Gmail

SUMARDI 1965 <sumardi.1965@fmipa.unila.ac.id>

[biodiv] Editor Decision

3 messages

Smujo Editors <smujo.id@gmail.com> Sat, Aug 8, 2020 at 9:20 AM
Reply-To: Smujo Editors <editors@smujo.id>
To: NETI YULIANA <neti.yuliana@fp.unila.ac.id>, Sumardi <sumardi.1965@fmipa.unila.ac.id>, EDO JATMIKO <edojatmiko95@gmail.com>, MENTARI ROSALINE <mentarirosaline@yahoo.com>, MUHAMMAD IQBAL <muhammadiqbal@gmail.com>

NETI YULIANA, Sumardi, EDO JATMIKO, MENTARI ROSALINE, MUHAMMAD IQBAL:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "The Potentially Lactic Acid Bacteria as an EPS Producing Starter from Yellow Sweet Potato Fermentation: an EPS Producing Starter from Yellow Sweet Potato Fermentation".

Our decision is: Revisions Required

Smujo Editors
editors@smujo.id

Reviewer C:

Your research is interesting and linear. But in translation into English, there are many compound sentences that are too long. I suggest a few changes. if you agree, you can copy the new sentence that I proposed.

There is one library that I did not find in references, namely Anino et al. 2014. Please complete.

Other comments you can read in the manuscript file.

Recommendation: Revisions Required

Reviewer G:

The manuscript entitled "Potentially lactic acid bacteria as an EPS producing starter from yellow sweet potato fermentation" is suitable for this journal. However, some revision should be done, to give the reader a better understanding why and how the study was performed:

Abstract:

line 12: "The results showed that the 72 hours of fermentation was found to have the highest EPS metabolites, the number of a LAB, the total lactic acid, and the lowest pH. " Before the word "number" and "total lactic acid" a word like "highest" or "lowest" should be included, as the reader does not know what is meant here.

Introduction:

There are some facts missing. The reader does not understand completely why this study is needed.

line 22: "Sweet potatoes that are processed into flour require a modification process to be more applicable flour for further processing". I think this needs more explanation, to highlight why your study is so important. How do you apply the sweet potatoe flour? Why does it need a modification process? What is not good about this flour and should be changed?

line 24: "Sweet potatoes flour made with lactic acid fermentation has better physicochemical properties and aroma" This is not detailed enough. How is the aroma changed? Are aroma compounds degraded or new aroma compounds formed? what exactly is changed with the physiochemical properties?

And overall, why was sweet potatoe used? A few facts about sweet potatoes and their properties would be useful.

line27: "Many authors have explored the role of the EPS in strengthening some flour and or starch properties (Patel and Prajapati, 2013; Karasu and Ermis, 2019)." What do you mean with strengthening, do mean improving? How were these properties improved?

Materials and Methods:

The materials and methods are not descrided detailed enough.

line 55: solid to liquid ratio? Amount of solution? How many solutions? Did you have duplicates? The reader does not understand your experimental set up.

line 56: why was salt added? How much was added?

line 57: please decribe in detail, step by step, how your back-slopping and spontaneous fermentation experiments were performed. The reador does not understand it

line 66: "fermented with back-slopping". Please include a step by step instruction.

line 68: Why did your media contain CaCO3?

line 68: "The colonies identified by the presence of clear zones around each colony were randomly". Why is there a clean zone?

line 68: "streaking plates": what are streaking plates?

line 73: What are slants?

line 100: was there a reference performed? A medium without inoculation and fermentation and just with the isolation of EPS to make sure that not some parts of the medium were isolated and and were mistakenly assumed as EPS.

Results and Discussion

line 149: "The activity of lactic acid bacteria allegedly caused this": in line 126 you said, that starch degradation happens during fermentation and it was by caused by natural occuring amylases? So are the changes of the starch granules due to microorganisms or enzymes?

The manuscript is not containing analysis for the identification of the isolated starters. We don't know about their GRAS-status and if they are safe. I think this should be a part of the discussion. Also, in the introduction it was said, that these starters are needed for the improvement of the flour, in regard to their technofunctionality and aroma. This should be also discussed in the discussion or an outlook should be included.

Recommendation: Revisions Required

Biodiversitas Journal of Biological Diversity

C-6418-Article Text-26016-1-4-20200714.doc
709K

NETI YULIANA <neti.yuliana@fp.unila.ac.id> Tue, Sep 20, 2022 at 12:55 PM
To: SUMARDI 1965 <sumardi.1965@fmipa.unila.ac.id>

[Quoted text hidden]

C-6418-Article Text-26016-1-4-20200714.doc
709K

NETI YULIANA <neti.yuliana@fp.unila.ac.id> Tue, Sep 20, 2022 at 12:55 PM
To: SUMARDI 1965 <sumardi.1965@fmipa.unila.ac.id>

---------- Forwarded message ---------
From: **NETI YULIANA** <neti.yuliana@fp.unila.ac.id>
Date: Tue, Aug 18, 2020 at 10:47 PM
Subject: Re: [biodiv] Editor Decision
To: Smujo Editors <editors@smujo.id>
Cc: <editors@smujo.id>

Dear Smujo Editors

Enclosed we submit the revised version of our manuscript.
The response to reviewer G is also attached. Pls find the attachments
Thank you

Best regard
[Quoted text hidden]

2 attachments

C-6418-Article Text-26016-1-4-20200714_(Revised Version 180820) by author.doc
717K

2020 (NY) RESPONSE TO THE REVIEWER 17 August 2020.docx
21K

Gmail **SUMARDI 1965 <sumardi.1965@fmipa.unila.ac.id>**

[biodiv] Editor Decision

3 messages

Smujo Editors <smujo.id@gmail.com> Wed, Aug 26, 2020 at 5:31 PM
Reply-To: Smujo Editors <editors@smujo.id>
To: NETI YULIANA <neti.yuliana@fp.unila.ac.id>, SUMARDI <sumardi.1965@fmipa.unila.ac.id>, EDO JATMIKO <edojatmiko95@gmail.com>, MENTARI ROSALINE <mentarirosaline@yahoo.com>, MUHAMMAD IQBAL <muhammadiqbal@gmail.com>

NETI YULIANA, SUMARDI, EDO JATMIKO, MENTARI ROSALINE, MUHAMMAD IQBAL:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Potentially lactic acid bacteria as an EPS producing starter from yellow sweet potato fermentation".

Our decision is to: Accept Submission

Smujo Editors
editors@smujo.id

Biodiversitas Journal of Biological Diversity

Smujo Editors <smujo.id@gmail.com> Wed, Aug 26, 2020 at 5:32 PM
Reply-To: Smujo Editors <editors@smujo.id>
To: NETI YULIANA <neti.yuliana@fp.unila.ac.id>, SUMARDI <sumardi.1965@fmipa.unila.ac.id>, EDO JATMIKO <edojatmiko95@gmail.com>, MENTARI ROSALINE <mentarirosaline@yahoo.com>, MUHAMMAD IQBAL <muhammadiqbal@gmail.com>

NETI YULIANA, SUMARDI, EDO JATMIKO, MENTARI ROSALINE, MUHAMMAD IQBAL:

The editing of your submission, "Potentially lactic acid bacteria as an EPS producing starter from yellow sweet potato fermentation," is complete. We are now sending it to production.

Submission URL: https://smujo.id/biodiv/authorDashboard/submission/6418

Smujo Editors
editors@smujo.id

[Quoted text hidden]

NETI YULIANA <neti.yuliana@fp.unila.ac.id> Tue, Sep 20, 2022 at 12:53 PM
To: SUMARDI 1965 <sumardi.1965@fmipa.unila.ac.id>

[Quoted text hidden]

Potentially lactic acid bacteria as an EPS producing starter from yellow sweet potato fermentation

Abstract. Potentially lactic acid bacteria as a starter for EPS production from yellow sweet potato fermentation were determined. The fermentation profile and partial characteristics of isolated lactic acid bacteria were used as considerations. The fermentation of yellow sweet potato was performed in the back-slopping and spontaneous procedure and was paid attention to the hours of 0, 24, 48, and 72. The results showed that the 72 hours of fermentation was found to have the highest EPS metabolites, the number of a LAB, the total lactic acid, and the lowest pH. The 72 hours of fermentation also seemed to cause a significant change in the sweet potato starch granules morphology. The potential starter, therefore, can be taken from the yellow sweet potato fermentation either spontaneously or by a back-slopping method, at 72 hours. From the sixty selected LAB, the 34 strains showed an ability to produce EPS. Among these, eight strains exhibited the potential high production of EPS. They were capable of growth on 28-45°C and exhibited tolerance to 3-4% NaCl. The favorable carbon sources for the growth and production of EPS were isolated dependent.

Key words: back-slopping, EPS, lactic acid bacteria starter, spontaneous, sweet potato

Abbreviations : EPS (Exopolysaccharide), LAB (lactic Acid Bacteria).

Running title: EPS-producing lactic acid bacteria

INTRODUCTION

Sweet potatoes that are processed into flour require a modification process to be more applicable flour for further processing, and modification of sweet potato flour can be done through lactic acid fermentation (Yuliana et al., 2014; Amajor et al., 2014; Liao and Wu, 2017). Sweet potatoes flour made with lactic acid fermentation has better physicochemical properties and aroma (Yuliana et al., 2014; Liao and Wu, 2017; Yuliana et al., 2017; Yulana et al., 2018). Besides, fermentation with the help of specific lactic acid bacteria can produce an exopolysaccharide (EPS), which is useful for improving the functional properties of flour. Many authors have explored the role of the EPS in strengthening some flour and or starch properties (Patel and Prajapati, 2013; Karasu and Ermis, 2019).

The lactic acid fermentation process can occur with the help of a starter of lactic acid bacteria (LAB). The progress in optimizing the factors of the growth of that desired lactic acid bacteria will influence the success of the lactic acid fermentation process. These factors will provide different conditions for each type of starter according to their environment to affect the fermentation. Besides, each microbial starter will show differences in growth patterns, the period needed to grow and adapt, and the metabolites produced (Anino et al., 2019; Kurniadi et al., 2019 Ajayi et al., 2016; Zajsek et al., 2013).

Therefore, among others, the starter culture is essential to determine the efficiency of the fermentation process. The uses of the culture will help obtain the specific changes in the chemical composition, nutritional value, and sensorial properties of the substrate. By helping with a starter culture, the fermentation process, and consistent quality product with improved hygiene could be optimized (Ajayi et al., 2016). Therefore, it is a challenge to find out the starter culture with EPS-producing properties, and the data related to growth and metabolites produced can be considered to assess the potential of the starter used.

Starter's culture can be obtained from a liquid of back-slopping fermentation or derived from a natural fermentation process. Isolation of microorganisms from these occurring processes has always been the most potent means for obtaining a useful culture. Back-slopping practice and the length of fermentation, drive the progress and outcome of fermentation processes as occurred on (back-slopped) sourdough (De Vuyst et al., 2017). The type of technology applied, such as back-slopping procedures, will ascribe to the selected predominance specific lactic acid bacteria (Vrancken et al., 2017).

