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Comparative influence of salinity and temperature on cassava flour product by *Lactobacillus plantarum* and *Lactobacillus acidophilus* during single culture fermentation

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Abstract. *Frediansyah A, Kurniadi M. 2016. Comparative influence of salinity and temperature on cassava flour product by* Lactobacillus plantarum *and* Lactobacillus acidophilus *during single culture fermentation. Nusantara Bioscience 8: 207-214.* *Lactobacillus plantarum* (FNCC 0027) and *Lactobacillus acidophilus* (FNCC 0051) were used as a single culture in cassava fermentation. The effect of salinity (0.5, 1, 2 and 3% (v/v)) and temperatures (4, 25, 30 and 40ºC) were investigated on the survival of these lactobacilli. In addition, fiber and moisture content were used to study the characteristic of cassava flour as a final product. About 6.98 cfu/g of *L. plantarum* and 7.02 cfu/g of L acidophilus were used as a single starter (t = 0 h) for cassava fermentation. After 15 h, the survival rate of both lactobacilli with the salinity of 0.5% and incubation at 40ºC were found to be at the highest compared with others. The temperature of incubation was showed in a degree-dependent manner in both reductions of pH to the substrate and fiber contents of flour product. However, it increased the survival of both *L. plantarum* and *L. acidophilus* in a degree of temperature-dependent manner. Both salinity and temperature did not give significant effect to further changing of moisture content. The fermentation of cassava could improve proximate composition, cyanide content and physical properties of cassava flour product.

Keywords: Cassava, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, salinity, temperature
Abbreviations: CF: crude fiber, DF: dietary fiber, MRSA: deMan Rogosa and Sharpe agar, MRSB: deMan Rogosa and Sharpe broth, CSM: skim milk from cow, LAB: lactic acid bacteria

INTRODUCTION

Cassava is one of the promising carbohydrate sources for Indonesian people since Indonesia is the 4th largest cassava producer worldwide with the total production up to 20 million tons per year (Ministry of Agriculture of Republic of Indonesia 2009). However, it is often considered as a food source for poor people. Most Indonesian people still depend on rice and wheat flour as carbohydrate sources. Unfortunately, the increase of their consumptions is incommensurate to their productions nationally. The rice consumption is 140 kg/capita/person (Indonesia-Investment 2006) and wheat flour is 18.5 kg/capita/years (USDA Foreign Agricultural Service 2014). The recent fact, Indonesia is the 3rd largest country for global rice production, but it is still the world's 7th largest rice importer (Indonesia-Investment 2006). Moreover, the presence of wheat flour still depends on import from western countries (USDA Foreign Agricultural Service 2014).

With the total population of about 260 million, the country needs sufficient food supply. Indonesia has other carbohydrate sources such as cassava. In the food industry, cassava can be processed into high-value products. These products are dried cassava chips, tapioca, sweetener (i.e., sorbitol), and modified cassava flour (Frediansyah et al. 2012; Oboh et al. 2002). Modified cassava flour is a recent trend for the Indonesian food industry. It is gluten-free and can substitute white flour to produce several food products, such as bread, pastries, and noodles. Gluten-free materials are good sources for people who have celiac disease, a genetically disorder on low tolerance of gluten ingestion. The presence of gluten in celiac people may damage the mucosa of the small intestine and produce a variety of sign and symptoms (La Vieille et al. 2016; Adriaanse et al. 2016).

The key for modified cassava flour production is culture starter. It contains large number of single or various microorganisms to accelerate the fermentation process. The culture will produce an extracellular enzyme which is important to breakdown the cassava's cell wall and to hydrolyze polysaccharide into simple sugar. Those bacterial enzymes including cellulase, hemicellulase, amylase and pectinase (Frediansyah 2011; Frediansyah and Sudiana 2013; Wahono et al. 2015; Adetunji et al. 2016). Lactic acid bacteria, is generally recognized as safe microorganism, is naturally found in nature. It has the ability to adapt and present in various environmental conditions including food matrices such as vegetables, fruits, dairy products, and meat; environment such as water, soils, and river; and a human mucosal surface such vagina, gastrointestinal tract and oral cavity. It is also widely used in food fermentation both on large industry or home industry. *Lactobacillus plantarum* has been found to

be the most dominant bacteria found during natural fermentation of cassava. In contrast, *Lactobacillus acidophilus* is very limited (Oyedeji et al. 2013). There are many types of starter which have been applied and some of them have been patented (Gunawan et al. 2015; Kostinek et al. 2007; Leroy and De Vuyst 2004). Previous studied showed that *L. plantarum* could be used as single culture starter for modified cassava flour production (Frediansyah et al. 2012). However, the study of fermentation conditions such as salinity and temperature and the correlation to the final products are still limited. Therefore, the objective of this research is to analyze the effect of salt concentration and temperature incubation on fermentation process of cassava by *L. plantarum* and *L. acidophilus* and to analyze some parameters of its product, including dietary fiber, crude fiber, moisture, and ash.

