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The growth dynamics, chemical, amylographic profile and granular morphology changes on cassava pulp fermentation

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Received: May 23, 2019 Accepted: October 05, 2019 Published: December 31, 2019	Abstract Solid-state fermentation using <i>Saccharomyces cerevisiae</i> is the most optimal method to increase protein content and reduce cyanide content of cassava pulp to use it as a food ingredient. This research aimed to evaluate the effect of fermentation time on the growth dynamics of <i>S. cerevisiae</i> , chemical, amylographic profile and granular morphology changes on cassava pulp fermentation. The results of the study revealed that <i>S. cerevisiae</i> was able to grow logarithmic up to 72 hours of fermentation with starch and dietary fiber as a nutrition source and causes changes in viscosity profile and granular morphology. The increase in the total number of cells (viable cell and dead cell) will increase single cell protein biomass, which will increase the protein content to 7.07% and reduce cyanide content to 8.78 ppm.
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Introduction

Cassava is one of the essential commodities in Indonesia with total production in 2015 reached 21,790,956 tons (BPS, 2016). Most of the cassava is used as raw material for tapioca industry. Cassava pulp (cassava bagasse) is the main by-product of tapioca industry, which can reach 60% of the total cassava raw material (FAO, 2001). According to Pandey et al. (2000), cassava pulp is a fibrous residue compared to other agricultural residues has the potential to be converted through bioprocess because it has a low ash content and it does not require any pretreatment. The possibility of cassava pulp to be used as food is mainly based on the high of starch content and fibrous residue in the form of dietary fiber, which were 43.1% db (dry basis) and 47.1% db respectively (Raupp et al., 2004). Dietary fiber on cassava pulp is composed of pectin (10.11%), hemicellulose (21.8%) and cellulose (6.31%) (Nurdjanah and Elfira, 2009). The obstacle faced if cassava pulp will be used as food is high cyanide content because most of the cassava used as a raw material in the tapioca industry is high-cyanide cassava. The high-cyanide cassava has a high level of cyanide in the form of cyanogenic glycosides (linamarin and lotaustralin) when hydrolyzed release hydrocyanic acid, a toxic compound for humans (Etsuyankpa et al., 2015). Besides cyanide content, another obstacle that needs to be overcome if cassava pulp will be used as food is low protein content (Ubalua, 2007).

The fermentation process is the most potential process applied to increase the protein content of cassava pulp Based on the and reduce its cyanide content. characteristics of semi-solid cassava pulp, the fermentation process can be carried out through a solid-state fermentation process. According to Ezekiel and Aworh (2013), compared to other fermentation methods, solid-state fermentation is the most suitable method for increasing the protein content of cassava pulp because it is relatively inexpensive and efficient. Saccharomyces cerevisiae, a cheap and nonpathogenic saprophytic aerobe, is the most potential microbe used in solid-state fermentation using cassava and its by-product as raw material. Oboh and Akindahunsi (2003), reported that solid-state fermentation using S. cerevisiae would increase the protein content of cassava flour and garri by 10.9% and 6.3% respectively.

Research on increasing the protein content of cassava pulp for feed purposes has been carried out by Kaewwongsa et al. (2011). According to Kaewwongsa et al. (2011), solid-state fermentation of cassava pulp using S. cerevisiae will improve feed quality as reflected in an increase in protein content up to 24.7% and an increase in undegradable protein rumen. The solid-state fermentation of cassava pulp for food needs using S. cerevisiae has been reported by Hidayat et al. (2018). The results of the study by Hidayat et al. (2018) showed that fermentation of cassava pulp would increase the protein content (0.92% to 6.98%)and reduce cyanide content (30.52 ppm to 8.87 ppm). Increasing protein content and decreasing cyanide content to a safe level for consumption of 8.87 ppm indicates that solid-state fermentation has the potential to be applied to improve the characteristics of cassava pulp flour in the context of its use as food. FAO/WHO recommends that the safe limit for cyanide intake from food is 10 mg HCN/kg (Codex Alimentarius, 1989). Although research on increasing the potential of cassava pulp as food has been carried out, information about the growth dynamics of S. cerevisiae and changes that occur during the fermentation process has not been reported. Information about the growth dynamics of S. cerevisiae and changes that occur during the fermentation process is vital for the context of the application of technology on an industrial scale. In the fermentation process of cassava pulp, fermentation time is an essential factor that will determine the growth dynamics of S. cerevisiae and chemical changes, amylographic, and morphological changes of fermented cassava pulp flour during the

fermentation process. This research aimed to evaluate the effect of fermentation time on the growth of *S. cerevisiae*, chemical change, amylographic profile and granular morphology changes on cassava pulp fermentation.