Commented [A1]: The progress in optimizing the growth factors of desired lactic acid bacteria will influence the success of the lactic acid fermentation process.

Commented [A2]: I did not find in the references

So far, research into the lactic fermentation process of sweet potatoes by using starter culture from back-slopping and spontaneous fermentation methods have been reported (Yuliana et al., 2014; Amajor et al., 2014; Ajayi et al., 2016; Yuliana et al., 2017; Yuliana et al., 2018). However, the research on the potential sources of the LAB starter, especially for possibly EPS production of both fermentation methods, is not widely known. Therefore, this study investigated the potentially lactic acid bacteria producing EPS from the fermentation of yellow sweet potato (back-slopping and spontaneous) since such microorganisms are expected to be useful as a starter to modify sweet potato flour further.

Commented [A3]: The sentence is too long. It's better to replace it: Therefore, this study aims to investigate lactic acid bacteria that have the potential to produce EPS from fermentation of yellow sweet potato (back-slopping and spontaneous). These bacterial isolates are expected to be used as a starter to further modify sweet potato flour

MATERIALS AND METHODS

Plant material and fermentation of yellow sweet potatoes

The main ingredient used in this study was a yellow sweet potato purchased at the traditional market in Bandar Lampung, Indonesia. The sweet potatoes were washed, peeled, sliced, and added to glassware containing a boiled solution of salt-sugar and were left at room temperature. The sweet potato slices were fermented with two procedures, namely back-slopping and spontaneous fermentation. Each experimental unit was repeated three times. Observations on total LAB (Yuliana et al., 2013), and biochemical changes: pH, total acidity as % of lactic acid, total glucose of supernatant (phenol-sulphuric method), and amount of crude exopolysaccharide (Razack et al., 2013), were ~~made~~ done at 0 ~~hours~~ (H0), 24 ~~hours~~ (H24), 48 ~~hours~~ (H48), and 72 hours (H72). A significant change in sweet potato starch granule at a selected hour of fermentation was also performed using scanning electron microscopy. Based on the above results, the potential lactic acid bacteria were then isolated and further observed for the candidate of exopolysaccharides (EPS)-producing lactic acid bacteria

Isolation of lactic acid bacteria (LAB)

The fresh yellow sweet potato slices were added to glassware containing a boiled solution of salt-sugar, fermented with back-slopping until 72 hours, and then used as microbial sources. The sample was taken, and bacterial isolation was performed by plating on MRS agar (Oxoid) containing 0.1% CaCO3 and was then incubated at 30 °C for 48h. The colonies identified by the presence of clear zones around each colony were randomly selected by streaking plates containing MRS agar, and purified by ~~re~~plating on MRS-agar plates. Colonies were reselected, plated on MRS agar, and incubated again at 30°C ± 2 °C for 24 hours. Gram staining, endospore, and catalase testing were performed for these selected colonies. Microorganisms categorized as lactic acid bacteria (gram-positive, negative endospore, and no catalase production) were purified on MRS agar and preserved at 4°C on MRS agar slants.

Screening of exopolysaccharide-producing LAB

Screening of EPS-producing LAB followed procedure based on the mucoid formation on the disc (Paulo et al., 2012), with little modification. The culture medium used for the screening of EPS-producing LAB was MRS agar supplemented with 10% skim milk, 3% sucrose, and 1% yeast extract. Four µL of culture from each selected were inoculated to the sterile filter paper (5 mm Ø) and put in Petri dishes (80 mm Ø) containing the culture medium agar. After 48h incubation, EPS production was confirmed based on the formation of a thick slime (mucoid) colony around the discs. The precipitation of this mucoid substance after mixing in absolute alcohol was also performed (Paulo et al., 2012). The mucoid zone diameter was measured, and the dry precipitate was weighed to assess the crude EPS production by the isolates. The saturation of the discs was fixed by observing the mucoid substance of the selected sample in scanning electron microscopy (SEM). An array of fiber polysaccharides was revealed as EPS (Paulo et al., 2012). Following a screening of EPS-producing bacteria, selected bacteria with potential high EPS yield were tested for their survival at various temperatures (10 and 45ºC), and ~~various~~ NaCl concentrations (3-10%). The temperature and salt endurance tests were performed to determine the ability of bacterial growth at different temperatures and different salt concentrations, indicated by either turbidity or sediment formation in the media. Beside~~s~~, endurance tests, their ability to produce EPS on various sugar mediums were also performed.

Commented [A4]: I suggest this sentence be changed to: Their endurance (resistance?) to temperature and salt is determined based on turbidity or sediment formation in the media. In addition, their ability to produce EPS in various sugar media is also carried out.

Potentially EPS-producing LAB on various sugar medium

The various medium conditions changing the type of sugar (sucrose, glucose, galactose, and lactose) were used for culturing the selected isolates. One mL of each isolate was inoculated into tubes containing 9 mL of nonfat skim medium (skim milk 10%, yeast extract 1%, and sugar 1%). These tubes were incubated at 28±2ºC for 24 hours. The EPS assessment followed procedure by Razack et al. (2013) with modification of the TCA precipitation step. After being incubated, the cells were removed by centrifugation at 4ºC at 15.000 rpm for 30 min. The supernatant was ~~then~~ taken for further purification by dissolving in 80% TCA and stirring for 30 minutes. The precipitated protein was removed by centrifugation at 15.000 rpm 30 minute~~s~~ 4ºC, and the supernatants were precipitated by adding an equal volume of absolute cold ethanol. The mixture was stored overnight at 4ºC. The precipitated EPS was ~~then~~ recovered by centrifugation at 4ºC at 15.000 rpm for 30 min, dried at 60ºC, and ~~then~~ its weight was determined as crude EPS.

Data analysis

The data generated on biochemical changes and total LAB were subjected to analysis of variance (ANOVA) and orthogonal polynomial contrast. Differences were reported at a significance level of 0.05.

RESULTS AND DISCUSSION

LAB and biochemical changes during fermentation

The value of pH, total acidity, crude EPS, residual reducing sugar, and lactic acid bacteria (LAB) are presented in Table 1. During fermentation, there was an increase in both treatments in total lactic acid, EPS, total LAB, residual reducing sugar, and a decrease in the pH. At the end of fermentation (72 hours), the back-slopping has a higher value of total acidity, crude EPS, reducing sugar, and a total of the LAB than those in spontaneous fermentation. Vice versa, the pH of the solutions in back-slopping was lower than that in spontaneous fermentation. The range of pH values achieved in both spontaneous and back-slopping treatments was not different from the results reported by previous studies (Yuliana et al., 2018; Oloo et al., 2014). A decrease in pH during fermentation indicates organic acid accumulation, especially lactic acid (Ohtman et al., 2017: Thorat et al., -2017; Zhao et al., 2018).

Table 1: LAB and biochemical and changes during Fermentation

	Time of fermentation (hour)	LAB (log cfu/mL)	pH	Crude EPS (g/mL)	Acidity (%)	Reducing sugar (mg/mL)
Spontaneous	0	0.00 ± 0.00	6.43 ± 0.40	0.15 ± 0.05	0.06±0.01	24.70 ± 1.45
	24	6.79 ± 0.36	4.44 ± 0.17	0.53 ± 0.09	0.68±0.31	29.47 ± 3.91
	48	7.43 ± 0.25	3.95 ± 0.09	0.75 ± 0.09	1.22±0.19	35.70 ± 3.42
	72	7.03 ± 0.44	3.87 ± 0.21	0.95 ± 0.06	1.44±0.12	44.01 ± 2.05
	trendline	quadratic	quadratic	linier	linier	linier
Back-slopping	0	8.21 ± 0.07	4.50 ± 0.26	0.76 ± 0.02	0.81±0.25	28.35 ± 1.38
	24	8.21 ± 0.11	3.87 ± 0.09	0.81 ± 0.03	0.99±0.12	33.42 ± 1.08
	48	8.59 ± 0.19	3.67 ± 0.06	1.19 ± 0.09	1.44±0.15	39.12 ± 0.73
	72	8.68 ± 0.14	3.57 ± 0.08	1.45 ± 0.11	1.89±0.13	49.49 ± 1.89
	trendline	quadratic	quadratic	linier	linier	linier
Comparison	Spontaneus vs Back-slooping	*	*	*	*	ns

Note: * significant at 0.05 by orthogonal contrast, ns = not significant

Commented [A5]: If you are doing analysis of varian, please include a notation if there is a real difference.

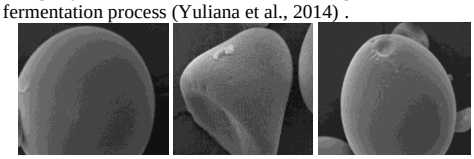
During fermentation, the LAB grows by utilizing a source of sugar as energy sources (Vidra et al., 2017; Bintsis, 2018) that can come from the degradation of starch in yellow sweet potato tissue. In 100 g of fresh material, a yellow sweet potato contains starches of 65.41% (dry basis) (Rodrigues et al., 2016). Amylase enzymes degrade yellow sweet potato starch during fermentation into polymers with shorter chains (simple sugars). Aside from LAB (Petrova et al., 2013), amylase was reported naturally on sweet potatoes (Ramakhrisnan and Rathnasamy, 2016). The LAB then uses simple sugar (glucose) as a source of energy or nutrition for its growth. In this fermentation, a rapid increase in reducing sugar in the substrate was observed in the 72 hours fermentation time, indicating that at this point, there was a faster growth rate of LAB (Table 1). An increase of LAB during the fermentation process with the back-slopping procedure was higher than those in natural treatment. The growth pattern of the LAB at the beginning of fermentation (0 hours) was possibly in the significant amount, and then it increased the next day until hour 72.

During LAB development, the exopolysaccharide was secreted into the extracellular matrix (Zeidan et al., 2017). The crude EPS content of fermented yellow sweet potato in all treatments increased linearly during fermentation with the back-slopping treatment (1.07%) was lower than a natural rate (0.9%). The differences were probably contributed by the types of the LAB that grow during the fermentation process. EPS production with BAL is influenced by the type of inoculum or is a strain-dependent property (Leory and De Vuyst, 2016). In addition to inoculum, EPS production was also fermentation time-dependent. This study's results are in line with previous research, which showed an increase of EPS by lactic acid bacteria with a rise in incubation time (Onilude et al. 2013; Maalej et al., 2014; Rawal et al., 2016). The profile of this fermentation suggests that the potential LAB could be obtained from the back-slopping method, mainly at 72 hours of fermentation. This incubation time, therefore, was selected as the reference for the next step of the experiment.

Granular Starch of Sweet Potatoes

Fermentation changes the structure of starch granules. Figure 1 shows the appearance of yellow sweet potato starch granules (controls without fermentation) that are not perforated. Meanwhile, yellow sweet potato starch granules experienced a change in shape at the end of fermentation time (t = 72 hours), which suffered degradation in two different fermentation treatments. Similar results were reported on fermented yellow sweet potato starch granules by *Lactobacillus plantarum* (Liao and Wu, 2017). Changes in starch granules were also reported during the spontaneous fermentation of white sweet potatoes (Yuliana et al., 2014). According to Liao and Wu (2017), the lengthy treatment of fermentation

destroyed the crystal structure of sweet potato starch and significantly affected the crystalline and amorph parts. The activity of lactic acid bacteria allegedly caused this. The size of starch granules in the white sweet potato fermentation process could change after the fermentation process (Yuliana et al., 2014) .

