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Growth kinetics of Saccharomyces cerevisiae and tape yeast on the cassava pulp fermentation

Growth kinetics of Saccharomyces cerevisiae and tape yeast on the cassava pulp fermentation

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Abstract. *Saccharomyces cerevisiae* is the most commonly used yeast in the fermentation process on high starch/carbohydrate substrates, for example, cassava pulp, a by-product from the tapioca industry. In addition to the pure culture of *S. cerevisiae*, the fermentation process of cassava pulp can be done using *tape* yeast (a consortium of yeast, fungal and bacteria). The objective of this research was to study the growth kinetics of *S. cerevisiae* and *tape* yeast on cassava pulp fermentation. The growth kinetics of *Saccharomyces cerevisiae* and *tape* yeast was observed through cells number, rate of starch degradation, rate of dietary fiber degradation, rate of cyanide degradation, and rate of protein formation. The research showed that the *S. cerevisiae* pure culture could grow better during cassava pulp fermentation compared to *tape* yeast, which is reflected by the logarithmic growth rate (up to 72 h versus 48 h). The difference in the growth rate between *S. cerevisiae* pure culture and *tape* yeast will cause a difference in starch degradation rate (73.02 mg/h versus 65.09 mg/h), dietary fiber degradation rate (87.33 mg/h versus 21.09 mg/h), cyanide degradation rate ($92.57.10^{-2}$ ppm/h versus $97.49.10^{-2}$ ppm/h), protein formation rate (48.92 mg/h versus 50.08 mg/h).

Keywords: *cassava pulp fermentation, food, growth kinetics, S. cerevisiae, tape yeast*

1. Introduction

Saccharomyces cerevisiae, a cheap and non-pathogenic saprophytic aerobe, is the most commonly used yeast in the fermentation process on high starch/carbohydrate substrates [1]. One of the high starch by-products that have the potential to increase its economic value is cassava pulp/cassava bagasse. Cassava pulp is a by-product from the tapioca industry, which still contains starch at 43.1% db (dry base) [2].

Fermentation of cassava pulp using *S. cerevisiae* was reported by Hidayat et al. [3] for food and by Kaewongsa et al. [4] for feed purposes. The fermentation process of cassava pulp for food is primarily aimed at increasing the protein content and reducing the cyanide content to the safe limit for consumption [3]. According to Codex Alimentarius [5], the safe limit for cyanide intake from food is 10 mg HCN/kg.

Fermentation of cassava pulp by *S. cerevisiae* for food needs is carried out with a solid-state system that will increase protein content and reduce the cyanide content to the safe limit for consumption [3]. Hidayat et al. [3], carried out the fermentation process of cassava pulp using *S. cerevisiae* without adding nutrients from the outside so that the nutrition of *S. cerevisiae* growth was solely based on the degradation process of the components in cassava pulp. During fermentation of cassava pulp, *S. cerevisiae* will degrade starch, dietary fiber, and cyanide as the main sources of its growth nutrients. *Saccharomyces cerevisiae* will produce amylase, which will hydrolyze starch into simple component [1]. Linamarase also will produce to decompose cyanides into non-toxic compounds [6]. The growth of *S. cerevisiae* will increase the amount of biomass cell that accumulates as a single cell protein [1] and will increase the protein content of cassava pulp. The single-cell protein of *S. cerevisiae* has high digestibility and no negative hematological effects [7].

The use of *S. cerevisiae* pure starter cultures on cassava pulp fermentation is relatively less practical so that other alternative starter cultures are needed, for example, yeast tape [8], instant yeast [9] or biomass of palm wine [10]. *Tape* yeast is the most interesting starter culture to study, considering that traditionally in Indonesia, it has been widely used in the cassava *tape*, glutinous rice *tape*, and *brem* processing [11]. *Tape* yeast is a dry starter culture consisting of microbial consortium in the form of yeast (*S. cerevisiae*), fungal (*Mucor*, *Rhizopus,* and *Amylomyces*) and cocci bacteria [12]. *Tape* yeast is made from a mixture of rice flour, spices, water, and sugarcane extract [13]. Merican and Queeland [13], reported that the *tape* yeast contained fungal counts of 8 x 10^7 – 9 x 10^8 cells/g, yeast counts of 3 x 10^6 – 3 x 10^7 cells/g and bacteria counts of 10^3 cells/g. The dominant bacteria in *tape* yeast is lactic acid bacteria, i.e., *Pediococcus pentosaceus*, *Enterococcus faecium*, *Lactobacillus curvatus*, *Weisella confuse,* and *W. paramesenteroides* [14].

