali Province. The medium used is cellulolytic medium with the following composition: KH_2PO_4, K_2SO_4, NaCl, $FeSO_4$, NH_4NO_3, $MnSO_4$, Carboxymethylcellulose (CMC) and agar. Meanwhile, for screening tests with the method Gram's Iodine use KI dan I_2. Primer 27F (5'-AGAGTTT GATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3').

Preparation of solid cellulolytic medium for isolation and screening of bacteria that have the potential to degrade cellulose is by dissolving 1 g KH_2PO_4, 0,5 g K_2SO_4, 0,5 g NaCl, 0,5 g $FeSO_4$, 1 g NH_4NO_3, 0,01 g $MnSO_4$, 10 g CMC, 20 g agar in 1000 mL distilled water. pH the medium was set to 7.0 (neutral) and sterilized with temperature 121°C for 15 minutes. The liquid cellulolytic medium is prepared in the same way as a solid cellulolytic medium but without agar.

2.2. Sampling and isolation of cellulose-degarding bacteria

Soil samples were taken from three different locations, namely in Batu Gianyar Village forest, Kutuh Jimbaran Village forest, and Suwung TPAS. For sampling in the forest, it is carried out by taking the soil from the wood while the sampling at the landfill is done by taking the soil from the trash. The method of taking soil samples is done randomLy at a depth of 0-10 cm. Soil samples are put into a plastic container and labeled and then stored in a cool box with a temperature of 4ºC during the trip from the field to the laboratory.

Bacterial isolation is done by the dilution method. Soil samples as much as 5 g, then put into a closed bottle which contained 45 mL of sterile peptone water (0.1%). From the 9 mL of culture remained after sub-cultivation, 100 mL was serially diluted up to 10^{-5} and 100 mL of each dilution was spread on cellulolytic agar plates supplemented with 0.1% (w/v) CMC, then incubated at 30ºC for 96 hours [15]. The selection criteria for isolates are based on the color, shape, elevation, edges, and size of the colony [16].

The purification of isolates was carried out by quadrant streak method, which moved the colonies that grew separately and had different characteristics from the shape, color, and morphology, then inoculated on a new solid cellulolytic medium and incubated at 30ºC for 96 hours. Separately grown

colonies in the last quadrant were transferred back to the new cellulolytic medium with the same method to obtain pure isolates [17]. After obtaining pure isolate, it was then inoculated on a test tube which contained a new liquid cellulolytic medium containing 1% CMC and incubated at 30ºC for 96 hours in a shaker with a speed of 100 rpm. After growing, 1000 µL was transferred into a 3 mL bottle containing 1 mL of 40% glycerol solution (as a culture stock) and stored in a freezer at -70ºC.

2.3. Screening of cellulolytic bacteria

The cellulolytic bacterial screening was carried out using Gram's Iodine method with cellulolytic medium containing 1% CMC which is cellulose derivative, so it can be used to test the ability of isolates to degrade cellulose [17]. Bacterial isolates obtained from culture enrichment were taken as much as 10 µL grown in a petri dish containing solid cellulolytic medium containing 1% CMC and incubated at 30ºC for 96 hours.

After incubation, petri dishes that had been overgrown with bacterial isolates were added a sterile Gram's Iodine solution (2.0 g KI and 1.0 g I_2 in 300 mL distilled water) as much as 5 mL by pouring evenly throughout the selective media surface and left for 5 minutes. Gram's Iodine solution is removed from the petri dish. Cellulolytic bacteria can be known by looking at and measuring the diameter of the clear zone formed around the growing colonies. The striking color difference between the hydrolyzed portion after the addition of Gram's Iodine in the media due to Gram's Iodine solution produces a bluish black complex that reacts with cellulose so that the clear zone is more clearly visible. Based on the diameter of the clear zone (cm) produced, the ability of isolates to degrade cellulose can be grouped into three, namely low ability (0.5-1.9), medium (2.0-3.9) and high ability (≥ 4.0)[18]. Four isolates showing the diameter of the largest clear zone were selected and used for further testing.

2.4. Cellulose degradation testing

Testing the cellulose degradation ability of cellulolytic bacteria isolates in this study is by using FPase test method (Filter Paperase). The bacterial strain in the culture stock is rejuvenated on a liquid cellulolytic medium. Rejuvenation results from the culture stock were inoculated into Erlenmeyer containing 150 mL of cellulolytic medium containing 1% CMC, and incubated at room temperature for 96 hours and shaken out at a speed of 100 rpm. After that, centrifuged (5,000 rpm for 20 minutes, 2 times washing repetition) and adjusted to OD_{660}, 5. One milliliter isolate cell was transferred into Erlenmeyer containing 20 mL liquid cellulolytic medium without CMC which already contained filter paper (Whatman no. 1) with size 1 x 6 cm^2 and with a certain weight. Then incubated at room temperature for 10 days at a shaker with a speed of 100 rpm [19]. The filter paper after incubated, washed with distilled water and then drying at 103ºC in the oven for several hours until the weight was constant and then weighed. The results of the difference between the filter paper before incubation and after incubation showed the level of cellulose degradation of the isolates tested. The isolates selected in this study were those that had the highest degradation rate in the filter paper test (which was indicated by the highest loss of filter paperweight).

2.5. Species identification

Isolates were identified based on physical standards and biochemical tests, including motility, Gram staining, methyl red (MR) testing, Voges-Proskauer (VP) tests, activities of catalase, oxidase, urease, and arginine dihydrolase, tests for nitrate reduction, indole production, utilization citrate, and gas production from glucose. Tests of carbon sources (D-lactose, D-Glucose, D-Fructose, D-Maltose, Mannose, Xylose, D-rhamnose, D-Mannitol, and D-Sorbitol) were used to evaluate carbon utilization. Except for the gelatinase activity test (which was carried out at 20°C), all tests were carried out at 28°C in the right medium and carried out according to the standard method [20].

Bacterial isolates were grown in LB medium (Tryptone, 10 g/L; yeast extract, 5 g/L; NaCl, 10 g/L) and set the pH to 7.0 and incubated at 30°C for 48 h. The cultures were centrifuged at 10,000×g for 1 min, and the supernatant was removed. DNA extraction was performed using a Cell/Tissue Genomic DNA Extraction Kit (BioTeke Corporation, Beijing, China) according to the manufacturer's instructions, and the genomic DNA was stored at −80°C until further analysis. Bacterial universal primers 27F (5'-

AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') were used to amplify the 16S rDNA from genomic DNA. Polymerase chain reaction (PCR) was performed in a thermocycler (MyCycler, Bio-Rad, USA). Each reaction mixture (50 µL) contained 5 µL of 10× reaction buffer without $MgCl_2$, 1.5 mM $MgCl_2$, 0.2 µM of each primer, 0.2 mM of each dNTP, 2.5 U of Taq DNA polymerase (TaKaRa Biotechnology (Dalian) Co., Ltd., China), and 25 ng of template DNA. The amplification was performed as follows: initial denaturation for 5 min at 94°C, 35 cycles each of denaturation for 30 s at 94°C, annealing for 30 s at 55°C, and primer extension for 1.5 min at 72°C, and a final extension for 10 min at 72°C. The PCR products were checked by gel electrophoresis in 1.2% (w/v) agarose gels, stained with ethidium bromide (10 mg/mL) and cleaned using an EasyPure Quick Gel Extraction Kit (Transgenic Biotech, China) according to the manufacturer's instructions [20].

3. Results and Discussion

3.1. Isolation of Cellulolytic Bacteria

Isolation of bacteria from the soil originating from the forest of Batu Village, Gianyar Regency, the forest of Kutuh Jimbaran Village, Badung Regency and the Final Waste Disposal Site (TPAS) of Suwung Village, Badung Regency, Bali Province was carried out by using selective media, namely 1% cellulolytic medium containing CMC, to select bacteria that degraded cellulose.

Based on the results of the isolation, 20 isolates of bacteria were able to grow and colonize on a solid cellulolytic medium containing 1% CMC. The isolates consisted of 6 isolates from Batu Gianyar samples, 6 isolates from Kutuh Jimbaran samples and 8 isolates from the Suwung TPAS samples.

3.2. Isolate Purification

Purification of bacterial isolates was carried out by using quadrant streak. Each petri dish at each dilution was taken from bacterial colonies that grew separately and showed different characteristics before being streaked on a sterile solid cellulolytic medium containing 1% CMC. Furthermore, the petri dish was incubated at 30°C for 96 hours. Purification was done 2 times with the same method to obtain pure isolates. The obtained pure isolates were then grown on a liquid cellulolytic medium which would be used as a culture stock for further testing [17].

Isolated bacterial colonies were coded (according to the sampling places) and numbers, namely BG01, BG02, BG03, BG04, BG05, BG06, KJ01, KJ02, KJ03, KJ04, KJ05, KJ06, TS01, TS02, TS03, TS04, TS05, TS06, TS07, TS08 and the morphologies were characterized in each colony including the forms, elevation, margins, color and size of the colony [16]. Characteristics of bacterial colonies that are able to grow on cellulolytic medium containing CMC (1%) are presented in Table 1.

Macroscopic observation of bacterial morphology on solid cellulolytic medium showed that most bacterial colonies were spherical in shape and only a small portion of them was irregularly shaped. The surface morphology of the isolates was mostly convex; the edges of the isolates were mostly flat, while the color of the bacterial morphology was mostly whitish and clear.

Furthermore, pure isolates were screened on solid cellulolytic medium containing 1% CMC by using the Gram's Iodine method to determine cellulolytic bacteria that were able to degrade cellulose as indicated by the formation of clear zones around the growing isolates.

Table 1. Characteristics of cellulolytic bacterial colonies

Isolate Code	Isolate Characteristics				
	Forms	Elevation	Margins	Color	Size (mm)
BG01	Irreguler	Convex	Entire	White	1
BG02	Circular	Convex	Entire	White	2
BG03	Circular	Convex	Entire	White	1
BG04	Circular	Convex	Undulate	White	3
BG05	Irreguler	Convex	Serrate	Clear white	3
BG06	Circular	Convex	Entire	Clear white	1

Isolate Code	Isolate Characteristics				
	Forms	Elevation	Margins	Color	Size (mm)
KJ01	Circular	Flat	Entire	White	1
KJ02	Circular	Flat	Lobate	Clear white	3
KJ03	Circular	Convex	Entire	White	2
KJ04	Irregular	Convex	Serrate	Clear white	1
KJ05	Circular	Convex	Entire	White	1
KJ06	Circular	Flat	Serrate	Yellow	1
TS01	Irregular	Flat	Undulate	White	3
TS02	Circular	Convex	Entire	White	1
TS03	Circular	Convex	Entire	Yellow	2
TS04	Circular	Convex	Entire	Clear white	3
TS05	Irreguler	Flat	Undulate	Clear white	2
TS06	Circular	Convex	Entire	White	2
TS07	Circular	Convex	Entire	White	1
TS08	Circular	Flat	Entire	White	1

Observations were made after inoculating isolates on solid cellulolytic medium and incubated at 30°C for 96 hours. BG, Batu Gianyar; KJ, Kutuh Jimbaran, and TS, TPAS Suwung.

3.3. Screening of cellulolytic bacteria

Screening tests on 20 isolates using the Gram's Iodine method showed that not all isolates growing on CMC media were able to form clear zones on the media. Only 6 isolates that produced extracellular cellulase enzymes were able to utilize carbon sources from CMC, while 14 other isolates that grew on CMC media did not undergo cellulose degradation around the isolates. Six isolates that produced extracellular cellulase enzymes could be shown by the formation of clear zones around the isolates (Figure 1). Balamurugan *et al.* [21], succeeded in isolating 25 cellulolytic bacterials from tea garden soil and only 5 isolates of them showed cellulolytic activity. Zin-Lay *et al.* [12], isolated cellulolytic bacteria from soil containing cow manure before obtaining 6 isolates capable of showing clear zones of 9 isolates undergoing screening tests.

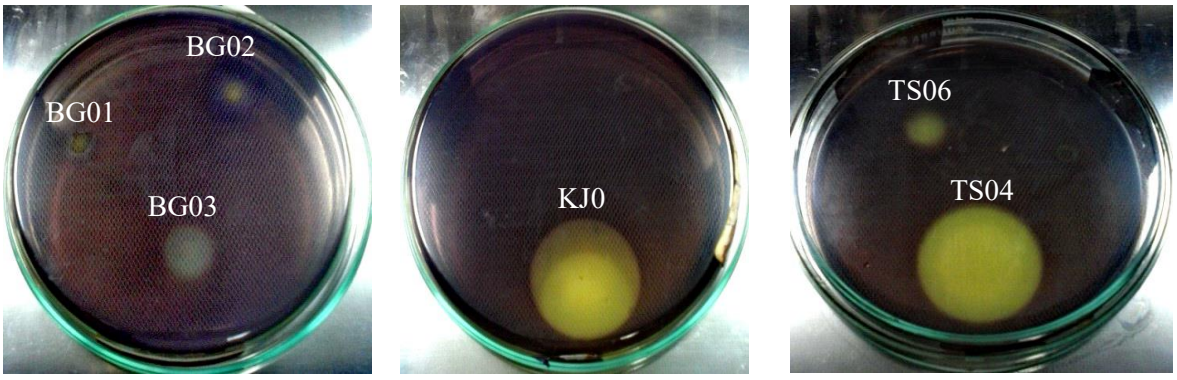

Figure 1. Hydrolyzing zones produced by bacterial strains on agar plates containing CMC after Gram's Iodine staining. Isolate BG01, BG02, BG03, KJ03, TS04, and TS06.

Based on the diameter of the clear zone of each bacterial isolate after being measured using calipers, it could be seen that the KJ03 isolate has the largest diameter of 36.9 mm. followed by KJ03 isolates, TS04 isolates, TS06 isolates, BG03 isolates, BG02 isolates and BG01 isolates which had a clear zone diameter of 34.1 mm, 23.9 mm, 19.2 mm, 12.4 mm and 11.03 mm respectively (Table 2). Photo of

cellulolytic bacterial screening results can be seen in Figure 1, data on the size of clear zones of each cellulolytic bacterial isolate can be seen in Table 2 and data on cellulolytic activity grouping based on the size of clear zones can be seen in Table 3.

Table 2. Clear zones of potential cellulolytic isolates

Isolate code	Clear zone (mm)
BG01	11.03 ± 0.05
BG02	14.40 ± 0.17
BG03	19.20 ± 0.17
KJ03	36.90 ± 0.10
TS04	34.10 ± 0.08
TS06	23.90 ± 1.15

Screening tests by measurement the clear zones area with the Gram's Iodine method.

Table 3. Data grouping cellulolytic activity based on the size of the clear zone

Soil sample site	Total isolate	Activity showed (%)	High activity (%)	Medium activity (%)	Low activity (%)	No Activity (%)
Batu Gianyar	6	3 (50.00)	0	0	3 (50.00)	3 (50.00)
Kutuh Jimbaran	6	1 (16.67)	0	1 (16.67)	0	5 (83.33)
TPAS Suwung	8	2 (25.00)	0	2 (25.00)	0	6 (75.00)
Total	20	6 (30.00)	0	3 (15.00)	3 (15.00)	14 (70.00)

Isolation, purification, and screening on solid cellulolytic medium containing CMC (1%). The grouping of activities is based on the diameter of the clear zone where activity is high (> 4 cm), medium (2.0-3.9 cm) and low (0.5-1.9 cm).

Table 4. Four isolates that have the largest clear zone

Isolate Code	Clear Zone (mm)
BG03	24.6 ± 0.06
KJ03	41.0 ± 0.10
TS04	38.5 ± 0.10
TS06	27.9 ± 0.13

The isolates that showed the largest clear zone in retesting with the Gram's Iodine method.

Although all bacteria isolated grew on 1% CMC cellulolytic medium, not all bacteria showed cellulolytic activity in clear zone testing [18]. From the testing of clear zones, only 6 (30.00%) isolates showed cellulolytic activity consisting of 3 isolates which showed low activity and 3 isolates which showed moderate activity. On the other hand, 14 (70.00%) other isolates did not show any cellulolytic activity because there was no clear zone around the isolate. It can be said that these bacterial isolates are only able to grow on medium containing cellulose but they are not able to degrade the cellulose.

Based on 6 isolates that produced clear zones on the test with Gram's Iodine before, it was re-tested for 4 isolates that showed the largest diameter of the clear zone. Among 4 isolates, the one showing the biggest clear zone's diameter would be used for further testing. The results of the repetition screening test

for the 4 isolates that produced the largest clear zone can be seen in Figure 2 and the data of the clear zone size can be seen in Table 4.

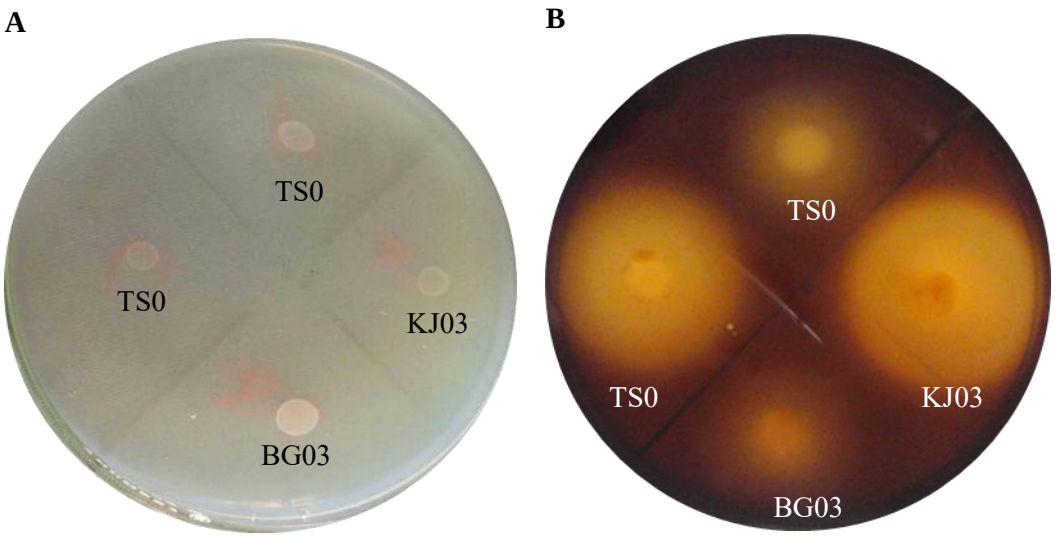

Figure 2. Photograph of 4 bacterial isolates that showed the largest clear zone on retesting with the Gram's Iodine method. A, isolates were grown on solid CMC medium before Gram's Iodine test; B, isolates showing clear zones around the colony after Gram's Iodine test. The formation of clearing zone around the colonies confirms the secretion of extracellular cellulose.

3.4. Cellulose degradation test

The tests were carried out on 4 bacterial isolates which produced the largest clear zone in the previous test. Cellulolytic bacterial isolates capable of showing the highest level of cellulose degradation could be seen from the damage and weight loss of the filter paper. The results of the cellulose degradation rate on filter paper after incubation in a shaker treatment with a speed of 100 rpm at room temperature for 10 days are shown in Figure 3.

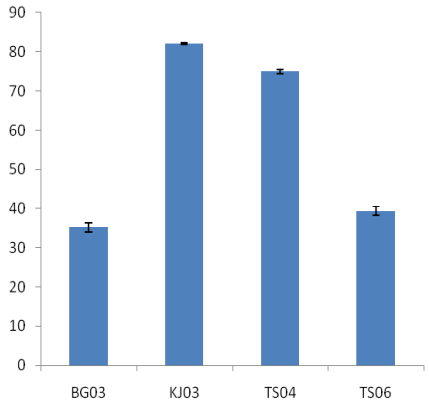

Figure 3. Cellulose degradation rates using filter paper. BG, Batu Gianyar; KJ, Kutuh Jimbaran; TS, TPAS Suwung.

Isolates that showed the highest level of cellulose degradation in the filter paper test could be seen from the damage and dry weight difference on filter paper (Whatman no. 1) (before and after incubation). The higher the level of destruction and the difference in weight of filter paper containing bacterial isolates, the greater the level of cellulose degradation produced by these isolates [19]. The selected isolates were isolates that were able to degrade the highest cellulose on filter paper.

From the observation of the level of cellulose degradation in filter paper showed that the level of cellulose degradation ranged from 36.13% to 82.25%. The highest level of cellulose degradation was 82.25% with an incubation's period of 10 days. While the lowest cellulose degradation rate was 36.13% with the same incubation time. Potential isolates having the highest level of cellulose degradation was a KJ03 isolate. The study of the incubation time, reported that the highest cellulase activity was obtained during a long incubation period of 10 days [22].

3.5. Morphological and physiological characteristics

All the strains were rod shaped Gram-negative, non-spore-forming, catalase negative, and non-motile. It could grow at 30°C on cellulolytic agar medium. The culture was positive for the utilization of citrate and Methyl Red (MR), but negative for the utilization urea, indole and Voges-Proskauer (VP). Morphology and physiological characteristic of isolates are presented in Table 5.

Table 5. Characteristic of cellulolytic isolates

Characteristic	Isolate Code			
	KJ03	BG03	TS04	TS06
Morphological characteristic				
Motility	Non motile	Non motile	Non motile	Non motile
Cell shape	Rod	Rod	Rod	Rod
Gram staining	Negative	Negative	Negative	Negative
Physiological characteristic				
Catalase test	–	–	–	–
Indole production	–	–	–	–
Methyl Red (MR)	+	+	+	+
Voges-Proskauer (VP)	–	–	–	–
Urease	–	–	–	–
Citrate	+	+	+	+

Biochemical tests: (+), positive reaction; (-), negative reaction

3.6. Species identification

From the results of cellulose degradation test, it was decided to first conduct DNA sequencing on all potential isolates that had the highest cellulose degradation ability to determine the species of these isolates. If the isolate with the highest performance was identified as a new species, then it was feasible to proceed with a further study in order to figure out its temperature, pH, and optimum growth period, and then characterized the rough enzyme produced. Sequencing results for each isolate were tested (data not shown).

DNA sequencing analysis in each isolate showed that the isolates had been identified before, where KJ03 isolates which had the highest performance were identified as species from *Klebsiella* sp. (data not shown), which was most likely identified as *K. quasipneumoniae*. isolates. This showed that the species from KJ03 could be ascertained as the member of the genus *Klebsiella* which is commonly found in the surrounding world and is a common flora among humans and animals, the ability, potential, pathogenic of which are known.

Meanwhile, isolates with the second best ability, TS04 isolates had also been identified most likely to come from *Klebsiella* sp., although there was still the possibility of originating from other genera such

as *Pseudomonas* or *Shigella*. While other isolates such as BG03 and TS06 having the ability to degrade cellulose which was inferior were most likely to come from other genera, although there was also the possibility of coming from the Genus *Klebsiella*. Pawar *et al.* [18] also reported that the members of *Enterobacter* and *Klebsiella* were observed by using culture-independent and -dependent methods by enrichment in carboxymethyl cellulose (CMC), indicating their dominance among the cellulolytic bacterial community in the Gastrointestinal (GI) tract of the snail. *Klebsiella oxytoca* recombinant strains have been employed in cellulose degradation and fermentation [23].

Wu *et al.* [24] reported that *Klebsiella* sp. HE1 strain isolated from hydrogen-producing sewage sludge was potential to produce H_2 and other valuable soluble metabolites (e.g., ethanol and 2,3-butanediol) from sucrose based medium and its production efficiency enhanced after increasing the initial culture pH to 7.3. The HE1 strain also produced ethanol (contributing to 29–42% of total of soluble microbial products). *K. pneumoniae* is often used for the production of 2,3-butanediol from cellulolytic materials because of its broad substrate spectrum (e.g., major sugars and uronic acid of cellulosic hydrolysate) [25, 26]. Therefore, fermentation with *Klebsiella* sp. has the advantage of producing a variety of valuable biofuels (e.g., ethanol and H_2) and industrial chemicals (e.g., 2,3-butanediol) [24].

From the data above, the phylogenetic tree of the identified samples (red box) can be made and seen in Figure 4 as follows:

Figure 4. Phylogenetic tree of the 16S rRNA of isolates. A bootstrap Neighbor Joining (NJ) method was used for the construction of phylogenetic trees using MEGA 5.0 program [27].

4. Conclusions

Based on the research, 20 isolates were grown on cellulolytic medium but only 6 isolates produced a clear zone with a diameter of 8 mm to 34 mm in the screening test by Gram's Iodine method. Four isolates that had the largest clear zone were tested further with Whatman no. 1. Each isolate that produced clear zones has a different ability to degrade cellulose. KJ03 isolates had the highest ability to degrade cellulose 82.08%. DNA sequencing analysis of the 4 best isolates was most likely the genus of *Klebsiella*, although

it might still be possible from other genera such as *Pseudomonas* or *Shigella*. KJ03 was identified as *Klebsiella quasipneumoniae*, TS04 identified as *Klebsiella* sp., TS 06 identified as *Klebsiella quasipneumoniae* and BG03 identified as *Klebsiella pneumoniae*

Acknowledgements
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Treatment of ammonia wastewater using membrane bioreactor: effect of activated sludge concentration and backwash

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Abstract. In this study, Membrane Bioreactor (MBR) was used to process synthetic ammonia wastewater. MBR consists of flat sheet polyethersulfone membranes and is assisted by the presence of activated sludge. The activated sludge acted as biologically degrade agent and the membrane served in filtration of the wastewater. The effect of various activated sludge concentrations were studied on the removal of ammonia from the wastewater. Furthermore, MBR system is also suffered by membrane fouling. The fouling cake could be removed by backwash. Two types of backwash liquid were studied, using pure water and addition of NaOCl compounds. The performance of MBR system were investigated e.g. Chemical Oxygen Demand (COD), Dissolve Oxygen (DO), Biological Oxygen Demand (BOD), Total Suspended Solids (TSS), flux, and pH. It was found that the maximum rejection of COD was 72%, with 357.14 mg /L of activated sludge concentration. The lowest BOD was 0.97 mg/L when the concentration of activated sludge was 660.38 mg/L. The higher concentration of activated sludge, then the higher rejection of COD and BOD. Furthermore, in backwash study, the addition of NaOCl was more effective in removing membrane fouling and produced good permeate, which rejection of COD was 85.7%, the BOD value was 0.69 mg/L, and higher water flux than backwash with pure water.

1. Introduction

The lack of clean water promotes the intensification treatment of water, including wastewater produced from domestic or inductrial processes. Wastewater treatment aiming at removal of contaminants or unwanted subtances prior to discharge in environment. Membrane technology used filtration concept to separate phases and potentially used for wastewater treatment [1][2]. Even though it is categorized as physical treatment, it also potentially combined with another treatment, such as biological treatment. The hybrid processes used by membrane bioreactor technology (MBR) incorporated physical and biological treatments of wastewater [3].

MBR technology get advantages on the ability of biological reaction to reduce contaminants, and membrane filtration on the ability to separate unwanted molecules from water. However, MBR as common membrane systems also suffered from fouling [4]. It is therefore important to control MBR fouling, for instance used physical cleaning [5]. In this study, MBR is used to process synthetic ammonia wastewater. The effect of different activated sludge concentration of ammonia reduction is reported, and the effect of backwashing liquid is also presented.