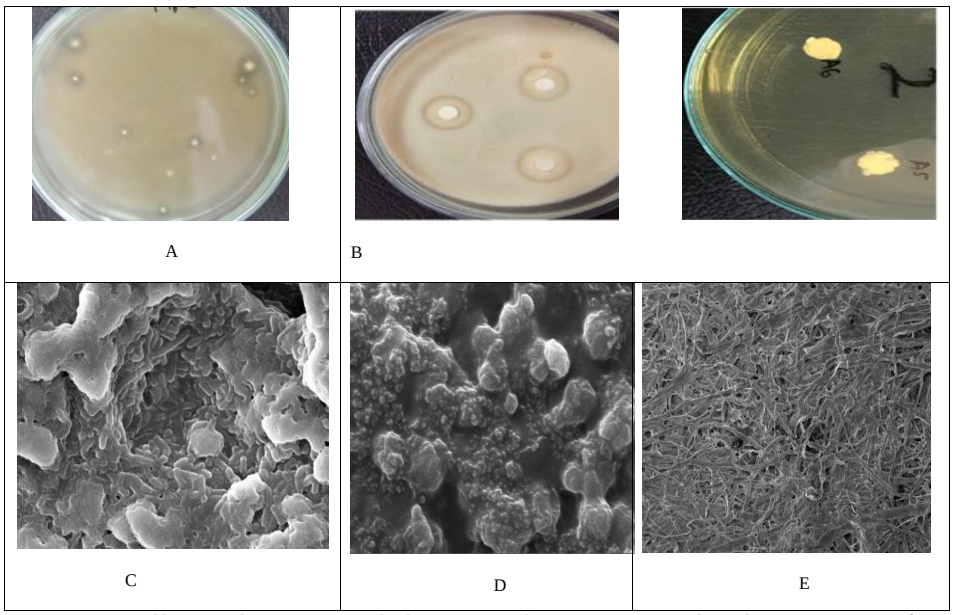


(a) (b) (c)

Figure 1. The appearance of yellow sweet potato starch granules (a) control, without fermentation, (b) Spontaneous 72 hours, (c) Back-slopping 72 hours.

Isolation and screening of EPS-producing lactic acid bacteria

Sixty isolates from back-slopping fermentation that had transparent zone colonies were selected as presumptive lactic acid bacteria. An example of these colonies is presented in figure 2A. All of these bacteria were confirmed lactic acid bacteria as all strains had Gram-positive, negative endospore, and negative catalase test. Besides, most of the strains were the creamy color with a circular form, entire margin, and flat elevation, the general colony performance of lactic acid bacteria. A previous study reported that the characteristic of the lactic acid bacteria was a white-round or oval colony, convex elevation, gram-positive bacteria, and a rod-shape (bacilli) (Astriani et al. 2018). Most of the isolates had rod-shaped cells (53 isolates), and the remaining was cocci-shape (7 isolates). Subsequent screening of these 60 lactic acid bacteria isolates resulted in 34 positive results (56.67%) that able to produce a polymer in the MRS modified medium. EPS production potential was indicated by a transparent or creamy material involving a mucoid colony (Figure 2B). The number of EPS by colonies was confirmed by the presence of crude EPS in the form of precipitation form. This formation has occurred after each mucoid was mixed in absolute alcohol. The EPS, matrix polysaccharides, confirmed by electron microscopy analyses, were presented in Figure 2C-E. Although the EPS-producing lactic acid bacteria were present at a low frequency, they could be used as starter cultures or adjuncts in mixed cultures to modify sweet potato flour.



A B C D E

Fig. 2 (A) Lactic acid bacteria colonies, (B) Mucoid colony, (C) Paper disc containing EPS-producing bacteria (×7000 magnification), D. Paper disc containing EPS only without bacteria cell (×7000 magnification), (E) Negative control, a paper disc without inoculation of bacteria (×1000 magnification)

Amongst 34 positive results isolates that able to produce a polymer in MRS modified medium, only six isolates (namely C2, A, E, N) having more than 1.4 mm diameter index of mucoid, and four isolates, namely L, P, Q, and V, produced crude EPS with high results (more than 0.1 g/ml) either on the agar medium containing skim milk-sucrose or on the MRSB medium containing 3% sucrose (Table 2). Meanwhile, the second high EPS-producing isolates (0.06-0.09 g/ml) were C2, A, E, F, L1, N, O, O1, W, X, and among this, it was only six isolates (namely C2, A,.E, N) having more than 1.4 mm diameter index of mucoid. Therefore, the eight strains (namely C2, A,.E, L, N, P, Q, and V) were selected for the subsequent experiment.

Commented [A6]: Six or four isolates?

Table 2. Diameter index of the mucoid colony and weight of EPS produced by isolates on LEL medium.

No	CODE	EPS Weight (g)	Index Diameter of mucoid (mm)	Number of colony
1	C4,A15 A1,A2,A3,A4,A5, A6,A7,A8,A14,	0.001-0.003	1.27-1.40	2
2	B3,B6,B7,C,C3,C8,Y,Q1,U	0.01-0.05	1.23-1.56	18
3	C2,A,E,F,L1,N,O,O1,W,X	0.06-0.09	1.27-1.58	10
4	Q,V	0.1-0.4	1.41	2
5	L,P	1.13-1.18	1.28-1.54	2
Total colony				34

Commented [A7]: Why do you use different unit for EPS weight? Above g/ml, but in this table only g

Based on a physiological test, most of these isolates grew well in the temperature of 28-45ºC, and the NaCl concentration of 3-4.5%. One isolate was slightly halophilic bacteria as they may grow at the concentrations of 3%, while the ~~rest~~ other isolates were moderately halophilic for being the ability for growth at 4 to 8% NaCl. The occurrence of halophilic lactic acid bacteria of this study is probably contributed to the origin of the product. These bacteria isolated from sweet potatoes pickle where 3-6% salt was added during its preparation. Based on the ability to grow in salt concentration media, bacteria may be grouped as slightly halophilic (2-5% NaCl), moderately halophilic (5-15% NaCl), and extremely halophilic for growth at a concentration of 15-30%) (Yadav et al., 2015). Tolerances to grow on media with varying NaCl salt levels are different depending on the genus of lactic acid bacteria (Ismail et al., 2018).

The endurance test for the growth temperature showed that all isolates grew well at 28ºC, and seven strains of these can survive until 45ºC, where four isolates among these can survive at temperatures of 50ºC. These results indicated that most of these isolates were thermophilic lactic acid bacteria. Isnawati and Trimulyono's (2018) study showed that lactic acid bacteria were able to grow at a range of 4 to 50º~~o~~C. The thermophilic lactic acid bacteria as an EPS producing were not surprising as reported by previous studies (Zhang et al., 2011; Panthavee et al., 2017; Khanal and Lucey, 2018).

Comparison of potentially EPS-producing LAB on various medium

The effect on different four carbon sources (glucose, galactose, lactose, and sucrose) on the production of exopolysaccharide (EPS) produced by selected isolates was investigated. The exciting result showed that four different carbon sources have varying degrees of a stimulatory effect on EPS production. Isolates A, E, L, N, Q, and V, had the maximum EPS yield when sucrose was employed. In contrast, an isolate of P and C2 were favorable to galactose, and lactose, and sucrose, respectively (Table 3). Several LAB produces polysaccharides that secreted into the environment as exopolysaccharides (EPS) (Zeidan et al., 2017). In this study, the favorable carbon sources for the growth and production of EPS were isolated dependent. According to EPS yield in Table 2, however, glucose was shown to sustain high rates in carbon conversion efficiency. The influence of the type of carbon sources on EPS production was confirmed by many authors (Rabha et al., 2012; Razack et al., 2013; Jaiswal et al., 2014). Amongst isolates, the highest yield of crude EPS was produced by V isolate (8.07 mg/ml) on glucose, following by P (6.60 mg/ml) on galactose and lactose, and N (6.20 mg/ml) on glucose medium respectively.

Table 3. The EPS (mg/ml) yield of selected isolates on carbon sources.

	EPS (mg/ml) yield on several Carbon Sources			
Isolates	Glucose	Galactose	Lactose	Sucrose
C2	1.73±0.13	3.40±0.21	4.00±0,09	4.20±0.10
A	4.40±0.26	0.73±0.08	0.00±0.00	1.93±0.23
E	3.80±0.16	2.67±0.35	0.00±0.00	1.20±0.13
L	4.30±0.12	3.40±0.03	2.73±0.01	3.47±0.25
N	6.20±0.16	0.10±0.00	0.20±0.00	2.40±0.33
P	5.27±0.37	6.60±0.16	6.60±0.46	4.07±0.29
Q	3.93±0.18	0.13±0.00	0.07±0.00	2.27±0.32
V	8.07±0.06	0.73±0.08	0.07±0.00	1.60±0.23

Note: The results are mean of triplicates determination ±standard deviation.

Commented [A8]: If you are doing analysis of varian, please include a notation if there is a real difference.

In conclusion, the potential starter can be taken from the yellow sweet potato fermentation by the back-slopping method, at 72 hours, as this showed the excellent value for the production of EPS metabolites, supported by the amount of LAB, total lactic acid and the lowest pH as well. Screening on the sixty strains of lactic acid bacteria isolated from fermented sweet potatoes pickle, 34 isolates were found to have EPS-producing ability. Amongst these, the eight highest EPS-producing strain was a promising possibility to be further developed as a starter. Most of these were favorable growth at 3% salt, glucose as carbon sources, and 28-45ºC of growth temperature.

ACKNOWLEDGEMENTS

The authors were very grateful to Universitas Lampung through Hibah Professor for funding this study.

REFERENCES

Ajayi OI, Ehiwuogu-Onyibe JI, Oluwole OB, Jegede AA, Salami TAI, Asieba GO, Chiedu IE, Suberu YL, Aba M, Dike EN, Jebor FN, Elemo GN. 2016. Production of fermented sweet potato flour using indigenous starter cultures. Afr. J. Microbiol. Res. 10: 1746-1758.

Amajor JI, Oti E, Ekeledo N, Omodamiro R, Amajor EE, Aniedu C. 2014. Studies on the characteristic properties of fermented, sun-dried orange-fleshed sweet potato flour. Niger. Food J. 32: 45-53.

Astriani A, Diniyah N, Jayus J, Nurhayati N. 2018. Phenotypic identification of indigenous fungi and lactic acid bacteria isolated from 'gatot' an Indonesian fermented food. Biodiversitas 19(3):947-954

Bintsis T. 2018. Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. AIMS Microbiol. 4:665-684

De Vuyst L, Van Kerrebroeck S, Leroy F. 2017. Microbial ecology and process technology of sourdough fermentation. Adv. Appl. Microbiol. 100: 49-160.