MATERIALS AND METHODS

Cassava tuber and media

Markonah cultivars, low-cyanide of white cassava tubers, were obtained from the local farm around Kudus, Central Java, Indonesia with diameter and length of 3-4 cm and 60-90 cm, respectively. CSM was purchased from Intisari Baker Smart, while MRSB and MRSA were obtained from Oxoid.

Bacterial culture and its preparation as a single culture starter

Lyophilized cells of *L. plantarum* FNCC 0027 and *L. acidophilus* FNCC 0051 were obtained from the culture collection of Biotechnology Laboratory, Graduate School of Biotechnology, Universitas Gadjah Mada, Yogyakarta, Indonesia. These freeze-dried cells were prepared by sub-culturing and growing the cells in micro-tube containing 1.5 mL MRSB which had been adjusted to pH 6.7. This is done two times to provide conditioned strain in MRS medium. And, the purity of culture was checked by gram staining and visualized under the optical microscopes. After incubation for 24 h at 37ºC, cell growth in the cultures were placed on MRSA by streak plate method and incubated at 37ºC for 48 h. The grown of single colony was harvested and centrifuged (10,000 x g, 2 min) with phosphate buffer saline. Supernatant was then discarded and cells were transferred into pasteurized medium contain skim milk and were incubated at 37ºC for 15 h. The temperature of pasteurization follows the modification method of Rynne et al. (2004). In brief, the CSM was mixed with starch (15:1) using hot plate stirrer. It was then pasteurized for 1 min at 77ºC. The initial concentration of *L. plantarum* FNCC 0027 and *L. acidophilus* FNCC 0051 after incubation was 6.98 and 7.02 log CFU/mL, respectively. These cultures were used as single culture starter for cassava fermentation.

Cassava chip production

Cassava chips were produced using the method as described by Frediansyah et al. (2012). In brief, fresh cassava tubers were peeled and cut into rounded pieces with a thickness of about 0.5 cm. The peeled tubers were then washed with distilled water.

Cassava fermentation

Submerged fermentation (involving soaking in water) was used for the initial step of cassava flour production using a method as described by Frediansyah et al. (2012) with minor modifications. In order to study the relationship between salinity and temperature during fermentation, completely randomized design has been applied. Several parameters were then measured including bacterial growth and physicochemical of the flour. Briefly, 150 g of cassava chips were soaked with 250 mL of distilled water in 1 L baker glass with various concentration of NaCl (0.5, 1, 2 and 3% (v/v)), followed by the addition of 1 mL single culture starter in 4 mL sterilized water. The mixture was then incubated at various temperature (4, 25, 30 and 40ºC).

Cassava flour production

Fermented chips were dehydrated using a drying machine at 30ºC for 18 h. Dried chips were then milled to produce a grayish-white flour and were sieved by 80 mesh sifter in order to achieve the standard size of commercial flour.

Determination of growth rate

Bacterial count was enumerated by taking 10 mL of filtrate from fermentation of cassava, serially diluted and stirred vigorously. Aliquots of 100 µl were transferred and placed onto MRSA, followed by incubation at 37ºC for 24 h. Cell survival was calculated as (Khem et al. 2016):

$$\% \text{ survival} = N/N_o \times 100$$

Where, N and N_o are number of cells after and before cassava fermentation in CFU/mL.

pH analysis

The pH of the filtrate obtained from fermentation was analyzed using Eutech PC 700 (Thermo Scientific, IL, USA). The pH meter was calibrated using pH 4.0 and 7.0 buffers before measurement.

Proximate analysis

Ash of cassava flour was determined by AOAC941.12 (2000). The moisture content of cassava flour was analyzed by moisture analyzer. About 1 g of flour were spread on an aluminum pan and the sample was heated to 100ºC and held until the mass changing into less than 1 mg for 90 s was achieved. Crude protein was determined using Kjeldahl method using a factor of 6.25 as described by Pearson (1973). Crude fat was determined by acid hydrolysis method as described by AOAC 922.06 (2005). Carbohydrate content was calculated by subtracting the percent of moisture, crude protein, crude fat, ash, and CF.