2. Materials and Methods

2.1 System description

The laboratory scale plant of MBR was used in this study. The schematic diagram of tangential flow MBR pilot system is shown in Figure1. The wastewater was filtered by polysulphone made flat sheet

membrane in the MBR unit. Wastewater was pumped to flow to the output hose and the permeate was produced.

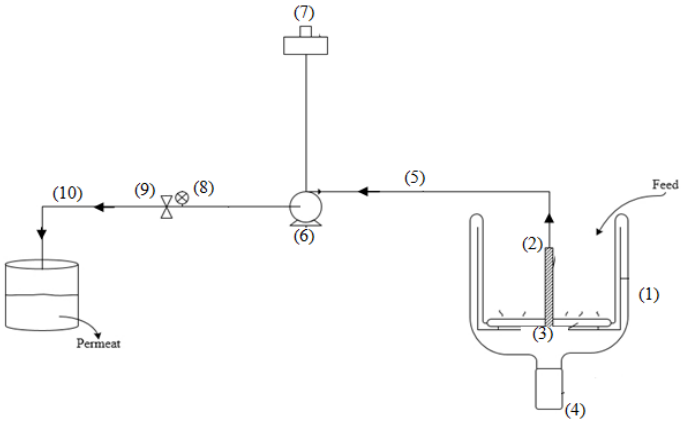

Figure 1. Schematic diagram of MBR pilot

A bioreactor (1) of 5000-6000 mL volume with module membrane (2) as separation media. Air pipe 2,5 mm pore (3) was connected to the aerator (4) as an oxygen supplier. Input hose (5) was connected with the water pump 0.48 Mpa (6) for sucked wastewater from bioreactor. This process was controlled by controll box (7). A manometer (8) was installed to measure the pressure of process flow. And it was connected to the valve (9) as a pressure controller in this filtration process. Output hose (10) to flow the permeate. After the filtration process, the quality of permeate could be determined.

2.2 Materials composition

The MBR was filled with activated sludge and synthetic ammonia wastewater. In this study, three various concentration of activated sludge were used and the process of backwash membrane comparing NaOCl solution and water. The material composition of this process is shown in Table 1.

Table 1. Materials composition

Materials	Case 1 (the effect of activated sludge concentration)	Case 2 (the effect of backwash with NaOCl)
Activated sludge	172.41 mg/L	870.67 mg/L (N)
	357.14 mg/L	870.67 mg/L (W)
	660.38 mg/L	
Ammonia wastewater	0.4 L	1 L (N)
		1 L (W)
Water	5.4 L	4 L (N)
	5.2 L	4 L (W)
	4.9 L	
NaOCl	-	10% (N)
		Pure water

Note:
(N): membrane backwash with NaOCl addition
(W): membrane backwash with pure water

2.3 Experimental condition

The acclimatization process of the activated sludge and wastewater were carried out for 3 days and occured in bioreactor. Then, this mixture was added with the water (Table 1) and the filtration process could be done. Case 1 is done to determine the effect of activated sludge concentration and the process took about 120 minutes. First experiment used activated sludge 172.41 mg/L, ammonia wastewater 0.4

L, and water 5.4 L. They were filtered by MBR for 30 minutes and the permeate was put into bottle to be tested (Table 2). Then, the second and third based on material composition in Table 1 and were filtered for 30 minutes each other.

Table 2. Experimental table

The Effect of Activated Sluge Concentration		The Effect of NaOCl Addition for Backwash MBR	
Sample	**Parameters**	**Sample in minutes**	**Parameters**
I	%COD, BOD	20' (N,W)	%COD, BOD
		40'(N,W)	%COD, BOD
II	%COD, BOD	**Backwash**	
		60'(N,W)	%COD, BOD
III	%COD, BOD	80'(N,W)	%COD, BOD
		Backwash	
Control variable	%COD, BOD	100'(N,W)	%COD, BOD
		Control variable	%COD, BOD

Meanwhile, the Case 2 to determine the effect of NaOCl addition for backwash MBR, there were 2 experiments in this study. First, the mixture of activated sludge and wastewater which has been aclimatized for 3 days was added with water 4 L. Then, the filtration process could be done. It took about 120 minutes and every 10 minutes the flux was measured. Also, in every 20 minutes the permeate was put into bottle to be tested (Table 2) and in every 40 minutes the MBR was backwashed with NaOCl. The second experiment, the same treatment was backwashed with pure water.

2.4 Analytical methods

COD was measured by Chromatography Test. The COD concentration would be converted to coefficient of COD rejection (%R). BOD was measured by BOD meter. The flux was measured by substitusing the data obtained into the flux formula.

3. Results and Discussion

3.1 Coefficient of Chemical Oxygend Demand Rejection (%R)

Membrane selectivity is a measure of the membrane's ability to hold or pass a certain species. The parameter used to measure membrane selectivity is the coefficient rejection. High rejection coefficient means good membrane performance because if the rejection coefficient is high then fewer substances are passed through the membrane [6]. The rejection coefficient is a fraction of solute which does not pass the membrane. The higher %R causes decreasing permeate concentration. It means the compound which pass through the membrane decreases.

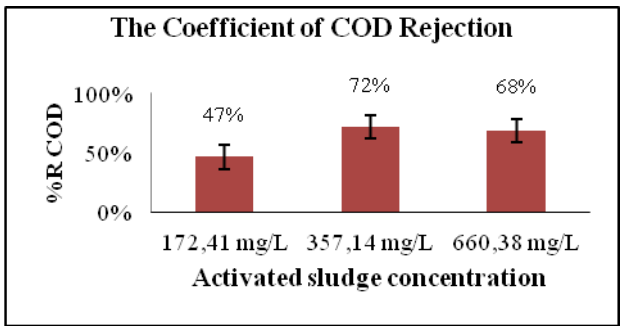

Figure 1. Coefficient of COD rejection (Case 1)

The effect of activated sludge concentration is shown in Figure 2. The concentration of activated sludge that most effectively reduces COD was on concentration 357,14 mg/L. In this concentration, COD could be decreased up to 72 %. The result is the activated sludge at the right concentration can reduce more COD, but if the concentration is to high then the decrease is not significant.

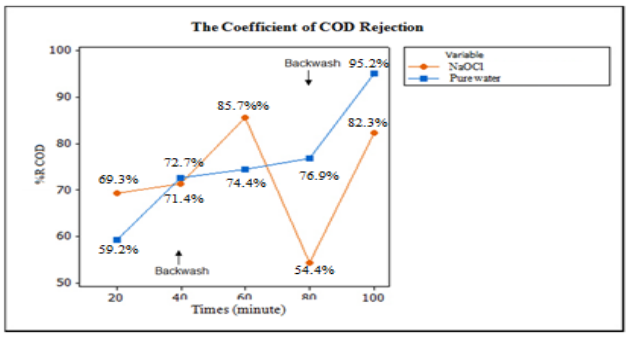

Figure 3. Coefficient of COD rejection (case 2)

The effect of NaOCl addition for backwash MBR is illustrated in Figure 3. The addition of NaOCl for backwashing MBR is more effective than backwashing MBR with pure water. Although, at 80 minutes %R decreased because there was a lot of fouling during the filtration process. But after backwash at 80 minutes, the coefficient of COD rejection increases significantly up to 82.3%. based on Figure3. backwashing membrane with pure water could not reduce COD concentration significantly.

3.2 Biological Oxygen Demand (BOD) Concentration

Biological Oxygen Demand (BOD indicates the amount of degraded organic matter biologically and oxygen used to oxidize organic matter. This BOD test is the standard in determining the quality of wastewater to be disposed of in any country [4]. In the BOD test, the procedure suggested by AOAC (Association of Official Analytical Chemists) is an incubation period of 5 days and is called BOD_5.

In this study, the effect of activated sludge concentration for BOD (Case 1) value based on Figure 4. is shown decreasing trend of BOD concentration. It means, the highest activated sludge concentration can reduce more BOD in the wastewater. This proves that the addition of activated sludge can reduce the BOD. Because activated sludge consists of microorganisms that can decompose and degrade organic compounds in wastewater.

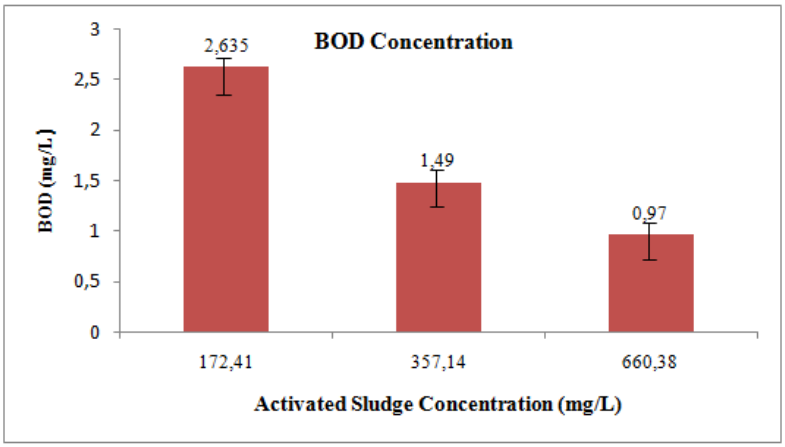

Figure 4. BOD concentration (Case 1)

The addition of NaOCl for membrane backwash caused reducing BOD concentration significantly as shown in Figure 5. Both of backwashing membrane with NaOCl addition and pure water could decrease the BOD, but NaOCl addition was more effective then backwash with pure water. In this study, the factor which influences the reducing of BOD was membrane performance because NaOCl addition can improve the membrane performance.

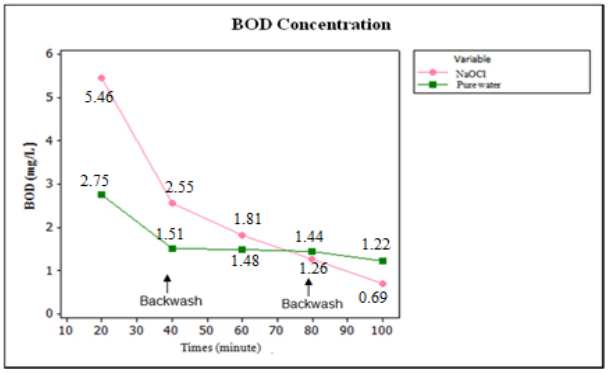

Figure 5. BOD concentration (Case 2)

3.3 Flux

Flux is important factor which influences membrane performance. If membrane performance is optimal, then the flux will be high. As shown in Figure 6. the addition of NaOCl could result fluxes more optimum then backwash with pure water. That's because NaOCl is able to reduce fouling which attached to the membrane pores. Based on Figure 6, there is decreasing trend of flux during the experiment caused by the longer filtering, the more viscosity of wastewater increases and the more fouling attached to the membrane pores.

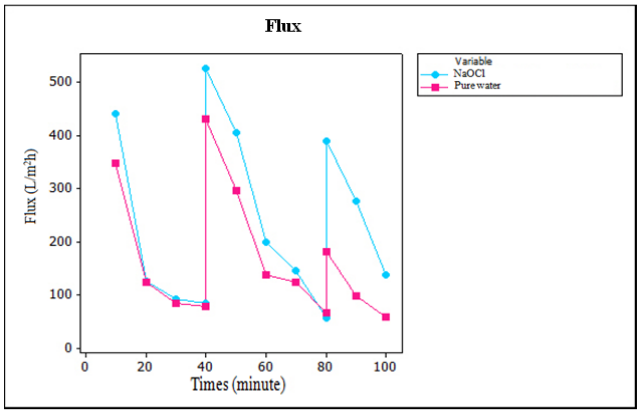

Figure 6. Flux behaviour (Case 2)

4. Conclusions

The optimum coefficient COD rejection was 72% with the activated sludge concentration was 357.14%. The highest activated sludge concentration can reduce more BOD in the wastewater. Activated sludge consists of microorganisms that can decompose and degrade organic compunds in wastewater. During the experiment, the flux tends to decrease due to a decrease in membrane performance. NaOCl is able to reduce fouling which attached to the membrane pores compared with water.

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The environmental impact risk of cow-hide tanning small - medium industries

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Abstract. The emergence of eco-friendly products has persuaded the tanning industries to improve their production processes. It was due to great amount of solid waste, gas emission and/or wastewater generated by the industries. These wastes trigger various environmental impacts; of those are GHG (Greenhouse Gas), acidification and eutrophication. The research was carried out in gate to gate scope. Then used purposive sampling to chose industry, Material, Energy, Toxicity (MET) to identify the material, energy and toxicity produced during the production and wastewater analysis to know pollutant content. Analysis on leather tanning industry showed that industries produced wastes and created impacts on a different level. Measured per 1.5 tons of salted-hide, industries generated 29.51 – 39.89 m^3 wastewater and then formed 1,528.86 - 1,749.14 kg (wet basis). Carcinogenic and toxics compound like sulfide, ammonia and Cr^{6+} were identified in industries' wastewater too in varying concentration in each step. These were also found in wastewater treatment plant (WWTP) outlet. Our analysis also revealed that industry B contributed more in GHG, acidification and eutrophication compared to industry A.

1. Introduction

Modern society envisions for a sustainable industrial development, particularly the tanning industry which is known to be a great contributor to environmental problems. Tannery produces a huge amount of wastes. Moreover, the wastes contain dangerous chemicals as tanneries utilize chemicals, which are essential in their processing.

In Indonesia, cow, goat and lamb are generally used as the raw material while solid waste and wastewater are both the main issue. Wastewater is still an urgent topic, especially for the small-medium industry. According to Bhargavi et al. [1], wastewater formed during the processing was about 30 – 35 liters/kg of skin, while to process 1 ton of skin would result 45 – 50 m^3 of wastewater [2].

Ahmed and Chowdhury [3] explained that wastewater from tannery contained a high number of organic compounds as well as the inorganics, such as chrome, chloride, ammonia, sulfide and sulfate. The dangerous contents of chemicals and inorganics can seriously affect the environment. Some notable impacts are GHG, eutrophication, acidification which come from the toxics of wastewater.

Weak control from the government to the quality standard of wastewater discharged by the industry to the environment encouraged tanneries to simply discharge it without any particular treatment. The fact that the higher-order stakeholders were seemingly careless was one special issue that required a massive attention from the government [4]. However, there was a possibility of the stakeholder being thoughtful but was constrained by the cost needed to handle the waste.

Tanning process as well the others also requires electricity for the machineries with varying power consumption and duration. Electricity utilization facilitates easier processing but also creates negative impacts to environment. The impacts include global warming, eutrophication and acidification [5-8]. According to Santoyo – Castelazo et al. [7], the most contributing energy generator source to the GHG came from fossil fuels, which were petroleum and coal.

Analysis on the environmental impacts of tanning industries is rarely conducted in Indonesia. The variances in the formulation, skin/hide type, machine power and processing duration in each industry were assumed to contribute differently to the environment. Thus, it is necessary to conduct the analysis of environmental impact potential from tanning processes.

2. Material and Method

2.1. Research scope

This work was an observational research conducted in cow-based tanneries in Magetan, East Java (industry A) and Garut, West Java (industry B), Indonesia. This research studied the manufacture/tanning process (Figure 1). We conducted the estimation of solid waste, wastewater, GHG, acidification and eutrophication from tanning processes in each industry.

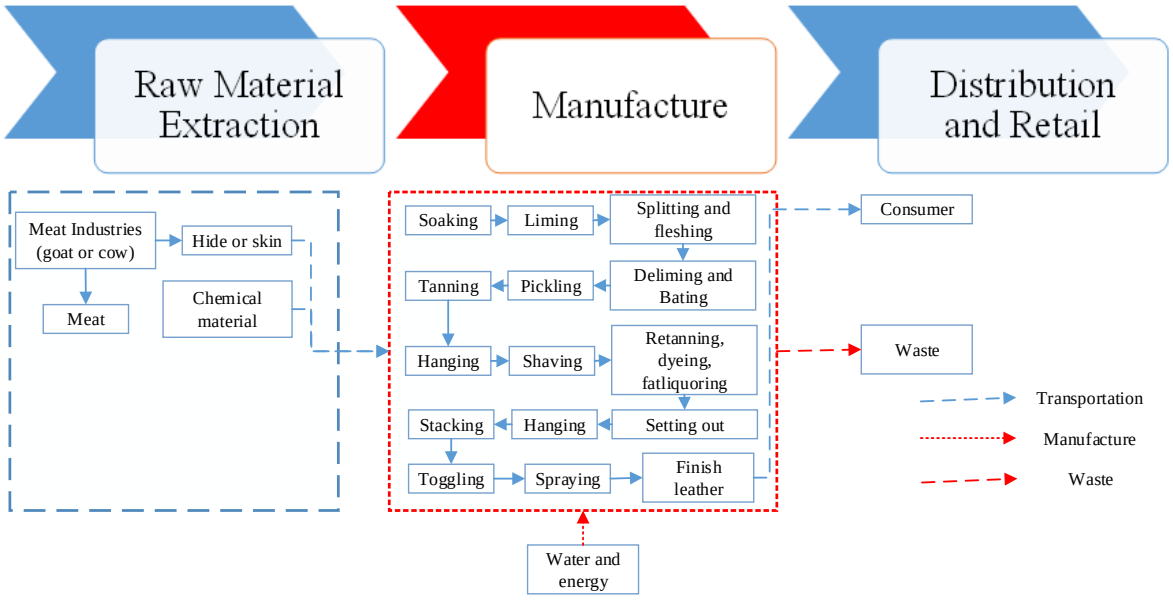

Figure 1. Research scope

2.2. Data collection

The research was conducted with purposive sampling as the technique to select the research subject (tannery) [9]. MET (Material, energy and toxicity) matrix was employed to identify the material, energy and toxicity produced during the production [10]. Data were obtained by interview to stakeholders in industry and literature review and the data were processed to estimate the input dan output, the energy utilization during processing as well as to identify the toxicity in tanneries. Wastewater sampling was done at the processes of: soaking; liming/unhairing; deliming/bating; tanning; and retanning, dyeing and fatliquoring. The estimation of environmental impacts such as GHG, acidification and eutrophication was measuared manually with energy/electricity basis and wastewaster as the input data. In this research, solid waste from the whole tanning process was not converted into GHG, acidification and eutrophication estimation since the solid waste was utilized as the input (raw material) of third party industry.

2.3. Wastewater characterization

All samples were measured for its pH, TSS (Total Suspended Solids), TDS (Total Dissolved Solids), BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand), Chrome hexavalent (Cr^{6+}), ammonia and sulfide [11].

2.4. Environmental impact assessment

Environmental impact assessment (EIA) was conducted to investigate the environmental consequences of the tanning industries. The assessment classified the parameters into Greenhouse gas (GHG), acidification and eutrophication.

2.4.1. Greenhouse gas (GHG). The estimation of GHG in this research was conducted by analyzing the contents of CO_2 and CH_4 from the wastewater and the use of energy (electricity and LPG). The formula to estimate could be seen in Table 1.

Table 1. Formula to GHG

Sources of emissions	Formula	Reference
Electicity	$Emission\ CO_2 = Q_E\ x\ FE$	[12][13]
LPG	$Emission\ CO_2 = Q_{LPG}\ x\ Q\ x\ FE$	[12
	$Emission\ CH_4 = Q_{LPG}\ x\ Q\ x\ FE$	
Wastewater	$Emission\ CH_4 = V_w\ x\ C\ x\ FE$	[14]

According IPCC (2006a), CH_4 (methane) has a GWP (Global Warming Potential) value of 23. As the value of GWP calculation is converted into comparable CO2 equivalents, the equation is as follows:

$$1\ kg\ CH_4 = 23\ kg\ CO_{2\ eq}$$

2.4.2. Acidification. The pollutants which can cause acidification are SO_2, NO_x and NH_3. The source these pollutants in this case of tannery is electricity. The estimation of SO_2 emission followed this equation (Table 2):

Table 2. Formula to Acidification

Sources of emissions	Formula	Reference
Electricity	$Emission\ SO_2 = Q_E\ x\ FE$	[13]
	$Emission\ NO_X = Q_E\ x\ FE$	[13]

All analysis results (SO_2, NO_x, NH_3) were converted into $SO_{2\ eq}$ according to the equation in Heijungs et al. [15] as follows:

$$1\ kg\ NO_x = 0.7\ kg\ SO_{2\ eq};\ 1\ kg\ NH_3 = 1.88\ kg\ SO_{2\ eq}$$

2.4.3. Eutrophication. The estimation of GHG in this research was conducted by analyzing the contents of PO_4^{3-} from the wastewater and the use of energy (electricity and LPG) (Table 3).

Table 3. Formula Eutrophication

Sources of emissions	Formula	Reference
Electricity	$Emission\ NO_X = Q_E\ x\ FE$	[12][13]
LPG	$Emission\ NO_X = Q_{LPG}\ x\ FE$	[12]
Wastewater	$Emission\ PO_4^{3-} = V_w\ x\ C\ x\ FE$	[16]

To estimate nitrogen emission, the number of NH_3 obtained was multiplied by 1.21589 to form NH_3-N, based on the respective molecular mass. According to EPA [17], each one NH_3 mole forms one mole of nitrite (Equation 1) and one nitrite mole forms one mole of nitrate (Equation 2).

$$NH_3 + O_2 \longrightarrow NO_2^- + 3H^+ + 2e^- \quad (1)$$
$$NO_2^- + H_2O \longrightarrow NO_3^- + 2H^+ + 2e^- \quad (2)$$

All analysis results (NO_x and NH_3) were converted into $PO_4^{3-}{}_{eq}$ according to the equation as follows [15]:

$$1\ kg\ NO_x = 0.13\ kg\ PO_4^{-3}{}_{eq};\ 1\ kg\ PO_4^{-3} = 1\ kg\ PO_4^{-3}{}_{eq}$$

3. Result and Discussion

3.1. *Waste identification in the tanneries*

Waste is indisputable byproduct from leather processing. Wastewater and solid waste were formed during leather processing. The high amount of wastewater was due to the high needs of water in each process [18]. Buljan et al. [19] explained that the total of water consumption in leather tannery was as much as 3,500% of the weight basis of hide. The result showed that industry A generated wastewater as much as 29.51 m^3 and industry B was 39.89 m^3 per 1.5 tons of salted hide (Table 4). According to Shunqing et al. [20], the amount of wastewater in tannery were 35 – 40 m^3 per ton hide. Therefore, the amount of wastewater in industry A and B were less than previous research.

Table 4. Wastewater and solid waste from tannery with the basis of 1.5 tons salted-hide

Process	Industry A		Industry B	
	Wastewater (m^3)	Solid waste (kg)	Wastewater (m^3)	Solid waste (kg)
Beamhouse	24.28	1,484.10	29.80	1,251.44
Tanning	2.26	261.19	2.11	277.42
Posttanning	2.55	0.00	7.84	0.00
Finishing	0.43	3.85	0.14	11.88
Total	29.51	1,749.14	39.89	1,528.86

Meanwhile, solid waste formed in industry A was 1,749.14 kg (wet basis) while industry B was 1,528.86 kg (wet basis) per 1.5 tons of salted hide. In both industries, the most solid waste formed was in beamhouse process (Tabel 4). Kanagaraj et al. [2] explained that 1 ton of hide processed into leather would form solid waste as much as 800 kg and the high amount solid waste generated in beamhouse was as much as 80%, tanning 19% and finishing 1%. 1 kg of hide would generate 0.7 kg solid waste, or it was only 30% of hide converted to leather [21].

Based on Table 4, each industry generated wastewater with different amounts. Wastewater was formed in soaking; liming; deliming and bating; tanning and retanning; and dyeing and fatliquoring (Figure 2). The analysis result showed that wastewater formed along processing contained high number of pollutans (Table 5). According Madhan et al. [22], much wastewater were formed in beamhouse and 60 -70% pollutans were generated there.

Table 5. Analysis result of wastewater parameter

Process	The rate value of each parameter							
	COD (g/l)	BOD (g/l)	TSS (g/l)	TDS (g/l)	Ammonia (mg/l)	Sulfide (mg/l)	Cr^{6+} (mg/l)	pH
Soaking	7.64	2.52	1.28	41.60	187.8	30.24	0.00	8.4
Liming	7.68	3.01	2.80	18.40	95.4	646.4	0.00	11.9
Deliming and bating	1.61	0.65	3.81	39.51	4,701.5	56.95	0.00	8.5
Tanning	5.93	1.05	1.71	62.85	109.6	16.41	0.62	4.1
Retanning, dyeing, fatliquoring	17.16	2.98	3.07	18.41	156.3	16.9	2.09	4.2
Outlet[a]	0.19	0.06	0.23	11.12	59.4	1.02	0.03	8.2
Wastewater quality standard*	0.11	0.05	0.06	-	0.5	0.8	-	6-9

Note: [a] outlet of tannery A; * wastewater quality standard referring to KLH [25]

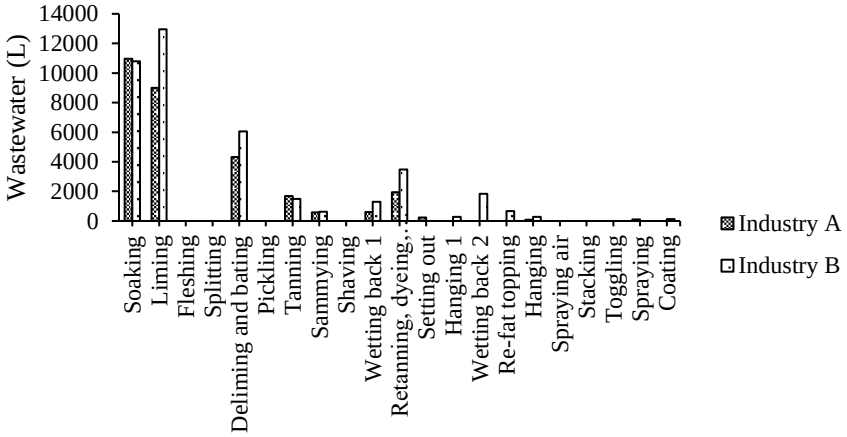

Figure 2. Distribution graph for the wastewater in the tanning process

The result of both industry showed that a lot of solid waste was generated in splitting (Figure 3). Solid waste of hide processing can be divided into two types, which are non-chrome and solid waste with chrome. Non-chrome solid waste is solid waste which is not exposed or has yet to be exposed by chrome (tanning chemical). It was generated in fleshing and splitting. Meanwhile, solid waste with chrome is one exposed by chrome, found in shaving. The amounts of non-chrome solid waste were greater than solid waste with chrome. Industry A and B formed solid waste with chrome as much as 261.19 kg (wet basis) and 277.42 kg (wet basis), respectively, while non-chrome solid waste formed was as much as 1,484.10 kg (wet basis) and 1,251.44 kg (wet basis), respectively. Thus far, non-chrome solid waste is sold to third party to gain more profit while chromic solid waste is given without charge to the third party. The solid waste can be used for crafting handcrafts or other products.