Ismail YS, Yulvizar C, Mazhitov B. 2018. Characterization of lactic acid bacteria from local cow´s milk kefir. IOP Conference Series: Earth and Environmental Science 130 (1): 012019. DOI: 10.1088/1755-1315/130/1/012019

Isnawati, Trimulyono G. 2018. Temperature range and degree of acidity growth of isolate of indigenous bacteria on fermented feed. The 2nd International Joint Conference on Science and Technology (IJCST) IOP Conference Series: Journal of Physics: Conference Series 953 (2018) 012209 DOI:10.1088/1742-6596/953/1/.

Jaiswal P, Sharma R, Sanodiya BS, Bisen PS. 2014. Microbial exopolysaccharides: Natural modulators of dairy products. J. Appl. Pharm. Sci. 4:105-109. DOI: 10.7324/JAPS.2014.40119ISSN 2231-3354.

Khanal SN, Lucey JA. 2018. Effect of fermentation temperature on the properties of exopolysaccharides and the acid gelation behavior for milk fermented by *Streptococcus thermophilus* strains Dgcc7785 and St-143. J. Dairy Sci. 101: 3799-3811.

Karasu EN, Ermis E. 2019. Determination of the effect of exopolysaccharide (EPS) from *Lactobacillus brevis* E25 on adhesion of food powders on the surfaces, using the centrifuge technique. J. Food Eng. 242:106-114.

Kurniadi M, Arsyad MF, Sar AM, Nurhayati R, Wiyono T. 2019. The effect of fermentation duration and starter concentration on physico-chemical properties of modified sorghum flour by *Lactobacillus plantarum* FNCC 0027. Food Res.3: 441 -447.

Leroy F, De Vuyst L. 2016. Advances in production and simplified methods for recovery and quantification of exopolysaccharides for applications in food and health 1. J. Dairy Sci. 99:3229–3238.

Liao L, Wu W. 2017. Fermentation effect on the properties of sweet potato starch and its noodle's quality by *Lactobacillus plantarum*. J. Food Process Eng. 40: e12460.

Maalej H, Hmidet N, Boisset C, Buon L, Heyraud A, Nasri, M. 2014. Optimization of exopolysaccharide production from *Pseudomonas* stutzeriAS22 and examination of its metal-binding abilities. J Appl Microbiol. 118:356--367

Othman M. Ariff AB, Rios-Solis L, Halim M. 2017. Extractive fermentation of lactic acid in lactic acid bacteria cultivation: a review. Front. Microbiol . 8:1-7

Oloo BO, Shitandi A, Mahungu S, Malinga JB, Ogata BR, 2014. Effects of lactic acid fermentation on the retention of beta-carotene content in orange-fleshed sweet potatoes. Int. J. Food Stud. 3: 13-33.

Onilude AA, Olaoye O, Fadahunsi IF, Owoseni A, Garuba O, Atoyebi T. 2013. Effects of cultural conditions on dextran production by *Leuconostoc* spp., Int. Food Res. J. 20: 1645-1651.

Panthavee W, Noda M, Danshiitsoodol N, Kumagai T, Sugiyama M. 2017. Characterization of exopolysaccharides produced by thermophilic lactic acid bacteria isolated from tropical fruit of Thailand. Biol Pharm Bull. 40: 621-629

Patel A, Prajapati JB. 2013. Food and health applications of exopolysaccharides produced by lactic acid bacteria. Adv. Dairy Res. 1:1-7**.**

Paulo EM, Vasconcelos MP, Oliveira IS, Affe HMJ, Nascimento R, de Melo IS, Roque MRA, de Assis SA, 2012. An alternative method for screening lactic acid bacteria for the production of exopolysaccharides with a rapid confirmation. Food Sci.Technol 32: 710-714

Petrova P, Petrov K, Stoyancheva G. 2013. Starch-modifying enzyme of lactic acid bacteria, structure, properties and application. Starch/Starke 65:34-37

Rabha R, Nadra R-S, Ahmaed B. 2012. Effect of some fermentation substrates and growth temperature on exopolysaccharide production by *Streptococcus thermophilus* BN1. Int J Biosci Biochem Bioinforma **2**: 44-47

Ramakrishnan S, Rathnasamy SK. 2016. β amylase purification from sweet potato (*Ipomoea batatas*): Reverse micellar extraction versus ammonium sulphate precipitation. Der Pharmacia Lettre 8:118-125

Rawal PM, Chauhan PB, Prajapati H, Gahlou M. 2016. Evaluation of cultivation condition for enhanced production of exopolysaccharide by bacterial isolate P 11 under submerged culture condition. Int. J. Adv. Res. Biol. Sci. 3:183-190

Razack SA, Velayutham V, Thangavelu V. 2013. Medium optimization for the production of exopolysaccharide by Bacillus subtilis using synthetic sources and agro wastes. Turk. J. Biol 37: 280-288.

Rodrigues, N. da R., Barbosa Junior, J. L., Barbosa, M. I. M. J. 2016. Determination of physico-chemical composition, nutritional facts and technological quality of organic orange and purple-fleshed sweet potatoes and its flours. Int. Food Res. J. **23**: 2071-2078

Thorat SPP, Sadawarte SK, Sawate AR, Machewad GM. 2017. Original studies on the effect of fermentation on physicochemical properties of vegetables and preparation of sauce. Int J Curr Microbiol Appl Sci.6: 3537-3545

Vidra VA, Tóth AJ, Németh A. 2017. Lactic acid production from cane molasses. Liquid Waste Recovery 2: 13–16

Vrancken G, Rimaux T, Weckx S, Leroy F, De Vuyst L. 2011. Influence of temperature and back slopping time on the microbiota of a type I propagated laboratory wheat sourdough fermentation. Appl. Environ. Microbiol. **77**: 2716–2726**.**

Yadav D, Singh A, Mathur N. 2015. Halophiles-a review. Int J Curr Microbiol Appl Sci. 4: 616-629

Commented [A9]: Above is written 2017

Yuliana N, Nurdjanah S, Sugiharto R, Amethy D. 2014. Effect of spontaneous lactic acid fermentation on physicochemical properties of sweet potato flour. Microbiol. Indones. 8: 1-8

Yuliana N, Nurdjanah S, Setyani S, Novianti D. 2017. Improving properties of sweet, potato composite flour: Influence of lactic fermentation. AIP Conference Proceedings 1854, 020040. https://doi.org/10.1063/1.4985431

Yuliana N, Nurdjanah S, Dewi YR. 2018. Physicochemical properties of fermented sweet potato flour in wheat composite flour and its use in white bread. "Int. Food Res. J.25: 1051-1059.

Yuliana N, Nurdjanah S, Margareta M. 2013. The effect of a mixed-starter culture of lactic acid bacteria on the characteristics of pickled orange-fleshed sweet potato (*Ipomoea batatas* L.). Microbiol Indones **7**: 1-8

Zajsek K, Gorsek A, Kolar M. 2013. Cultivating conditions effects on kefiran production by the mixed culture of lactic acid bacteria imbedded within kefir grains. *Food Chem.* 139: 970-977

Zeidan AA, Poulsen VK, Janzen T. Buldo P, Derkx PMF, Øregaard G, Neves AR. 2017. Polysaccharide production by lactic acid bacteria: from genes to industrial applications. FEMS Microbiol. Rev. 41(Supp_1): S168–S200

Zhao J, Dong Z, Li J. 2019. Effects of lactic acid bacteria and molasses on fermentation dynamics, structural and nonstructural carbohydrate composition and in vitro ruminal fermentation of rice straw silage. Asian Austral J Anim. 32:783-791. DOI: https://doi.org/10.5713/ajas.18.0543

Zhang Y, Li S, Zhng C, Luo Y, Zhang H, Yang Z. 2011. Gowth and exopolysaccharide production by *Lactobacillus fermentum* F6 in skim milk. Afr. J. Biotechnol. 10: 2080-2091

Commented [A10]: Above is written 2018

SUBMISSION CHECKLIST

Ensure that the following items are present:

The first corresponding author must be accompanied with contact details:

	Give mark (X)
• E-mail address	x
• Full postal address (incl street name and number (location), city, postal code, state/province, country)	x
• Phone and facsimile numbers (incl country phone code)	x

All necessary files have been uploaded, and contain:

• Keywords	x
• Running titles	x
• All figure captions	x
• All tables (incl title and note/description)	x

Further considerations

• Manuscript has been "spell & grammar-checked" Better, if it is revised by a professional science editor or a native English speaker	x
• References are in the correct format for this journal	x
• All references mentioned in the Reference list are cited in the text, and vice versa	x
• Colored figures are only used if the information in the text may be losing without those images	x
• Charts (graphs and diagrams) are drawn in black and white images; use shading to differentiate	x

Potentially lactic acid bacteria as an EPS producing starter from yellow sweet potato fermentation

Abstract. Potentially lactic acid bacteria as a starter for EPS production from yellow sweet potato fermentation were determined. The fermentation profile and partial characteristics of isolated lactic acid bacteria were used as considerations. The fermentation of yellow sweet potato was performed in the back-slopping and spontaneous procedure and was paid attention to the hours of 0, 24, 48, and 72. The results showed that the 72 hours of fermentation was found to have the highest EPS metabolites, the most upper the number of a LAB, the highest total lactic acid, and the lowest pH. The 72 hours of fermentation also seemed to cause a significant change in the sweet potato starch granules morphology. The potential starter, therefore, can be taken from the yellow sweet potato fermentation either spontaneously or by a back-slopping method, at 72 hours. From the sixty selected LAB, the 34 strains showed an ability to produce EPS. Among these, eight strains exhibited the potential high production of EPS. They were capable of growth on 28-45ºC and exhibited tolerance to 3-4% NaCl. The favorable carbon sources for the growth and production of EPS were isolated dependent.

Key words: back-slopping, EPS, lactic acid bacteria starter, spontaneous, sweet potato

Abbreviations : EPS (Exopolysaccharide), LAB (lactic Acid Bacteria).