Although *tape* yeast has been used on the various fermentation process of cassava product [11], its application as the starter on cassava pulp fermentation has not been reported. The potential use of tape yeast on cassava pulp fermentation can be observed by comparing its growth kinetics with *S. cerevisiae* pure starter cultures which include cells number, rate of starch degradation, rate of dietary fiber degradation, rate of cyanide degradation, and rate of protein formation. The objective of this research was to study the growth kinetics of *Saccharomyces cerevisiae* and *tape* yeast on cassava pulp fermentation.

2. Materials and Methods

2.1 Materials

Cassava var. Kasetsart was obtained from cassava farmers in Margomulyo Village, Jati Agung District, South Lampung Regency. Pure culture of *S. cerevisiae* ATCC 9763 was purchased from the culture collection of microbiology laboratory of Bogor Agricultural University. The chemicals used were Starch (GR, Merck), maltose (Sigma M5885), dinitrosalicylic acid (DNS, Sigma D-0550), glucose (Sigma G8270), termamyl enzyme (α-amylase, Sigma A-4862), pepsin enzyme (Sigma P7000), amyloglucosidase enzyme (Sigma A-9913), pancreatin enzyme (Sigma P-1750) obtained from PT Elo Karsa, Jakarta.

2.2 Methods

Pure culture preparation. Pure culture of *S. cerevisiae* was regenerated on Potato Dextrose Agar (PDA) slants agar and incubated at room temperature for four days. The spore suspension was prepared by adding 9 ml of sterilized water to slants agar and agitated at high speed for about 1 minute. The spore suspension of *S. cerevisiae* used contained as much as 10^{10} cells/ml.

Fresh cassava pulp preparation. Preparation of fresh cassava pulp begins with the stages of sorting cassava, then peeled with abrasion method (inner skin was not peeled), added water 20 times the volume of cassava, and pressed until the pressurized water was clear. Cassava pulp then soaked for ± 2 hours while stirring occasionally and pressed again to separate the water.

The fermentation process of cassava pulp. The starter in the form of *Saccharomyces cerevisiae* pure culture (2% v/w) or tape yeast (2% w/w), was mixed evenly with cassava pulp mash. Thus put into a plastic jar container with a hollow cover. Fermentation was carried out at room temperature for

fermentation time according to treatment (0 hours, 24 hours, 48 hours, 72 hours, 96 hours, and 120 hours).

Cassava pulp drying and grinding process. Drying of fermented cassava pulp mash was done using a cabinet dryer at 50°C for 5-6 hours followed by grinding until 80 mesh cassava flour was obtained.

Chemical component analysis. Analysis of chemical components was carried out on fermented cassava pulp flour, including starch content, dietary fiber content, protein content, and cyanide content. The protein was measured according to the methods of AOAC International [15] in the form of crude protein (Nx 6.25); dietary fiber was determined by the enzymatic method using termamyl, pepsin, and pancreatin enzymes [16]; total starch was measured by spectrophotometric method [17] with slight modification by using DNS [18]; cyanide content was determined using the alkaline titration [19]. The analysis was carried out in three replicates, and the data obtained were reported as mean ± SD.

Growth kinetics analysis. Growth kinetics analysis includes the cells number, rate of starch degradation, rate of dietary fiber degradation, rate of cyanide degradation, and rate of protein formation. Observation of cells number was done by spread plate method after the fermentation time was reached. One gram of fermented cassava pulp mash were suspended on physiological saline solution 0.85% in 10^{-6} to 10^{-12} dilution factor and cultured on Potato Dextrose Agar (PDA). The Petri dishes of PDA were incubated at the temperature room for 3-4 days. Analysis of starch degradation rate (/), dietary fiber degradation rate (/), cyanide degradation rate (/) and protein formation rate (/) were done by comparing the difference each component at the initial of fermentation (0 h) and end of fermentation (120 h).

Statistical analysis. The data of chemical composition were analyzed by two-way analysis of variance (ANOVA) using SPSS 16.0 software and continued with the least significant difference (LSD) test. The significance of the differences was defined as $P<0.05$.