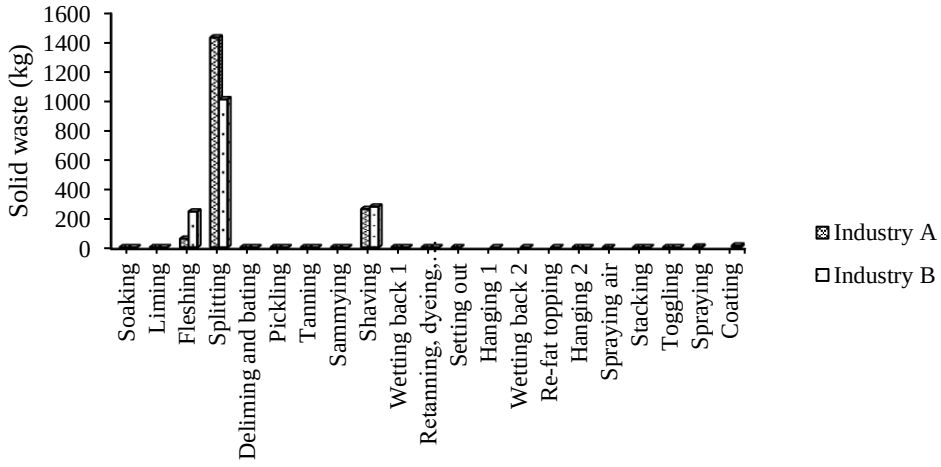

Figure 3. Distribution graph for the solid waste in the leather tanning process

3.2. *Identification of energy consumtion in the tanneries*

Analysis on the tanneries' energy consumption revealed that industry A consumed 632.08 kWh per batch, far greater than 441.64 kWh-consuming per batch industry B (Table 6). However, in the processes of beamhouse, tanning and postanning, industry A utilized less energy than industry B. The largest distinction was found in finishing process, where industry A used LPG and the energy usage was converted into 336.37 kWh. Other factors influencing the energy consumption were machine power and processing duration (different formulation). Our analysis identified that setting out process in industry A required the most energy. Meanwhile, the processes in industry B demanding greater energy use were tanning and retanning, dyeing and fatliquoring (Figure 4).

Table 6. Total energy utilization

Process	Industry A (kWh)	Industry B (kWh)
Beamhouse	110.80	145.16
Tanning	91.27	122.90
Posttanning	55.77	104.55
Finishing	374.24	69.03
Total	632.08	441.64

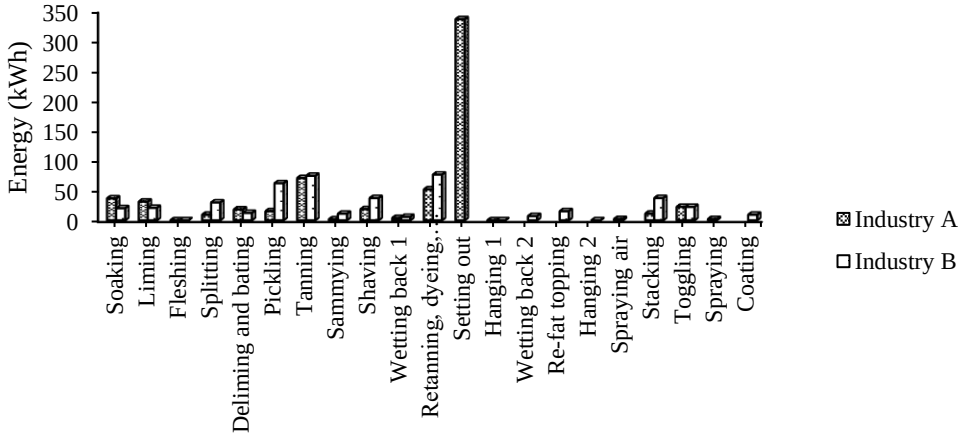

Figure 4. Distribution graph for the energy in the leather tanning process

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Table 7. Machineries identification in tanneries

Identification		Measurement		
Machinery	Energy source	Power (kW)	Industry A (hour)	Industry B (hour)
Concrete mixer	Electricity	7.46	27.01	37.25
Splitting	Electricity	14.92	3.00	2.00
Sammying	Electricity	11.19/*11.00	1.00	*1.00
Shaving	Electricity	29.84	2.00	1.25
Setting out	Electricity/* LPG	10.00	*2 12kg-LPG cans	-
Stacking/milling	Electricity	11.19/*7.46	1.00	5.00
Toggling	Electricity	7.46	3.00	3.00
Spraying	Electricity	2.57/*11.19	0.83	*0.83
Pump	Electricity	1.10	10.55	14.59

Analysis on the survey conducted revealed that almost every process of the whole tanning processes utilized machineries, of those were concrete mixer, water pump, splitting machine, sammying machine, setting out/vacuum machine, shaving machine, buffing machine, spraying machine and toggling machine (Table 7). As Table 7 shows, there are machines in industry A and B with similar power usage and those with different power as well, as well as industry B which had longer duration of machinery work

According to Soesanto [5] and Lubis [6], energy utilization of petroleum, coal, gas and nuclear will contribute to the environment damage by pollution and coal power plant were the one impacting to the greenhouse effect most negatively, as well as to acidification and eutrophication [8]. Hence, it is unquestionable that the extensive energy use in tanning industry plays a negative part in the environmental perspective.

3.3. *Toxicity in the tanneries*

The presence of toxic and carcinogenic chemicals created wastes with similar characteristics, particularly the wastewater, disturbing the environmental balance. Some of the chemicals were sodium chloride, sulfate, various organics and inorganics, as well as toxic metallic compound [23]. Other than those compounds, tannery A and B also generated sulfide, ammonia and Cr^{6+} (Table 5).

Our analysis exhibited most sulfides were generated at unhairing/liming as much as 646.40 mg/l. Meanwhile, other processes which were also identified to contain sulfide were soaking (30.24 mg/l), deliming/bating (56.95 mg/l), tanning (16.41 mg/l) and retanning, dyeing and fatliquoring (16,9 mg/l). The use of Na_2S in liming process was responsible to the high number of sulfide. Sulfide was also formed due to the presence of sulfate. In the wastewater with low oxygen concentration, sulfate will be reduced into sulfide [24]. According to the regulation of KLH [25], the limit of sulfide to be discharged into the environment is 0.8 mg/l. Thus, it was clear that the tanneries did not satisfy the quality standard. The analysis on WWTP outlet showed that the wastewater there did not comply with the regulation as well, as much as 1.04 mg/l of sulfide. High concentration of sulfide can cause a toxic effect to the environment [26-29].

Ammonia could induce eutrophication and acidification as well as decreasing the dissolved oxygen level in the water [30-33]. Analysis on the wastewater of tannery A and B showed the presence of ammonia. Most of the ammonia was formed in deliming/bating process, about 4,701.5 mg/L while in the other process it ranged from 95.4 – 187.8 mg/L (Table 9). Ammonia was formed mostly in deliming/bating due to the use of ammonium sulfate. In other processes, ammonia was formed because of protein hydrolysis by either acid or base catalyst, also because of the use of bactericide containing ammonia. According to Wang et al. [34], bactericide contains 14,5 mg/g ammonium nitrogen. High amount of ammonia in wastewater would impede the work in WWTP to reduce it upon discharge [34]. This was proved by the wastewater analysis on WWTP outlet showing ammonia content (59.4 mg/l) exceeding the regulation value of 0.5 mg/l [25].

Chrome is a chemical used in tanning process and is carcinogenic. Its presence in the wastes will endanger the life form, especially human as it induces cancer [35]. Generally, chrome presents in the form of Cr^{3+}; however, it is also possible for it to be converted into Cr^{6+} with 100 times toxicity than Cr^{3+} [37]. The accumulation of Cr^{6+} in vital organ could damage metabolism function as it possessed carcinogenic, mutagenic and teratogenic effects [37]. Table 5 shows that chrome hexavalent was formed in tanning (0.62 mg/l) and retanning, dyeing and fatliquoring (2.09 mg/l).

3.4. *Analysis on environmental impact*

Table 8 shows our analysis results where industry B generated greater environmental damage compared to industry A.

Table 8. Environmental impact in tanning industry

Category	Industry A	Industry B
GHG (kg $CO_{2\ eq}$ per 1.5 tons of raw skin)	1,515.58	1,689.56
Eutrophication (kg $PO_4^{-3}{}_{eq}$ per 1.5 tons of raw skin)	7.82	9.95
Acidification (kg SO_{2eq} per 1.5 tons of raw skin)	3.12	4.99

GHG is one factor affecting global warming. According to IPCC [14], the gasses considered to be GHG and responsible for the global warming effect are CO_2, CH_4, and N_2O. CO_2 has a 50% contribution while CH_4 contributes as much as 20% of the total GHG. Analysis result revealed that industry A and B contributed to the GHG emission as much as 1,515.58 kg $CO_{2\ eq}$ and 1.689,56kg $CO_{2\ eq}$ respectively, per 1.5 tons of salted-hide in 1 production batch (Table 6). Figure 4 exhibits the great contribution of soaking; liming; setting out; and retanning, dyeing, fatliquoring to the GHG emission by industry A and also shows the contribution of industry B to GHG emmsion, mainly found in the processes of soaking; liming; pickling; tanning; and retanning, dyeing, fatliquoring.

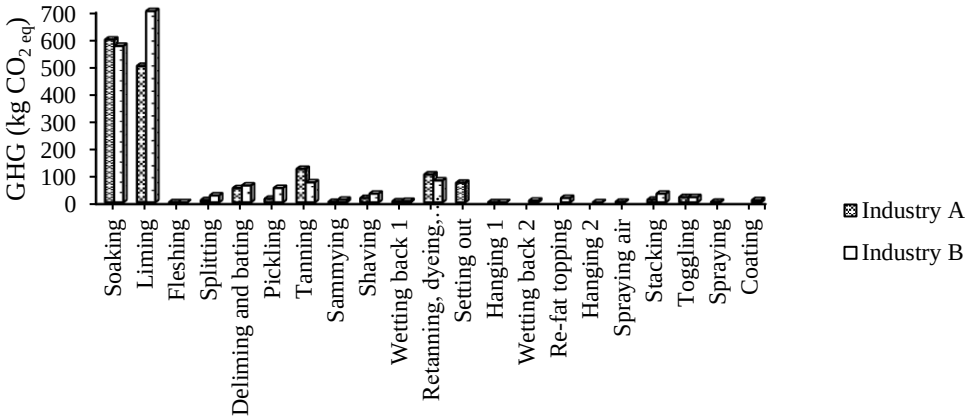

Figure 4. GHG distribution in the leather tanning process

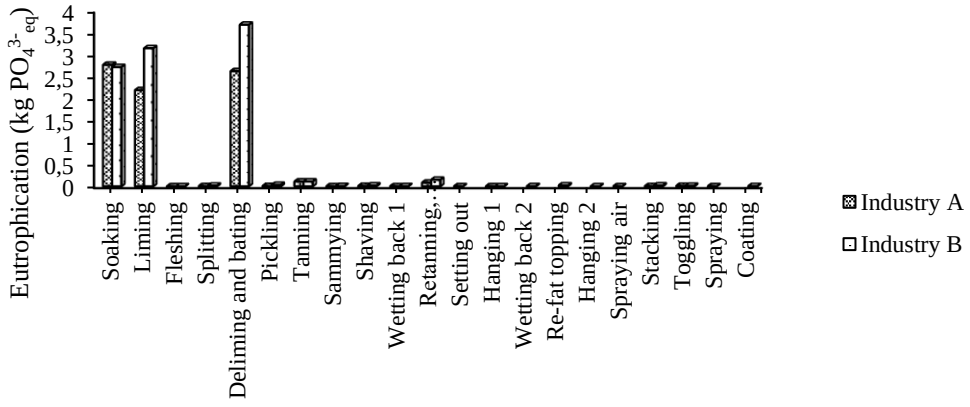

Figure 5. Eutrophication distribution in the leather tanning process

Eutrophication is a phenomenon in water ecosystem due to over-nutrient situation of phosphor and nitrogen [38]. The introduction of phosphor and nitrogen into water body caused a disturbance in the ecosystem [39] and a decrease in water quality. Based on our analysis, industry B (9.95 kg $PO_4^{3-}{}_{eq}$) resulted in greater eutrophication than industry A (7.82 kg $PO_4^{3-}{}_{eq}$) per 1.5 tons of salted-hide (Table 8). In both industry, soaking, liming and deliming and bating were the processes responsible for eutrophication, as there were a lot of skin components (nutrient) were removed along with the wastewater (Figure 5).

Acidification is a factor affecting acid rain [40] with SO_2 (Sulfur dioxide) and NO_x (nitrogen oxide) being the main reason of it [41]. Analysis showed that industry A contributed 3.12 kg SO_{2eq} to acidification while industry B impacted more, as much as 4.99 kg SO_{2eq} per 1.5 tons of salted-hide (Tabel 8). The processes of soaking; liming; tanning; and retanning, dyeing and fatliquoring were the most contributing processes to acidification in industry A while in industry B they were pickling; tanning; shaving; and retanning, dyeing and fatliquoring (Figure 6).

Our analysis exhibited a quite significant difference between each industry's contributions to varying categories of environmental impact due to the differences of machine power, duration of machine utilization, wastewater produced, pollutants in wastewater, ammonia content in wastewater and the process flow. These distinctions were also due to the difference in funtionality of leather product.

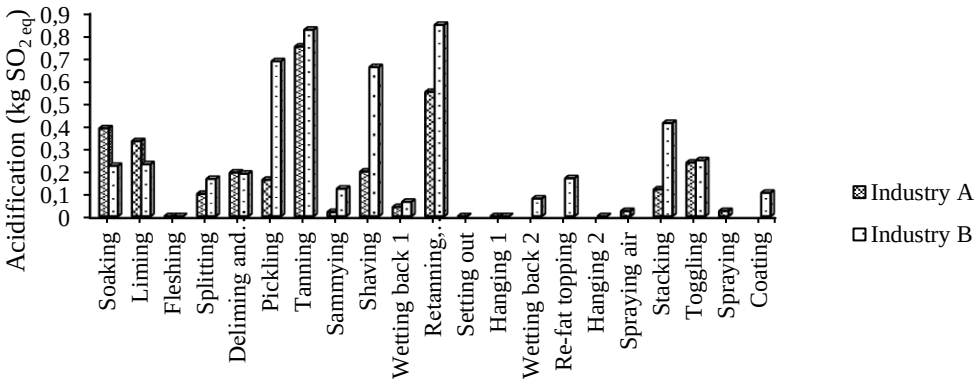

Figure 6. Acidification distribution in the leather tanning process

4. Conclusion

Our research concluded that tanning industry is greatly potential to impact the environment negatively. Each industry contributed differently; measured per 1.5 tons of salted-hide, industry A resulted in a fewer amount of wastewater compared to industry B. With the same basis, industry A produced more solid waste in comparison to industry B. The wastewater in tannery A and B were both identified as toxic and carcinogenic due to the content of sulfide, ammonia and Cr^{6+} with varying concentration in each process output. Those compounds were also found in WWTP outlet. Our analysis also revealed that industry B contributed more in GHG, acidification and eutrophication compared to industry A in the same basis.

Acknowledgments

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Study of Factors relating to community behavior in disposing household waste in the illegal disposal site in West Denpasar District

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Abstract: The urban populated conditions with narrow land lead to an increase in the amount of waste generation as residual activity. West Denpasar District is one of the districts that have the highest population density in Denpasar. The volume of waste generated in the area in 2010 reached 922 m^3/day and increased to reach 1001 m^3/day in 2014. Increasing the amount of waste generated causing waste management system was not optimum. One of the impacts is the emergence of illegal disposal site. The emergence of illegal disposal site was not only because of not proper waste management system but also due to community's behavior on waste handling. The study aims to recognize factors related to community behavior in disposing of household waste at illegal disposal site in West Denpasar District. The study uses analytic observational research design, using quantitative approach with cross sectional design. The sample of this research is 100 household selected by probability sampling technique type proportionate stratified random sampling. The data collection of behavior, education, knowledge, attitude, income level and availability of facilities is done by using structured questionnaires. The results obtained 70 respondents (70%) have bad behavior in waste management while 30 respondents (30%) have good behavior in waste management. Based on bivariate analysis between education and behavior obtained p value = 0.402 with α = 0.05, for income level with behavior obtained value p = 0.000 with α = 0.05, for knowledge with behavior obtained value p = 0.826 with α = 0.05, for attitudes with the behavior obtained p value = 0.001 with α = 0.05 and the availability of means obtained p value = 0.001. Meaning that there was no relationship between education and knowledge with the behavior of waste management in West Denpasar District. On the other hand, it was found that there was a relationship between income level, attitude and availability of facilities with waste management behavior.

1. Introduction

West Denpasar District is part of Denpasar City area which has an area of 24.13 km^2 with a population of 259,790 inhabitants. In 2016, West Denpasar District was ranked first with the highest population density of 10,798 km^2 compared to districts of South Denpasar, North Denpasar and East Denpasar [1]. Large urban populations with high population densities will produce larger volumes of garbage [2]. Based on data of Department of Environment and City Cleanliness of Denpasar, the volume of waste generated in West Denpasar District in 2010 reached 922 m^3/day and increased to reach 1.001 m^3/day in 2014 [3]. Increasing the amount of waste generated resulting in waste management system is not optimal. Based on the Guidelines for Determination of Minimum Service Standard for Spatial Planning, Ministry of Settlement and Public Works, it is explained that the coverage of urban garbage service is 80% of urban/municipal population [4].

The coverage of waste management in Denpasar City in 2015 which conducted by Department of Environment and City Cleanliness of Denpasar reaches 50% of the total population [5]. The absence of minimum service standards, low public awareness that likes to behave garbage carelessly, and the

reluctance to dispose of garbage in places that have been provided cause things that have a negative impact on the environment [6].

Based on a preliminary survey conducted in West Denpasar District, found 30 locations classified as illegal waste dumps, i.e. empty areas, alongside the river and on the roads. Until now people still disposes their waste to those area. Therefore, it is need to study factors related to community behavior in disposing household waste at the illegal disposal site in West Denpasar District.

2. Materials and Methods

The research uses analytic observational research design, using quantitative approach with cross sectional design. Population in this research is all household in District of West Denpasar that is 37,823 households (HH). Determination of the number of samples from the entire population using the Slovin formula [7]. On the basis of these considerations, the number of samples in this study as many as 100 households. Sampling method used is probability sampling technique type proportionate stratified random sampling. Based on the technique obtained the number of each sample per village is as follows: Padangsambian Klod 12 HH, Pemecutan Klod 16 HH, Dauh Puri Kauh 10 HH, Dauh Puri Klod 6 HH, Dauh Puri 6 HH, Dauh Puri Kangin 5 HH, Pemecutan 10 HH, Tegal Harum 6 HH, Tegal Kerta 9 HH, Padangsambian 13 HH and Padangsambian Kaja 7 HH.

The data collection of behavior, education, knowledge, attitude, income level and availability of facilities is done by using structured questionnaire. The analysis used in this study using quantitative methods. Univariate analysis is done to obtain the description of each variable, presented descriptively in the form of frequency distribution table of each variable. Bivariate analysis is intended to see the relationship of independent variables with dependent variable. Analysis of this research data using Chi Square test with degree of accuracy 95% ($\alpha = 0.05$).

3. Results and Discussion

3.1 Univariate Analysis

Variabel	Frequency	Percentage
Gender		
Female	83	83%
Male	17	17%
Level of Education		
Low	43	43%
High	57	57%
Income		
Low	45	45%
Middle	55	55%
Knowledge		
Not Proper	45	45%
Proper	55	55%
Attitude		
Negative	49	49%
Positive	51	51%
Availability of facilities		
Inadequate	52	52%
Adequate	48	48%
Behavior		
Bad	70	70%
Good	30	30%

3.2 Bivariate Analysis

Table 1. Bivariate analysis

Variabel	Attitude				A	*P Value*
	Bad		Good			
	∑	%	∑	%		
Education						
Low	32	32.0	11	11.0	0.05	0.402
High	38	38.0	19	19.0		
Income						
Low Income	43	43.0	2	2.0	0.05	0.000
Middle Income	27	27.0	28	28.0		
Knowledge						
Not Proper	32	32.0	13	13.0	0.05	0.826
Proper	38	38.0	17	17.0		
Attitude						
Negative	42	42.0	7	7.0	0.05	0.001
Positive	28	28.0	23	23.0		
Availability of facilities						
Inadequate	44	44.0	8	8.0	0.05	0.001
Adequate	26	26.0	22	22.0		

Respondents who have low education as many as 43 people (43%) and who have higher education as many as 57 people (57%). Based on the result of bivariate test between education variable and the behavior of society in disposing garbage at Illegal Disposal Site obtained p value = 0.402 (p> 0.05) meaning Ho accepted, hence there is no significant correlation between education with society behavior in throwing garbage. Research finding is in line with Damayanti's [8] research on factors related to trader behavior in disposing of garbage in the Central Sekura market. The result of statistical test p value = 0.492 (p> 0.05), which states there is no significant correlation between education and respondent behavior in throwing garbage at Sekura Central Market. The lack of correlation between educational level and behavior is contradictory to Green theory [9] in Damayanti [8] which states that education is one of the supporting factors for the behavior of a person. A person is not only influenced by an understanding of something but can be influenced by consistency in attitude. A person who is inconsistent in attitude, when he states agree on something, but he shows an attitude that is not supportive in the form of behavior.

Respondents who are on low income category are 45 people (45%) and those who were in middle income as many as 55 people (55%). Based on the result of bivariate test between income level variable and society behavior in disposing garbage at Illegal Disposal Site obtained p value = 0.000 (p <0.05) which means Ho is rejected, hence there is significant correlation between income level with society behavior in throwing garbage at Illegal Disposal Site in West Denpasar District.

The number of respondents who are on low income category and have bad waste processing behavior as much as 43 respondents while those who have good behavior as much as 2 respondents. Low income people will result in the outbreak of attention between the necessity to meet the needs of household life with the necessity to perform other activities. Such as the necessity to manage and maintain the cleanliness of life from the negative impacts of the environment is less healthy and dirty. Furthermore, as many as 27 respondents who have enough income to have bad waste management behavior, while 28 respondents who have enough income have good waste management behavior. Based on the results of interviews that have been done, people who have enough income but have bad behavior due to heavy work, no time, opportunity and reluctant to spend the cost to process waste in the home and environment. Respondents assume that the waste is just enough to throw it on the empty land or roadside and there are already other people who will burn the garbage.

Furthermore, research finding is in line with Darmawan's research [10] which examines the analysis of the behavior of housewives on the river banks of Citampian in managing household waste in Bendungan and Ciawi Villages. The results obtained by p value is 0.029 (p <0.05) which shows that income has a positive influence on the behavior of managing household waste.

Respondents who have less knowledge are 45 people (45%) and respondents who have good knowledge are 55 people (55%). Based on the results of the bivariate test between the variables knowledge of the behavior of people in disposing of waste in an illegal TPS obtained p value = 0.826 (p> 0.05), which means that Ho is accepted, meaning there is no significant relationship between the knowledge of the behavior of people in the household trash at Illegal Disposal Site in West Denpasar District. An assessment of the level of knowledge is based on an understanding of trash, garbage understanding, kind of garbage, waste sources, diseases caused by the trash and the consequences if carelessly discarded rubbish (into empty fields, alongside roads, river bank).

Good knowledge about waste management because in West Denpasar carry out waste management through the Waste Bank system which can improve community knowledge about waste management. Although knowledge of solid waste management is good, not all respondents who have good knowledge behave well in waste management. Respondents who are well-informed but the behavior of their waste management is not good because simply the respondents does not want to bother with garbage problems.

In addition, research finding is in accordance with Mulasari [11] regarding the relationship between the level of knowledge and attitudes towards community behavior in processing waste in Padukuhan Hamlet, Sidokarto Village, Godean District, Sleman Regency, Yogyakarta. P value obtained = 0.429 with $\alpha = 0.05$, which means there is no relationship between the level of knowledge with people's behavior in processing waste, this study also states that not all respondents who have good knowledge have good behavior in waste management.

In other perspectives, respondents who had negative attitudes were 49 people (49%) and respondents who had a positive attitude were 51 people (51%). Based on the results of the bivariate test between the variables of attitudes with people's behavior in disposing of garbage at Illegal Disposal Site obtained p value = 0.001 (p <0.05), which means that Ho is rejected, so there is a significant relationship between attitudes with people's behavior in disposing of garbage. Respondents who have a negative attitude tend to behave worse in disposing of trash than respondents who are positive. Negative attitude of the community is influenced by the waste collection, which mostly done only twice a week, especially when there are religious ceremonies which are carried out less frequently. This is what encourages people to burn trash or throw garbage on vacant land around the house. Most respondents also have an attitude of not supporting the implementation of sanctions for everyone who throws garbage on vacant land or rivers.

Respondents with inadequate facilities and bad behavior in disposing of household waste in Illegal Disposal Site amounted to 44 respondents. The facilities most respondents do not have are trash bins equipped with lids, this is due to the assumption that providing trash bins is not a priority in their lives. In addition, respondents also said that the transport of waste is infrequent and the existence of official polling stations that are far from home, so that the waste owned by the respondents accumulates, if left to take longer, it causes odor. This causes respondents to prefer to burn or dispose of garbage on vacant land.

The results of this study in accordance with the theory of Lawrence Green [9] in Intan [12] say that one that influences behavior is the enabling factor. The enabling factor is a factor that includes a variety of skills and resources, where skills and resources are important points needed to make changes in health behavior. The availability of a trash can is one of the enabling factors that indirectly gives messages to people to dispose of their trash in place so that the environment becomes clean and free of disease. The existence of waste disposal facilities in many places will make it easier for the community to dispose of garbage. The unavailability of garbage disposal facilities makes it easy for people to dispose of garbage in any place.

4. Conclusion

Based on research finding and analysis some conclusion can be drawn. Firstly, the behavior of respondents in West Denpasar sub-district in disposing of household garbage in illegal TPS is still relatively high, out of 100 respondents surveyed, 70% of respondents still throw litter (to vacant land, on the side of a highway, on a riverbank). Secondly, there is no significant relationship between the level of education and the behavior of the community in disposing of household garbage in illegal polling stations in West Denpasar ($p = 0.402$).

Furthermore, it can be seen that there is a significant relationship between the level of income and the behavior of the community in disposing of household garbage in illegal polling stations in West Denpasar ($p = 0.000$). Other things that can be seen is no meaningful relationship between knowledge and community behavior in disposing of household garbage at illegal polling stations in West Denpasar ($p = 0.826$). On the other hand, there is a significant relationship between attitudes with people's behavior in disposing of household garbage at illegal polling stations in West Denpasar ($p = 0.001$). Finally, it can be concluded that there is a significant relationship between the availability of facilities and the behavior of the community in disposing of household garbage in illegal TPS in West Denpasar ($p = 0.001$).