CF and DF analysis

Crude fiber (CF) content was analyzed using determined by gravimetrically-chemical digestion method

as described by Williams and Starkey (1982). In brief, 3 g of flour sample (Wo) was mixed with 200 mL of 0.25 N of sulfuric acid in beaker glass containing boiling chips. After it reached the boiling point, the mixture was then boiled under reflux on the crude fiber boiling apparatus. Then, it is followed by the addition of 407.42 g/L of sodium hydroxide solution. The mixture was then boiled for 30 min. The solution was subsequently filtered through sintered silica crucible, washed free of alkali with boiling water, and the residue was then transferred to the filter crucible. The crucible was dried for 1 h at 100ºC and weighted (W1), followed by cooling process in desiccators and placed overnight in a muffle furnace at 600ºC. The residual ash was cooled in oven overnight at 105ºC, followed by cooled to room temperature in desiccators and reweighed (W2). The percentage of CF was calculated with formula as described below:

$$\% CF = [(W_1-W_2)/Wo] \times 100$$

Total DF and insoluble DF content were analyzed by an enzyme with gravimetric method as described by Prosky et al. (1992) with minor modifications. Briefly, 0.5 g of flour samples were digested with thermophilic alpha-amylase (Sigma) for 25 min at pH 6 with boiling temperature and left for cooling. After cooling, the flour sample was adjusted to pH 4.6 and incubated with amyloglucosidase at 60ºC for 30 min. Then, precipitated and pre-weighed containing celite (Sigma) was used to filter the phase digested by the enzymes. The residue was then washed with acetone and ethanol. Soluble DF was determined in the combined filtrate and washing solution from Insoluble DF step as described above. Total DF was assumed as the sum of insoluble DF and soluble DF fractions.

HCN, whiteness and viscosity analysis

Hydrogen cyanide (HCN) content was determined by spectrophotometric alkaline picrate method according to Williams and Edwards (1980). Absorbance was measured at 510 nm. Whiteness degree was measured using Minolta Chroma Meter as described by Wheat Marketing Center (2004). Five grams of cassava flour was placed on the attachment of the granular material and compacted. The Chroma Meter was then inserted into granular material attachment followed by measurement. Viscosity was analyzed using a method by Mosha and Svanberg (1983) and Wheat Marketing Center (2004) with slight modifications. Seven grams of cassava flour was added with 25 mL of distilled water. The slurry mixture was then constantly homogenized in a water bath (100ºC) for 30 min. The beaker glass containing cassava flour was then transferred to a new water bath at 45ºC. The spindle number 2 from Brookfield DV-E viscometer at 45ºC was used. The effect of stirring speed was determined at 12 rpm.

Statistical analysis

All the triplicate data were subjected to analysis of variance (ANOVA). The mean was then separated using Tukey's HSD ($p \leq 0.05$).

RESULTS AND DISCUSSION

Effect of salinity and temperature on bacterial cell survival and pH of cassava medium

The combination of salinity (0 to 3%) and temperature of incubation (4 to 40ºC) were employed in the experimental design using *L. plantarum* and *L. acidophilus* as a single culture starter. These experiments were chosen to assess whether the difference in temperature and salinity of fermentation cassava medium of *L. plantarum* and *L. acidophilus* affected bacterial growth and the pH. The survival of bacterial cell and pH after 15 h and cassava fermentation at five different NaCl concentrations and four different incubations are presented in Table 1 and Table 2 and the result of bacterial cell survival of *L. plantarum* and *L. acidophilus* ranged from 86.10-118.48% and 88.58-124.64%, respectively. The survival of some strains is greater than 100% due to the optimal condition for their growth. In addition, the time, up to 18h, is still in the range of lag phase. The pH of a substrate after 15 h fermentation was 4.61-6.87 for *L. plantarum* and 3.17-6.54 for *L. acidophilus.* Temperature and salinity affected significantly ($p < 0.05$) to both bacterial cell survival and pH medium after 15 h. The treatment with 0.5% NaCl before fermentation showed higher bacterial cell survival on both *L. plantarum* and *L. acidophilus*. Higher salinities reduced bacterial survival, although they were non-lethal. Arab et al. (2016) stated that maximum viability of *L. acidophilus* LA-5 was shown at medium with 0.5% NaCl/KCl. The suitable temperature for survival was 40ºC for both *L. plantarum* and *L. acidophilus,* while at lower temperatures the survival was reduced without lethality. The finding is in line with Park and Lim (2015); Kim et al. (2009) and Karthikeyan and Santosh (2009). The optimum incubation temperature for bacterial survival depended on salinity (40ºC for both *L. plantarum* and *L. acidophilus* at 0.5%). In the absence of salt, both lactobacilli were able to survive normally, although the survival was not optimum as in 0.5%.