Running title: EPS-producing lactic acid bacteria

INTRODUCTION

White sweet potato (SP) is important food crop that has potential to be used in composite flour for substitute the wheat. The naturally sweet potato flour, however, has some drawbacks properties. ~~such as s~~Slightly dark color (Trejo-González et al., 2014), low viscosity (Aprianita et al., 2009), less swelling power (Yuliana et al., 2014), and low loaf volume ~~and~~ when applied to bread products~~, the loaf volume is low~~ -(Amal, 2015), make ~~. Also,~~ the utilization of ~~level of~~ sweet potato flour ~~utilization~~ as wheat substituted flour acceptable for consumers is still low. Thus, sweet potatoes that are processed into flour require a modification process to be more applicable flour for further processing. Modification of sweet potato flour can be done through lactic acid fermentation (Yuliana et al., 2014; Amajor et al., 2014; Liao and Wu, 2017). Sweet potatoes flour made with lactic acid fermentation has better physicochemical properties and aroma (Yuliana et al., 2014; Liao and Wu, 2017; Yuliana et al., 2017; Yulana et al., 2018). Amylolyctic enzymes and acid action during lactic fermentation could change the structure of the starch granules (Diaz et al., 2018; Guo et al., 2019). Changes of the starch granule could lead to a modification in fermented SP starch or flour properties such as increasing peak viscosity and water-binding capacity, which in turn increasing the capacity of starch to swell quickly (Yuliana et al., 2014; Ajayi et al., 2016). Fermentation also improves the whiteness of SP flour by reducing component factors causing the browning (Yuliana et al., 2018). In term of the aroma, in addition to organic acid, the functional groups such as hydroxyl, aldehydes, alcohol, and carboxyl are present in the fermented samples of SP flour (Ajayi et al., 2019). Besides, fermentation with the help of specific lactic acid bacteria can produce an exopolysaccharide (EPS), which is useful for improving the functional properties of flour. Many authors have explored the role of the EPS in strengthening some flour and or starch properties such as increase binding and water holding capacity, improve viscosity, stabilize gel structure and improve rheological parameters -(Patel and Prajapati, 2013; Karasu and Ermis, 2019) ~~.~~

The lactic acid fermentation process can occur with the help of a starter of lactic acid bacteria (LAB). The progress in optimizing the growth factors of desired lactic acid bacteria will influence the success of the lactic acid fermentation process. These factors will provide different conditions for each type of starter according to their environment to affect the

fermentation. Besides, each microbial starter will show differences in growth patterns, the period needed to grow and adapt, and the metabolites produced (Anino et al., 2019; Kurniadi et al., 2019 Ajayi et al., 2016; Zajsek et al., 2013).

Therefore, among others, the starter culture is essential to determine the efficiency of the fermentation process. The uses of the culture will help obtain the specific changes in the chemical composition, nutritional value, and sensorial properties of the substrate. By helping with a starter culture, the fermentation process, and consistent quality product with improved hygiene could be optimized (Ajayi et al., 2016). Therefore, it is a challenge to find out the starter culture with EPS-producing properties, and the data related to growth and metabolites produced can be considered to assess the potential of the starter used.

Starter's culture can be obtained from a liquid of back-slopping fermentation or derived from a natural fermentation process. Isolation of microorganisms from these occurring processes has always been the most potent means for obtaining a useful culture. Back-slopping practice and the length of fermentation, drive the progress and outcome of fermentation processes as occurred on (back-slopped) sourdough (De Vuyst et al., 2017). The type of technology applied, such as back-slopping procedures, will ascribe to the selected predominance specific lactic acid bacteria (Vrancken et al., 2011).

So far, research into the lactic fermentation process of sweet potatoes by using starter culture from back-slopping and spontaneous fermentation methods have been reported (Yuliana et al., 2014; Amajor et al., 2014; Ajayi et al., 2016; Yuliana et al., 2017; Yuliana et al., 2018). However, the research on the potential sources of the LAB starter, especially for possibly EPS production of both fermentation methods, is not widely known. Therefore, this study aims to investigate lactic acid bacteria that have the potential to produce EPS from fermentation of yellow sweet potato (back-slopping and spontaneous). These bacterial isolates are expected to be used as a starter to further modify sweet potato flour

MATERIALS AND METHODS

Plant material and fermentation of yellow sweet potatoes

The main ingredient used in this study was a yellow sweet potato purchased at the traditional market in Bandar Lampung, Indonesia. The sweet potatoes were washed, peeled, sliced, and added to glassware containing a boiled solution of 3% salt-1% sugar and were left at room temperature. The sweet potato slices were fermented with two procedures, namely back-slopping and spontaneous fermentation. A starter, prepared by fermenting diced sweet potato in sterilized bottles containing 3% saline and 1% (w/w) sucrose solution for seven days at 37°C (Yuliana et al., ~~and Nurdjanah,~~ 2018~~09~~), was added the lot for a back-slopping procedure. The inoculation rate was 10% based on the volume of the fermentation solution. Another lot that ~~there was~~ no starter addition was prepared as spontaneous~~ly~~ fermentation. ~~The sweet potatoes were washed, peeled, sliced, and added to glassware containing a boiled solution of salt-sugar and were left at room temperature. The sweet potato slices were fermented with two procedures, namely back-slopping and spontaneous fermentation.~~ Each experimental unit was repeated three times. Observations on total LAB (Yuliana et al., 2013), and biochemical changes: pH, total acidity as % of lactic acid, total glucose of supernatant (phenol-sulphuric method), and amount of crude exopolysaccharide (Razack et al., 2013), were done at 0 (H0), 24 (H24), 48 (H48), and 72 hours (H72). A significant change in sweet potato starch granule at a selected hour of fermentation was also performed using scanning electron microscopy. Based on the above results, the potential lactic acid bacteria were then isolated and further observed for the candidate of exopolysaccharides (EPS)-producing lactic acid bacteria

Isolation of lactic acid bacteria (LAB)

The fresh yellow sweet potato slices were added to glassware containing a boiled solution of salt-sugar, fermented with back-slopping until 72 hours, and then used as microbial sources. The sample was taken, and bacterial isolation was performed by plating on MRS agar (Oxoid) containing 0.1% CaCO3 and was then incubated at 30°C for 48h. The colonies identified by the presence of clear zones around each colony were randomly selected by streaking plates containing MRS agar, and purified by plating on MRS-agar plates. Colonies were reselected, plated on MRS agar, and incubated again at 30 °C ± 2°C for 24 hours. Gram staining, endospore, and catalase testing were performed for these selected colonies. Microorganisms categorized as lactic acid bacteria (gram-positive, negative endospore, and no catalase production) were purified on MRS agar and preserved at 4°C on MRS agar slants.

Screening of exopolysaccharide-producing LAB

Screening of EPS-producing LAB followed procedure based on the mucoid formation on the disc (Paulo et al., 2012), with little modification. The culture medium used for the screening of EPS-producing LAB was MRS agar supplemented with 10% skim milk, 3% sucrose, and 1% yeast extract. Four µL of culture from each selected were inoculated to the sterile filter paper (5 mm Ø) and put in Petri dishes (80 mm Ø) containing the culture medium agar. After 48h incubation, EPS production was confirmed based on the formation of a thick slime (mucoid) colony around the discs. The precipitation of this mucoid substance after mixing in absolute alcohol was also performed (Paulo et al., 2012). The mucoid zone diameter was measured, and the dry precipitate was weighed to assess the crude EPS production by the isolates. The saturation of the discs was fixed by observing the mucoid substance of the selected sample in scanning electron microscopy (SEM). An array of fiber polysaccharides was revealed as EPS (Paulo et al., 2012). Following a

Commented [A1]: I did not find in the references

Author: Has been added in list of reference

Commented [U2]: Revision has been done

Formatted: Font color: Custom Color(RGB(46;116;181))

Formatted: Font color: Custom Color(RGB(46;116;181))

Formatted: Font color: Custom Color(RGB(46;116;181))

screening of EPS-producing bacteria, selected bacteria with potential high EPS yield were tested for their survival at various temperatures (10 and 45ºC), and NaCl concentrations (3-10%). Their endurance to temperature and salt is determined based on turbidity or sediment formation in the media. In addition, their ability to produce EPS in various sugar media is also carried out.

Potentially EPS-producing LAB on various sugar medium

The various medium conditions changing the type of sugar (sucrose, glucose, galactose, and lactose) were used for culturing the selected isolates. One mL of each isolate was inoculated into tubes containing 9 mL of nonfat skim medium (skim milk 10%, yeast extract 1%, and sugar 1%). These tubes were incubated at 28±2ºC for 24 hours. The EPS assessment followed procedure by Razack et al. (2013) with modification of the TCA precipitation step. After being incubated, the cells were removed by centrifugation at 4ºC at 15.000 rpm for 30 min. The supernatant was taken for further purification by dissolving in 80% TCA and stirring for 30 minutes. The precipitated protein was removed by centrifugation at 15.000 rpm 30 minutes 4ºC, and the supernatants were precipitated by adding an equal volume of absolute cold ethanol. The mixture was stored overnight at 4ºC. The precipitated EPS was recovered by centrifugation at 4ºC at 15.000 rpm for 30 min, dried at 60ºC, and its weight was determined as crude EPS.

Data analysis

The data generated on biochemical changes and total LAB were subjected to analysis of variance (ANOVA) and orthogonal polynomial contrast. Differences were reported at a significance level of 0.05.

RESULTS AND DISCUSSION

LAB and biochemical changes during fermentation

The value of pH, total acidity, crude EPS, residual reducing sugar, and lactic acid bacteria (LAB) are presented in Table 1. During fermentation, there was an increase in both treatments in total lactic acid, EPS, total LAB, residual reducing sugar, and a decrease in the pH. At the end of fermentation (72 hours), the back-slopping has a higher value of total acidity, crude EPS, reducing sugar, and a total of the LAB than those in spontaneous fermentation. Vice versa, the pH of the solutions in back-slopping was lower than that in spontaneous fermentation. The range of pH values achieved in both spontaneous and back-slopping treatments was not different from the results reported by previous studies (Yuliana et al., 2018; Oloo et al., 2014). A decrease in pH during fermentation indicates organic acid accumulation, especially lactic acid (Ohtman et al., 2017: Thorat et al., 2017; Zhao et al., 2018).

Table 1: LAB and biochemical changes during Fermentation

	Time of fermentation (hour)	LAB (log cfu/mL)	pH	Crude EPS (g/mL)	Acidity (%)	Reducing sugar (mg/mL)
Spontaneous	0	0.00 ± 0.00	6.43 ± 0.40	0.15 ± 0.05	0.06±0.01	24.70 ± 1.45
	24	6.79 ± 0.36	4.44 ± 0.17	0.53 ± 0.09	0.68±0.31	29.47 ± 3.91
	48	7.43 ± 0.25	3.95 ± 0.09	0.75 ± 0.09	1.22±0.19	35.70 ± 3.42
	72	7.03 ± 0.44	3.87 ± 0.21	0.95 ± 0.06	1.44±0.12	44.01 ± 2.05
	trendline	Quadratic*	Quadratic*	Linier*	Linier*	Linier*
Back-slopping	0	8.21 ± 0.07	4.50 ± 0.26	0.76 ± 0.02	0.81±0.25	28.35 ± 1.38
	24	8.21 ± 0.11	3.87 ± 0.09	0.81 ± 0.03	0.99±0.12	33.42 ± 1.08
	48	8.59 ± 0.19	3.67 ± 0.06	1.19 ± 0.09	1.44±0.15	39.12 ± 0.73
	72	8.68 ± 0.14	3.57 ± 0.08	1.45 ± 0.11	1.89±0.13	49.49 ± 1.89
	trendline	Quadratic*	Quadratic*	Linier*	Linier*	Linier*
Comparison	Spontaneous vs Back-slooping	*	*	*	*	ns

Note: * significant at 0.05 by orthogonal polynomial contrast, ns = not significant

Commented [U3]: * significant at 0.05 by orthogonal polynomial contrast

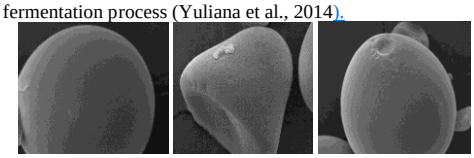
Commented [A4]: If you are doing analysis of varian, please include a notation if there is a real difference.