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Community behaviour in single-use plastic bottles consumption in term of increasing plastic pollution mitigation

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Abstract. Since plastic is considered as one of hazardous material causing negative impacts in the environment, various ways to educate community regarding impact of plastics pollution and the behaviour on plastics consumption have been continuously conducted. Furthermore, the relationship between culture and the increasing plastic waste based on community behaviour in single-use plastic bottles consumption have been investigated in the term of mitigate the increasing of plastic pollution. The research purposes is to investigate how many single-use plastic bottles with polyethylene terephthalate (PET) base material have used in daily activity and how are the community manage it as a waste. Hence, to achieve those aim, we collected data from 100 housewives with various background of education and social economic, age, lifestyle and family members. About 7 home companies that works in plastic waste collector also the most important part in the data collecting to support the data of plastics consumption. From the data investigation, we have almost 80 % of housewife reported 1-4 single-use plastic bottle each day for their family member consumption and the rest 20% uses more than 4 single-use plastics bottles. From 88 respondent only 10 % housewife collect the plastic bottles separately in the trash bin, it means that almost 90 % of house wife did not manage their garbage properly. Reducing consumption single - use plastic bottles by replace it with refill water bottle is one solution of many solution to decrease the plastics garbage in this environment. The survey result showed that increasing of Single-use plastic bottles waste in the environment is much influenced by the behaviour of community in consumption and waste management.

1. Introduction

Many studies about plastics phenomenon have been conducted and negative impact of plastics pollution have been understood in the term of recovery the environment from plastics pollution itself. Recently, Plastics are ubiquitous in the environment and need to get more attention regarding on mitigate the impact of plastics pollution [1][2]. Due to the durability of plastic, relatively cheap and long-lasting form of packaging for food products and beverages, the production of plastic increase in line with the increasing of plastic consumption in other hand those plastic characteristic become a big problem when it is dumped and accumulated in environment.

Indonesian society is a very diverse society in the economic and educational level. Community understanding of the dangers of plastic and plastic waste is not shared by all individuals. Communities living in urban areas tend to choose a practical lifestyle so that the selection of food and beverage products by utilizing disposable plastic packaging is the right alternative choice to support daily activities. This causes the amount of plastic waste to increase from year to year as the number of population increases and the number of new products using disposable plastics [3]. Less than half of the bottles bought in

2016 were collected for recycling and just 7% of those collected were turned into new bottles after recycle processes the rest is used as a raw material for making toys and household items that are not valuable [4] instead most plastic bottles produced end up in landfill or in the ocean. Plastic products in Indonesia are used by a wide array of industries such as the food and beverage packaging industry which accounts for 60%, building and household appliances for 15%, the automotive sector for 8% and the remainder by other sectors including agriculture and horticulture [5]

Single-use plastic bottles are essential part of our society consider to its characteristic easy to carry, portable enough, strength, plenty size and model, cheap and easy to get. Most plastic bottles used for mineral water and soft drinks are made from polyethylene terephthalate (PET) polymer base material, which is highly recyclable. This type of plastic is suggested to use as a single-use plastic regarding the risk of this plastic type can cause bacteria to grow if reuse it [6]. But as their use soars across the globe, efforts to collect and recycle the bottles to keep them from polluting the oceans [7], since only 20% of plastic water bottles are recycled and the rest 80% end up in landfills [8]. In other hand there is no other material that can replace plastic packaging such as single –use plastic bottles for beverage products make a strong reason that plastic packaging has become a vital component in people's lives especially in recent decades where plastic packaging has become very popular, undermining the market shares of glass or tin.

The most important point as a key to educate people in term to rise their awareness up on plastic management and consumption is knowing the impact of plastic for the environment and the health risk of plastic itself. The durability of plastic causes plastic is accumulated in a long time in the environment, furthermore the plastic which in the manufacturing process involves various chemical compounds also has the ability to adsorb, release and distribute pollutants to and from the environment [9] and increasing of plastic contamination supported by degradation process of plastic itself due to the chemical release process (leaching) and fragmentation of macro plastic being micro plastic as a degradation product [1][10] While specifically, the components contained in single-use plastic bottles made from PET such as Bisphenol A (BPA), phthalates and brominated flame retardants [11] have health risks for humans.

However, to solve the problem of plastic waste in the environment by cleaning up or dumping plastic waste are not best choices while the recycling process will increase carbon emissions therefore a more appropriate way is by reduce the production and consumption of plastics, especially single-use plastics [13]. The fact that commercial use of plastics as a packaging material is a cheapest one in supporting various industrial and daily necessity should encourage the government to tax plastic material in the term to reduce the plastic consumption. In 2002, Ireland became the first country to introduce a tax on plastics. This is known as Plastics tax and was found to reduce plastic bag consumption by 90%. In other hand, Janice Wung mentioned that the bottled water market in Indonesia has seen increasing competition due to exploding bottled water consumption [13], it showed that government policy have no significant impact in the way of reducing single-use plastic bottles consumption in Indonesia as mentioned in GBG Indonesia 2014 that in Indonesia, the plastic consumption per capita increased to over 17 kg per year.

Study on the relationship between people's behavior in consuming plastic and the increase in the amount of plastic waste needs to be done in order to get the right solution in the process of mitigate increasing the impact of pollution of plastic waste. A journal that examines the cultural relations of an area with people's behavior writes that people's behavior in handling waste is strongly influenced by the habits of the majority of individuals around them, the level of education, employment, age of activities outside the home, television programs watched and even types of social communities that followed [14]

The purpose of this reasearch is to study more about the relationship between social-cultural-influenced people's behavior towards plastic consumption and plastic waste management in order to mitigate an increase in the amount of single-use plastic bottles with polyethylene terephthalate (PET) base material waste in the environment by survey and questionnaire distribution method.

2. Material and Methods

In accordance with waste management law No.18/2008, Indonesia government stipulate policies and strategies for reduce waste landfill and the handling of waste that is difficult to decompose naturally in environment such as plastic. However, the habits and lifestyles of Indonesian community are not

supported by adequate facilities for waste handling and education about the hazardous of plastic waste causes government policies do not play a role properly.

To obtain a real description of single – use plastic bottles with Poly Ethylene Terephthalate (PET) base material consumption, the approach to objects is conducted by surveying plastic garbage collection locations, interview with stakeholders who work as plastic waste collector and distributed questionnaires to respondents with a similar economic and education backgrounds influenced by age. The questionnaire distribution aims to obtain data on the use and handling of single-use plastic bottles where each respondent will represent the amount of consumption in one family with the diverse of family members in the range of 3-5 family members. The questionnaire consisted of 10 questions divided into three parts, which measured part I. Background information, part. II. Consumption behavior and part III. Plastic waste management. Most questions focused in single-use plastic bottles, since the research concerned in this type of plastic. Stakeholders selected in the survey are plastic waste collectors who have worked for more than 1 year and collected large quantities of Single-use plastic bottles that representing each area in Semarang which are expected can provide an overview of the single-use plastic bottles quantities that have been consumed by the surrounding communities and it can estimated the single use plastic bottles number are not entered the environment globally.

3. Result and Discussion

Government interventions in the term to reduce single-use plastic waste has already conducted in Indonesia, included by education, facilitation and some regulation, but yet the maximum achievement seems not adequate as expected. The most obstacle play important role in this situation is changing community habit and behaviour in Single –use plastic consumption by involving social culture. Hence, to find out how people's behavior in the consumption of Single-use plastic bottles, the investigation was conducted using questionnaire instruments distributed to 88 respondents. From about 88 respondents who had the same economic and educational background the rest ages as independence variable, the results showed (Figure 1) that in the age range of 25-35 and 36 -45 years most respondents used plastic bottles around 2-4 pieces per day, this was supported by data on the number of percentages (Figure 2) of plastic bottles usage without involving the age factor, where most respondents (43.18%) using plastic bottles about 2-4 pieces per day and the remaining 36.36% using plastic bottles less than 2 pieces per day and as much as 20.45% used more than 4 pieces per day. It is showed that almost 80% used single-useplastic bottles not more than 4 and even not less than 1 perday in their house. If for one house they use about 4 pieces per day or less for all family member where one family have about 3-5 familys member it means each person consumes only 1 plastic bottle or less. One plastic bottle has an average volume of 330-500 mL, this volume is not sufficient for the amount of drinking water needed per day for each individual, therefore the use of single-use to supply water needs is not a primary necessity, hence , replacing by reuseable plastic bottles instead os single-use plastic bottles is very possible to be socialized and practised by community. Moreover, it can be concluded with certainty that consumption behaviour in single-use plastic bottles can still be changed with better alternatives to replace it.

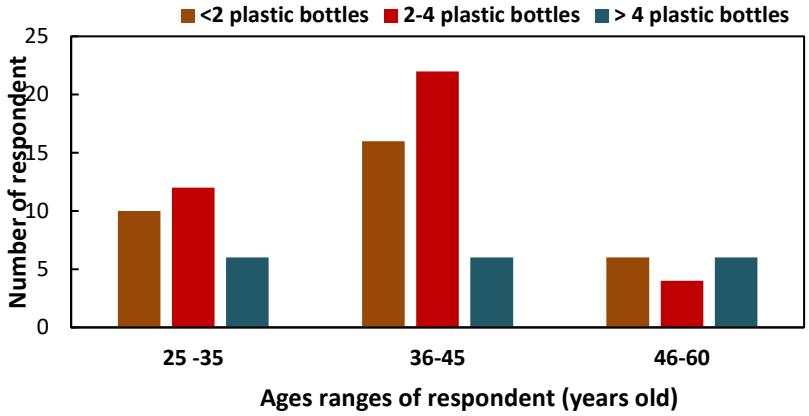

Figure 1. Single-use plastic bottles consumption influenced by ages

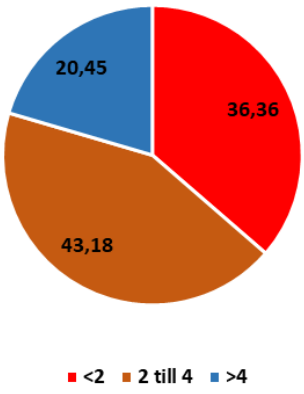

Figure 2. Persentage of respondent in Single-use plastic bottles consumption

The data of single-use plastic bottles consumption is supported by the survey results of 7 respondents who are work as a plastic waste collector (Pengepul sampah), the survey results can be seen in the following table (Table 1)

Table 1. Survey data of single-use plastic bottles obtained by plastic collector company

Age of Respondent (years)	Age of plastic collector company (years)	The most collected plastic waste	The amount of plastic bottle (kg/day)
65	27	- Single-use plastic bottles - Plastic carrier bags	20 kg
54	20	- Single-use Plastic bottles	100 kg
35	6	- Single-use plastic bottles	<10 kg
27	3	- Single-use plastic bottles - Plastic carrier bags	300 – 400 kg
28	3	- Single-use plastic bottles	100 kg
69	22	- Single- use plastic bottles	100 kg
25	2	- Single- use plastic bottles	400 kg

Identifiying the single-use plastic bottles consumption must be supported by the data of the plastic waste amount that have been collected by collector such as ragpicker (pemulung) and waste collector company. Through interviews, 7 plastic waste collectors obtained information that on average 100 kg of plastic waste per day have been collected, mostly is single-use plastic bottles with PET base material. Single-use plastic bottles is obtained from household, office and public areas collected most by ragpicker and only a few of it is obtained from family members who have separated plastic waste in their house. This shows that public awareness in separating plastic waste is still low from the expectation, so it takes certain people who work as ragpicker to be able to separate plastic waste from landfills.

4. Conclusion

To overcome the problem the integration of all parties, government, citizens and private will be required. The most important thing is changing the paradigm that plastic waste especially Single –Use plastic bottles in the environment will decrease in line with consumption of plastic it self, whereas recycle and degradation procesess take the next role for the waste that by perforced be in place as a result of consumption because of necessity. Changing the community behaviour by strategies and alternatives to reduce single-use plastic bottles for instant with reuseable plastic bottles instead of single-use plastic bottles and introduce plastic tax to be a suitable solution, meanwhile educated community with a right framework about plastic still have to be conducted.

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Nitrogen use efficiency (NUE) for oil palm seedlings cultivation under tropical soil

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Abstract. Oil palm is the most important commodity crop in Malaysia, and planted about 5.71 million hectare.Therefore, it is important for effective management of oil palm in term of nutrient management, especially nitrogen (N). Management of N is important because it highly influence oil palm growth and yield production. However, N lossess are often high in tropical soils due to denitrification, volatilization and leaching processes. A potential management of N is through coated urea as slow release fertilizer. A study was conducted to determine the efficacy of coated urea to improve nitrogen-use efficiency (NUE) and N uptake can be increased. Subsequently, reduction in fertilizer cost and increase in yield of oil palm. A total of six coated urea have been studied, namely; common urea (U), geopolymer coated urea(GeoU), biochar coated urea (BioU), copper coated urea (CuU), zinc coated urea (ZnU), and copper-zinc coated urea (CuZnU). Nursery study was conducted to evaluate the effects of coated urea on crop growth and nitrogen uptake in mineral soil and peat. In mineral soil, plant that have been treated by BioU have most significant effects on growth performance which increase plant height and dry weight. BioU also increase N uptake and NUE. In peat soil, CuZnU treatments have increase growth performance such as plant height and dry weight. CuZnU treatments increase N uptake. The percentage of NUE noted to be supportive of N uptake with regards to plant age, and this can be attributed to the fact that nitrogen in coated urea are readily and slowly available for plant uptake over a given period of time. BioU have showed the best performance in mineral soil while CuZnU treatment showed the best effect in peat.

1. Introduction

In Malaysia, oil palm plantations were established a top of highly weathered tropical soils; inherent poor nutrient availability due to high precipitation with large leaching losses. As that, tremendous amounts of fertilizers were required for crop improvement. Various studies indicated that most of the N loss from the plant-soil system through several pathways such as mineralization, ammonia volatilization and leaching (Rao *et. al.*, 1993; Owens and Bonta, 2004; Yan *et. al.*, 2003).

Methods for improving crops nutrient-used efficiency (NUE) have been proposed in many literatures. Application of coated urea have promising prospects to improve the NUE, these through coating with natural materials and/or urease inhibitors. Application of coated urea can improve plant growth and quality, increase nutrient use efficiency and can reduce the surface runoff and leaching losses compared to urea.

However, cost of fertilizer fluctuating according for world trade. As that, there is a need to manage fertilizers management efficiently. According to Watson (2009), selecting fertilizers which have ability to slow the hydrolysis process, prevent ammonia, and increase the NUE delivery. Thus, there is lack of "know-how" on its applications for oil palm seedlings in compared to standard urea usage. With the correct selection of fertilizers used, the farmer's able to optimize their profitability and reduce environmental degradation.

At the same time, lack of the reproducibility between laboratories study with the actual field performance of coated urea were hard to justify. Therefore, *in-situ* assessments need to be conducted in order to get a better view.

2. Material and Methods

2.1 Experimental site

This study was conducted in open area farm of Universiti Putra Malaysia (2 ˚59' 59' N, 101˚42'25 E) with temperature ranging from 24-33°C daily. The randomized complete block design (RCBD) with six (6) treatments of four (4) replications each. Two (2) types of soils were used in this experiment with the duration of nine months. The size of plots were 13.7 m (length) x 17.5 m (width).

2.2 Plant materials and preparation

Three (3) months oil palm seedlings were used as planting materials and obtained from Sime Darby Group. The *psifera* GH500 seedlings variety was used in this study, which cross-breed between Elite *Deli Dura* with second generation of *Psifera* BM119. These seedlings were transplanted into polybag containing 15 kg of mineral soil and 10 kg of peat soil respectively. Total of 144 experimental units was used in this study. Planting distance between each of the polybags within the block was 1m each. The seedlings were watered twice daily using sprinkle whilst weeding was done manually.

2.3 Fertilizer Treatment Application

Six (6) treatments were used in this study; 1) typical urea (U), 2) geopolymer urea (GeoU), 3) Biochar coated urea (BioU), 4) copper coated urea (CuU), 5) zinc coated urea (ZnU), and 6) copper-zinc coated urea (CuZn). The treatment was applied in an interval period of three (3) months; December 2105, March 2016 and June 2016.

2.4 Soil Sampling

As much as 20 g of peat samples were taken in three (3) phases; 6,9 and 12 months old from each polybag using a sampling auger. These samples were used to determine total nitrogen (N) by using CNS analyzer (CNSTruMac Determinators version 1.1x). Mineral soil sample was air dried, passed through a 2 mm mesh sieve whilst peat fresh soil was directly analyzed.

2.5 Plant growth measurement

Plant height was recorded every three (3) months interval, September 2015, March 2016 and June 2016 using 1 m ruler measured from soil surface to the highest petiole tip. Chlorophyll value were count every three (3) months interval. The seedling were harvested in three (3) phases; 6, 9 and 12 months old. Aerial of plants was sampled and the weight was recorded. Harvested plants were oven-dried at 60 ^{0}C temperature for 72 hours.

2.5 Total N and Nitrogen Uptake

The dried and grinded samples were weighted for 2 mg and being analyzed for carbon and nitrogen using CNSTruMac Determinators version 1.1x. Total nitrogen uptake was calculated by multiplying total N (%) with dry matter weight (g).

2.6 Computations for nitrogen use efficiency (NUE)

The recovery of nitrogen in crop system normally being represented by NUE. The formula was derived from (Dobermann, 2005) and as shown below:

$$\frac{\text{N uptake treatment} - \text{N uptake no fertilizer (blank)}}{\text{N fertilizer application}} \times 100\%$$

2.7 Statistical Analysis

Data were analyzed using of analysis of variance (ANOVA) for means separation. The different means in the treatments were compared using Tukey test. All the analyses were conducted using Statistical Analysis System Software (SAS) version 9.

3. Results and Discussion

3.1 Aerial dry weight

Aerial dry weight for the first phase was shown significant effect ($P<0.05$) of fertilizer treatments with type of fertilizer in the field. Analysis of variance indicates that there was significant interaction between fertilizer treatments and soils on aerial dry weight.

Among all treatments in minerals soils, the highest aerial dry weight was obtained from BioU compare to other treatment. These followed by (18.58 g polybag^{-1}) CuU, (16.18 g polybag^{-1}) GeoU, (13.44 g polybag^{-1}) CuZnU and (11.66 g polybag^{-1}) ZnU. Zinc coated (ZnU) and copper-zinc coated (CuZnU) showed lowest aerial dry weight due to N availability caused by the delayed release of N (Grant *et.al*, 2012). While in peat soil, CuZnU showed better respond of aerial dry weight, followed by other coated treatments.

For the 9 months old of seedlings, aerial dry weight has shown positive effect to all coated urea. Asides from the fertilizer treatment effects, the effect of fertilizer treatments with soils also significant during this stage. These indicated that fertilizer treatments also varies among the soils.

In the second phase, application with BioU and copper & zinc were showed high significantly different ($p \leq 0.05$) in aerial dry weight of the value of 136.48 g/polybag and 131.62 g/polybag as compare control treatment (78.24 g/polybag) respectively. However, treatment of ZnU also has shown high value of aerial dry weight (121.98 g/polybag) in this phase for mineral soil.

Whilst, on the peat soil, highest mean of aerial dry weight was the plant that grown under ZnU and BioU which was 78.88 g/polybag and 65.51 g/polybag as compared to GeoU, CuU, CuZn and U. The lowest significant in the upper dry weight was urea (control). Nevertheless, GeoU, CuU and CuZnU has no significant with urea (control) but it shown a good performance since the result was similar either between split application or one application. Therefore, all of treatments showed slow release of N except typical urea.

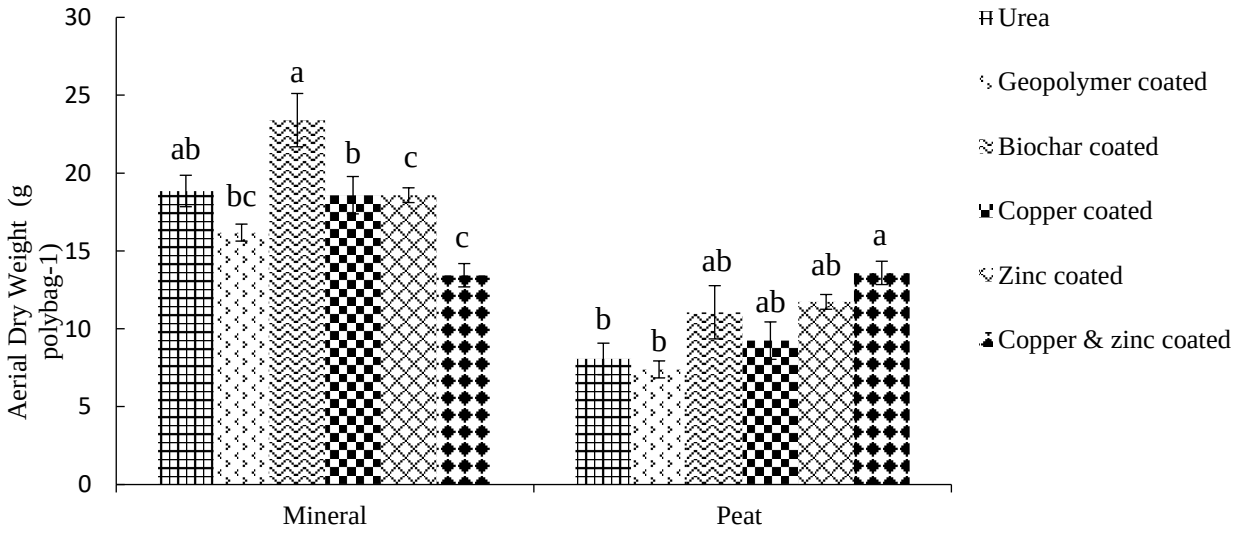

Figure 1. Effect of coated urea on aerial dry weight of oil palm seedlings of 6 months old

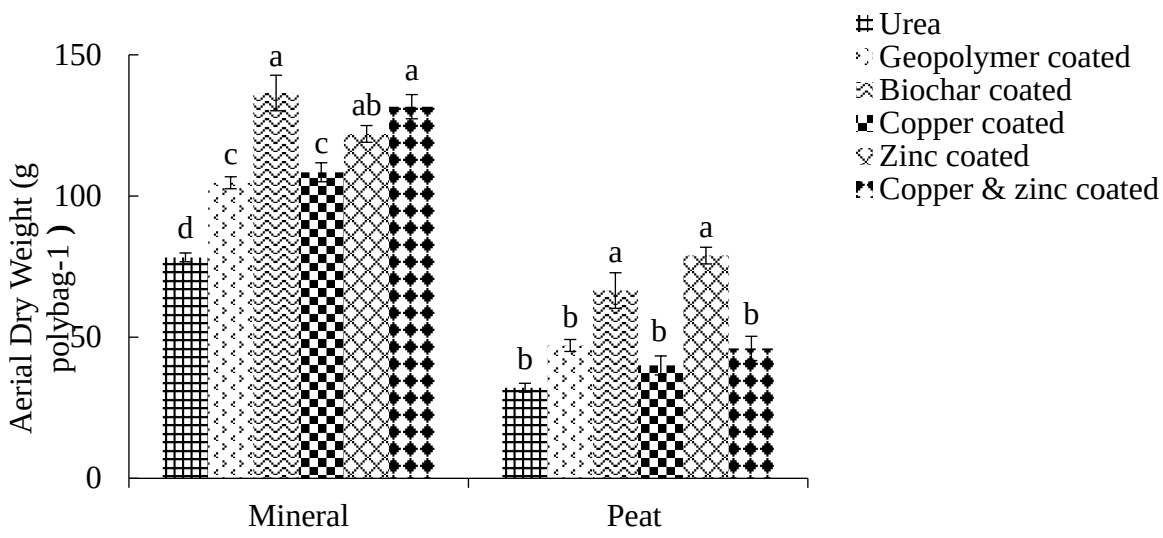

Figure 2. Effect of coated urea on upper dry weight of oil palm seedlings of 9 months old

Aerial dry weight were significantly ($p < 0.05$) increased by all coated urea treatments compared to control, for mineral and peat soil accordingly. The interactions between fertilizer treatments and soil also shown significantly effect during12 months old.

Among all treatments in minerals soil, the highest aerial dry weight was obtained from ZnU (586.42 g polybag^{-1}) and BioU (453.72 g polybag^{-1}) treatment, closely followed by CuZn (368.93 g polybag^{-1}), GeoU (366.06 g polybag^{-1}), CuU (306.09 g polybag^{-1}) and U (161.98 g polybag^{-1}).However, all coated urea have shown high value of aerial dry weight compared to control which CuU has most high value followed by CuZnU, BioU, ZnU, GeoU and urea as a control.

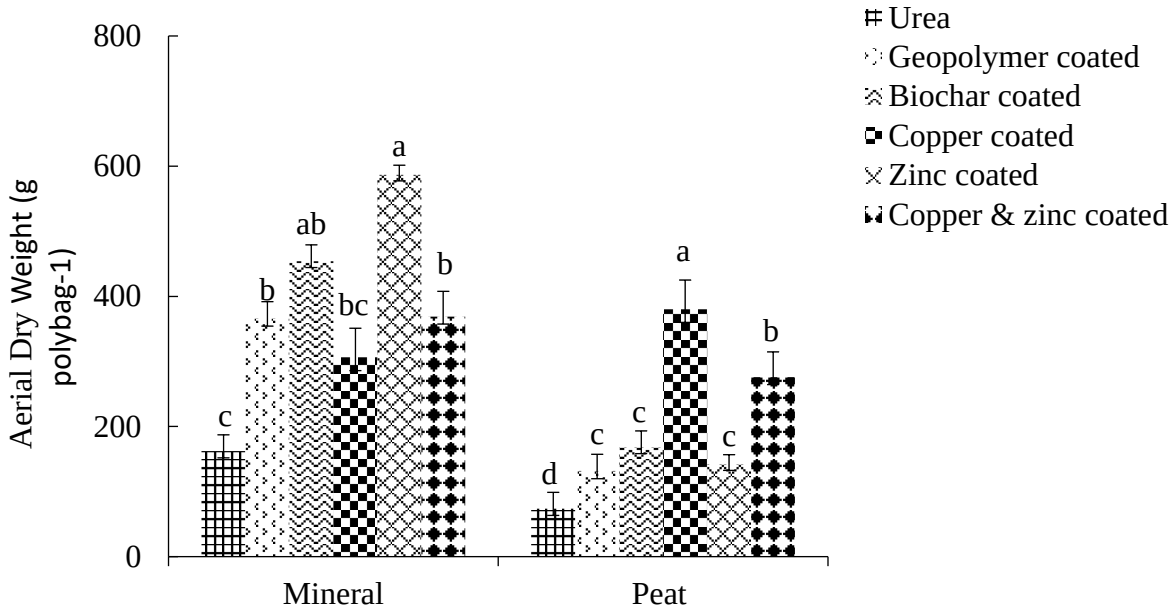

Figure 3: Effect of coated urea on upper dry weight of oil palm seedlings of 12 months old

Generally, all coated urea treatments gave better response compared to urea as a control in both type of soils. From the study, even with one-off application of coated urea treatments comparable result with

three times application of urea (control) were observed. Application of coated urea in full dosage synchronize with nutrient demand of oil palm seedlings as slow release properties of nitrogen from the coated. Previous study on Napier grass showed that control release fertilizer was about 40 % more efficient in term of dry matter yield production compared to conventional fertilizer such as compound and straight fertilizer (Low, 2009). According to Trenkel (1997) polymer-coated urea tends to have a more predictable release pattern which resulted in yields similar to or greater than those with soluble N sources.

3.2 N uptake

The N contents in the first phase was shown significant effect ($P<0.05$) of fertilizer treatment and type of soils in the field. Analysis of variance indicates that there was significant interaction between fertilizer treatment and soils on nitrogen uptake.