Effect of single culture starter with salt and temperature treatment on final moisture content and ash

The moisture content is a valuable constituent in flour product which provides an indication of shelf stability. The treatment using salinity and incubation temperature on single culture starter during bioprocess of cassava did not affect significantly to the moisture content of cassava flour. The water content of cassava flour product with *L. plantarum* was 8.28-9.04% and 8.33-8.85% dry basis moisture for *L. acidophilus* as presented in Table 1 and Table 2. The result showed that the water content of cassava flour products depends on dehydration process of chips and milling process during cassava flour production. This is an agreement with Ojokoh et al. (2014) who also reported that the water content of flour products depends on the temperature and duration of the drying process. Ngamnikom and Songsermpong (2011) reported that different types of grinding were also contributed to the flour moisture. However, cassava flour products had less than 10% moisture. According to Frediansyah et al. (2012),

it was lower than the water content of modified cassava flour. It also was lower than on wheat flour (10.74%) and arrowroot flour (11.37%) (USDA 2016). The maximum moisture content of modified cassava flour according to SNI 7622-2011 is 13%. The low water content means a good keeping in quality of the flour products (Ojokoh et al. 2014; Syamaladevi et al. 2016).

The ash content of cassava flour product with *L. plantarum* and *L. acidophilus* as a single culture starter was 0.46-2.88% and 0.71-4.74%, respectively (Table 1 and Table 2). These values decreased significantly as the increasing temperature in various salinities of media. The ash values observed in this study (at temperature 30ºC) were similar in range with ash content of fufu reported by Sobowale et al. (2007). Reduced ash content has been reported in *Afzelia africana* flour (Igbabul et al. 2014), in fermented *Parkia biglobosa* seed (Ojewumi et al. 2016), and in fufu fermentation (Sobowale et al. 2007). However, increased ash content has been reported in fermented maize (Gernah et al. 2011) and fermented mung bean (*Vigna radiata*) using spontaneous and back-slopping fermentation (Onwurafor et al. 2014).

Effect of single culture starter with NaCl and temperature treatment on dietary fiber and crude fiber

The treatment using NaCl and incubation temperature on both *L. plantarum* and *L. acidophilus* during bioprocess of cassava has affected significantly to the CF and DF of cassava flour products. CF contents ranged from 0.99-4.18% for *L. plantarum* and 1.13-5.18% for *L. acidophilus* (Table 1 and Table 2). Increasing the temperature of each cassava medium containing single culture bacteria (*L. plantarum* or *L. acidophilus*) decreased significantly to CF contents of cassava flour products. Ojokoh et al. (2014) and Ojewumi et al. (2016) also reported a reduction in CF with fermentation. The total DF and insoluble DF contents followed a similar trend. The temperature of incubation showed a degree-dependent manner in the reduction of DF. Total DF and insoluble DF content ranged from 3.79-9.26 and 3.13-8.56%, respectively, for *L. plantarum* and 3.76-9.37% and 3.29-8.24, respectively, for *L. acidophilus* (Table 1 and Table 2). The DF is defined as the part of food materials which are neither digested nor absorbed by small intestine track and reaches the colon as a substrate for fermentative micro-flora.

The reduction of CF and DF content in the cassava flour products could be to attribute to the enzymatic breakdown of the fiber during fermentation by lactic acid bacteria (Ojokoh et al. 2013; Adetunji et al. 2016). Both *L. plantarum* and *L. acidophilus* utilized carbohydrate and non-digestible plant component (fiber) for their growth and metabolism (Nout 1991), thus improving food utilization efficiency and nutritional quality. This is an agreement with the findings of many researchers such as Sobowale et al. (2007), who reported that fermentation decreased the concentration of fiber as a result of utilization by microbes.