Material and Methods

Chemicals

Cassava var. Kasetsart was obtained from cassava farmers in Margomulyo Village, Jati Agung District, South Lampung Regency. Pure culture of *S. cerevisiae* 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 P-7000), amyloglucosidase enzyme (Sigma A-9913), pancreatin enzyme (Sigma P-1750) obtained from PT Elo Karsa, Jakarta.

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

Suspension of *S. cerevisiae* as much as 2% (v/b) 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

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by grinding until 80 mesh cassava flour was obtained.

Characteristics analysis

Analyzes were carried out on fermented cassava pulp and fermented cassava pulp flour. Observation of cell number was done by spread plate method after the fermentation time was reached. Analysis of fermented cassava pulp flour includes chemical characteristics (starch content, dietary fiber content, cyanide content and protein content), granular morphology, and amylographic profile. Total starch was analyzed based on Goni et al. (1997) method with slight modification by using DNS; dietary fiber was determined by the enzymatic method (Asp et al., 1983); the cyanide content was determined using the alkaline picrate method (Etsuyankpa et al., 2015); the crude protein was measured according to the methods of AOAC International (1999). Analysis of granular morphology was conducted with the scanning electron microscope (SEM) method using scanning electron microscope (SEM) type SEM ZEISS EVO MA 10; and analysis of amylographic profile was carried out by the brabender method using brabender viscograph type Brabender GmbH & Co.KG. The analysis of cell numbers and chemical characteristics were carried out in three replicates and the data obtained were reported as mean \pm SD.

Statistical analysis

The data of cell numbers and chemical characteristics were analyzed by one-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.

Results and Discussion

Microbial growth

The growth dynamics of *S. cerevisiae* from 0 hours to 120 hours of fermentation is presented in Fig. 1. The growth dynamics was obtained by observing the cell numbers presented in the form of a log of cell numbers. Observation of cell numbers was done by calculating viable cell number by spread plate method. There was a significant difference (p<0.05) of the cell number between different fermentation time. The results showed that *S. cerevisiae* was able to live in the media of cassava pulp without any prior treatment and addition of carbon and nitrogen nutrients. The previous study suggested that cassava pulp has advantages as a fermentation substrate in comparison

to other crop residues because it does not require pretreatment and can efficiently metabolize by the microorganism (Pandey et al., 2000).

Three phases of growth were distinctly seen, i.e., the lag phase, growth phase (exponential phase), and mortality phase (death phase); even though, the stationary phase of S. cerevisiae was not clear noted (Fig. 1). The death phase of S. cerevisiae started at 72 h. The cell number increase to 72 h fermentation time showed that S. cerevisiae was able to grow and utilize starch and dietary fiber on cassava pulp media. During fermentation, S. cerevisiae will produce extracellular enzymes that will decompose starch and dietary fiber as nutrients for its growth (Oboh and Akindahunsi, 2003). Since the fermentation period of 72 hours, the available nutrients begin to be out of balance with the number of viable cells so that S. cerevisiae begins to enter the phase of death. This condition also shows that up to the duration of 120 h fermentation, S. cerevisiae was still carrying out metabolic processes even though the nutrients available are inadequate.



Figure-1: The effect of fermentation time on the growth of *S. cerevisiae* on cassava pulp fermentation

Starch content

The change of starch content from 0 hours to 120 hours of fermentation is presented in Table 1. During the fermentation process of cassava pulp, starch was the primary nutrient source for *S. cerevisiae*. In the fermentation process of high starch products, starch is a significant source of nutrients (Yuliana et al., 2016). The ANOVA showed that the fermentation time significantly (p<0.05) affect the starch content. The fermentation process up to 120 hours will reduce starch content from $56.29 \pm 0.81\%$ to $47.53 \pm 0.93\%$ (Table 1). The lower starch content with the longer fermentation process associated with its use as a

nutrition source. *Saccharomyces cerevisiae* will produce amylase, which will hydrolyze starch into a simpler component (Oboh and Akindahunsi, 2003). Decreased starch content was in line with the increase in the cell number of *S. cerevisiae* up to 72 hours of fermentation.

Dietary fiber content

The result of the dietary fiber content during cassava pulp fermentation is presented in Table 1. The other component found in high amounts on cassava pulp is fiber in the form of dietary fiber of 47.1% db (Raupp et al., 2004), that is composed of pectin (10.11%), hemicellulose (21.8%), and cellulose (6.31%) (Nurdjanah and Elfira, 2009). Pectin is a soluble dietary fiber, whereas cellulose and hemicellulose are insoluble dietary fiber (Chawla and Patil, 2010). Dietary fiber content of cassava pulp flour on various fermentation time is shown in Table 1. The fermentation time significantly (p<0.05) affect the dietary fiber content. The fermentation process up to 120 hours will reduce dietary fiber levels from 27.92 \pm 0.27% to 17.44 \pm 0.63%. The lower dietary fiber content with the more extended the fermentation process was related to the metabolic activity of Saccharomyces cerevisiae, which will elaborate the dietary fiber into a digestible component. Dietary fiber content decline was in line with the increase of cell number until the fermentation time was 72 hours.