During fermentation, the LAB grows by utilizing a source of sugar as energy sources (Vidra et al., 2017; Bintsis, 2018) that can come from the degradation of starch in yellow sweet potato tissue. In 100 g of fresh material, a yellow sweet potato contains starches of 65.41% (dry basis) (Rodrigues et al., 2016). Amylase enzymes degrade yellow sweet potato starch during fermentation into polymers with shorter chains (simple sugars). Aside from LAB (Petrova et al., 2013), amylase was reported naturally on sweet potatoes (Ramakhrisnan and Rathnasamy, 2016). The LAB then uses simple sugar (glucose) as a source of energy or nutrition for its growth. In this fermentation, a rapid increase in reducing sugar in the substrate was observed in the 72 hours fermentation time, indicating that at this point, there was a faster growth rate of LAB (Table 1). An increase of LAB during the fermentation process with the back-slopping procedure was higher than those in natural treatment. The growth pattern of the LAB at the beginning of fermentation (0 hours) was possibly in the significant amount, and then it increased the next day until hour 72.

During LAB development, the exopolysaccharide was secreted into the extracellular matrix (Zeidan et al., 2017). The crude EPS content of fermented yellow sweet potato in all treatments increased linearly during fermentation with the back-slopping treatment (1.07%) was lower than a natural rate (0.9%). The differences were probably contributed by the types of the LAB that grow during the fermentation process. EPS production with BAL is influenced by the type of inoculum or is a strain-dependent property (Leory and De Vuyst, 2016). In addition to inoculum, EPS production was also fermentation time-dependent. This study's results are in line with previous research, which showed an increase of EPS by lactic acid bacteria with a rise in incubation time (Onilude et al. 2013; Maalej et al., 2014; Rawal et al., 2016). The profile of this fermentation suggests that the potential LAB could be obtained from the back-slopping method, mainly at 72 hours of fermentation. This incubation time, therefore, was selected as the reference for the next step of the experiment.

Granular Starch of Sweet Potatoes

Fermentation changes the structure of starch granules. Figure 1 shows the appearance of yellow sweet potato starch granules (controls without fermentation) that are not perforated. Meanwhile, yellow sweet potato starch granules experienced a change in shape at the end of fermentation time (t = 72 hours), which suffered degradation in two different fermentation treatments. Similar results were reported on fermented yellow sweet potato starch granules by *Lactobacillus plantarum* (Liao and Wu, 2017). Changes in starch granules were also reported during the spontaneous fermentation of white sweet potatoes (Yuliana et al., 2014). According to Liao and Wu (2017), the lengthy treatment of fermentation destroyed the crystal structure of sweet potato starch and significantly affected the crystalline and amorph parts. The activity of lactic acid bacteria allegedly caused this. The size of starch granules in the white sweet potato fermentation process could change after the fermentation process (Yuliana et al., 2014).

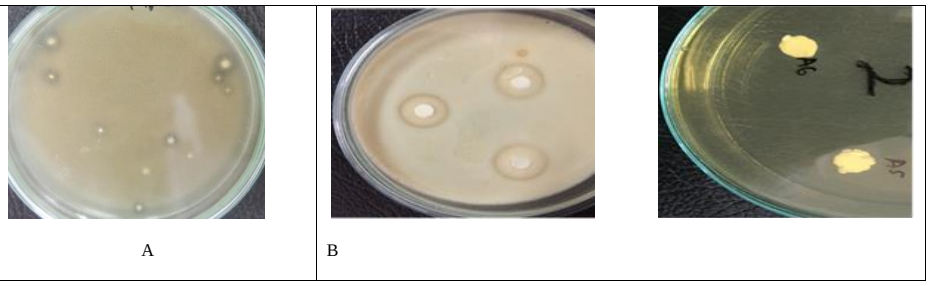


(a) (b) (c)

Figure 1. The appearance of yellow sweet potato starch granules (a) control, without fermentation, (b) Spontaneous 72 hours, (c) Back-slopping 72 hours.

Isolation and screening of EPS-producing lactic acid bacteria

Sixty isolates from back-slopping fermentation that had transparent zone colonies were selected as presumptive lactic acid bacteria. An example of these colonies is presented in figure 2A. All of these bacteria were confirmed lactic acid bacteria as all strains had Gram-positive, negative endospore, and negative catalase test. Besides, most of the strains were the creamy color with a circular form, entire margin, and flat elevation, the general colony performance of lactic acid bacteria. A previous study reported that the characteristic of the lactic acid bacteria was a white-round or oval colony, convex elevation, gram-positive bacteria, and a rod-shape (bacilli) (Astriani et al. 2018). Most of the isolates had rod-shaped cells (53 isolates), and the remaining was cocci-shape (7 isolates). Subsequent screening of these 60 lactic acid bacteria isolates resulted in 34 positive results (56.67%) that able to produce a polymer in the MRS modified medium. EPS production potential was indicated by a transparent or creamy material involving a mucoid colony (Figure 2B). The number of EPS by colonies was confirmed by the presence of crude EPS in the form of precipitation form. This formation has occurred after each mucoid was mixed in absolute alcohol. The EPS, matrix polysaccharides, confirmed by electron microscopy analyses, were presented in Figure 2C-E. Although the EPS-producing lactic acid bacteria were present at a low frequency, they could be used as starter cultures or adjuncts in mixed cultures to modify sweet potato flour.



A B

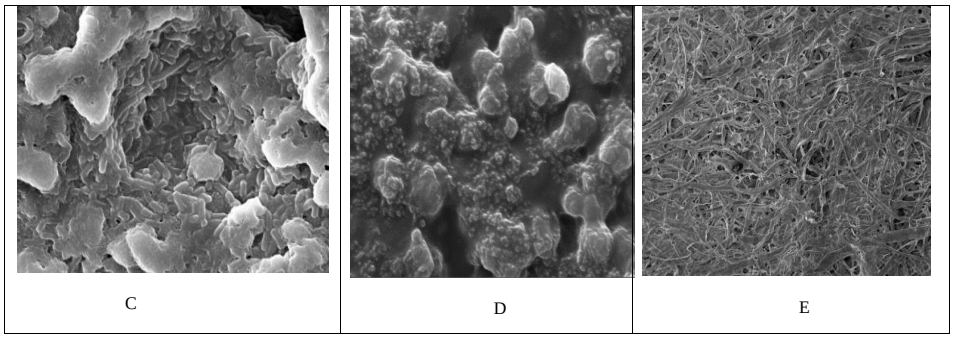


Fig. 2 (A) Lactic acid bacteria colonies, (B) Mucoid colony, (C) Paper disc containing EPS-producing bacteria (×7000 magnification), D. Paper disc containing EPS only without bacteria cell (×7000 magnification), (E) Negative control, a paper disc without inoculation of bacteria (×1000 magnification)

Amongst 34 positive results isolates that able to produce a polymer in MRS modified medium, only four isolates (namely C2, A, E, N) having more than 1.4 mm diameter index of mucoid, and four isolates, namely L, P, Q, and V, produced crude EPS with high results (more than 0.1 g/ml) either on the agar medium containing skim milk-sucrose or on the MRSB medium containing 3% sucrose (Table 2). Meanwhile, the second high EPS-producing isolates (0.06-0.09 g/ml) were C2, A, E, F, L1, N, O, O1, W, X, and among this, it was only four isolates (namely C2, A, E, N) having more than 1.4 mm diameter index of mucoid. Therefore, the eight strains (namely C2, A,.E, L, N, P, Q, and V) were selected for the subsequent experiment.

Table 2. Diameter index of the mucoid colony and weight of EPS produced by isolates on LEL medium.

No	CODE	EPS Weight (g/ml)	Index Diameter of mucoid (mm)	Number of colony
1	C4,A15 A1,A2,A3,A4,A5, A6,A7,A8,A14,	0.001-0.003	1.27-1.40	2
2	B3,B6,B7,C,C3,C8,Y,Q1,U	0.01-0.05	1.23-1.56	18
3	C2,A,E,F,L1,N,O,O1,W,X	0.06-0.09	1.27-1.58	10
4	Q,V	0.1-0.4	1.41	2
5	L,P	1.13-1.18	1.28-1.54	2
Total colony				34

Based on a physiological test, most of these isolates grew well in the temperature of 28-45ºC, and the NaCl concentration of 3-4.5%. One isolate was slightly halophilic bacteria as they may grow at the concentrations of 3%, while the other isolates were moderately halophilic for being the ability for growth at 4 to 8% NaCl. The occurrence of halophilic lactic acid bacteria of this study is probably contributed to the origin of the product. These bacteria isolated from sweet potatoes pickle where 3-6% salt was added during its preparation. Based on the ability to grow in salt concentration media, bacteria may be grouped as slightly halophilic (2-5% NaCl), moderately halophilic (5-15% NaCl), and extremely halophilic for growth at a concentration of 15-30%) (Yadav et al., 2015). Tolerances to grow on media with varying NaCl salt levels are different depending on the genus of lactic acid bacteria (Ismail et al., 2018).

The endurance test for the growth temperature showed that all isolates grew well at 28ºC, and seven strains of these can survive until 45ºC, where four isolates among these can survive at temperatures of 50ºC. These results indicated that most of these isolates were thermophilic lactic acid bacteria. Isnawati and Trimulyono's (2018) study showed that lactic acid bacteria were able to grow at a range of 4 to 50ºC. The thermophilic lactic acid bacteria as an EPS producing were not surprising as reported by previous studies (Zhang et al., 2011; Panthavee et al., 2017; Khanal and Lucey, 2018).

Comparison of potentially EPS-producing LAB on various medium

The effect on different four carbon sources (glucose, galactose, lactose, and sucrose) on the production of exopolysaccharide (EPS) produced by selected isolates was investigated. The exciting result showed that four different carbon sources have varying degrees of a stimulatory effect on EPS production. Isolates A, E, L, N, Q, and V, had the maximum EPS yield when sucrose was employed. In contrast, an isolate of P and C2 were favorable to galactose, and

Commented [U5]: Correction: Four isolates

Commented [A6]: Six or four isolates?

Commented [U7]: Correction: g/ml

Commented [A8]: Why do you use different unit for EPS weight? Above g/ml, but in this table only g

lactose, and sucrose, respectively (Table 3). Several LAB produces polysaccharides that secreted into the environment as exopolysaccharides (EPS) (Zeidan et al., 2017). In this study, the favorable carbon sources for the growth and production of EPS were isolated dependent. According to EPS yield in Table 2, however, glucose was shown to sustain high rates in carbon conversion efficiency. The influence of the type of carbon sources on EPS production was confirmed by many authors (Rabha et al., 2012; Razack et al., 2013; Jaiswal et al., 2014). Amongst isolates, the highest yield of crude EPS was produced by V isolate (8.07 mg/ml) on glucose, following by P (6.60 mg/ml) on galactose and lactose, and N (6.20 mg/ml) on glucose medium respectively. With this high yield, these isolates can be developed as good starters for EPS production. Therefore, further research is necessary to elucidate the EPS effect on functionality and aroma of fermented SP flour with these potential starters.