3. Results and Discussion

3.1 Cells Number

The growth of *S. cerevisiae* pure culture and *tape* yeast from 0 hours to 120 hours of fermentation is presented in Fig. 1. On *tape* yeast treatment there are four phases of growth were distinctly seen, i.e., the lag phase, growth phase (exponential phase), stationary phase, and mortality phase (death phase); while in *S. cerevisiae* pure culture just three phases, i.e., the lag phase, growth phase (exponential phase), and mortality phase (death phase). The exponential phase in the pure culture treatment occurred up to 72 h, while on the tape yeast treatment was reached at 48 h. This study showed that on the pulp cassava substrate, *S. cerevisiae* could grow better than *tape* yeast.

Better growth of pure culture compared to *tape* yeast showed that compared to fungal and bacteria, *S. cerevisiae* was able to grow better in cassava pulp media on solid-state fermentation. *Tape* yeast is a microbial consortium, composed of *S. cerevisiae*, fungal, and bacteria [13]. This better growth shows that *S. cerevisiae* can more intensively degrade the components of cassava as a nutritional source of its growth. During fermentation, *S. cerevisiae* will produce extracellular enzymes that will decompose starch and dietary fiber as nutrients for its growth [1].

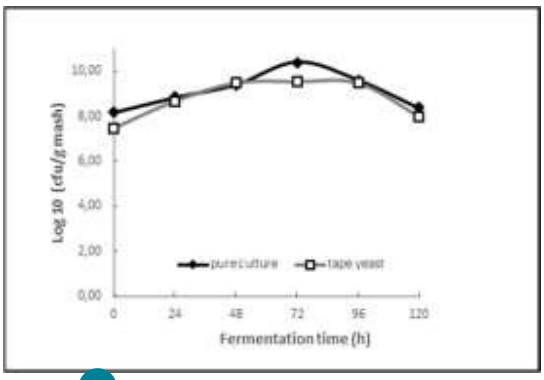

Fig. 1. The effect of fermentation time and starter culture on the cell number of fermented cassava pulp

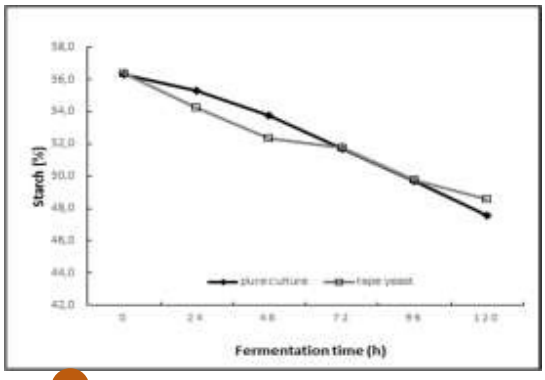

Fig. 2. The effect of fermentation time and starter culture on the rate of starch degradation of fermented cassava pulp

3.2 Chemical Composition

The results of ANOVA analysis and LSD test (Table 1) show that there are no significant differences (P> 0.05) between starter culture treatment in starch and protein content during the cassava pulp fermentation, but there are significant differences (P ≤ 0.05) for dietary fiber and cyanide parameters. The pure culture and *tape* yeast treatments respectively reduced starch content from 56.29% to 47.53% and 56.35% to 48.54%, increasing the protein content from 1.20% to 7.07% and 1.21% to 7.22%. In the other hand, the treatment of pure culture and *tape* yeast respectively will reduce the dietary fiber content from 27.92% to 17.44% and from 27.47% to 24.93% and reduce the cyanide content from 19.89% to 8.78% and from 20.87% to 9.17%.

Table 1. Chemical composition of fermented cassava pulp on various starter culture and fermentation time (mean ± SD).