Among all treatments in minerals soils, the highest N uptake was obtained from BioU, followed by CuU and GeoU. However, all coated urea have shown significantly effect on 9 and 12 months old of oil palm seedlings compared to urea (control). This was associated to coated urea treatments act as control released with ability to slow down hydrolysis process and supply nitrogen into plant in available form. Hence, coated urea reduced loss of nitrogen in the process of evaporation and leachate. This may significantly reduce the risk of environmental contamination.

In peat soil, CuZnU, BioU and ZnU showed that there was no significant difference. However, in the first phase there was significantly higher value of N uptake for CuZnU, followed by BioU and ZnU treatments respectively. While in the second phase (9 months old), all coated urea treatments gave better N uptake compare to control urea. Besides that, plant that contains CuZnU and CuU has highly significant effect on N uptake for 12 months old.

In general plant that has been treated by copper & zinc has significantly effects on N uptake in all phases of peat soil. Peat soil material contained high total N, but still unable to provide sufficient amount of N to support for plant growth. On top of that, peat soil inherently deficient in micronutrients particularly for copper and zinc (Tie and Kueh, 1979). However, copper and zinc as trace elements in peats failed to ameliorate the deficiency problem due to reaction with inorganic and organic compounds within the soils (James and Barrow, 1981). Nitrogen, copper and zinc are very important elements in order to meet the nutrient requirements of crops in peat environment. Thus, coating of urea with micronutrient (acts as control release fertilizer) especially copper and zinc potentially helps in slow release of nitrogen as well as micronutrients.

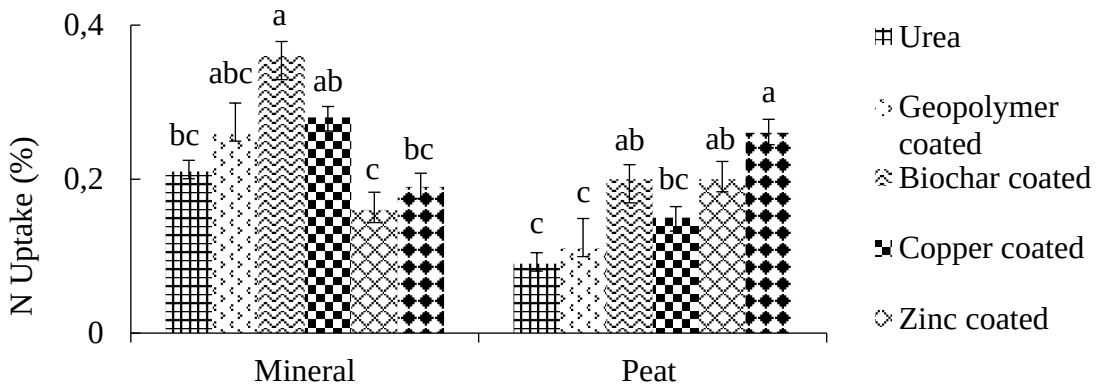

Figure 4. Effect of coated urea on N uptake of oil palm seedlings from 6 months old

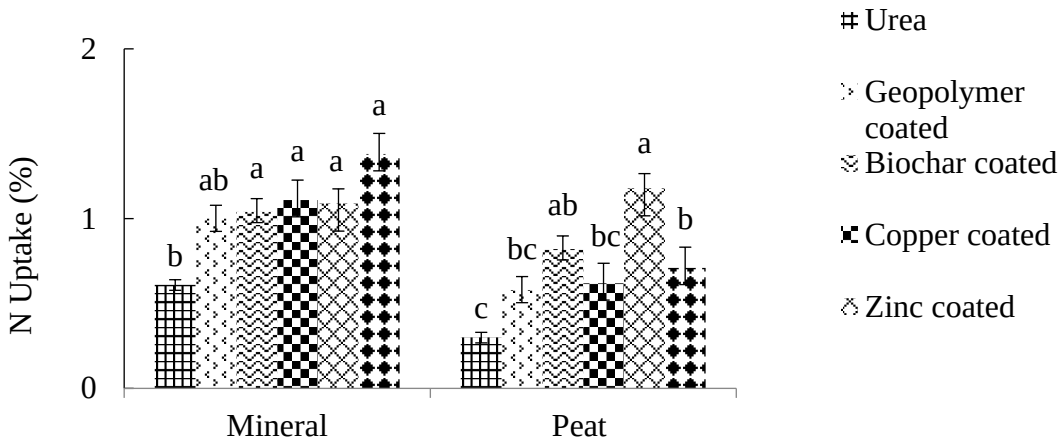

Figure 5. Effect of coated urea on N uptake of oil palm seedlings from 9 months old

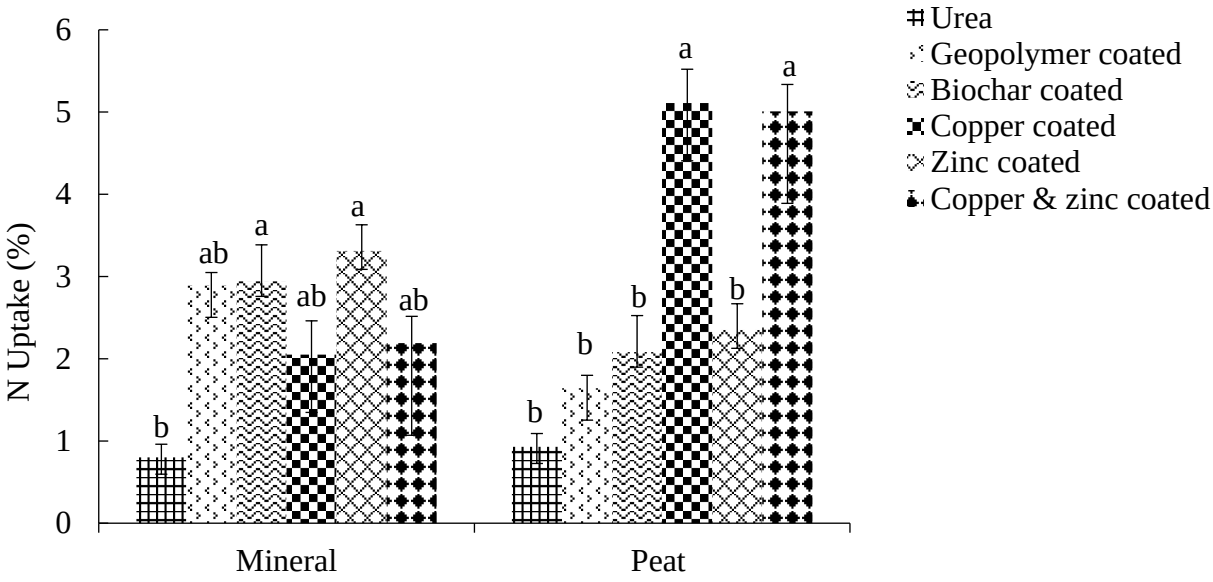

Figure 6. Effect of coated urea on N uptake of oil palm seedlings from 12 months old

3.3 Nitrogen use efficiency

Nitrogen use efficiency (NUE) is prediction of ability for nitrogen fertilizers to supply N source to the plant. NUE in the all phases were shown significant effect ($P<0.05$) with fertilizer treatments and type of soils in the field. Analysis of variance indicated that significant interaction between fertilizer treatments and soils on NUE were observed.

All coated urea treatments portrayed effects on NUE in all phases of mineral soils. Biochar coated (BioU) shown highest percentage of NUE in mineral soil followed by CuU And GeoU for the first three months. However, in the second and third phases all coated urea were more efficient. Some of the coated urea has low NUE in the first phase due to thickness of fertilizer which enable release of nutrients over an extended period of time (Sempeho *et. al.,* 2014). According to Shaviv (2001), the release of nutrients in the initial stage was very slow due water absorption process on coating materials, later releases constantly over given time

The plants treated with coated urea materials influenced NUE percentage in the first phase and second phases in peat soils set. Copper-zinc coated (CuZnU) have high percentage of NUE (5.76%) in the first phase, ZnU for the second phase whilst CuZnU and CuU for third phase compare to urea.

The relationships between NUE and N uptake in plant shows positive correlation ($p \leq 0.05$). However in peat soil, the relationship between NUE with plant chlorophyll, plant height, dry weight and N uptake are fairly strong positive relation ($p \leq 0.05$) (Appendix). These correlations shows that N source from fertilizer treatments was able to improve N contents in plant. Thus, increase in chlorophyll value, plant height and dry weight of the plant accordingly.

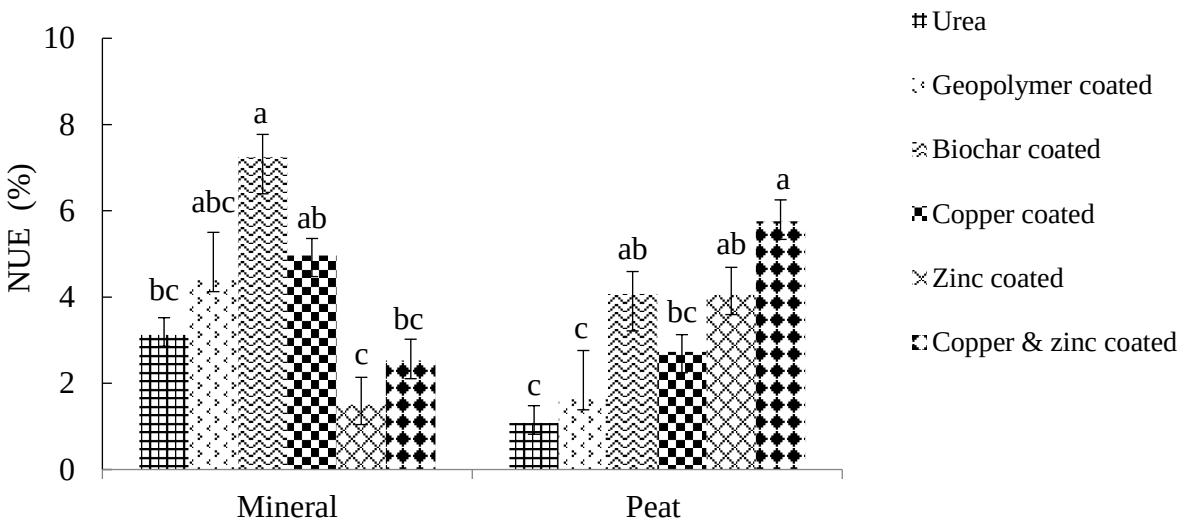

Figure 7. Effect of coated urea on NUE of oil palm seedlings from 6 months old

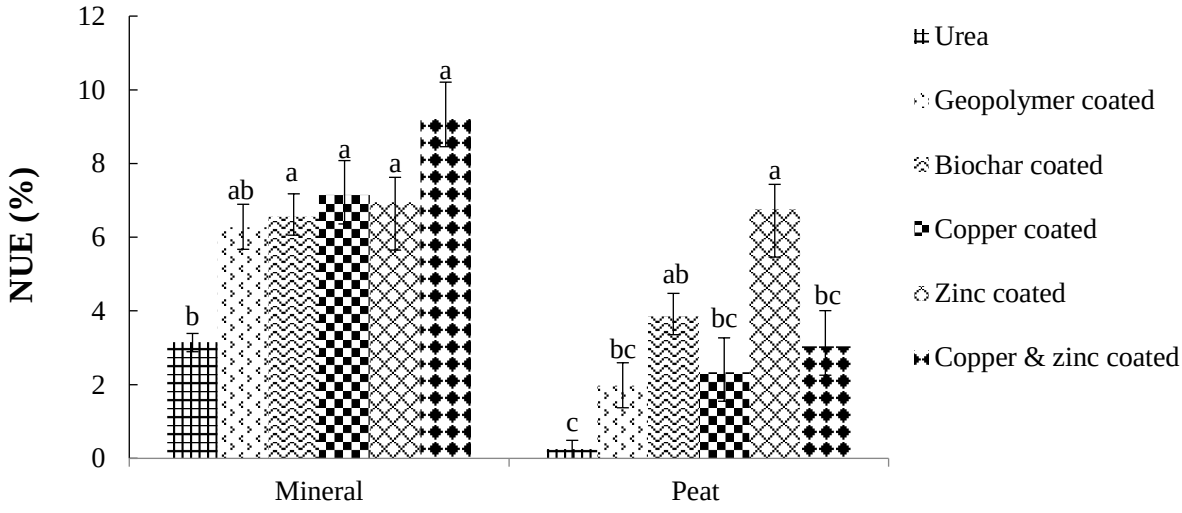

Figure 8 Effect of coated urea on NUE of oil palm seedlings from 9 months old

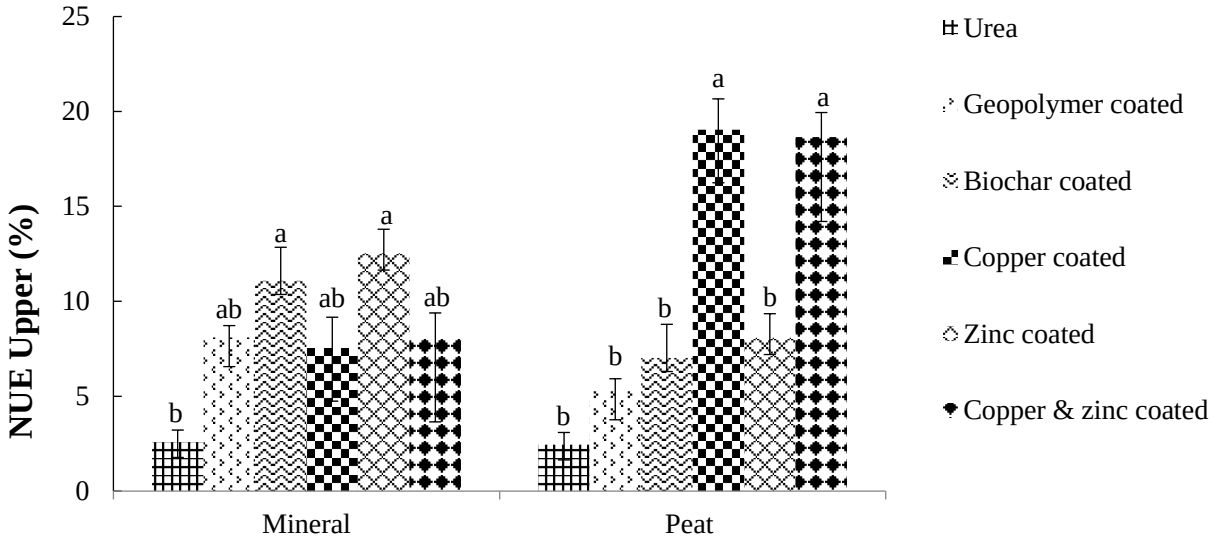

Figure9 Effect of coated urea on NUE of oil palm seedlings from 12 months old

4. Conclusion

The application of coated urea portrayed ability to improve the growth of oil palm seedling and promote NUE under nursery establishment. All coated urea treatments showed increase in plant height both soils and all phases. These were attributed to occurrence of root injury which lead to increase in plant growth (Low, 2009).

Coated urea treatments have characteristic in releasing nutrients gradually for efficient delivery and uptake. Whilst, typical urea easily dissolved and leached which potentially jeopardizing stable plant growth.

The dry matter weight was found significantly influenced from coated urea application for all growing phases. These could be attributed to plant physiological effect and its inhibition to urea hydrolysis in soils and nitrogen loss. The application of coated urea helps to improve the percentage NUE.

For comparison between coated urea treatments, BioU and zinc was the optimum treatments for the mineral soil. However, all micronutrients coated shows a better performance in peat soils. The additions of Cu and Zn left a positive impact on the physiological phenomenon of plant by increasing availability of N. In conclusion, coated urea was effectively reduce N loss and improve growth performance in oil palm seedlings with one applications rather than three applications.

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Effect of season on feeding behavior of Bali cattle that kept in oil palm plantation with semi-intensive system

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Abstract. Ecosystem damage and degradation of biodiversity often become conflicts for oil palm plantations. Integration of cattle-palm oil is considered to be able to overcome the problem of biodiversity, but the comfort of livestock still needs attention. Information about the influence of the season on the comfort of livestock in oil palm plantations is the aim of this study. Environmental conditions and feeding behavior during grazing were the basic parameters of livestock comfort. The study was conducted in the oil palm plantation at Rokan Hulu, Riau province in December-February (rainy season) and August (dry season). The colonies of Bali cattle with semi-intensive maintenance (grazing 8 am - 5 pm) were observed for their feeding behavior (browsing, feeding forage, and feeding oil palm leaves). Environmental conditions include temperature and relative humidity than with calculations obtained the temperature humidity index (THI). The results showed that Bali cattle were in heat stress conditions in both seasons. Cattle showed different feeding behavior from the two seasons during grazing in oil palm plantations. In dry season cattle had more browsing time ($P<0,01$) and feeding timeless ($P<0,01$). The lack of forage in the dry season makes feeding timeless and longer for browsing. This is confirmed by the higher percentage of feeding oil palm leaves time in the dry season ($P<0.01$). Seasonal differences did not have a significant impact on the level of Bali cattle comfort but affect feeding behavior while grazing in oil palm plantations.

1. Introduction

Indonesia is one of the countries with the largest area of oil palm plantations in the world. The development of the palm oil industry continues to be massive every year and is predicted to continue to grow for the next few years. The Director-General of Plantations (Dirjen Perkebunan) in 2017 noted that the area of oil palm plantations had reached 12.3 million hectares, a figure that was 3 times that of 2000. The expansion of oil palm plantations was not separated from the conversion of land functions, both other plantations, and forests. The conversion of forests to oil palm plantations has a severe impact on biodiversity [1, 2]. This then becomes a conflict that is burdensome for the sustainability of oil palm plantations. The existence of cattle can be an alternative to increase biodiversity in oil palm plantations. Livestock grazing can help increase biodiversity by increasing habitat heterogeneity, providing habitat, and new food for invertebrate animals which are then eaten by larger animals [3]. Maintenance of cattle in oil palm plantations can have a positive impact on oil palm plantations and would not cause damage if managed properly [4]. The semi-intensive system allows livestock to be rotated in a controlled manner in the area of oil palm plantations so that it becomes an alternative method of maintenance. However, regardless of the benefits gained by oil palm plantations through the integration of cattle, studies on the comfort of livestock need to be done. The season would have an impact on the comfort of livestock due to changes in environmental conditions. Feeding behavior is one indicator of the comfort of livestock kept in oil palm plantations [5]. This study makes the comfort of livestock in different seasons (rainy and

dry season) as the main information that wants to be known through parameters of environmental conditions and feeding behavior while grazing in oil palm plantations.

2. Materials and Metods

The research was conducted in the oil palm plantation at Rokan Hulu, Riau Province. Livestock used are Bali cattle colonies consisting of bull, cows, and calf. The data collection was carried out by 56 days of observation in November 2016 - February 2017 (rainy season) and August 2018 (dry season). Observations were made on one of the different cows in the colony. Colonies are kept semi-intensively with grazing time 8 am to 5 pm. The basic source for feed was forage at oil palm plantation area with nutrients containing Dry Matter (DM) 22,29%, Crude Protein (CP) 10,67%, Crude Fiber (CF) 36,85%, and total digestible nutrient (TDN) 54,37% [5] without supplemental feeding. Parameters observed include environmental conditions (temperature and humidity) and feeding behavior (browsing, feeding forage, and feeding oil palm leaves). Temperature humidity index (THI) each season was calculated, considering the average environmental temperature and relative humidity of the time in the study was conducted, applying the proposition equation [6] :

$$THI = \left(0.8\ x\ T + \left(\frac{RH}{100}\right) x\ (T - 14{,}4) + 46{,}4\right)$$

Where THI is the temperature-humidity index; T is the mean temperature expressed in ∘C; and RH is the average relative humidity expressed in percentage. Unfortunately, wind speed and solar radiation were not considered. Animal heat stress indications are based on the THI table [6]. The collected data were compiled in Microsoft Excel rather than independent analysis T-test by using SPSS 20 programs.

3. Results and Discussion

3.1. Environmental condition

Table 1. Environmental condition of oil palm plantation

Parameter	Rainy Season	Dry season	Sig.
Temperature (°C)	33.45±2.22	34.22±2.67	NS
Relative humidity (%)	68.88±8.17	50.66±3.79	*
Temperature Humidity Index	86.16±2.15	86.38±0.60	NS

Temperature Humidity Index calculated according to Garcia-Ispierto et al. (2007)- see the chapter called Materials and Methods. The different values are indicated * (P<0.05) in a row or difference is not significant (NS).

Environmental conditions become external factors that would have an impact on livestock productivity. Suitable conditions provide comfort for livestock so as to support maximum productivity. Environmental conditions (temperature and humidity) in the oil palm plantation environment in the rainy and dry seasons were shown in Table 1. The temperature of oil palm plantations in both seasons was not significantly different (P>0.05), while the relative humidity was significantly different (P<0.05). Oil palm plantations still had high temperatures during grazing (during the day) even in the rainy season. The high-temperature environment has a high impact on THI and is categorized as Heat stress, where the heat stress indicator was indicated by the THI value> 74 [6]. Even so according to Maulana et al. [7] Bali calves that were kept semi-intensively in oil palm plantations could still show good performance and were not different from cattle that are kept with different systems and location.

3.2. Influence of the season on feeding behaviour of Bali cattle

Comfortability with environmental conditions would be expressed by livestock through normal behavior [8]. Environmental conditions that were not significantly different (temperature and relative humidity) in the dry and rainy seasons need to be reviewed from feeding behavior as a form of expression shown by cattle during grazing in oil palm plantations. Cattle grazed for 8.44 - 9.13 hours a day in the rainy and dry

seasons showed that feeding behavior was significantly different (P<0.01). In the dry season, cattle spend more time browsing with low for feeding. This is caused by limitations of forage in oil palm plantations during the dry season. Prawiradiputra et al. [9] stated that high rainfall in the rainy season has a significant impact on increasing productivity of fresh forages compared to the dry season. The high availability of forage in the rainy season allows cattle to feed longer. The low availability of forage in the dry season was indicated by a significant increase (P<0.01) of the percentage of time spent by cattle to consume oil palm leaves, Figure 1. Oil palm leaves were a byproduct of oil palm plantations which could be an alternative source of forage feed for cattle during grazing . In the future, research on forage production in oil palm plantations in the dry and rainy seasons needs to be done to complete the information needed.

Table 2. Influence of season on Bali cattle feeding behavior kept in oil palm plantation

Parameters	Rainy Season	Dry Season	Sig.
Total grazing (h/d)	9.13±0.11	8.44±0.13	**
Browsing (h/d)	1.13±0.47	4.52±1.26	**
Feeding (h/d)	7.28±0.84	3.48±1.38	**
Forage (h/d)	5.80±1.77	1.24±1.16	**
Oil palm leaves (h/d)	1.48±1.06	2.25±0.69	NS

The different values are indicated ** (P<0.01) in a row or difference is not significant (NS).

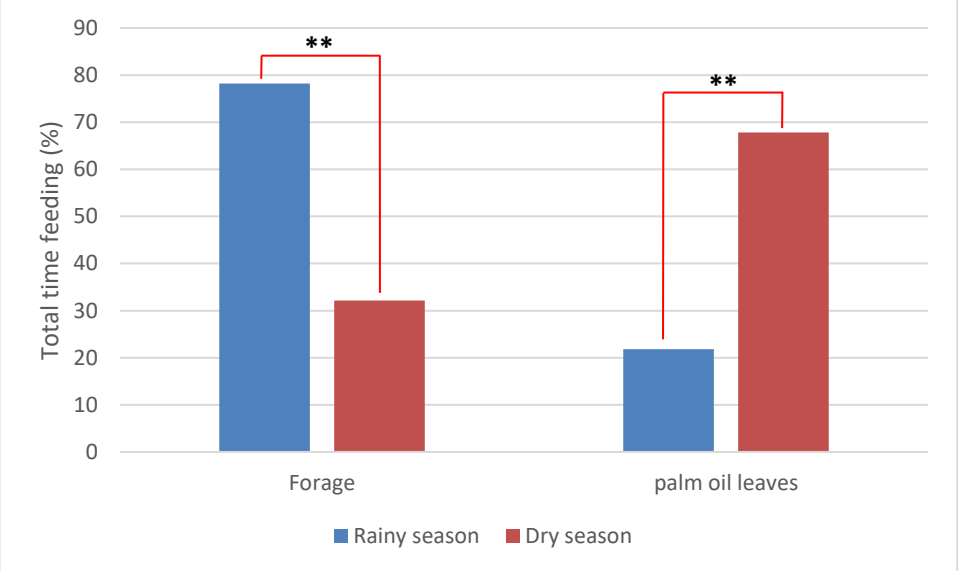

Figure 1. Percentage of feeding forage and oil palm leaves from the total feeding time in the different season. The different values are indicated ** (P<0.01)

4. Conclusions

The different seasons (dry and rainy) had less impact on Bali cattle comfortability in terms of THI but had an impact on the feeding behavior during grazing in oil palm plantations with the semi-intensive system.

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and cattle-oil palm plantation integration research team, Faculty of Animal Science, Universitas Gadjah Mada.

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The influence of halal product assurance laws on product development in indonesia

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Abstract. The Government has issued Law Number 33 the year 2014 on the halal product assurance, which aims to provide protection and guarantee on the halalness of products consumed and used by the community. Therefore the need for research on the effect of halal assurance law on product development in Indonesia. This study aims to determine the impact of the act on the company in development product. Collecting data using a non-probability sampling method and then distributing questionnaires to respondents spread in Indonesia territory. The method used in this research is multiple linear methods and SWOT methods. The results of this study indicate that with the enactment of halal guarantee law, 35% affect the company in developing products and taking care of halal certification. The government has also established an organizing body to guarantee halal products to assist producers in obtaining halal certification. Some strategies that must be done by the government in implementing the law of halal guarantee, among others; establishing certification bodies in collaboration with educational and research institutions, conducting coaching to producers, simplifying the process of handling, and protecting producers from imported products.

1. Introduction

The Government has issued Law Number 33 the year 2014 on the halal product assurance, which aims to provide protection and guarantee on the halalness of products consumed and used by the community [1]. The implementation of halal product assurance aims to provide comfort, security, safety, and certainty of the availability of halal products for the community in consuming and using products, as well as increasing added value for businesses to produce and sell halal products [2]–[6]. This shows that products that enter, circulate, and are traded in the territory of Indonesia must be halal certified. A product is an output obtained from a production process (transformation) and value-added carried out on raw materials [6], [7]. Products (products) according to Kotler & Armstrong, (2014) are everything that can be offered to the market to get attention, be bought, used, or consumed that can satisfy desires or needs [8]. According to (Philip Kotler, 2016), states that design is the totality of features that affect the appearance and function of certain products according to what the customer implies. Product development is a series of activities that start with analyzing perceptions of market opportunities and then ending with the stages of production, sale, and delivery of products [3]. Therefore, the need for research on the effect of halal assurance law on product development in Indonesia. This study aims to determine the effect of the act on the company in product development [4].