Table 3. The EPS (mg/ml) yield of selected isolates on carbon sources.

Isolates	EPS (mg/ml) yield on several Carbon Sources			
	Glucose	Galactose	Lactose	Sucrose
C2	1.73±0.13	3.40±0.21	4.00±0,09	4.20±0.10
A	4.40±0.26	0.73±0.08	0.00±0.00	1.93±0.23
E	3.80±0.16	2.67±0.35	0.00±0.00	1.20±0.13
L	4.30±0.12	3.40±0.03	2.73±0.01	3.47±0.25
N	6.20±0.16	0.10±0.00	0.20±0.00	2.40±0.33
P	5.27±0.37	6.60±0.16	6.60±0.46	4.07±0.29
Q	3.93±0.18	0.13±0.00	0.07±0.00	2.27±0.32
V	8.07±0.06	0.73±0.08	0.07±0.00	1.60±0.23

Note: The results are mean of triplicates determination ±standard deviation.

In conclusion, the potential starter can be taken from the yellow sweet potato fermentation by the back-slopping method, at 72 hours, as this showed the excellent value for the production of EPS metabolites, supported by the amount of LAB, total lactic acid and the lowest pH as well. Screening on the sixty strains of lactic acid bacteria isolated from fermented sweet potatoes pickle, 34 isolates were found to have EPS-producing ability. Amongst these, the eight highest EPS-producing strain was a promising possibility to be further developed as a starter. Most of these were favorable growth at 3% salt, glucose as carbon sources, and 28-45ºC of growth temperature.

ACKNOWLEDGEMENTS

The authors were very grateful to Universitas Lampung through Hibah Professor for funding this study.

REFERENCES

Ajayi OI, Ehiwuogu-Onyibe JI, Oluwole OB, Jegede AA, Salami TAI, Asieba GO, Chiedu IE, Suberu YL, Aba M, Dike EN, Jebor FN, Elemo GN. 2016. Production of fermented sweet potato flour using indigenous starter cultures. Afr. J. Microbiol. Res. 10: 1746-1758.

Ajayi OI, Okedina TA, Samuel AE, Asieba GO, Jegede AA, Onyemali CP, Ehiwuogu-Onyibe J, Lawal AK, Elemo GN. 2019. Evaluation of starter culture fermented sweet potato flour using FTIR spectra and GCMS chromatogram. Afr. J. Microbiol. Res. 13(1):1-13

Amajor JI, Oti E, Ekeledo N, Omodamiro R, Amajor EE, Aniedu C. 2014. Studies on the characteristic properties of fermented, sun-dried orange-fleshed sweet potato flour. Niger. Food J. 32: 45-53.

Amal AM. 2015. Quality evaluation of wheat-sweet potato composite flours and their utilization in bread making. Int. J. Adv. Res. Biol. Sci. 2: 294–303.

Anino C, Onyango A, Imathiu S, Maina J. Effect of lactic acid bacteria starter cultures on vitamin and oligosaccharide composition of milk extracted from three common bean (L) varieties. J. Food Res. 8(3):103-110

Aprianita A, Purwandari U, Watson B, Vasiljevic T. 2009. Physico-chemical properties of flours and starches from selected commercial tubers available in Australia. Int. Food Res. J. 16:507-520

Astriani A, Diniyah N, Jayus J, Nurhayati N. 2018. Phenotypic identification of indigenous fungi and lactic acid bacteria isolated from 'gatot' an Indonesian fermented food. Biodiversitas 19(3):947-954

Bintsis T. 2018. Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. AIMS Microbiol. 4:665-684

De Vuyst L, Van Kerrebroeck S, Leroy F. 2017. Microbial ecology and process technology of sourdough fermentation. Adv. Appl. Microbiol. 100: 49-160.

Díaz A. Dini C, Viña SZ, García MA. 2018. Technological properties of sour cassava starches: Effect of fermentation and drying processes. Lwt Food Sci. Technol. 93: 116–123

Guo L, Tao H, Cui B, Janaswamy S. 2019. The effects of sequential enzyme modifications on structural and physicochemical properties of sweet potato starch granules. Food Chem. 277, 504–14.

Ismail YS, Yulvizar C, Mazhitov B. 2018. Characterization of lactic acid bacteria from local cow´s milk kefir. IOP Conference Series: Earth and Environ mental Science 130 (1): 012019. DOI: 10.1088/1755-1315/130/1/012019

Isnawati, Trimulyono G. 2018. Temperature range and degree of acidity growth of isolate of indigenous bacteria on fermented feed. The 2nd International Joint Conference on Science and Technology (IJCST) IOP Conference Series: Journal of Physics: Conference Series 953 (2018) 012209 DOI:10.1088/1742-6596/953/1/.

Jaiswal P, Sharma R, Sanodiya BS, Bisen PS. 2014. Microbial exopolysaccharides: Natural modulators of dairy products. J. Appl. Pharm. Sci. 4:105-109. DOI: 10.7324/JAPS.2014.40119ISSN 2231-3354.

Khanal SN, Lucey JA. 2018. Effect of fermentation temperature on the properties of exopolysaccharides and the acid gelation behavior for milk fermented by *Streptococcus thermophilus* strains Dgcc7785 and St-143. J. Dairy Sci. 101: 3799-3811.

Commented [U9]: Additional text has been made

Commented [A10]: If you are doing analysis of varian, please include a notation if there is a real difference.

Author:

ANOVA was only performed to biochemical data of first step of experiments (table 1).

Formatted: Font color: Custom Color(RGB(46;116;181))

Formatted: Font color: Custom Color(RGB(46;116;181))

Formatted: Font color: Custom Color(RGB(46;116;181))

Formatted: Font color: Custom Color(RGB(46;116;181))

Formatted: Font color: Custom Color(RGB(46;116;181))

Formatted: Font color: Custom Color(RGB(46;116;181))

Formatted: Font color: Custom Color(RGB(46;116;181))

Formatted: Indent: Hanging: 0,5 cm

Karasu EN, Ermis E. 2019. Determination of the effect of exopolysaccharide (EPS) from *Lactobacillus brevis* E25 on adhesion of food powders on the surfaces, using the centrifuge technique. J. Food Eng. 242:106-114.
Kurniadi M, Arsyad MF, Sar AM, Nurhayati R, Wiyono T. 2019. The effect of fermentation duration and starter concentration on physico-chemical properties of modified sorghum flour by *Lactobacillus plantarum* FNCC 0027. Food Res.3: 441 -447.
Leroy F, De Vuyst L. 2016. Advances in production and simplified methods for recovery and quantification of exopolysaccharides for applications in food and health 1. J. Dairy Sci. 99:3229–3238.
Liao L, Wu W. 2017. Fermentation effect on the properties of sweet potato starch and its noodle's quality by *Lactobacillus plantarum*. J. Food Process Eng. 40: e12460.
Maalej H, Hmidet N, Boisset C, Buon L, Heyraud A, Nasri, M. 2014. Optimization of exopolysaccharide production from *Pseudomonas* stutzeriAS22 and examination of its metal-binding abilities. J Appl Microbiol. 118:356--367
Othman M. Ariff AB, Rios-Solis L, Halim M. 2017. Extractive fermentation of lactic acid in lactic acid bacteria cultivation: a review. Front. Microbiol . 8:1-7
Oloo BO, Shitandi A, Mahungu S, Malinga JB, Ogata BR, 2014. Effects of lactic acid fermentation on the retention of beta-carotene content in orange-fleshed sweet potatoes. Int. J. Food Stud. 3: 13-33.
Onilude AA, Olaoye O, Fadahunsi IF, Owoseni A, Garuba O, Atoyebi T. 2013. Effects of cultural conditions on dextran production by *Leuconostoc* spp., Int. Food Res. J. 20: 1645-1651.
Panthavee W, Noda M, Danshiitsoodol N, Kumagai T, Sugiyama M. 2017. Characterization of exopolysaccharides produced by thermophilic lactic acid bacteria isolated from tropical fruit of Thailand. Biol Pharm Bull. 40: 621-629
Patel A, Prajapati JB. 2013. Food and health applications of exopolysaccharides produced by lactic acid bacteria. Adv. Dairy Res. 1:1-7**.**
Paulo EM, Vasconcelos MP, Oliveira IS, Affe HMJ, Nascimento R, de Melo IS, Roque MRA, de Assis SA, 2012. An alternative method for screening lactic acid bacteria for the production of exopolysaccharides with a rapid confirmation. Food Sci.Technol 32: 710-714
Petrova P, Petrov K, Stoyancheva G. 2013. Starch-modifying enzyme of lactic acid bacteria, structure, properties and application. Starch/Starke 65:34-37
Rabha R, Nadra R-S, Ahmaed B. 2012. Effect of some fermentation substrates and growth temperature on exopolysaccharide production by *Streptococcus thermophilus* BN1. Int J Biosci Biochem Bioinforma **2**: 44-47
Ramakrishnan S, Rathnasamy SK. 2016. β amylase purification from sweet potato (*Ipomoea batatas*): Reverse micellar extraction versus ammonium sulphate precipitation. Der Pharmacia Lettre 8:118-125
Rawal PM, Chauhan PB, Prajapati H, Gahlou M. 2016. Evaluation of cultivation condition for enhanced production of exopolysaccharide by bacterial isolate P 11 under submerged culture condition. Int. J. Adv. Res. Biol. Sci. 3:183-190
Razack SA, Velayutham V, Thangavelu V. 2013. Medium optimization for the production of exopolysaccharide by Bacillus subtilis using synthetic sources and agro wastes. Turk. J. Biol 37: 280-288.
Rodrigues, N. da R., Barbosa Junior, J. L., Barbosa, M. I. M. J. 2016. Determination of physico-chemical composition, nutritional facts and technological quality of organic orange and purple-fleshed sweet potatoes and its flours. Int. Food Res. J. **23**: 2071-2078
Thorat SPP, Sadawarte SK, Sawate AR, Machewad GM. 2017. Original studies on the effect of fermentation on physicochemical properties of vegetables and preparation of sauce. Int J Curr Microbiol Appl Sci.6: 3537-3545
Trejo-González AS, Loyo-González AG, Munguía-Mazariegos MR. 2014. Evaluation of bread made from composite wheat-sweet potato flours. Int. Food Res. J. 21: 1683-1688
Todar K. 2020. Lactic acid bacteria. http://textbookofbacteriology.net/lactics.html. Accessed by August 17, 2020
Vidra VA, Tóth AJ, Németh A. 2017. Lactic acid production from cane molasses. Liquid Waste Recovery 2: 13–16
Vrancken G, Rimaux T, Weckx S, Leroy F, De Vuyst L. 2011. Influence of temperature and back slopping time on the microbiota of a type I propagated laboratory wheat sourdough fermentation. Appl. Environ. Microbiol. **77**: 2716–2726**.**
Yadav D, Singh A, Mathur N. 2015. Halophiles-a review. Int J Curr Microbiol Appl Sci. 4: 616-629
Yuliana N, Nurdjanah S, Sugiharto R, Amethy D. 2014. Effect of spontaneous lactic acid fermentation on physicochemical properties of sweet potato flour. Microbiol. Indones. 8: 1-8
Yuliana N, Nurdjanah S, Setyani S, Novianti D. 2017. Improving properties of sweet, potato composite flour: Influence of lactic fermentation. AIP Conference Proceedings 1854, 020040. https://doi.org/10.1063/1.4985431
Yuliana N, Nurdjanah S, Dewi YR. 2018. Physicochemical properties of fermented sweet potato flour in wheat composite flour and its use in white bread. "Int. Food Res. J. 25: 1051-1059.
Yuliana N, Nurdjanah S, Margareta M. 2013. The effect of a mixed-starter culture of lactic acid bacteria on the characteristics of pickled orange-fleshed sweet potato (*Ipomoea batatas* L.). Microbiol Indones **7**: 1-8
Zajsek K, Gorsek A, Kolar M. 2013. Cultivating conditions effects on kefiran production by the mixed culture of lactic acid bacteria imbedded within kefir grains. *Food Chem.* 139: 970-977
Zeidan AA, Poulsen VK, Janzen T. Buldo P, Derkx PMF, Øregaard G, Neves AR. 2017. Polysaccharide production by lactic acid bacteria: from genes to industrial applications. FEMS Microbiol. Rev. 41(Supp_1): S168–S200
Zhao J, Dong Z, Li J. 2019. Effects of lactic acid bacteria and molasses on fermentation dynamics, structural and nonstructural carbohydrate composition and in vitro ruminal fermentation of rice straw silage. Asian Austral J Anim. 32:783-791. DOI: https://doi.org/10.5713/ajas.18.0543
Zhang Y, Li S, Zhng C, Luo Y, Zhang H, Yang Z. 2011. Gowth and exopolysaccharide production by *Lactobacillus fermentum* F6 in skim milk. Afr. J. Biotechnol. 10: 2080-2091