Effect of fermentation on proximate composition, HCN and physical properties of cassava flour

Fermentation by both *L. plantarum* and *L. acidophilus* resulted in an increase in crude protein content (2.75-3.23/3.70%) whereas there was a decrease in content of crude fat (0.48-0.23/0.33%), carbohydrate (87.23-86.21/85.36%), ash (1.75-0.46/0.83%), and fiber (2.37-0.99/1.01%), however, the moisture content was in steady state as showed in Table 3. There was an increase in crude protein content after cassava fermentation. A similar trend has been made by Gernah et al. (2011), Amankwah et al. (2009) who reported an increase in crude protein content of maize. Onwurafor et al. (2014) and Amadou et al. (2014) were also reported that the increase of protein content had been observed in mung bean and foxtail millet (*Setaria italica*) flour, respectively. In contrast, reduced protein content has been reported in fufu flour (Sobowale et al. 2007). The increasing of crude protein with fermentation could be due to an increase in the number of lactic acid bacteria and its metabolic activities (Ojokoh et al. 2013). In addition, Amankwah et al. (2009) reported that proteolytic activities by lactic acid bacteria were increased during fermentation. *L. plantarum* (Fadda et al. 2002; Khalid and Marth 1990) and *L. acidophilus* (Kabadjova-Hristova et al. 2006; Bergamini et al. 2009; Fung and Liong 2010) had the ability to degrade the protein in their substrate to an amino acid or other simple peptides. Furthermore, their activities could improve functionality and protein composition of flour products (Taylor et al. 2016; Skudra et al. 1998). However, the protein content of fermented cassava flour was lower than Jerusalem artichoke tubers (6.36%; Cieśliket al. 2011), wheat flour (9.8%; Akubor and Badifu 2004) and pearl millet (11.4%; Oshodi et al. 1999). Consequently, cassava flour cannot be considered as valuable of protein sources.

As regard to the crude fat content, the fermented cassava flour had lower crude fat content than the unfermented. This is in agreement with Safitri (2014), Ojewumi et al. (2016) and Onwurafor et al. (2014). The decrease may be due to the breakdown of fatty acid and glycerol during fermentation. It has resulted in the production of taste, aroma, and odor. Another possibility was releasing the fat from cassava cells due to the presence of fermentative bacteria. The fat content of cassava was in range of 0.74 to 1.49% (Emmanuel et al. 2012). The reduction of lipid content increased the shelf life of flour product. However, it would not be a good source of oil. Lipid peroxidation could be observed by typical rancid aroma is one of parameter that could be used as shelf life prediction, quality parameter and safety implications (López-Duarte and Vidal-Quintanar 2009).

The carbohydrate content of fermented cassava flours (86.95 and 86.36%) was lower compared to unfermented (87.55 %). The reduction was due to the utilization of some sugars for the growth and various metabolic activity by *L. plantarum* and *L. acidophilus*. The downward in carbohydrate level due to the presence of lactic acid bacteria agrees with the work of Refstie et al. (2005). The decrease may also be attributed to the conversion of carbohydrate to glucose. Furthermore, it will use as an energy source for lactic acid bacteria and further converted to lactic acid (John et al. 2007). The high amount of carbohydrate of both fermented and unfermented cassava flour could be used as a good source of energy.

Table 1. Effect of salinity and temperature on fermentation activity and characteristic of cassava flour using *L. plantarum*