2. Mehod

This study aims to determine the effect of the act on the company in development product. Collecting data using a non-probability sampling method and then distributing questionnaires to respondents spread

in Indonesia territory. The method used in this research is multiple linear methods and SWOT analysis [9], [10]. Multiple regression analysis used to know how significant influence of independent variable. F test is used to test the feasibility of the model or to test whether the model substructure used is substantial or not, so it can be ascertained whether the model can be used to predict the effect of independent variables together on the dependent variable. Test T This test is performed to know the significant influence of the three independent variables partially done by comparing the value of t arithmetic (sig t) with t-table / probability t-count (sig t) with significant level (F = 5%) [11, 12].

3. Result and Discussion

The understanding of food and beverage producers about Law Number 33 the year 2014 on the halal product assurance is still very low, only national or international scale companies that already understand the law on the halal guarantee system. Even the majority of small industries did not know about law number 33 the year 2014, so they did not know if the products produced must have halal certificates. The obligation for halal certification for products circulating and traded in the territory of Indonesia comes into force on October 17, 2019. In Law number 33 the year 2014, products are goods and services related to food, beverages, drugs, cosmetics, chemical products, products. Biology, genetically engineered products, as well as used goods that are used, applied or utilized by the community. Halal products are products that have been declared halal by Islamic law. A halal certificate is the recognition of the halalness of a product issued by BPJPH based on a written halal fatwa issued by the Majelis Ulama Indonesia (MUI).

The results of this study indicate that with the enactment of halal guarantee law, 35 affect the product and taking care of halal certification. So that with the obligation for producers to produce halal products, the company must immediately develop products by the halal production process. The process of halal products is a series of activities to ensure the halal of products including the supply of materials, processing, storing, packaging, distributing, selling, and presenting products. Whereas what is meant by a material is an element used to make or produce a product. The government has also established an organizing body to guarantee products to assist producers in obtaining halal certification.

Many obstacles faced by companies in meeting Law Number 33 the year 2014, among others, have not understood and understood the roles and functions of the law for companies, less competent human resources in the process of managing halal certificates, the absence of socialization to small companies, expensive, difficult processing of permits. To carry out halal product assurance, the government established the Halal Product Assurance Organizing Agency hereafter abbreviated as BPJPH. Halal Certificate is a Halal certificate of a product issued by BPJPH. In organizing halal product assurance. (1) BPJPH is authorized to formulate and establish halal product assurance policy. (2) creating norms, standards, procedures, and criteria, halal product assurance; publish and revoke the Halal Certificate and Halal Label on Products. (3) to register Halal Certificate on Product overseas; socialization, education, and publication of Halal Products; accrediting LPH. (4) Halal Auditor register; conduct monitoring of halal product assurance; conducting Halal Auditor guidance. (5) Doing cooperation with the institution and overseas in the field of halal product assurance.

Conducting the halal control of products from raw materials to finished products, because the materials used in the Process of Halal Product (PPH) consist of raw materials, processed materials, additives, and auxiliary materials from animal, plant, microbes, or materials produced through chemical processes, biological processes, or genetically modified processes which is lawful according to Islamic sharia. The location, place, and equipment of PPH shall be separated by the method of slaughtering, processing, storage, packaging, distribution, sale and presentation of non-halal product which is kept clean and sanitary, free from unclean, and free from non-halal materials.

The results of in-depth interviews with the food and beverage industry, as well as experts, produce strengths, weaknesses, opportunities, and threats of a halal product assurance which will be done SWOT analysis. SWOT analysis results obtained some strategies that must be done by the government in implementing the law of halal guarantee, among others; establishing certification bodies in collaboration

with educational and research institutions, conducting coaching to producers, simplifying the process of handling, and protecting producers from imported products.

4. Conclusion

The results of this study indicate that with the enactment of halal guarantee law, 35% affect the company in developing products and taking care of halal certification. The government has also established an organizing body to guarantee halal products to assist producers in obtaining halal certification. Some strategies that must be done by the government in implementing the law of halal guarantee, among others; establishing certification bodies in collaboration with educational and research institutions, conducting coaching to producers, simplifying the process of handling, and protecting producers from imported products.

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Microbial diversity of naturally fermented Sumbawa mare's milk using next-generation sequencing: a preliminary study

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Abstract. This study aimed to investigate the bacterial and yeast diversity in naturally fermented Sumbawa mare's milk compared to naturally fermented cow's milk through a next-generation sequencing approach, and evaluate the quality of fermented mare's milk based on the presence of pathogenic or undesirable microorganisms. Bacterial and yeast density of naturally fermented mare's milk have been compared to naturally fermented cow's milk. Microbial density determined using plate count agar (total aerobic bacteria), de Mann Rogosa Sharpe agar (lactic acid bacteria) and yeast peptone dextrose agar supplemented with streptomycin 50 ppm (yeast). Nutritional content and acidity level of each fermented milk sample were also elucidated. Genomic DNA was extracted using FastDNA Spin (MPBIO) for bacteria and i-genomic Soil (iNtRON) for yeast. The total DNA was further analyzed using illumina high-throughput sequencing (paired-end reads). Microbial density of fermented cow's milk was higher than those in mare's milk, because a low acidity level was observed in fermented mare's milk. Interestingly, microbial diversity in fermented mare's milk was higher than those in fermented cow's milk as revealed by NGS approach. Fermented mare's milk was dominated by *Lactococcus* for bacteria and family Dipodascaceae for yeast. *Lactobacillus* and *Candida* were the predominant genera of bacteria and yeast, respectively, in fermented cow's milk. Furthermore, family Enterobacteriaceae was the second largest taxon in both fermented milk with relative abundance around 20%. Milk type determined the microbial composition and diversity. The presence of family Enterobacteriaceae in both fermented milk warrants special attention in improving the hygiene of manufacturing process.

1. Introduction

Fermented mare's milk, one of special commodity from Sumbawa Island, Province of West Nusa Tenggara, Indonesia, is made naturally without addition any starter cultures. The fresh milk is poured in a clean plastic container and then let it stand at room temperature for several days. The nutritional content of mare's milk is different with cow's milk. Mare's milk is easily digested with the nutritional content is almost similar to human milk. The optimum ratio of whey protein and casein has become the mare's milk is very suitable for infant diets [1].

Recently, the indigenous microflora of Sumbawa mare's milk were studied based on dependent-culture method. As a result, some important and the uncultured microbes were not thoroughly investigated. The investigation of indigenous mare's milk microflora was still limited on lactic acid bacteria (LAB) with probiotic properties [2-4]. Information on the other microbial groups such as fungi/ yeast has not been reported. The interaction between LAB and yeast in the naturally fermented milk products cannot be ignored [5-7]. Lactic acid bacteria in koumiss, product from Central Asia, play an essential role in developing flavor, texture and acidity level as well as bring some health benefits, such as probiotics. Meanwhile, the ability of yeast in this product in fermenting lactose into alcohol has resulted a unique flavor [8].

Therefore, a metagenomic approach is essential to be elucidated to obtain comprehensive information of microbial groups in the fermented mare's milk. Next-generation sequencing (NGS) involving illumina

high-throughput sequencing is the most applied in the metagenomic approach to profile the microbial diversity. In this research, the microbial diversity in naturally fermented Sumbawa mare's milk was investigated compared to naturally fermented cow's milk, and the quality of fermented mare's milk based on the presence of pathogenic or undesirable microorganisms was also evaluated.

2. Materials and Methods

2.1 *Sample Collection*

Fresh mare's milk from Sumbawa was collected form a horse farm in Dompu Regency, Province of West Nusa Tenggara. Mare's and cow's milk was fermented naturally in a clean plastic container, and then incubated in room temperature for five days. The samples transported to Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya for further analyzed.

2.2 *Microbial Isolation*

Microbial isolation was performed by transferred 25 mL of fermented milk samples into 225 mL of saline water (NaCl 0.85%) served as 10^{-1} dilution. Serial dilution was conducted by taking 1 mL from each dilution until 10^{-6}. Samples from each dilution (0.1 mL) were inoculated into respective culture media on Petri dishes in duplicates. Media of plate count agar (PCA) for total bacteria, de Mann Rogosa and Sharpe (MRS) agar for lactic acid bacteria and yeast peptone dextrose (YPD) agar supplemented with streptomycin 50 ppm for yeast. The incubation condition was conducted for each microbial group: 30^{o}C for 24-48 h, 37^{o}C for 24-48 h, 30^{o}C for 48-72 h for total bacteria, LAB and yeast, respectively.

2.3 *Determination of Nutritional Content and Acidity Level*

Nutritional content (total protein, fat content, carbohydrate, total sugar, fiber, water content and ash) and acidity level of naturally fermented mare's and cow's milk was measured according to AOAC method.

2.4 *Extraction of Genomic DNA and High-Throughput Analysis*

To remove lipids, proteins and salts from fermented milk samples, 1 mL of fermented mare's milk or 1 g of fermented cow's milk were firstly emulsified with 9 mL of sterile trisodium citrate buffer (2%), and then incubated at 37^{o}C for 5 min. After that, the mixture was homogenized using vortex at maximal speed for 1 min (fermented mare's milk) or 5 min (fermented cow's milk). Different initial treatment among samples because the texture of fermented mare's milk was liquid compared to fermented cow's milk. Next, the samples were centrifuged at 12000 g for 1 min at room temperature [9]. The pellet was used for total DNA extraction using DNA extraction kit by following the manufacture's instructions. i-genomic Soil kit (iNtRON) was used to extract fungal/ yeast genome, while FastDNA Spin kit (MPBIO) for extracting bacterial genome. The quality and quantity of total DNA were checked using nanodrop spectrophotometer (ratio A260/ 280).

The next-generation sequencing analysis was performed at Macrogen Inc., South Korea using illumina (MiSeq) platform (paired-end reads). Primers used in this sequencing were V3-V4 region for bacteria (41F: 5'-CCTACGGGNGGCWGCAG-3';805R: 5'GACTACHVGGGTATCTAATCC-3') [10], and for fungi/ yeast was ITS1-F (CTTGGTCATTTAGAGGAAGTAA and ITS2-R (GCTGCGTTCTTCATCGATGC) [11]. In order to remove poor quality sequences, raw reads were filtered and further analyzed using QIIME v.1.9.1 to produce diversity profiles.

3. Results and Discussion

3.1 *Microbial Density and Nutritional Content*

The total bacteria, lactic acid bacteria (LAB) and yeast were isolated and enumerated using specific media. In general, the density of microbes in fermented cow's milk was higher than those in fermented mare's milk (Table 1). This is because the pH of fermented cow's milk was higher (pH: 5.04) compared with fermented mare's milk (pH: 3.61). The lower acidity level inhibited the microbial growth, especially total bacteria and lactic acid bacteria. The number of yeast in either fermented mare's milk or cow's milk was lower compared to the number of total bacteria and LAB. Specifically, the yeast density of fermented

mare's milk (1.25 x 10^2 CFU/mL) was relatively lower than those in fermented cow's milk (4.75 × 10^2 CFU/mL).

Nutritional contents between fermented mare's milk and cow's milk were slightly different especially in terms of carbohydrate, total sugar, calcium, fiber and ash (Table 1). Carbohydrate content of fermented mare's milk was higher than in fermented cow's milk. Otherwise, the higher content of calcium, fiber and ash was reported in fermented cow's milk. This higher ash content correlated with the content of fiber and mineral salt in cow's milk. According to Uniacke-Lowe, Huppertz, & Fox [1], mare's milk has a lower mineral salt compared to cow's milk, so that mare's milk is suitable in replacing human milk for pediatric diet.

Table 1. Microbial density, nutritional content and acidity level of naturally fermented milk

Parameter	Fermented mare's milk	Fermented cow' s milk
Total bacteria (CFU/mL)	9.83 × 10^5	1.46 × 10^7
LAB (CFU/mL)	6.1 ×10^5	8.9 × 10^6
Yeast (CFU/mL)	1.25 × 10^2	4.75 × 10^2
Total protein (%)	2.32	2.38
Total carbohydrate (%)	2.13	1.71
Fat (%)	1.73	1.92
Total sugar (%)	3.76	2.48
Calcium (ppm)	177. 66	216. 05
Water content (%)	89.97	87. 67
Fiber (%)	0.14	0.20
Ash (%)	0.29	0.73
pH	3.61	5.04

3.2 *Bacterial Community Profile*

The total sequences as a result of illumina (MiSeq) high-throughput sequencing (paired-end) were filtered and yielded 250115 and 174748 sequences for fermented mare's milk and fermented cow's milk, respectively. The sequence reduction during quality control step by 31% (fermented mare's milk) and 18% (fermented cow's milk) has been performed to increase the sequence quality before continuing to the diversity profile analysis. The bacterial diversity in fermented mare's milk was higher (108 OTUs) than in fermented cow's milk (70 OTUs) (Table 2).

In phylum level, Firmicutes and Proteobacteria were found in both fermented milk samples (Figure 1A). Phylum of Bacteroides and Actinobacteria were only found in fermented mare's milk although with a lower relative abundance (below 1%). In fermented mare's milk, 12 families were obtained, with family Streptococcaceae (52.9%) was mostly found and followed by family Enterobacteriacae (31.8%) and Lactobacillaceae (5.6%). The rest was the family level with relative abundance less than 5%. Conversely, in fermented cow's milk, the dominated family was Lactobacillaceae (53%) and followed by Enterobacteriacae (25.8%) and Streptococcaceae (10.8%) (Figure 1B). It was not all sequences were able to classify until family level. There were two orders, which were Bacillales and Lactobacillales as the maximal identified groups.

Table 2. Information on sequence analysis in naturally fermented milk

Samples	Observed OTUs		Number of sequence reads		Number of filtered passed sequences	
	16S	ITS	16S	ITS	16S	ITS
Fermented mare's milk	108	39	250115	191131	172247	163981
Fermented cow's milk	70	31	174748	160723	143380	138283
Total	178	70	424863	351854	315627	302264

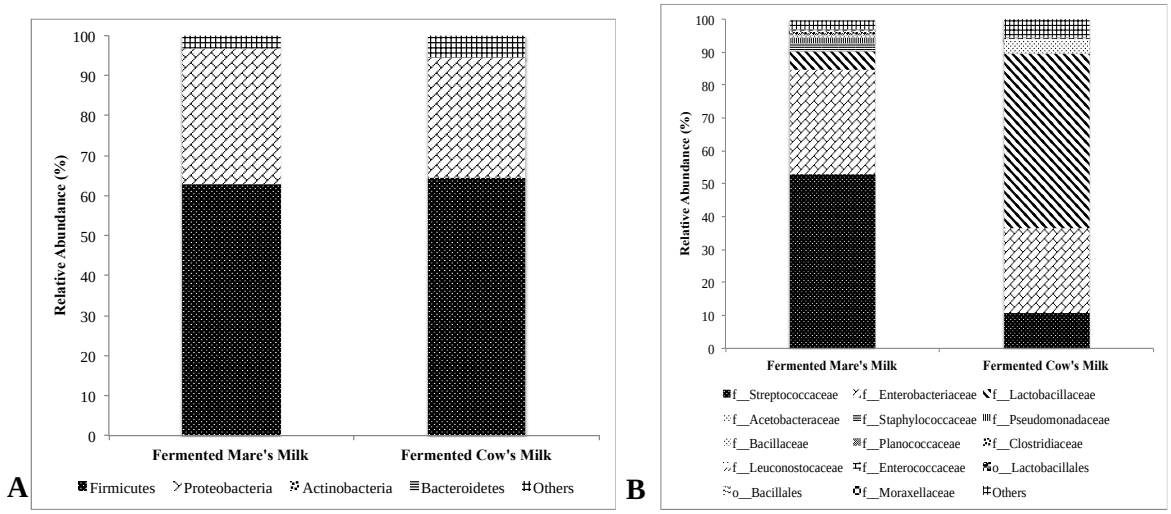

Figure 1. Relative abundance of bacteria sequence read at the phylum level (A) and order/ family level (B)

The number of bacterial genus obtained from fermented mare's milk was 26 genera (including 10 families and two order), and in fermented cow's milk was 13 genera (including two families and one order) (Figure 3A). For the relative abundance above 5%, in fermented mare's milk, from the highest to the lowest percentage *Lactococcus* (36.2%), family Enterobacteriaceae (24%), *Streptococcus* (15.8%), *Citrobacter* (7.5%) and family Lactobacilliaceae (5.5%). In koumiss, *Streptococcus* occupied the second largest after *Lactobacillus* [12]. Meanwhile, fermented cow's milk was dominated by genus *Lactobacillus* (52.8%), and then followed with Enterobacteriaceae (22.5%) and *Lactococcus* (10.7%). Genus *Lactobacillus* also dominated tarag, a naturally fermented cow's milk from Mongolia and Northern China [13]. Besides *Citrobacter*, the members of family Enterobacteriaceae detected in fermented mare's milk with relative abundance less than 5% were *Erwinia*, *Enterobacter*, *Klebsiella*, and *Proteus.* Of five genera, only *Erwinia* was not found in fermented cow's milk. The presence of family Enterobacteriaceae with a relatively high proportion (around 20%) has become an indicator of unhygienic condition and contamination from either fecal materials, dairy farm or production area [14, 15]. Acetic acid bacteria which also frequently observed in naturally fermented milk products was only detected in fermented cow's milk by 3.4% representing family Acetobacteraceae and 0.7% for genus *Acetobacter.*

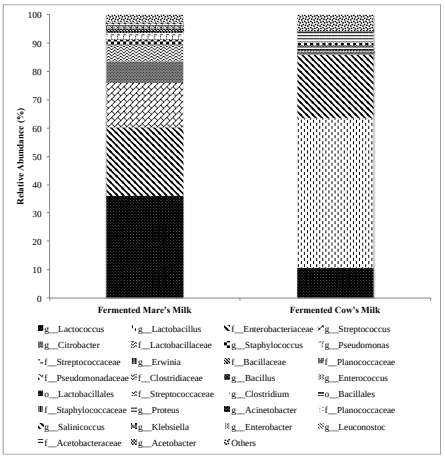

Figure 3. Relative abundance of bacteria sequence read at the genus/ family level

3.3 Fungal/ Yeast Community Profile

The total of sequence read resulted from NGS analysis was 191131 sequences (fermented mare's milk) and 160723 sequences (fermented cow's milk). After filtering step has been performed, sequences were reduced by 163981 (14.2%) in fermented mare's milk, while in fermented cow's milk by 138283 sequences (14%). Overall, a higher level of fungal/ yeast diversity was shown in fermented mare's milk than in fermented cow's milk with values of 39 and 31 OTUs, respectively (Table 2).

A total of two phyla (Ascomycota and Basidiomycota) were found in both fermented milk samples. At phylum level, either fermented mare's milk or cow's milk were dominated by phylum Ascomycota (84.6% for fermented mare's milk and 6.9% for fermented cow's milk) (Figure 4). Relative abundance below 5% was grouped as others representing taxa member outside of the two phyla. Ascomycotina was also a dominant phylum in home-made yogurt from Xinjiang Ugyur, China [16].

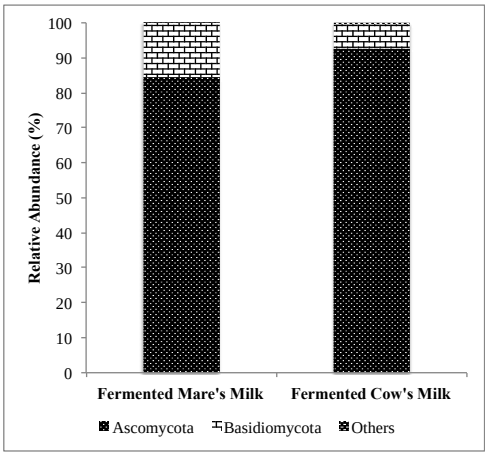

Figure 4. Relative abundance of fungal/ yeast sequence read at phylum level

A different fungal/ yeast composition was observed at the family level (Figure 5A). The number of family (including one order) in fermented mare's milk exceeded (four families: Dipodascaceae 53.2%; Saccharomycetales fam Incertae sedis 16.7%; Trichosporonaceae 13.4%; Saccharomycetaceae 7.4%, and one order: Saccharomycetales 5.2%) the family number in fermented cow's milk (four families: Saccharomycetales fam Incertae sedis 60.2%; Debaryomymycetaceae 31.4%; Tremelales fam Incerta sedis 4.4%; and Sporidiobolales fam Incerta sedis 1.7%). The family number among these fermented milk products was similar; however, Saccharomycetales found in the fermented mare's milk was the order of these families, except Trichosporonaceae.

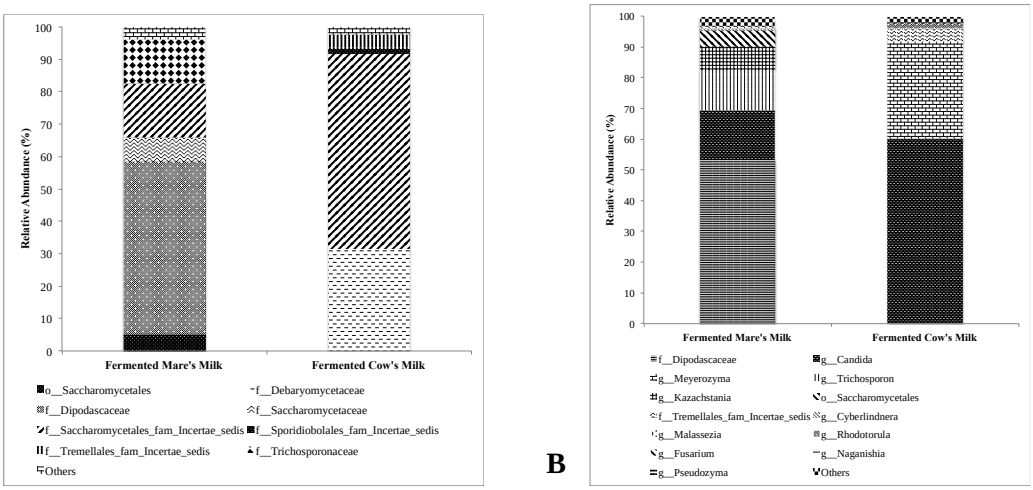

Figure 5. Relative abundance of fungal/ yeast sequence read at the order/ family level (A) and genus/ family level (B)

The sequences of fungal/ yeast DNA classified until genus level by 62.5% (five genera, one family and one order) for fermented mare's milk, while in fermented cow's milk was 75% (six genera and one family) (Figure 5B). With relative abundance more than 5%, three taxa level were found in fermented mare's milk, i.e family Dipodascaceae (53.2%), genus *Candida* 16.1%, genus *Trichosporon* 13.4%, genus *Kazachstania* 7.4% and order Saccharomycetales 5.2%. *Dipodascus geotrichum*, a member of Dipodascaceae family was frequently found in kefir grains [17]. *Trichosporon* was also commonly detected in milk samples, but its presence indicates a low hygiene standard [13]. *Kazachstania unispora* was also detected in koumiss [8]. In fermented cow's milk, *Candida* was a dominant genus with relative abundance by 60.2%, and then followed by genus *Meyerozyma* by 31.4%. The presence of *Candida* was reported in naturally fermented milk products of Indonesia, such as *Candida stelimalicola* in dadih [5], and *Candida* sp. in dangke [18].

4. Conclusion

Microbial diversity of fermented mare's milk was higher than fermented cow's milk, affected by milk type and acidity level. Microbial composition of both fermented milk was different, especially the predominant microflora. Fermented mare's milk was dominated by genus *Lactococcus*, and family Dipodascaceae represented the mostly found yeast. Meanwhile, in fermented cow's milk, *Lactobacillus* and *Candida* were the predominant bacterial and fungal/ yeast genera, respectively. Family Enterobacteriaceae was detected in both fermented milk with the second largest abundance. Coliform bacteria are member of this family indicating the quality of these products urgently required to be improved.

Acknowledgments

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Evaluation of noni (Morinda citrifolia L.) juice waste with *Indigofera zollingeriana* in ration by observing the fermentability and digestibility

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Abstract. The noni (*Morinda citrifolia* L.) juice industry produces fibre waste from processing noni fruit into juice which is consumed by human as a traditional medicine. Based on our previous research, 15% of waste noni juice (NJW) could be added as an alternative forage to replace 25% of napier grass (NG) in ration of lactating dairy goat that contained 60% forage and 40% concentrate. The availability of this waste is restricted by noni juice demand by human, so that the use of NJW should be combined with other raw material in rations. The aim of this study was to obtain several ration formulas for lactating dairy goat by combined the use of NJW, *Indigofera zollingeriana* (Iz) and napier grass that were evaluated through in vitro method. This study used a randomized block design with 3 rumen fluid collection as a groups and 5 treatments of ration, *i.e.* R0 (control) = 60% NG + 40% concentrate (C); R1 = 45% NG + 15% NJW + 40% C; R2 = 50% NG + 5% NJW + 5% Iz + 40% C; R3 = 45% NG + 7.5% NJW + 7.5% Iz + 40% C; R4 = 40% NG + 10% NJW + 10% Iz + 40% C. The measured variables were total bacteria and protozoa population, fermentability (total VFA and NH_3 concentration), and digestibility (dry and organic matter). The data were analyzed using ANOVA and the differences were tested with the Duncan test. The results showed that addition of NJW and indigofera in rations very significantly increased the fermentability (total VFA and NH_3 concentration) ($P<0.01$), and were not influence the microbes population and digestibility. It can be concluded that several rations formula for lactating dairy goat with ratio 60% forage and 40% concentrate are able to formulate by use the waste of noni juice (NJW) as much as 5 to 10%, indigofera 5 to 10%, napier grass 40 to 50%, and 40% concentrate.

1. Introduction

Based on the data obtained from Statisitik Peternakan dan Kesehatan Hewan [1], the goat population in Indonesia over a period of 5 years continues to increase every year by an average of 2.85% from 2011 as many as 16.946 million head in 2015 as many as 18.880 million head. One of them is a dairy goat. Peranakan Etawa (PE) Goat is one type of goat that is widely used as a milk producer. According to Sodiq and Abidin [2], the average production of PE goat milk in Indonesia is around 2-3 liters / head / day. PE goat breeders are able to produce up to 200 days in a year if managed properly so that this type of goat has the potential to be developed. However, the lack of forage is one of the inhibiting factors, especially during the dry season, which requires farmers to use unconventional feeds such as straw, beans and waste from plantations or agricultural / plantation products.

Indonesia has many plants that can be used not only as traditional medicines for humans but also as feed for livestock. *Morinda citrifolia* L. or noni fruit is one of the plants that can grow easily in tropical regions such as Indonesia and Malaysia [3]. According to statistical data in 2003 Noni was cultivated in 15 provinces covering 23 hectares with a production of about 1,910 tons and increased to 73 hectares in 2004 with a production of 3,509 tons. Noni juice waste (NJW) which is a waste of noni juice production

still contains bioactive compounds such as polyphenols and saponins [4]. Some of diseases that caused by pathogenic bacteria such as worms, mastitis, infection etc. usually can be cured by certain herbal that contain bioactive compound as substitute antibiotics. The use of herbal or plants in daily animal ration will avoid animal from such as diseases. According to Fakhirudin [5], the use of NJW up to 15% can replace the use of elephant grass forage by 25% in the lactation dairy goat ration. However, it is important to note that the use of NJW more than level of 15% in the ration can reduce the concentration of total VFA and NH_3, but not reduce the total bacterial population and protozoa population. Nowadays, the availability of NJW is restricted due to relate to human demand to noni juice as herbal medicines. To solve that problem, the use of NJW should be combined with other forage or other materials of concentrate to maintain the function of bioactive compound of NJW for supporting animal health. Addition the source of protein feed such as indigofera (*Indigofera zollingeriana*) will be one of solution. According to Abdullah and Suharliana [6], the production of dry matter (DM) of total indigofera reaches 51 tons of dry forage / ha / year with a 60-day defoliation interval can produce high quality forage. Thus, indigofera plants are potential plants to be developed, especially in areas with dry climates as one of the leading fodder crops.