Treatment	Starch (% db)	Dietary fiber (% db)	Cyanide (ppm)	Protein (% db)
Pure cultures, fermentation time 0 h	56.29 ± 0.81 a	27.92 ± 0.27 a	19.89 ± 0.70 a	1.20 ± 0.04 e
Pure cultures, fermentation time 24 h	55.28 ± 0.66 ab	26.84 ± 0.50 b	12.03 ± 1.07 de	2.77 ± 0.07 d
Pure cultures, fermentation time 48 h	53.76 ± 0.97 bc	23.44 ± 0.22 d	9.94 ± 0.98 fg	4.44 ± 0.44 c
Pure cultures, fermentation time 72 h	51.68 ± 0.92 c	22.17 ± 0.33 e	9.53 ± 0.75 g	6.19 ± 0.05 b
Pure cultures, fermentation time 96 h	49.69 ± 0.21 cd	18.53 ± 0.85 f	8.87 ± 0.82 g	6.98 ± 0.29 a
Pure cultures, fermentation time 120 h	47.53 ± 0.93 e	17.44 ± 0.63 g	8.78 ± 0.67 g	7.07 ± 0.10 a
Tape yeast, fermentation time 0 h	56.35 ± 0.46 a	27.47 ± 0.67 ab	20.87 ± 1.32 a	1.21 ± 0.34 e
Tape yeast, fermentation time 24 h	54.24 ± 0.94 b	26.69 ± 0.61 b	17.21 ± 1.83 bc	2.52 ± 0.31 d
Tape yeast, fermentation time 48 h	52.35 ± 0.18 bc	26.26 ± 0.33 bc	15.98 ± 1.91 c	4.80 ± 0.88 c
Tape yeast, fermentation time 72 h	51.78 ± 0.99 c	26.04 ± 0.20 bc	13.84 ± 1.12 d	6.30 ± 0.32 b
Tape yeast, fermentation time 96 h	49.76 ± 1.36 cd	25.64 ± 0.35 c	11.57 ± 1.18 ef	7.18 ± 0.16 a
Tape yeast, fermentation time 120 h	48.54 ± 1.05 de	24.93 ± 0.34 c	9.17 ± 1.12 g	7.22 ± 0.38 a

Values with the same letters are not significantly different (P > 0.05)

3.3 Rate of starch degradation

Starch is the main source of nutrition for microbial on cassava pulp fermentation. In the fermentation process of high starch products, starch is a significant source of nutrients [20]. Starch will be degraded to a simple component and used as a carbon source by microbes so that the amount of starch will decrease during the fermentation process. There was a difference in starch degradation rate ($\partial S/\partial T$) between pure culture (73.02 mg/h) and yeast tape (65.09 mg/h). The higher of the starch degradation rate of the pure culture show that compared to *tape* yeast, *S. cerevisiae* pure culture is better able to

degrade starch in cassava pulp media (Fig. 2). *Saccharomyces cerevisiae* will produce amylase, which will hydrolyze starch to a simple component [1]. The ability of *tape* yeast to degrade starch is mainly related to the ability of fungal to produce amylolytic enzymes [21].

3.4 Rate of dietary fiber degradation

Cassava pulp contains dietary fiber of 47.1% [2], which consists of pectin (10.11%), hemicellulose (21.8%), and cellulose (6.31%) [22]. Dietary fiber is a structure that covers the starch matrix in cassava pulp. The fermentation process opens up the fiber structure, so starch becomes more available for microbes [23]. The research shows that there is a difference in the dietary fiber degradation rate ($\partial DF/\partial T$) between pure culture and tape yeast, i.e., 87.33 mg/h for pure culture and 21.09 mg/h for tape yeast (Fig. 3). This fact showed that during cassava pulp fermentation, the *S. cerevisiae* pure culture was better able to produce enzymes that degrade dietary fiber (pectinase and cellulase) than *tape* yeast.

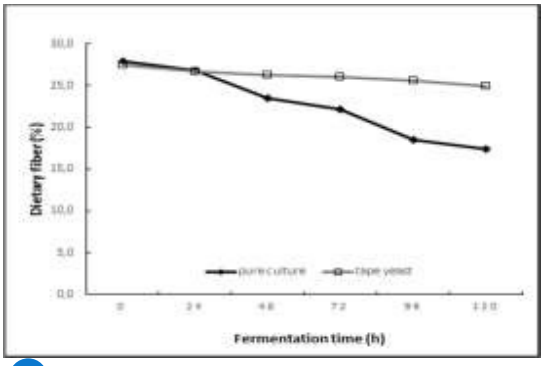

Fig. 3. The effect of fermentation time and starter culture on the rate of dietary fiber degradation of fermented cassava pulp