Treatment		Fermentation activity	Cassava flour analysis (moist base)						
Salinity (%)	Temp (ºC)	Bacterial survival (%)	pH	Moisture (%)	DF (%)			CF (%)	Ash (%)
					Insoluble	Soluble	Total		
0	4	95.56	6.75 ± 0.16^{e}	8.55 ± 0.10^{a}	7.90 ± 0.08^{ef}	1.36 ± 0.04^{g}	9.26	3.19 ± 0.05^{gh}	2.75 ± 0.06^{ij}
	25	100.43	5.69 ± 0.04^{cd}	8.43 ± 0.12^{ab}	6.37 ± 0.44^{cde}	1.40 ± 0.06^{g}	7.76	2.75 ± 0.17^{cf}	2.32 ± 0.04^{fg}
	30	111.17	5.65 ± 0.05^{cd}	8.36 ± 0.05^{a}	5.20 ± 0.55^{b}	0.68 ± 0.10^{abc}	5.88	1.75 ± 0.09^{b}	1.39 ± 0.04^{bc}
	40	116.76	4.83 ± 0.07^{a}	8.28 ± 0.08^{a}	3.30 ± 0.06^{a}	0.59 ± 0.03^{a}	3.89	1.05 ± 0.06^{a}	0.65 ± 0.05^{ab}
0.5	4	96.13	6.73 ± 0.13^{e}	8.44 ± 0.11^{ab}	7.75 ±0.12^{f}	0.33 ± 0.10^{g}	9.08	3.16 ± 0.14^{gh}	2.65 ± 0.10^{f}
	25	102.87	5.73 ± 0.08^{cd}	9.04 ± 0.25^{b}	6.41 ± 0.44^{de}	1.32 ± 0.01^{g}	7.74	2.71 ± 0.06^{efg}	2.24 ± 0.08^{f}
	30	117.62	5.69 ± 0.04^{cd}	8.59 ± 0.17^{ab}	5.63 ± 0.05^{bcd}	0.80 ± 0.07^{cde}	6.43	2.17 ± 0.12^{c}	1.65 ± 0.10^{d}
	40	118.48	4.61 ± 0.04a	8.88 ± 0.31^{ab}	3.13 ± 0.05^{a}	0.66 ± 0.05^{ab}	3.79	0.99 ± 0.02^{a}	0.46 ± 0.04^{ab}
1	4	93.12	6.82 ± 0.08^{e}	9.00 ± 0.51^{bab}	7.65 ± 0.12^{f}	1.43 ± 0.02^{g}	9.08	3.21 ± 0.05^{gh}	2.88 ± 0.11^{j}
	25	95.99	5.83 ± 0.14^{cd}	8.45 ± 0.11^{ab}	6.32 ± 0.14^{cde}	1.03 ± 0.08^{f}	7.35	2.55 ± 0.10^{ef}	2.18 ± 0.06^{f}
	30	96.99	5.71 ± 0.05^{cd}	8.84 ± 0.36^{ab}	5.59 ± 0.35^{bc}	0.74 ± 0.13^{abc}	6.33	2.19 ± 0.07^{c}	1.74 ± 0.07^{cde}
	40	100.43	5.31 ± 0.13^{b}	8.44 ± 0.10^{ab}	3.66 ± 0.09^{a}	0.75 ± 0.05^{abc}	4.41	1.56 ± 0.11^{b}	1.23 ± 0.07^{b}
2	4	89.11	6.87 ± 0.05^{e}	8.48 ± 0.25^{ab}	8.14 ± 0.53^{ef}	1.27 ± 0.04^{g}	9.34	3.32 ± 0.13^{gh}	2.75 ± 0.23^{ij}
	25	89.26	5.86 ± 0.09^{cd}	8.53 ± 0.18^{ab}	7.85 ± 0.33^{f}	1.29 ± 0.06^{g}	9.14	3.15 ± 0.08^{h}	2.74 ± 0.12^{hj}
	30	89.54	5.72 ± 0.02^{cd}	8.59 ± 0.16^{ab}	7.56 ± 0.16^{cde}	0.85 ± 0.04^{def}	8.41	2.82 ± 0.14^{ef}	2.39 ± 0.08^{fgh}
	40	90.40	5.58 ± 0.05^{c}	8.56 ± 0.17^{ab}	6.30 ± 0.06^{f}	0.89 ± 0.09^{def}	7.19	2.40 ± 0.13^{cd}	2.10 ± 0.06^{ef}
3	4	86.68	6.86 ± 0.09^{e}	8.68 ± 0.10^{ab}	8.56 ± 0.03^{f}	1.38 ± 0.11^{g}	9.94	4.18 ± 0.06^{i}	3.38 ± 0.28^{j}
	25	88.58	5.84 ± 0.12^{d}	8.63 ± 0.17^{ab}	7.51 ± 0.07^{e}	1.46 ± 0.07^{g}	8.98	2.99 ± 0.19^{fg}	2.70 ± 0.36^{hij}
	30	88.58	5.81 ± 0.03^{d}	8.49 ± 0.13^{ab}	6.64 ± 0.31^{b}	0.99 ± 0.02^{ef}	7.63	2.69 ± 0.06^{efg}	2.26 ± 0.02^{hij}
	40	86.10	5.51 ± 0.06^{cd}	8.67 ± 0.18^{ab}	5.28 ± 0.05^{b}	0.95 ± 0.05^{def}	6.23	2.20 ± 0.07^{c}	2.02 ± 0.06^{def}

Note: Different letters in each column are statistically significant of each other (Tukey's HSD, $p \leq 0.05$). Each data (Mean ± SD) was calculated from an average of three independent replicates

Table 2. Effect of salinity and temperature on fermentation activity and characteristic of cassava flour using *L. acidophilus*