According to Apdini [7], the use of indigofeed in goat rations showed an increase in milk production up to 26% and an increase in feed efficiency of 15-23% and nutritional efficiency of 5-9%. Based on those advantages, the aim of this research was to obtain several ration formulas for lactating dairy goat by combined the use of NJW, *Indigofera zollingeriana* (Iz) and napier grass that were evaluated through in vitro method.

2. Materials and Methods

The ration used in this study consisted of napier grass and concentrate with a ratio of 60:40. Other raw material for rations consisted of napier grass, tempe waste, soybean meal, coconut meal, corn and $CaCO_3$. The ingredients are mixed according to the ration formulation which refers to the nutritional needs of mid-lactating dairy goats according to NRC [8], namely 12-17% CP and 53-66% TDN. The composition of research rations is presented in Table 1, and the nutrient content of the research ration is presented in Table 2.

Table 1. Composition of research rations (%)

Item	R0	R1	R2	R3	R4
Ingredients					
Napier grass	60	45	50	45	40
Noni juice waste (NJW)	0	15	5	7.5	10
Indigofera	0	0	5	7.5	10
Tempe waste	10	2	10	10	10
Corn	17	17	17	17	17
Soybean meal	10	15	10	10	10
Coconut meal	0	3	0	0	0
$CaCO_3$	1	1	1	1	1
Premix	0.5	0.5	0.5	0.5	0.5
DCP	1.5	1.5	1.5	1.5	1.5

2.1 In vitro Fermentation Test

Fermentative digestion in vitro for 4 hours using the Tilley and Terry [9] method to test the microbial population of rumen (bacteria and protozoa) and fermentability testing (total VFA and NH3 concentrations). The treatment ration of 0.5 grams was put into a fermenter tube with 40 ml of McDougall solution and 10 ml of rumen liquid added. Samples were incubated for 4 hours under anaerobic conditions at 39ºC in a water bath shaker. The sample was taken as much as 0.5 ml for the calculation of the total bacterial population, the sample was also taken as much as 1 ml for the calculation of the protozoa

population. The fermented product is then given a saturated $HgCl_2$ solution to stop the fermentation process. The fermenter tube was then centrifuged (5000 rpm, 15 minutes), then the supernatant was taken and used to measure NH3 concentration using the Conway Microdifusion method [10] and total VFA by steam distillation technique [10].

Table 2. Nutrient content of the research rations (%)

Rations	CP	EE	CF	TDN[1]	Ca	P	Lignin
R0	13.18	3.62	26.50	58.55	1.14	0.65	3.62
R1	14.37	5.01	24.02	60.95	1.08	0.60	6.28
R2	14.36	3.86	25.77	59.80	1.11	0.64	3.98
R3	14.96	3.98	25.41	60.43	1.10	0.63	4.15
R4	15.55	4.10	25.05	61.06	1.09	0.62	4.33

Note: R0 = 60% Napier grass (NG) + 0% Noni Juice Waste (NJW) + 0% Indigofera (Iz) + 40% Concentrate (C); R1 = 45% NG + 15% NJW + 0% Iz + 40% C; R2 = 50% NG + 5% NJW + 5% Iz + 40% C; R3 = 45% NG + 7.5% NJW + 7.5% Iz + 40% C; R4 = 40% NG + 10% NJW + 10% Iz + 40% C; [1]Calculation is based on Sutardi (1980) equation; CP: crude protein; EE: crude fat; CF: crude fiber; TDN: total digestible nutrients; Ca: calcium; P: phosphor.

2.2 In vitro Digestibility

Measurement of the digestibility coefficient of dry matter and organic matter (DMDC and OMDC) was carried out by the Tilley and Terry [9] modification methods of Sutardi [11]. The procedure starts from anaerobic fermentative digestion process such as the implementation procedure for microbial rumen testing and fermentability, only the incubation is carried out for 48 hours, then continued with the enzymatic digestion process with the pepsin enzyme aerobically for 48 hours. The fermentative digestion process was carried out with 2 drops of saturated HgCl2 solution and centrifuged (5000 rpm for 15 minutes), the supernatant was removed and the residue plus 0.2% pepsin-HCl solution and the mixture was incubated for 48 hours. The mixture is then filtered with Whatman No. 41 with the help of a vacuum pump. The filtrate is then put into a porcelain cup of known weight, then dried in an 105ºC oven for 24 hours with a cup for determination of dry matter content. After weighing the dry weight, then the cup is put back into the 600ºC furnace to determine the ash content and organic matter. Dry matter and organic matter levels are used to determine the digestibility coefficient of dry and organic ingredients.

2.3 Viability Test Bacteria and Protozoa

The calculation of total bacterial population was carried out by 1 ml of rumen liquid inserted into 9 ml of Butterfiled's Phosphate Buffered diluent to obtain 1:10 dilution. After that, the sample is homogenized so that a sample solution with a 10-1 dilution is obtained. From a dilution of 10-1, further dilution is carried out (10-2, 10-3, 10-4, and so on as needed) by means of 1 ml of the previous solution taken using a sterile pipette into 9 ml of diluent. Then the sample is homogenized with vortex. As much as 1 ml of each desired dilution is piped into a sterile petri dish in duplicate. Then the NA (Nutrient Agar) / PCA (Plate Count Agar) media is poured into a petri dish which has been given a sample of 15-20 ml. After the media is mixed with the sample, the cup is inserted into the jar of anoxomat in an upside down position with anaerobic conditions. Protozoa population calculations were carried out by the methods of Ogimoto and Imai [12]. The calculation is done with 2 drops of rumen liquid sample which has been mixed with thryphan blue formalin saline (TBFS) in a 1: 1 ratio in the counting chamber. TBFS solution was made from a mixture of 4% formalin plus 0.9% physiological NaCl salt solution in 100 ml of solution. The total protozoa calculated from 16 counting chamber boxes with a microscope magnified 10 times.

2.4 Statistical Analyses

The experimental design used was a randomized block design with 5 treatment rations and 3 rumen fluid groups. The data obtained were analysed by analysis of variance (ANOVA). If the treatment is significant, then the data is tested further using the Duncan test.

3. Results and Discussion

3.1 The fermentabiliy (NH_3 concentration and total VFA)

The addition of noni juice waste (NJW) and indigofera very significantly affected ammonia concentration (P <0.01). Based on the results obtained, R3 treatment produces the highest concentration compared to the treatments R1, R2, and R4. While the R0 treatment gives the lowest concentration results than the R1 to R4 treatment. The addition of NJW and indigofera increased NH3 concentrations until level of 7.5%, and then decreased due to increasing level of lignin in ration. High lignin levels can reduce feed utilization by rumen microbes because lignin will bind cellulose and hemicellulose so that microbes are difficult to degrade protein. The NH3 concentration needed to support maximum rumen microbial growth is 4-12 mM with an optimum concentration of 6-8 mM [13]. The results of NH3 concentration in this study averaged between 6.56-8.28 mM.

Table 3. The fermentability (NH_3 concentration and total VFA)

Rations	NH_3 concentration (mM)*	Total VFA (mM)*
R0	6.56±1.03C	106.88±4.21C
R1	7.37±1.39B	116.18±5.55B
R2	7.57±1.34B	143.71±6.44A
R3	8.28±1.76A	149.42±5.65A
R4	7.56±1.52B	141.64±5.75A

Note: R0 = 60% Napier grass (NG) + 0% Noni Juice Waste (NJW) + 0% Indigofera (Iz) + 40% Concentrate (C); R1 = 45% NG + 15% NJW + 0% Iz + 40% C; R2 = 50% NG + 5% NJW + 5% Iz + 40% C; R3 = 45% NG + 7.5% NJW + 7.5% Iz + 40% C; R4 = 40% NG + 10% NJW + 10% Iz + 40% C; * Means with different superscripts in the same column differ highly significant (P<0.01).

The total VFA concentration in this study showed that the addition of NJW and indigofera on ration was very significant (P <0.01). Based on the results obtained, R2, R3 and R4 treatments produced the same higher concentration compared to control and also R1. The addition of NJW and indigofera increased the concentration of total VFA. In this study, VFA concentrations of all treatments were about 106.88-149.42 mM on average. According to Suryapratama [14], the amount of VFA production is good to meet the rumen microbial synthesis which is around 80-160 mM. This study showed the results of VFA concentration seem proportional to NH3 concentration, so that this pattern might able to support the synthesis of protein microbes highly.

3.2. Microbial population and in vitro digestibility

Analysis of various treatments of addition of NJW and indigofera had no significant effect (P> 0.05) on the protozoa population and total bacterial population. The results of measurements of goat rumen fermentation in the form of total bacterial population and protozoan population can be seen in Table 4.

There was not any difference among the treatment rations for microbes population and also digestibility. Total bacterial population according to Tilman et al. [15] which reached 9 log CFU ml^{-1}. This value is higher than the total bacterial population of the study which is only around 6.15-6.35 log CFU ml^{-1}. Whereas according to Dehority [16], the normal condition of protozoa population in goats can reach 6 log cells ml^{-1} rumen fluid. The protozoan population in this study was lower in the range of 4.60-4.76 log cells ml^{-1}. The bacterial population can be influenced by several factors such as feed type, protozoa predation properties [17], VFA, pH [18], and NH3 concentrations [19]. Rumen bacteria need more or less ammonia for 80% growth process [20]. The content of DM and protein also affects the growth of bacterial populations. According to van Saun [21], bacteria contain 60% protein which has high quality and digestibility. While the protozoa population can be influenced by the protein content in the ration [18].

Table 4. Microbial population and *in vitro* digestibility

Rations	Rumen Microbial Populations (log CFU ml^{-1})*		Digestibility (%)	
	Total bacteria	Protozoa	Dry matter	Organic matter
R0	6.35±0.07	4.76±0.07	68.37±1.65	67.50±1.80
R1	6.31±0.15	4.60±0.04	70.62±1.00	69.84±1.07
R2	6.18±0.09	4.60±0.17	68.34±2.43	67.37±2.36
R3	6.25±0.11	4.70±0.05	70.38±2.05	69.49±1.95
R4	6.15±0.07	4.60±0.04	69.56±0.89	68.58±1.16

Note: R0 = 60% Napier grass (NG) + 0% Noni Juice Waste (NJW) + 0% Indigofera (Iz) + 40% Concentrate (C); R1 = 45% NG + 15% NJW + 0% Iz + 40% C; R2 = 50% NG + 5% NJW + 5% Iz + 40% C; R3 = 45% NG + 7.5% NJW + 7.5% Iz + 40% C; R4 = 40% NG + 10% NJW + 10% Iz + 40% C.

The results of analysis of variance showed that addition of NJW and indigofera in ration of dairy goats in each treatment had no significant effect on dry matter and organic matter digestibility. The average dry matter digestibility in this study was 68.34-70.62%, while the average organic matter digestibility was 67.37-69.84%. The digestibility value of this study is quite high compared to the digestibility value according to Sutardi [13] which ranges from 50-60%. The results of the above study indicate that the increase or decrease in the digestibility of dry matter rations is directly proportional to the increase or decrease in digestibility of organic matter. Factors that influence the digestibility of feed ingredients according to McDonald et al. [22], namely the composition of feed ingredients, composition comparisons between feed ingredients with other feed ingredients, feed treatment, enzyme supplementation in feed, livestock, and feeding levels. The digestibility value of each treatment is relatively the same due to similar of the nutrient content of each treatment ration.

4. Conclusions

Based on the results of this study it can be concluded that several rations formula for lactating dairy goat with ratio 60% forage and 40% concentrate are able to formulate by use the waste of noni juice (NJW) as much as 5 to 10%, indigofera 5 to 10%, napier grass 40 to 50%, and 40% concentrate.

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The usage of information and communication technology in improving bio-business performance: a case of vegetable farming in Indonesia

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Abstract -This paper aims to analyze the roles of information and communication technology (ICT) in bio-business activities. The motivation of this study is the fact that vegetable intensive vegetable farming is a risky bio-business. A low threshold of risk aversion is one of the critical factors for successful bio-business. The risk comes from economic and natural elements. Another potential risk is because vegetables are perishable products, which relate to post-harvest losses. Bio-business players require ICT to plan their business. In this study, farmers were selected as the subject because the intensive vegetable farming is more profitable than other crops. Data were compiled from field surveys in vegetable production regions of Indonesia. This study used a structural equation modeling (SEM). Using SEM can measure direct and indirect effects of multiple endogenous variables in simultaneous regression equations. The results show that ICT provide potential roles in bio-business. The usage of ICT can increase profit of the business via mediations of high sales and reasonable price. The usage of ICT enabled the bio-business players to increase sales, because of access to market information and improved agronomic technology, access to credit and customers. Even though ICT has been available and affordable, the use of mobile-phone was still low. The bio-business players should be encouraged to use the device. Specialized training on the use of mobile-phone is one of the best ways.

1. Introduction

Vegetable sector plays an important role in the bio-economy of Indonesia. Indonesia gained US$17.15 million in 2014 from seasonal vegetables [1]. However, Indonesia also imports several vegetables. There was still a deficit, which indicated that the total value of imported vegetables was higher than of exported ones. The amount of deficit in 2014 reached double that value of export [2].

The production of vegetables grew by an average of 8% per annum, to cover almost one million hectares with an average yield of 9.6 tons per hectare. This excludes nearly 31 million tons of mushrooms. Chili production accounts for 20% of the land currently used for vegetable production but produces only 12% of the total vegetable output due to low average yields. In comparison, cabbage and potato use just 6.3% and 6.8% respectively of vegetable land and have much higher yields, resulting in substantial production volumes. The main vegetables in Indonesia (besides mushrooms) and their average yields are cabbages (22.4 t/ha), chili (4.7 to 6.4 t/ha), potato (16.4 t/ha), shallot and onions (8.8 t/ha), and tomato (12.6 t/ha). Among the vegetables grown in Indonesia, chili is the highest in terms of acreage and volume of production. Figure 1 shows the trend of five major vegetable in Indonesia.

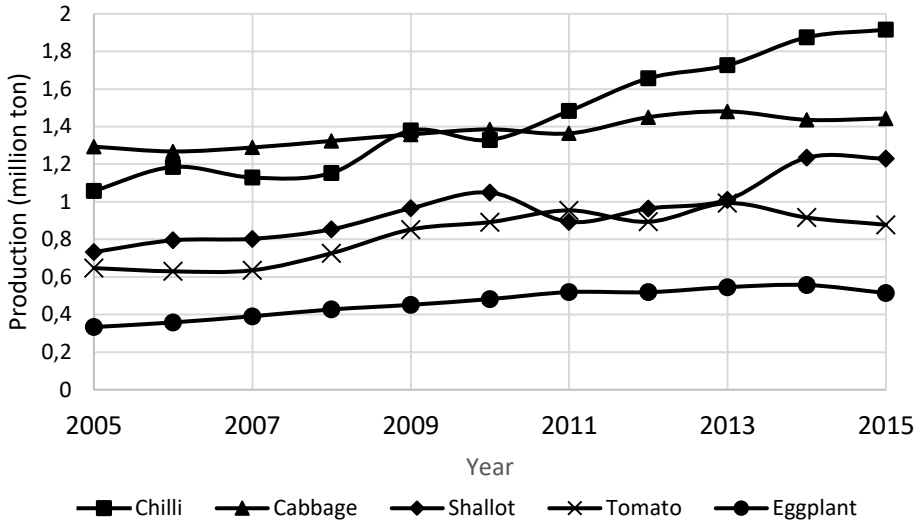

Figure 1. Trend of vegetable production [3]

In a bio-business, decisions on timely land preparation, planting, weeding, irrigation, harvesting, storage, and marketing have always been central concerns to stakeholders. information and communication technology (ICT) especially mobile telephones can speed the way farmers in rural areas get, exchange and manipulate information. They rework the way farmers interact with markets and cities. A variety of innovations that integrate ICTs into the dissemination of agricultural information to farmers have been developed at local, national and regional levels. They are increasingly enabling farmers to focus, search and extract useful and up-to-date market information. Because of its potential to upgrade this old rural farming problem an evaluation of its usage among farming communities becomes necessary. It is what this study aimed at by reviewing the role of ICT and its practical contributions to agriculture and rural development in Indonesia. The objective of this study is to analyze the usage of ICT in bio-business management, and assess its impact on bio-business performance.

Table 1. Definition, measurement, and summary of selected variables

Variables	Definition	Measure
***Endogenous variables*:**		
Use of ICT	Use of mobile-phones in farming business activities	dummy: 1=yes; 0=no
Price	Prevailing farm-gate price perceived by farmers	dummy 1= low; 3=high
Sales	Percentage vegetables sold for commercial to total harvest	percentage
Profit	Net revenue gained from a hectare of farming	million IDR
***Exogenous variables*:**		
Age	Age of household head	year
Education	Length of formal education of household head	year
Experience	Experience on vegetable farming system	year
Training	Participation in agricultural training program	dummy: 1=yes; 0=no

Source: field surveys

2. Materials and Methods

The fundamental concept of this study is a hypothesis that an introduction of mobile-phone, as a part of ICT, can potentially affect business performance. In this case, the usage of ICT indirectly affect profit through mediation of sales and price of the products. Following a concept of supply [4], the volume of sales is affected by the price of product. Simultaneously, the use of ICT is dependent on the producers' human capital, which characterized by age, education, experience and training. This concept is formulated

in a multi-path analysis or a multiple simultaneous equation model. Structural equation modeling is used to estimate the model. The definition and measurement of variables are presented in Table 1, and the proposed model is expressed in Figure 2.

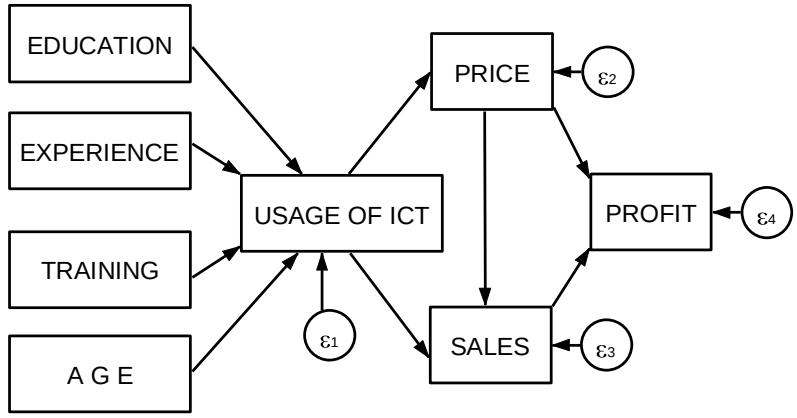

Figure 2. Proposed model of analysis

The null hypothesis is that every path (represented by an arrow) has statistically no effect on the corresponding variable. The alternative hypothesis is that at least one path has a statistically significant effect on the corresponding variable. The hypotheses were tested at least 90% confidence interval. The model was estimated using STATA ver. 13. The results were presented in standardized form such that the impact of each factor is comparable to others.

This study is based on a cross-section data set collected from some field surveys of farm households in the major vegetable production areas of Indonesia. The surveys were conducted during 2014-2015, using structured questionnaires. A purposive stratified random sampling approach was followed at a farm level.

SEM is preferable to other usual methods because it reduces multicollinearity and bias [5]. This tool performs test models with multiple endogenous variables and also using several regression equations simultaneously. SEM is a very powerful multivariate technique that is a specialized version of the analytical method and enables researchers to measure direct, indirect and total effects of variables on others [6].

3. Result and Discussion

The result of estimation is presented in Figure 3. All paths are statistically significant except the path of price to profit. The R-squared is 0.85, and χ^2 is 1850.64 which is very significant. The usage of ICT in bio-business was significantly affected by farmers' characteristics. The older ones were less likely to use ICT. It is logical that older players faced a difficulty of a modern device like mobile-phone. Education, skill, and training positively affected ones to use ICT. This means that advanced human capital makes acceptance to modern technology including ICT. The same case in some African countries. Among others, in Kenya where young farmers are more exposed to modern technology and they are more likely to make direct telephone calls to buyers or surf the internet to search for new markets or to understand the current market trends; and educated farmers are more likely to adopt use of information and communication technology than old and uneducated ones [7]. As well in Ethiopia, young and educated household heads have higher probability of owing mobile-phone than old and uneducated household heads.

The sales of bio-business were positively driven by the use of ICT and farm-gate price received by the producers. This particular finding is theoretically understandable. The ICT led to high sales because the producers could do many things to increase the production (and eventually sales) by employing ICT. By sing ICT, producers can access modern agronomic technologies leading to a high performance of chili

cultivation [8]. This study also shows that the usage of ICT directly and indirectly improves sales via mediation of price. Higher price leading to higher sales fits a theory of supply, where the producers responded the high price by increasing production. Profit generated from bio-business was only significantly determined by sales of vegetables. The model suggests that higher sales led to higher profit. This phenomenon is economically understandable. The price did not provide direct contributions to the profit, but it gave indirect impact on profit through sales.

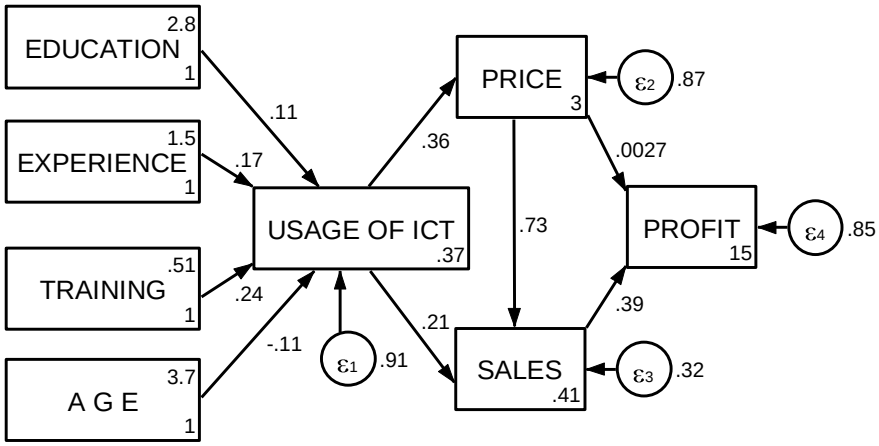

Figure 3. Estimated result of analysis

This finding fits the previous studies, among others, by Aker and Mbiti [9], Aker and Fafchamps [10] and Tack and Aker [11]. The use of ICT can potentially affect households' endowments – as well as their production and trade entitlements, and hence their agricultural outcomes and well-being – in many ways. First, ICT could potentially reduce farmers' search costs, thereby allowing them to obtain price information in a greater number of markets and sell in the market with the highest price net of transport costs. Second, in the absence of selling in a different market, improved access to information could potentially improve farmers' bargaining position against traders, thereby allowing them to negotiate a higher sales price. Third, mobile technology could potentially enable farmers to conclude a sale using a mobile-phone, thus reducing uncertainty associated with selling in a distant market. Fourth, if information technology increases the prices that farmers receive, and agricultural production is price elastic, then this would increase the production of such commodities in the future

Vegetable-based bio-business has been able generated profit through farm management. Based on a study of Mariyono [12] the profit per hectare for chili, eggplant, tomato and yard-long bean was higher than that of other cereal crops. Eggplant and big chili contributed an almost similar share of profit, which accounted for around 45%. This means that in relative term, both vegetables were superior compared to other vegetables. Yard-long bean had the lowest net return, which accounted for 15%. Small chili and tomato provided similar shares, which accounted for 30%. Note that the main component of material input was agrochemicals that have been introduced since the Green Revolution [13]. The use of agrochemicals needs a wise decision because it can potentially lead to health and environmental problem [14, 15]. Introduction improved technology can reduce the use of agrochemicals without reducing the production [16]. It is also important that market infrastructures and credit facilities should be provided to encourage farmers engage bio-business, based on vegetable farming [17, 18].

4. Conclusion

This study shows that the ability of bio-business players can improve the business performance by utilizing ICT. The bio-business performance increased because of better price and higher sales of product compared to those who did not use ICT. Furthermore, the high price received by the producers led to high sales. This is consistent to the law of supply stating that producers will respond high price by increasing

production to sell. By utilizing ICT, the producers can potentially access agronomic technology, networks, markets and institutions necessary to improve production. The eventual impact of ICT usage was high profit. In this case, the increase in profit is considered indirect impact through the mediations of price and sales.

Policy implications are drawn for up-scaling and making it more affordable for the end users. Strengthen producers' capacities to undertake sustainable rural transformation processes by accessing all useful market information and agronomy through ICT innovations in agricultural research and development in developing countries. Increase access to ICTs for producers and strengthen the knowledge and leverage the use of ICTs will support bio-business productivity in Indonesia, and other developing countries in general.

With the growing importance of information technology for bio-business, the government through the ministry of agriculture carries out policies in the use of information technology to improve the results of agriculture and the welfare of farmers. One of its policies is that the agricultural department in cooperation with private sectors needs to provide agricultural commodity price information services that can be accessed via cellular phone in the form of content. Through this cooperation, farmers can obtain information more quickly and easily and get content that is useful for agriculture.

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Biological aspects of green mussels *Perna viridis* (Linnaeus, 1758) cultivated on raft culture in Pasaran Coastal Waters, Lampung Province, Indonesia

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Abstract. Green mussel (*Perna viridis*) is widely distributed in Indonesia. In Lampung province, the green mussel has cultivated on floating raft method surrounding the coastal water of Pasaran island. Green mussel's meat is one of the cheap and important protein sources from marine resources. However, information on the cultivation and some biological aspects of green mussels are rarely reported from Pasaran island, even though the commodity has been cultivated since 2012. It is an essential step to develop and culture intensively. This study aims of monitoring the growth performance, body condition index (BCI) and gonadal profiles of green mussel cultivated from Pasaran island. Differentiating between males and females species is simple, by using the color observation of meat. Females are reddish while males are gray to brown. The traditional fisherman harvested of the mussel ranging from 6-8 months after cultivation. The growth rate between males and females is not significantly different but BCI values. The differences among ages affected to the total weight, BCI, meat weight, shell length, thickness, width, and weight ratio. Shell length and weighing of males 55.7 mm and 10.38 g at the age of 6 months respectively while in females 57.3 mm and 9.82 g. The female BCI value doubled for males at the six months after cultivation (1.63 and 0.84). Gonads begin to be found at the age of 3 months with gonadal maturity starting at 4-6 months.
Keywords: growth, BCI, gonad, green mussel, raft culture

1. Introduction

The green mussel (*Perna viridis*) is distributed in the Indo-Pacific region, extending from Japan to New Guinea and from the Persian Gulf to South Pacific islands [1].In tropical countries such Indonesia, green mussels are widely distributed on almost all coastal of Indonesian islands, disperse from Sumatera in Malacca Strait, Lampung Strait, Sunda Strait, while in Java island located in Lada bay, Jakarta Bay, Java sea, and Indian ocean. In eastern of Indonesia found in Nusa Tenggara coastal waters, Makassar Strait to Ambon Bay [2-5].