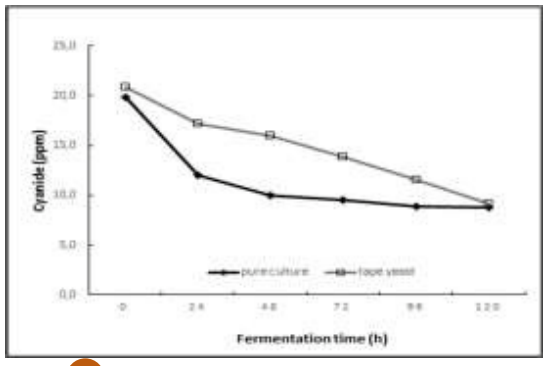

Fig. 4. The effect of fermentation time and starter culture on the rate of cyanide degradation of fermented cassava pulp

3.5 Rate of cyanide degradation

Cyanide in cassava is in the form of cyanogenic glucosides (CNG), which are composed of 96% linamarin (96%) and 4% lotaustralin [24]. Hydrogen cyanide, the form of cyanide that is watersoluble and volatile, will be formed if CNG is brought into contact with glycosidase and hydroxynylene lyases (upon tissue disruption) and degraded into cyanohydrins, hydrogen cyanide and ketones [25]. The removal of cyanide of cassava pulp during fermentation is due to the utilized CNG through assimilatory degradation with the release of hydrogen cyanide and ammonia [26].

The cyanide degradation process is presented in Fig. 4, with the degradation rate ($\partial Cn/\partial T$) $92.57.10^{-2}$ ppm/h for pure culture and $97.49.10^{-2}$ ppm/h for *tape* yeast. This research showed that *tape* yeast (a consortium of yeast, fungal, and bacteria) was able to degrade cyanide better than the pure culture. Murugan et al. [26] reported that bacteria have a better ability to degrade CNG. Conversely to this research, Lambri et al. [24] reported that compared to other microbes, *S. cerevisiae* was able to degrade CNG more effectively.

3.6 Rate of protein formation

Increased protein content during the cassava pulp fermentation was related to the formation of microbial biomass (single cell protein) [1]. The higher the biomass that was formed, the higher the protein content in cassava pulp. Compared with the cell number data in Fig. 1, it can be seen that the protein content of fermented cassava pulp would continue to increase until the length of fermentation was 120 h, although the viable cell data had been reduced since the fermentation period had been 72 h for pure culture and 96 h for *tape* yeast. This fact showed that cell biomass was a combination of the number of viable cells and dead cells. The increased protein content of cassava pulp flour during the fermentation process using *Saccharomyces cerevisiae*, also reported by Kaewwongsa et al. [4].

The protein formation process is presented in Fig. 5 with degradation rate ($\partial P/\partial T$) 48.92 mg/h for pure culture and 50.08 mg/h for *tape* yeast. Higher rate of protein formation in *tape* yeast (a consortium of yeast, fungal, and bacteria) mainly due to fungal biomass containing mycelium has a greater weight than yeast biomass [21].

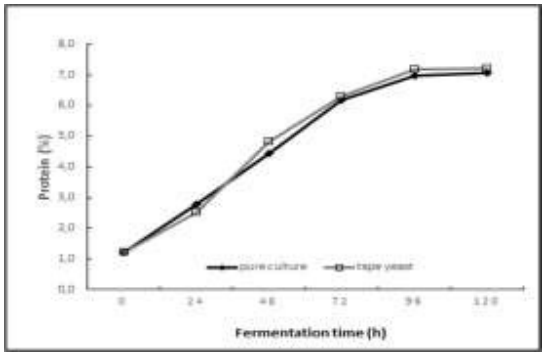

Fig. 5. The effect of fermentation time and starter culture on the rate of protein formation of fermented cassava pulp

4. Conclusions

Saccharomyces cerevisiae pure culture can grow better during cassava pulp fermentation compared to *tape* yeast (a consortium of yeast, fungal and bacteria), which is reflected by the logarithmic growth rate (up to 72 h versus 48 h).

There are no significant differences ($p > 0.05$) between starter culture treatment in starch and protein content during the cassava pulp fermentation, but there are significant differences ($P \leq 0.05$) for dietary fiber and cyanide parameters.

The difference in the growth rate between *S. cerevisiae* pure culture and *tape* yeast will cause differences in starch degradation rate (73.02 mg/h versus 65.09 mg/h), dietary fiber degradation rate (87.33 mg/h versus 21.09 mg/h), cyanide degradation rate ($92.57.10^{-2}$ ppm/h versus $97.49.10^{-2}$ ppm/h), protein formation rate (48.92 mg/h versus 50.08 mg/h).

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