Cyanide content

The change of cyanide content from 0 hours to 120 hours of fermentation is presented in Table 1. The ANOVA showed that the fermentation time significantly (p<0.05) affect the cyanide content. Until 120 hours of fermentation, cyanide content will decrease from $19.89 \pm 0.70\%$ to $8.78 \pm 0.67\%$ (Table 1). The lower the cyanide content by the more extended the fermentation process was related to the

decomposition of the component by the *S. cerevisiae* (Etsuyankpa et al., 2015; Gunawan et al., 2015). As growth increases, the metabolic activity of *S. cerevisiae* will produce linamarase enzymes that will decompose cyanides into non-toxic compounds (Etsuyankpa et al., 2015). Cyanide content of fermented cassava pulp flour of 8.87 ppm qualifies its use as food (Codex Alimentarius, 1989).

Protein content

The result of the protein content from 0 hours to 120 hours of fermentation is presented in Table 1. One of the main obstacles to the use of cassava products is their low protein content (Stupak et al., 2006). The protein content of cassava pulp flour on various fermentation time is shown in Table 1. The fermentation time significantly (p<0.05) affect protein content. The fermentation process up to 120 hours will increase protein content from $1.20 \pm 0.04\%$ to $7.07 \pm$ 0.10% (Table 1). The higher the protein content with the more prolonged the fermentation time was related to the higher number of the biomass of S. cerevisiae cells that formed. Biomass S. cerevisiae is a single cell protein that will increase protein content in fermented cassava pulp flour (Oboh and Akindahunsi, 2003). The single cell protein has high digestibility and had no negative hematological effect (packed cell volume, red blood cell counts and white blood cell counts) (Oboh and Akindahunsi, 2005). Compared with the cell number data in Fig 1, it can be seen that the protein content of fermented cassava pulp will 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. This fact showed that from 72 h fermentation, cell biomass was a combination of the number of viable cells and dead cells.

SD), 70 ury basis							
Fermentation time	Starch (%)	Dietary fiber (%)	Cyanide (ppm)	Protein (%)			
0 hours	56.29 ± 0.81 a	27.92 ± 0.27 a	19.89 ± 0.70 a	$1.20 \pm 0.04 \text{ e}$			
24 hours	55.28 ± 0.66 a	$26.84\pm0.50\ b$	12.03 ± 1.07 a	$2.77 \pm 0.07 \text{ d}$			
48 hours	$53.76 \pm 0.97 \text{ b}$	23.44 ± 0.22 c	$9.99\pm0.98~b$	$4.44\pm0.44~c$			
72 hours	$51.68 \pm 0.92 \text{ c}$	22.17 ± 0.33 d	$9.53 \pm 0.75 \text{ b}$	$6.19\pm0.05~b$			
96 hours	$49.69 \pm 0.21 \text{ d}$	$18.53 \pm 0.85 \text{ e}$	$8.87\pm0.82~c$	6.98 ± 0.29 a			
120 hours	$47.53 \pm 0.93 \text{ e}$	$17.44 \pm 0.63 \text{ f}$	$8.78 \pm 0.67 \ c$	7.07 ± 0.10 a			

Table-1: The effect of fermentation time on chemical composition of fermented cassava pulp flour (mean ± SD), % dry basis

Values with the same letters were not significantly different ($P \ge 0.05$)

The increase in protein content during cassava pulp fermentation was in line with the results of a study conducted by Olaoye et al. (2015), Adepoju et al. (2010), Owuamanam et al. (2010a, 2010b) during the fermentation process of the garri. The increased protein content of cassava pulp flour during the fermentation process using *S. cerevisiae*, also reported by Kaewwongsa et al. (2011).

Amylographic profile

The amylographic profile of cassava pulp flour from 0 hours to 120 hours of fermentation observed by the brabender method is presented in Table 2 and Fig. 2. The fermentation activity of *S. cerevisiae* up to 120 h will cause changes in the amylographic profile of cassava pulp flour for viscosity profile but does not affect the gelatinization temperature profile.