Formatted: Indent: Left: 0 cm, Hanging: 0,5 cm

Commented [A11]: Above is written 2017

Author: Has been revised

Commented [A12]: Above is written 2018

Author: has been revised

SUBMISSION CHECKLIST

Ensure that the following items are present:

The first corresponding author must be accompanied with contact details:	**Give mark (X)**
• E-mail address	x
• Full postal address (incl street name and number (location), city, postal code, state/province, country)	x
• Phone and facsimile numbers (incl country phone code)	x

All necessary files have been uploaded, and contain:

• Keywords	x
• Running titles	x
• All figure captions	x
• All tables (incl title and note/description)	x

Further considerations

• Manuscript has been "spell & grammar-checked" Better, if it is revised by a professional science editor or a native English speaker	x
• References are in the correct format for this journal	x
• All references mentioned in the Reference list are cited in the text, and vice versa	x
• Colored figures are only used if the information in the text may be losing without those images	x
• Charts (graphs and diagrams) are drawn in black and white images; use shading to differentiate	x

Reviewer G:

Reviewer Comments	Author Response
Line 12: "The results showed that the 72 hours of fermentation was found to have the highest EPS metabolites, the number of a LAB, the total lactic acid, and the lowest pH. " Before the word "number" and "total lactic acid" a word like "highest" or "lowest" should be included, as the reader does not know what is meant here	Has been revised: The results showed that the 72 hours of fermentation was found to have the highest EPS metabolites, the most upper the number of a LAB, the highest total lactic acid, and the lowest pH.
line 22: "Sweet potatoes that are processed into flour require a modification process to be more applicable flour for further processing". I think this needs more explanation, to highlight why your study is so important. How do you apply the sweet potatoe flour? Why does it need a modification process? What is not good about this flour and should be changed?	Has been revised: One of the potential raw materials used in the composite for substitute the wheat flour is white sweet potato (SP). However, the native sweet potato flour has a slightly dark color (Trejo-González et al., 2014), low viscosity, and less swelling power (Yuliana et al., 2014). When applied to bread products, the loaf volume is low (Amal, 2015). These drawbacks properties make the level utilization of SP flour in acceptable wheat substitution is still low. Thus, SP that are processed into flour requires a modification process to be more applicable flour for further processing.
line 24: "Sweet potatoes flour made with lactic acid fermentation has better physicochemical	Amylolyctic enzymes and acid action during lactic fermentation could change the structure of the starch granules (Diaz et al., 2018; Guo et al., 2019). Changes of the starch granule could lead to a modification in fermented SP starch or flour properties such as increasing peak viscosity and water-binding capacity, which in turn increasing the capacity of starch to swell quickly (Yuliana et al., 2014; Ajayi et al.,

properties and aroma" This is not detailed enough. How is the aroma changed? Are aroma compounds degraded or new aroma compounds formed? What exactly is changed with the physiochemical properties. And overall, why was sweet potatoes used? A few facts about sweet potatoes and their properties would be useful	2016). Fermentation also improves the whiteness of SP flour by reducing component factors causing the browning. In term of the aroma, in addition to organic acid, the functional groups such as hydroxyl, aldehydes, alcohol, and carboxyl are present in the fermented samples of SP flour (Ajayi et al., 2019)
line27: "Many authors have explored the role of the EPS in strengthening some flour and or starch properties (Patel and Prajapati, 2013; Karasu and Ermis, 2019)." What do you mean with strengthening, do mean improving? How were these properties improved?	Yes, strengthening mean improving. The properties were improved by increasing binding and water holding capacity, increase viscosity etc We have revised to make it the sentence clearer: Many authors have explored the role of the EPS in strengthening some flour and or starch properties such as increase binding and water holding capacity, improve viscosity, stabilize gel structure and improve rheological parameters (Patel and Prajapati, 2013; Karasu and Ermis, 2019)
Materials and Methods	
line 55: solid to liquid ratio? Amount of solution? How many solutions? Did you have duplicates? The reader does not understand your experimental set up.	There is no sentence about solid to liquid ratio on line 55 This is the sentence in line 55: ….The sweet potatoes were washed, peeled, sliced, and added to glassware containing a boiled solution of salt-sugar and were left at room temperature

line 56: why was salt added? How much was added?	Solution containing 3% sodium chloride and 1% sucrose. Salt addition is an important to encourage LAB growth in sweet potatoes fermentation.
line 57: please decribe in detail, step by step, how your back-slopping and spontaneous fermentation experiments were performed. The reador does not understand it line 66: "fermented with back-slopping". Please include a step by step instruction.	Answer for the line 57 & 66 The sweet potato slices were fermented with two procedures, namely back-slopping and spontaneous fermentation. A starter, prepared by fermenting diced sweet potato in sterilized 3% saline and 1% (w/w) sucrose solution for seven days at 37°C (Yuliana et al., 2018), was added to the lot for a back-slopping procedure. The inoculation rate was 10% based on the volume of the fermentation solution. Another lot that there was no starter addition was spontaneously fermentation
line 68: Why did your media contain CaCO3? line 68: "The colonies identified by the presence of clear zones around each colony were randomly". Why is there a clean zone?	Answer for the line 68 (CaCO3 and clear zone): The CaCO3 acts as a non-diffusible buffer that localizes the acid producers. Lactic acid bacteria produces organic acid during their growth specifically lactic acid (up to 90%). So, calcium carbonate/chloride is used as an indicator for acid-producing strains since it dissolved when interact with acid then a clear zone is observed. Please find the following links of reference papers for the support of answer. http://www.sciencedirect.com/science/article/pii/S0956713510002872 http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2672.2002.01573.x/full
line 68: "streaking plates": what are streaking plates?	by streaking plates we mean by streaking them onto agar plates. We has revised the sentence to make it clear:

	The colonies identified by the presence of clear zones around each colony were randomly selected by streaking them onto plates containing MRS agar
line 73: What are slants?	Solid growth media usually contains agar used in the following forms: agar plates, agar slants, and agar deeps. To make agar slants, melted agar is poured into a test tube and then allowed to solidify at a slant position.
line 100: was there a reference performed? A medium without inoculation and fermentation and just with the isolation of EPS to make sure that not some parts of the medium were isolated and and were mistakenly assumed as EPS.	Even though a medium of sweet potatoes in brine-sugar solution without inoculation, the lactic fermentation is occurred. In this experiment the number of total lactic acid bacteria is presented in Table 1. Others study, please find the following link of reference papers: The isolation of EPS followed the procedure referred to Razak et al (2013). Purification medium (supernatant) was taken by centrifugation at 4ºC at 15.000 rpm for 30 min to remove the cells, by dissolving in 80% TCA to precipitate the protein and further removed by centrifugation at 15.000 rpm 30 minutes 4ºC. The supernatants were then precipitated by adding an equal volume of absolute cold ethanol for extracting hydro soluble slime (crude EPS) Supporting reference please find the following paper: Extraction, isolation and purification of exopolysaccharide from lactic acid bacteria using ethanol precipitation method. Bangladesh Journal of Pharmacology 11(3):573, (2020)
line 149: "The activity of lactic acid bacteria allegedly caused this": in line 126 you said, that starch degradation happens during fermentation and it was by caused by natural occuring amylases? So are the changes of the starch granules due to microorganisms or enzymes? The manuscript is not containing analysis for the identification of the isolated	We assumed that the change of the starch granules due to of mainly microorganism (lactic acid bacteria) and also enzyme. It is clear that in line 123-125: Amylase enzymes degrade yellow sweet potato starch during fermentation into polymers with shorter chains (simple sugars). Aside from LAB (Petrova et al., 2013), amylase was reported naturally on sweet potatoes (Ramakhrisnan and Rathnasamy, 2016).

starters. We don't know about their GRAS-status and if they are safe. I think this should be a part of the discussion. Also, in the introduction it was said, that these starters are needed for the improvement of the flour, in regard to their techno functionality and aroma. This should be also discussed in the discussion or an outlook should be included.	Few fact on lactic acid bacteria has been added to discussion. Also the potential of LAB producing EPS that could be developed as a starter for fermentation to improve the SP flour. All of selected bacteria in this experiment were confirmed lactic acid bacteria. Lactic acid bacteria from food have a reputation of being 'generally recognized as safe' (GRAS). However, few LAB are phatogenic to human such as Streptococcus pyogenes, streptococcus pneumonia, and nonhemolytic oral streptococci that play a role in dental caries (Todar, 2020). http://textbookofbacteriology.net/lactics.html. Therefore, the next developments of these isolates as starters, safety aspects of LAB must be brought into focus. Additional revision has been made: Amongst isolates, the highest yield of crude EPS was produced by V isolate (8.07 mg/ml) on glucose, following by P (6.60 mg/ml) on galactose and lactose, and N (6.20 mg/ml) on glucose medium respectively. With this high yield, these isolates can be developed as good starters for EPS production. Therefore, further research is necessary to elucidate the EPS effect on functionality and aroma of fermented SP flour with these potential starters.

.