Treatment		Fermentation activity	Cassava flour analysis (moist base)						
Salinity (%)	Temp (ºC)	Bacterial survival (%)	pH	Moisture (%)	DF (%)			CF (%)	Ash (%)
					Insoluble	Soluble	Total		
0	4	99.43	6.37 ± 0.06^{gh}	8.65 ± 0.09^{ab}	8.24 ± 0.11^{h}	1.13 ± 0.12^{de}	9.37	3.74 ± 0.23^{e}	3.44 ± 0.10^{g}
	25	104.56	4.10 ± 0.05^{bc}	8.52 ± 0.08^{ab}	7.53 ± 0.07^{g}	1.45 ± 0.06^{f}	8.99	3.68 ± 0.17^{de}	3.37 ± 0.12^{g}
	30	122.79	3.87 ± 0.11^{b}	8.40 ± 0.03^{ab}	6.14 ± 0.44^{de}	0.56 ± 0.16^{ab}	6.70	1.48 ± 0.12^{abc}	1.24 ± 0.08^{b}
	40	123.36	3.17 ± 0.05^{a}	8.33 ± 0.03^{a}	3.40 ± 0.06^{a}	0.35 ± 0.06^{a}	3.76	1.01 ± 0.03^{de}	0.71 ± 0.04^{a}
0.5	4	98.15	6.31 ± 0.08^{gh}	8.55 ± 0.10^{ab}	7.68 ± 0.24^{g}	1.29 ± 0.06^{ef}	8.98	3.40 ± 0.15^{a}	3.18 ± 0.06^{fg}
	25	104.70	4.16 ± 0.11^{bc}	8.85 ± 0.13^{b}	6.31 ± 0.11^{e}	1.32 ± 0.01^{ef}	7.64	3.08 ± 0.15^{de}	2.70 ± 0.05^{e}
	30	123.65	3.37 ± 0.13^{a}	8.54 ± 0.12^{ab}	5.66 ± 0.17^{cd}	0.80 ± 0.05^{bc}	6.47	1.50 ± 0.04^{abc}	1.21 ± 0.04^{b}
	40	124.64	3.19 ± 0.02^{a}	8.57 ± 0.07^{ab}	3.29 ± 0.06^{a}	0.59 ± 0.05^{ab}	3.88	1.13 ± 0.15^{ab}	0.83 ± 0.05^{a}
1	4	98.15	6.36 ± 0.08^{gh}	8.57 ± 0.07^{ab}	7.55 ± 0.12^{g}	1.33 ± 0.03^{ef}	8.88	3.51 ± 0.06^{de}	3.23 ± 0.10^{fg}
	25	104.42	4.34 ± 0.11^{cd}	8.77 ± 0.02^{ab}	5.55 ± 0.10^{bc}	1.10 ± 0.11^{cde}	6.65	1.68 ± 0.15^{abc}	1.31 ± 0.06^{b}
	30	122.65	4.37 ± 0.17^{cd}	8.65 ± 0.10^{ab}	5.15 ± 0.04^{b}	0.74 ± 0.13^{b}	5.89	1.92 ± 0.05^{abc}	1.79 ± 0.07^{c}
	40	121.94	3.30 ± 0.11^{a}	8.73 ± 0.50^{a}	3.71 ± 0.14^{a}	0.85 ± 0.05^{bcd}	4.56	1.51 ± 0.12^{de}	1.23 ± 0.07^{b}
2	4	93.45	6.41 ± 0.06^{gh}	8.34 ± 0.09^{ab}	7.56 ± 0.11^{g}	1.37 ± 0.07^{ef}	8.92	3.34 ± 0.05^{de}	2.97 ± 0.07^{ef}
	25	96.30	4.89 ± 0.02^{e}	8.49 ± 0.06^{ab}	7.33± 0.11f^{g}	1.19 ± 0.04^{ef}	8.52	3.48 ± 0.14^{abc}	3.20 ± 0.03^{fg}
	30	107.12	4.61 ± 0.09^{cd}	8.48 ± 0.03^{ab}	7.43 ± 0.20^{g}	1.07 ± 0.14^{cde}	8.50	3.28 ± 0.26^{de}	3.04 ± 0.07^{f}
	40	107.26	4.26 ± 0.05^{c}	8.56 ± 0.10^{ab}	5.58 ± 0.20^{bc}	1.07 ± 0.12^{cde}	6.65	1.54 ± 0.02^{abc}	1.28 ± 0.03^{b}
3	4	90.31	6.54 ± 0.17^{h}	8.55 ± 0.10^{ab}	8.53 ± 0.14^{h}	1.48 ± 0.11^{f}	10.01	5.18 ± 0.95^{d}	4.74 ± 0.07^{h}
	25	91.45	6.38 ± 0.06^{gh}	8.58 ± 0.17^{ab}	7.35 ± 0.09^{fg}	1.44 ± 0.01^{f}	8.79	3.76 ± 0.22^{e}	3.40 ± 0.14^{g}
	30	88.58	6.11 ± 0.10^{g}	8.58 ± 0.12^{ab}	6.87 ± 0.06^{f}	1.23 ± 0.06^{ef}	8.10	2.90 ± 0.03^{d}	2.24 ± 0.08^{d}
	40	91.60	5.26 ± 0.06^{f}	8.54 ± 0.11^{ab}	5.29 ± 0.08^{g}	1.85 ± 0.23^{bc}	7.14	3.25 ± 0.19^{bc}	2.79 ± 0.03^{e}