According to Davy and Graham [6], green mussel farming in Indonesia began in the late 1970s with the first cultivation carried out in Jakarta and Banten Bay. Followed by cultivation in Belawan, North Sumatera and Surabaya, Java Sea [3], and Lampung Strait [7].

In Indonesia, the meat of green mussel is accustomed to consume and be an important species in aquaculture organism. Also, they were fast-growing and inexpensive protein source [8]. Green mussels generally lived near to the estuary, find out attached to the wood, bamboo, coral, ropes as their substrate through the byssus [9]. The seeds naturally attached to collectors [10], and ready to harvest in 6-7 months of cultivation [12].

The location of cultivation of green mussel in Lampung strait is in coastal waters of Pasaran Island which is located in the west of Teluk Betung district, Bandar Lampung City. According to Ali *et al*. [12], this location is an adequate environmental and carrying capacity to cultivate green mussels, since these

waters have low currents, and naturally available of green mussels seeds. The cultivation of green mussels adopts a raft culture system which is due to the ease of cultivation [8].

Biological characteristics of green mussel in Lampung strait have not been reported yet, as well as growth performance, morphometric, condition index performance, and gonad index where they have significant contribution in developing the future culture. This study aims to monitor the biological aspects of green mussel cultivated on the raft system in Pasaran Island waters during cultivation.

2. Materials and Methods

2.1. Study area and culture condition

The research located around the Pasaran island coastal waters (S050 27'54 524", E1050 15'39 468") in Lampung bay of Indonesia. The samples obtained from a floating raft culture mussel within raft dimension was 10x10 m2 [13].

The substrate used for green mussel seedling consisted of 200 cm of ropes in length and made from natural fibers. The ropes tied to bamboo crosses the surface of the seawater and at the end of the ropes tied with ballast. After 1-3 weeks of installation, the green mussel seeds will be tapped on the substrate and can be harvested at the age of 5-6 months [8, 12].

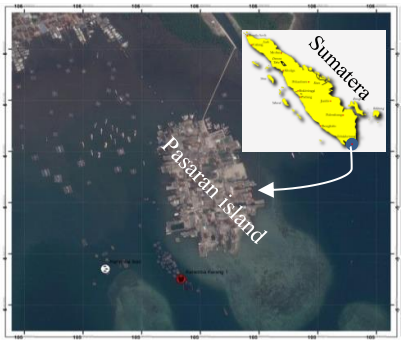

Figure 1. Map showing the mussel farming in Pasaran Island, Indonesia

2.2. Sampling

Sample specimens were taken in the morning time, after washing and weighing then followed by classifying of sex differentiation of *Perna viridis* based on monitoring the different coloration of the tissue and gonad [14]. Whereas the age determination based on the measurement of the shell length size and information from the local fisherman.

2.3. Morphometric analysis

Morphometric analysis conducted by weighing of total and tissues weight followed by measuring the shell dimension by calliper, including total length, width, and height (Figure 2), followed by identification of height ratio, width ratio, and weight ratio.

$$Breadth\ ratio = \frac{av.heigth\ (b)}{av.length\ (L)} \quad \text{.....(1)}$$

$$Width\ ratio = \frac{av.width\ (w)}{av.lenght\ (L)} \quad \text{.....(2)}$$

$$Weigh\ ratio = \frac{av.weight\ of\ meat\ (Wm)}{av.of\ total\ weigh\ (Tw)} \quad \text{.....(3)}$$

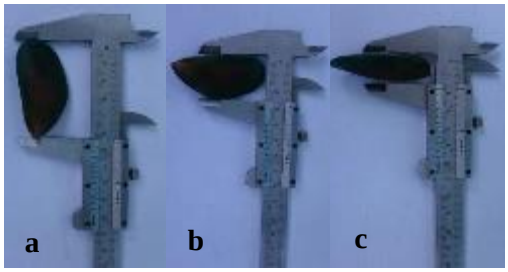

Figure 2. The measurement of the green mussel dimension, length (a), height (b), and width (c)

2.4. Growth

The growth of mussels was calculated by using the manual ruler, the dimension measured from anterior to posterior of shells in every age both males and females green mussel [13].

2.5. Shell length-height relationship

The allometric correlation between length and height calculated by Vakily's formula [15],

$W = aL^b$ …… (4)

Exp.: W= total weight (g), L= total length (cm), a, b= constant of linear regression

2.6. Body condition index (BCI)

The BCI was measured by calculated the dry weight of the soft tissue dried at 60°C until the weight remained constant divided by the dry weight of the shell [16].

2.7. Gonado somatic index (GSI) analysis

The gonad separated from the mussel's meat and weighing, and the GSI analyzed base on the histological characteristics of the gonad by using microscopy.

2.8. Data analysis

The statistical analysis was conducted on ANOVA followed by Duncan's all-pairwise-comparison test using SPSS software v2.40.

3. Results and Discussion

The green mussels farming carried out in Pasaran Island coastal waters began in 2012 by using floating raft culture. The farming location is close to the Way Belau estuary and located in the intertidal zone with a water depth of about 8 meters. The culture condition on Pasaran Island indicate an appropriate carrying capacity for green mussel with salinity 26-30 ppt, temperature 28-30°C, pH 7.5, and dissolved oxygen 5.1-5.6 $mg.L^{-1}$, brightness level ranging from 11.5-3.09 meters, current 0.09-0.16 $m.s^{-1}$, and the chlorophyll-a of the water were10.83 $mg.m^{-3}$ [12].

The cultivation process tends to be easy, including determining the location, making a raft system, cultivating, and harvesting. The site used in the manufacture of raft placed around 30-200 meters from the shoreline. A raft with a size of 10 x 10 m2 can produce fresh green mussels up to 3-4 tons, depending on the number of seedlings, the ages of harvest time, and the size of shells [8].

3.1. Determination of sex type and morphometric

Even though sexual dimorphism between both sexes is not easily distinguished externally as male and female of the *Perna viridis* species, however, the results of this study indicate several morphometric parameters that distinguish between male and female that can be observed visually. Statistically, male and female green mussel differed significantly ($p < 0.05$) on the parameters of height, width, width ratio, weight ratio while the height ratio did not have a significant effect ($p > 0.05$) (data was not shown).

Specifically, female mussels are longer but thinner than male shells at the same ages. In line with Villaluz et al., [17], that the differences in the body shape of the sexes of male mussels appear to have a smaller shell size and a wider width as shown by the distance of their ligament region to its umbo.

Whereas the female mussel has a larger shell size, the distance from posterior adductor to its umbo region. Based on observations of the weight of female and male shells tend to have a relatively equal total weight, as well as the meat weight, and the total length of the shell (p> 0.05).

Figure 3. Tissues color of males and females green mussel (*Perna viridis*) in various ages (1-6 months). The color of female mussels is reddish while male are grey to orange-brownish.

Furthermore, observations based on the color of female tissues tend to be reddish orange while males are creamy towards orange chocolate (Fig. 3). Determination sexes of mussels can be determined from the age of 4 weeks and more clearly as longer as cultivating period, until 12 weeks where the first found of gonads. Arshad et al. [14], explained that there are easy and specific ways in classifying female and male *Perna viridis* based on an internal morphology of the mantle and monitoring coloration of both male and female tissue, male are usually milky to creamy white than in females.

3.2. Growth rate

The total length of the green mussel can reach 56 and 57 mm in male and female respectively after being cultivated for six months. Observations on the differences of ages showed the increase in the size ($p < 0.05$). Sex differences did not show differences in total length of both type mussels ($p > 0.05$).

The growth rate of green mussels cultivated in the Pasaran coastal waters is relatively high and reaches 9.3 $mm.mo^{-1}$. Report from the Jakarta Bay shows an average monthly growth of 8 mm [18], on the Philippine seashore can reach 10 $mm.mo^{-1}$ [19], while growth rate of *Perna perna* in Brazil reached 8.6 $mm.mo^{-1}$ or able to reach 60 mm in seven months [20]. Urbano et al. [21], reported a smaller growth rate of *Perna viridis* shell length only 7.1 $mm.mo^{-1}$. The lowest growth rate in *Perna viridis* was in Hong Kong waters was recorded at a maximum growth rate of 5 $mm.mo^{-1}$ or only able to reach a size of 60 $mm.year^{-1}$, it caused the contaminated and unhealthy water on the cultivating area [22].

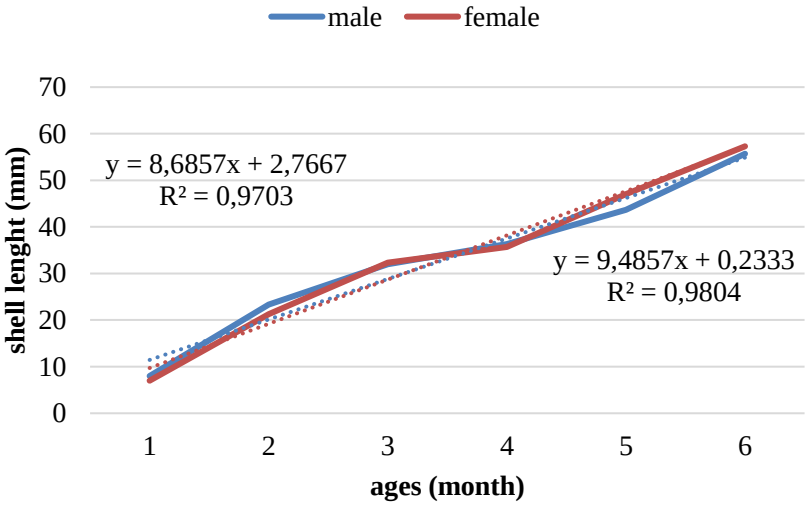

Figure 4. The growth rate of *Perna viridis*.

The tropical *Perna viridis* farming has a harvesting phase commences when the mussels reach minimum commercial size. An optimal harvest of marketable size is achieved after a culture period of 6 months [23][24], while Mohamed [11], recorded the harvestable sizes are reached within 4-6 months. The size of green mussels in Pasaran island waters can reach 57 mm in six months cultivation. Rajagopal et al. [25], *Perna viridis* showed marketable size on 50-60 mm in length. It was achieved within a culture of 6 months. However, in subtropical mussel farming, reach marketable size achieved only after a lengthy period of cultivation of 12-24 months [26]. This varies significantly according to species, geographic region, and cultivation method.

3.3. Shell length-height relationship

The relationship between the length and weight of male green mussels were Y = 2.0072x-2.5338 while female green mussels were Y = 1.7595x-1.921. By using these equation obtained b values of 1.38 and 1.14 respectively which showed that the green mussels type of growth was negative allometric, whereas the growth rate was more dominant than the weight.

Differences in b constant value between males and females show differences in the growth rate of both sexes. In accordance with Setyobudiandi [27], male mussel grows faster than females caused male mussels to have a greater b value than females [28].

Table 1. Constanta values of length-weight relationship

Sex	Sample	a	b	R^2
Male	180	0.18	1.38	0.8512
Female	180	0.15	1.14	0.8683

Expl.: a,b= constanta values, R^2= coefficient of correlation

3.4. Body condition index (BCI)

Differences of ages, sexes and both interactions have a significant effect ($p < 0.05$) on the BCI values. The BCI value of male mussels in the early stage was higher than female, although it was not significantly different. However, simultaneously overtaken from the third month, and in six months the BCI value of female have twice greater than males, which are 1.63 and 0.84 (Fig. 5)

It showed the nutrition of female is higher than male because of the possibility of the formation of the gonad which in this study occurred three months after cultivation. According to Wang et al. [29], BCI values are in line with the nutritional status of green mussels, the higher the BCI, the better the nutritional status of mussels. Meanwhile, Huhn et al. [16], explained that BCI is a positive linear function of phytoplankton abundance in the culture area.

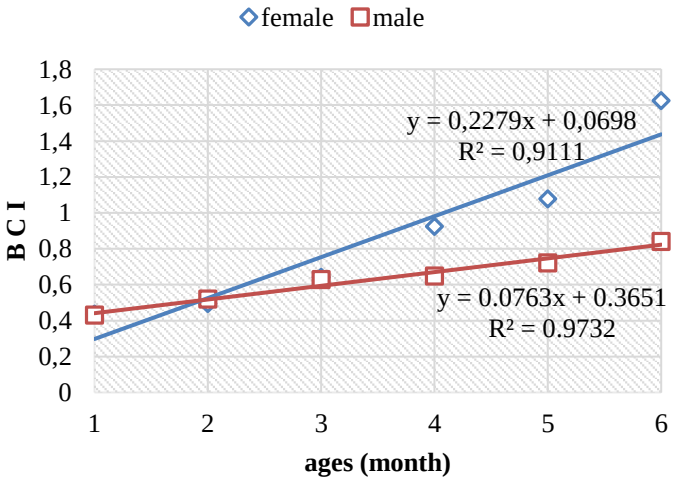

Figure 5. BCI's values of males and females of green mussels in different ages

3.5. Gonado somatic index (GSI) analysis

The gonadal observation performed three months after cultivation because the gonad appear for the first. It is showed not all the three-month-old mussel had gonad, whereas the 4, 5, 6 months had. The three months gonad appear tight and thickened and in the developmental phase, while on 4, 5 and 6 months are in the gonadal maturity phase seen from the increasing of gonadal size and widening cell wall.

According to McDonald et al. [30], there was a positive correlation between mussel size and stage of reproductive development. The initiating gonad recorded tissue development from 6.5 mm in length. Shell length at three-months-old reached 32 mm and increased up to 57 mm at the age of six months of cultivation.

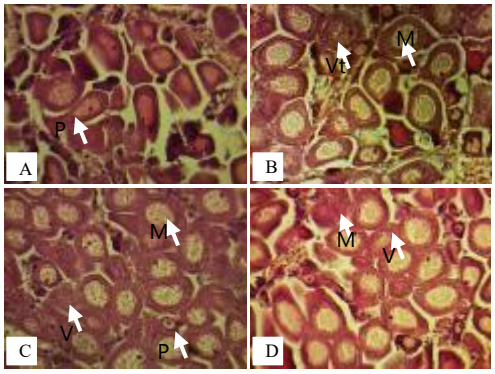

Figure 6. Green mussel gonads observed at different ages 3,4,5,6 months (a,b,c,d): PO-primary oocyte, V-Vitellogenic stage, and M-mature stage

Three-months-old mussel is mostly light yellow in mantle pattern of developing gonadal tissue clearly visible, which is based on visual assessment score developed by McDonald et al. [30], belongs to Stage 1, concluded to the early stage of gonad development, as shown occurred of the primary oocyte by the tight and thickness of the cell walls (arrows) (Fig. 6a). While the 4.5 and 6-months olds were in the phase of gonadal maturity seen from the increase in gonadal cell size and widening of the cell wall and the presence of vitellogenin and mature oocytes (Fig. 6b, c, d).

Mantle color tends to be opaque with gonadal tissues, dark orange to brick red gonadal tissue (reproductively mature). McDonald et al. [30], size and age have a positive correlation with gonad maturity. This also reinforces the information that the size of mature green mussels is in the range of 30-35 mm [18], while Siddall [31], reported *Perna viridis* becomes sexually mature at 15-30 mm in length, which equated to 2–3 months of age, and according to Yap et al. [19], the adulthood of the green mussel reached after 20-35 mm in length.

4. Conclusion

The green mussel cultivated on raft culture has different values of BCI between males and females and also has different values in shell width, thickness, width ratio, weight ratio, while differences of ages of cultivation affected to the total weight, BCI values, total length, width ratio, weight ratio. The interaction of age and sex affected to the value of BCI. The older the age of the mussel, the BCI value will be increased. BCI value in the female mussel has twice greater than the male at six months after cultivation. Gonads began to be found three months ages with gonadal maturity at 4-6 months of age.

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The production of whole sera equine Chorionic Gonadotropin (ECG) from Indonesian pregnant local horse towards estrus and pregnant cats

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Abstract. This research was conducted in two stages as follows. Isolation of horse serum proteins pregnant eCG Indonesian Local horse blood serum accumulated on 40-120 Days. The pregnant horse blood was collected cumulatively via jugular vein as much as 10 ml per horse. Then filtrated by chromatography techniques coloums CM Sephadex G-100. Then were centrifuged at 3000 rpm for 15 minutes at a temperature of 4 ºC with charcoal for eliminated of steroid hormone and extracted with the addition of absolute ethanol 1:1 in 4 ºC Ultra centrifuge were continued the precipitate diluted on PBS Concentration of eCG by using Bioassay technology Laboratory Horse Pregnant Mare Serum Gonadotropin, the result showed absorbancy OD 0.272 with the concentration 392.17 IU/ml. 5 cats as control group were injected of eCG 150 IU of folligon intervet Holland 82 hours after eCG they were injected of 100 IU hCG and 5 cats as treatment group were injected of 150 IU from whole serum prenat horse and 100 IU hCG chorullon. All of cats on normal cycle control groups and treatment not on estrus and pregnancies. The conclusion is not significant differences between eCG folligon intervet Holland (patent product) and Indonesian Product eCG Pregnant Local Horse at $p > 0.05$.

1. Introduction

The reproductive potential of a species has long been identified as the most important source of reproduction. Small animal studies have become the main engine of discovery in reproductive biology. In the early days of gonadotropin studies it was proved that transplanting anterior pituitary tissue from domestic species into productive animals stimulated indicative early development of puberty and other reproductive consequences [1]. All control of the hypothalamus to the hypophysis to control the production of gonadotropins from the pituitary in the anterior part of the pituitary by specific release action and inhibitory agents. In various domestic animal species FSH secretion - LH is controlled by two separate systems which are functionally responsible for the secretion of the gonadotropin protein hormone and stimulates the growth of germinanatip and endocrine in the ovary, and the follicle growth wave system that controls gonadotropin secretion, especially LH, which is short-lived, responsible for ovulation [2]. Equine chorionic gonadotropin (eCG) supports the development of follicles in the ovary, such as FSH [3]. Some follicles to become ovulated because eCG also functions as an activity like LH. The life

expectancy of eCG-producing cells appears to be strong, as the chorionic corset cells formed by pre-ectopic mares are able to maintain eCG secretion for 75-100 days, which is produced when pregnant horses [4].

Diversity can be divided into two senses, namely stetrility, namely cases of infertility that cannot be cured and infertility, which is a case of infertility that can be treated and still has recovery hopes, is a chain and cannot be separated from other reproductive sciences. Today reproductive disorders are the biggest cause of failure in increasing animal populations in Indonesia. One reproductive disorder due to hormonal factors. Reproductive disorders due to hormonal factors can be handled by improving the quality of animal feed and eliminating reproductive disorders due to these hormonal factors. This research offers a solution by producing Sedimentation and SDS - Page Techniques to determine the molecular weight and Pregnant Mare Serum Gonadotropin (eCG) levels. eCG can play a role in solving this problem in addition to improving the quality of animal feed and eliminating other reproductive disorders. This eCG is to overcome reproductive disorders due to hormonal factors where eCG has been known as a hormone that has FSH & LH like its activity. The eCG has the alpha and beta activity of the gonadotropin subunit which can be produced from 40 to 130 days of horse pregnancy produced by endometrial cups which can be found to resemble anterior hypophysis hormones. In addition, this invention also produces eCG Frozen Dry production from the local Indonesian pregnant Horse Serum to increase animals pregnancy This study relates to sedimentation techniques and SDS-Page to determine the molecular weight and Pregnant Mare Serum Gonadotropin (eCG) levels in local horses crossing between Sandel and Thourobreed horses, CBG2 and CBG4 Indonesia. This research is also related to the production of eCG Frozen Dry from the Indonesian Local pregnant Horse Serum to Increase cats This study relates to sedimentation techniques and SDS-Page to determine the molecular weight and Pregnant Mare Serum Gonadotropin (ECG) levels in local horses crossing between Sandel and Thourobreed horses, CBG2 and CBG4 Indonesia. This invention is also related to the production of ECG Frozen Dry from the Indonesian Local pregnant Horse Serum as FSH – LH like [5].

Goodrowe et al. [6] and Romano, J E. [7], have conducted research on domestic paint using a variety of various doses of eCG, FSH and hCG treatment with hCG at 250 IU, IM, or GnRH at 25 mcg, IM. Ovulation is reported to occur 25–27 days after hCG administration to cats and used eCG of 200 IU and 80 hours later given 100 IU of hCG. Using developmental competence of domestic cat follicular oocytes after fertilization in vitro [6] and [8] to know 0varian response in the estrus cat receiving varying dosages of hCG.

2. Materials and Methods

The horses of 40 to 130 days of pregnancy in Indonesia were taken from yugular blood venous in the neck area of 20 cc and the serum was separated cumulatively collected after cold centifugation and continued techniques were performed. ECG is a Gonadotropin hormone which is extracted from horse serum Biologically it is known to be identical with FSH - LH and is often called LH like [5].

The results achieved in this research are the isolation and identification of ECG from pregnant horse serum with the help of charcoal in ultra ECG serum centrifuges by centrifugation with 500 g 4°C CM and filtration with sephadex G-100 coloums chromatography. Perform characteristics of ECG protein identification with SDS-PAGE. Isolate and purify ECG proteins. Molecular weight of ECG SDS - Page molecular weight of 63, 43 and 28 kDa corresponds to the results of 12% sds page. This research was conducted in two stages as follows. Isolation of horse serum proteins pregnant eCG Indonesian Local horse blood serum accumulated on 40-120 Days. The pregnant horse blood was collected cumulatively via jugular vein as much as 10 ml per horse. Then filtrated by chromatography techniques coloums CM Sephadex G-100. Then were centrifuged at 3000 rpm for 15 minutes at a temperature of 4 ^{o}C with

charcoal for eliminated of steroid hormone and extracted with the addition of absolute ethanol 1:1 in 4 ºC Ultra centrifuge were continued the precipitate diluted on PBS [5].

5 cats as control group were injected of eCG 150 IU of folligon intervet Holland 82 hours after eCG they were injected of 100 IU hCG and 5 cats as treatment group were injected of 150 IU from whole serum prenat horse and 100 IU hCG chorullon. All of cats on normal cycle control groups and treatment not on estrus and pregnancies [5].

3. Result and Discussion

Concentration of eCG by using Elisa Bioassay technology Laboratory Horse Pregnant Mare Serum Gonadotropin, the result showed absorbancy OD 0.272 with the concentration 392.17 IU/ml.

Table 1. The level of eCG dengan elisa biotechnology assay technology of pregnant mare serum gonadotropin

Sampels	Y (OD)	OD	ECG (mIU/mL)
ECG	0.263	0.272	
	0.270		392.17
	0.282		

As research has been carried out by Goodrowe et al. [6] that domestic cats is injected with eCG of 200 IU and 80 hours later given 100 IU of hCG and oocyte collections of cat foicles carried out in aspiration, aspiration follicles appear to be able to form normal and functional CL and young live births after embryo transfer expressly demonstrate, for the first time, the competence of developing fertilized carnivorous oocytes in vitro. Five of the 6 cats that received 6 to 18 embryos became pregnant and were produced from 1 to 4 kittens / litters.

Estrus may be induced in cats with follicle-stimulating hormone (FSH) 2 mg or eCG, , IM, the first day, then 0.5–1 mg, IM, daily for 4 additional days. Recommended doses of human chorionic gonadotropin (hCG) range from 25 to 500 IU. The higher doses are more effective at inducing ovulation but may also result in oocyte degeneration. For cats with anovulation or for queens undergoing artificial insemination, ovulation of mature follicles (present on day 2 of estrus) may be induced by treatment with hCG at 250 IU, IM, or GnRH at 25 mcg, IM. Ovulation is reported to occur 25–27 hr after hCG administration to cats [7].

Table 2. The result of pregnancy detection via abdominal palpacy showed on the table below:

Treatment	Pregnant	Not-pregnant	Total
Control Group (CG):	5	0	5
150 IU eCG foligon + 100 IU Chorulon			
150 IU eCG Sera +100 IU Chorulon	5	0	5

Terri et al. [9], developmental Competence of Domestic Cat Embryos fertilized In vivo Versus In Vitro Use of *e*CG manifest advantageous in increasing the pregnancy rate for fixed time embryo transfer, independent of the cases employed for synchronization. Perhaps the most widespread use of *e*CG has been exploitation of its FSH activity in induction of estrus in immature animals and luteinizing hormone. In addition, *e*CG may induce supplementary ovulations as well as support the second wave of corpora lutea [1, 4].

The result of cats pregnancy from this research used palpation abdominals between control group and treatment group with injection *e*CG dosage of 150 IU foligon and from pregnant serum shows 10

pregnant cats. Variations in the incidence of estrus is most likely a reflection of differences in the ovarian follicles growth phase so that when after ovulatory follicles cause hCG that are not uniform maturity, ultimately could lead to ovulation at different timing. Several researches stated that about 82 hours after injected of hCG [1, 10]. Which is responsible for the process of folliculogenesis and ovulation. Lastly, the growth and maturation of follicles which produce estrogen, indicate symptoms of heat. The hormone estrogen system works to improve the sensitivity of female sex organs are characterized by changes in the vulva and transparent discharge [7]. The injection of eCG was accompanied by an increase in the percentage of oestrus detected. Oestradiol is the hormone responsible for oestrus behaviours with passive mounting activity being positively correlated with increased concentrations. It appears that the injection of eCG is accompanied by greater follicular growth, and hence an increase in the production of oestradiol encouraging better expression of heat [9, 10].

The hypothalamus is responsible for the control of release of gonadotrophins from the anterior pituitary by the action of specific releasing and inhibitory substances. These are secreted by the hypothalamic neurons and are carried from the median eminence of the hypothalamus by the hypothalamic-hypophyseal portal system. In the domestic species the secretion of FSH and LH is controlled by two functionally separate systems. These are the tonic episodic system, which is responsible for the continuous basal secretion of gonadotropin and stimulates the growth of both germinal and endocrine components of the ovary, and the surge system which controls the short-lived massive secretion of gonadotropin, particularly LH, responsible for ovulation. The pregnancy rate in tech is determined by the detection of estrus and the right time for mating. Lower pregnancy rate in control group is likely due to low progesterone levels during the luteal phase. The possibility of an early death of the embryo, which is a normal process of natural selection, often occur in one pregnancy of cats. One of the cause's early embryonic deaths is caused by a deficiency of progesterone. Cats are experiencing heat but not with ovulation, resulting in low levels of pregnancy at first estrus. The low pregnancy rate is likely due to abnormalities of fertilization, not every ovulation always followed by fertilization and not all fertilization produces by all normal pregnancy. [2, 9, 10].

4. Conclusion

Based on the results of this research, can be concluded that the *e*CG and hCG combination can increase the rate of pregnancy in domestic cat. Injection of *e*CG dosage of 150 IU and 100 IU intramuscular has shown no significant in cats pregnants. 5 cats as control group were injected of eCG 150 IU of folligon intervet Holland 82 hours after eCG they were injected of 100 IU hCG and 5 cats as treatment group were injected of 150 IU from whole serum pregnant horse and 100 IU hCG chorullon. The conclusion is not significant differences between eCG folligon intervet Holland (patent product) and Indonesian Product eCG Pregnant Local Horse towards estrus and pregnancies at $p > 0.05$.

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