Table-2: Amylographic profile of fermentedcassava pulp flour on various fermentation time

Fermentation time	PV (BU)	Trough (BU)	BV (BU)	FV (BU)	SB (BU)	PT (min)	P. Temp. (°C)
0 hours	183	152	31	202	57	30.42	92.8
24 hours	183	150	33	196	54	30.06	92.9
48 hours	159	120	39	169	53	29.26	93.3
72 hours	124	105	19	148	46	29.36	93.3
96 hours	87	59	28	94	39	27.00	90.2
120 hours	68	55	13	80	28	29.32	93.1

*PV = Peak viscosity, BV = Breakdown viscosity, FV = Final viscosity, SB = Setback, PT = Peak time P. temp. = Peak temperature, BU = Brabender unit

Fermentation up to 120 h will reduce peak viscosity (183 BU to 68 BU), trough viscosity (152 BU to 55 BU), breakdown viscosity (31 BU to 13 BU), final viscosity (202 BU to 80 BU) and setback viscosity (57 BU to 28 BU). This change in viscosity profile was mainly related to a decrease in starch content due to S. cerevisiae fermentation activity, from 56.29% to 47.53% (Table 1). This fact was also reported by Zaidul et al. (2007), which states that the increase in viscosity of wheat flour-sweet potato-cassava starch composite was associated with an increase in starch content. The decrease in starch content of cassava pulp flour during the fermentation process was related to the use of starch as a source of nutrition by S. cerevisiae. Compared with cassava flour, which has a peak viscosity of 900 BU (Hidavat et al., 2009) fermented cassava pulp flour has a lower peak viscosity (68 BU--183 BU). The low value of peak

viscosity shows that fermented cassava pulp flour has a low ability to expand during the heating process (Marta et al., 2016).

The research results also showed that the fermentation time did not affect peak temperature (90.2°C--93.3°C). This was in line with the research by Eduardo et al. (2013), who reported that the fermentation process and drying method did not affect the gelatinization temperature characteristics of cassava starch granules.



Figure-2: The effect of fermentation time on amylographic profile of fermented cassava pulp flour

Granular morphology

The granular morphology of cassava pulp flour from 0 hours to 120 hours of fermentation observed by SEM method is presented in Fig. 3. In the initial condition (fermentation time of 0 h), most starch granules were in a state that joins other starch granules and were covered by a fiber matrix. Covert starch granules by a fiber matrix cause imperfect starch extraction so that it still leaves starch in cassava pulp. According to Nurdjanah and Elfira (2009), dietary fiber of cassava pulp is composed of pectin (10.11%), hemicellulose (21.8%), and cellulose (6.31%). The activity of S. cerevisiae, which seeks to use starch as a source of nutrition, will produce cellulase enzymes which will cause damage and opening of the fiber matrix that envelops the aggregate of starch granules and causes liberation of starch. During the fermentation process, S. cerevisiae will produce various extracellular enzymes to metabolize the components of cassava pulp as a source of nutrition for its growth (Oboh and Akindahunsi, 2003). Enzymatic activity of decomposition of starch and dietary fiber as a source of nutrition causes S. cerevisiae to grow well until the fermentation time of 72 hours.

Damage to the fiber matrix structure by *S. cerevisiae* activity during the fermentation process was in line with the opinion of Sriroth et al. (2000) that enzymatic or physical methods can damage the fiber matrix in cassava pulp. This phenomenon was also reported by Eduardo et al. (2013) in the fermentation process of cassava flour. This shows that during the solid-state fermentation of cassava pulp, *S. cerevisiae*, in addition to producing enzymes that will hydrolyze starch, also produces enzymes that can decompose fiber components.

Starting from the 48-hours fermentation time, the liberated starch granules (coming out of the fiber matrix structure) began to break down into smaller granules. The longer the fermentation, the more granular breakdown occurs. Since 48 hours of fermentation, starch granules have started to appear not intact, cracked and broken into smaller granules. The process of breaking starch granules was strongly related to the use of starch as the primary nutrient source by S. cerevisiae which was shown by the increase in cell number to 72 hours of fermentation (Fig. 1). The more intensive the breakdown of granules with the more prolonged the fermentation process, also reported by Marcon et al. (2006) on fermentation of sour starch.



Figure-3: The Granular morphology of fermented cassava pulp flour on various fermentation time (*scanning electron microscope* method)

Conclusion

Saccharomyces cerevisiae was able to grow logarithmic up to 72 hours of fermentation with starch and dietary fiber as a nutrition source and causes changes in viscosity profile and granular morphology. The increase in the total number of cells (viable cell and dead cell) will increase single cell protein biomass, which will increase the protein content to 7.07% and reduce cyanide content to 8.78 ppm.

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Ir. Hidayat B: Author of the manuscript and advised on experimental design and technical aspect. Hasanudin U: Author of the manuscript and advised on the technical aspect. Akmal S: Author of the manuscript and conducted laboratory work. Muslihudin M: Author of the manuscript and

conducted laboratory work.