Note: Different letters in each column are statistically significant of each other (Tukey's HSD, $p \leq 0.05$). Each data (Mean ± SD) was calculated from an average of three independent replicates

Table 3. Effect of fermentation on proximate composition, antinutritional and other functional properties of cassava flour

	Proximate composition							Some functional properties	
	Moisture	Crude protein	Crude fat	Carbohydrate	Ash	CF	HCN	Whiteness	Viscosity
UF	8.87	2.75	0.48	87.55	0.35	1.12	5.5	87	142.4
FLP	8.88	3.23	0.28	86.95	0.66	0.99	<3	88	196.6
FLA	8.77	3.7	0.33	86.36	0.83	1.01	<3	89	192.8

Note: UF: unfermented cassava, FLP: fermentation of cassava using *L. plantarum* with treatment of 0.5% NaCl and temperature 40ºC, FLP: fermentation of cassava using *L. acidophilus* with treatment of 0.5% NaCl and temperature 40ºC

The ash and fiber content (0.66/0.83% and 0.99/1.01%, respectively) of fermented cassava flour were higher than unfermented cassava flour. The reduction of ash level in cassava flour could be as a result of perfect utilization of minerals during fermentation. The finding showed a similar after fermentation due to enzymatic breakdown. The trend is similar to the report by Aryee et al. (2006).

The presence of ash content is correlated with valuable mineral sources. The fiber content of cassava flour was decreased bacteria, then, utilized them as carbon sources and converted them into biomass of bacterial bodies. Lactic acid bacteria have been found to exhibit broad range for hydrolytic enzymes (Williams and Bank 1997). There are many types of simple carbon composition as a result of fermentation such as glucose, xylose, galactose, rhamnose, arabinose, and others. However, glucose could be found up to 85% by this process (Adetunji et al. 2016).

The moisture content was in steady state (Table 3). As explained previously, the moisture content depended on drying process during the production of cassava flour. The maximum acceptable level of moisture for flour product was 14% (Butt et al. 2004). The finding showed that both unfermented and fermented cassava flour had lower water content (8.77-8.88%). Lower moisture content in flour products has a correlation to longer shelf life. Oduro et al. (2009) explained that higher water content in flour could enhance spoilage and enzymatic deterioration.

The decrease of HCN content after cassava fermentation may be due to hydrolytic activities of *L. plantarum* or *L. acidophilus* (Table 3). In Indonesia, HCN content of cassava cultivars ranged from 9-234 ppm (Hidayat et al. 2000). The mean total of HCN from 179 cassava cultivars was 82 ppm (Hidayat et al. 2000). Djazuli and Bradbury (1999) reported that the total HCN from 29 cassava flour and other products was 54 ppm. However, Markonah cultivar is one of sweet cassavas with lower HCN content (Frediansyah et al. 2012). Based on the result, the residual cyanide in both fermented and unfermented cassava was below the recommended safe level of 10 mg/kg set by Indonesian authorities (SNI 7622-2011) and lower than the result on the study by Oboh et al. (2002). Agbor-Egbe and Mbome (2006) and Cardoso et al. (2005) explained that the reduction of the endogenous cyanic compound in cassava food could be done by fermentation.

The viscosity of fermented cassava flours (196.6 and 192.8 cP) was lower compared to unfermented (142.4 cP). The similar trend has also occurred in the degree of whiteness of cassava flour products (87 to 88/89). The increasing of viscosity could be as a result of an increase in the effective volume of the protein which generally results from increased molecular asymmetry brought about by a change from highly compact to an elongated random coil. However, it was above the SNI 7622-2011 (87).

To conclude, the temperature and salinity have a significant effect on the survival rate and acid production of both *L. plantarum* and *L. acidophilus*. The temperature showed a degree-dependent manner in the reduction of pH and in the elevation of bacterial survival rate. The optimum condition was found at 40ºC in 0.5% NaCl for bacterial growth. Physicochemical properties of cassava flour depend on the treatment (salinity and temperature) which had been applied during fermentation.

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