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Effect of biodegradation by Lactic Acid Bacteria on physical properties of cassava starch

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Abstract: Sour cassava starch is used as main ingredient in the manucfacture of food that can expand when baked. Lactic acid fermentation has been known to be responsible for the baking expansion properties. Many strains of lactic acid bacteria with different ability to degrade starch has been isolated and used as starter culture in this study. The fermentation effect was investigated with the purpose of establishing a relation between physicochemical changes on cassava starch granule and functional properties of starch. Some lactic acid fermented samples presenting baking property have been compared with lactic acidified and native cassava starch. All the samples were analyzed for its thermal properties and starch granule structure by using Differential Scanning Calorimeter and Scanning Electron Microscopy, respectively. Samples of fermented cassava starch have a higher baking expansion character (8.54-11.51 ml/g) than native starch (6.61 ml/g), which were associated with its lower gelatinization enthalphy and peak gelatinization temperature. The starch granule of all fermented and acidified samples had lost their surface smoothness due to external corrosion and provided a greater extent of starch modification.

Keywords: Sour cassava starch, lactic acid fermentation, thermal property, starch granule structure

Introduction

Cassava is important food crop in Indonesia and potential to be developed into flour or starch as the main ingredient of bread and similar products. But, the starch granule characteristics are needed to be changed so that the ability to expand during baking or frying can be enhanced. Cassava starch is produced from cassava root by extraction, washing, purification and drying. With the help of various modifications, the structure and properties of starch can be changed for diverse applications.

Sour cassava starch is a cassava starch modified by using a fermentative process to improve the specific baking expansion characteristic (Dufour *et al*., 1996). Lactic acid bacteria which produce lactic acid and have an amylolytic characteristics play an important role in the preparation of sour cassava starch. Amylolytic lactic acid bacteria (ALAB) could change the microstructure of starch and induce their amylography and viscosity characteristics (Plata-Oviedo and Camargo, 1998; Bertolini *et al*., 2000; Demiate *et al*., 2000). Due to the ability of their α-amylases to partially hydrolyze raw starch (Reddy *et al*., 2000), ALAB can ferment different types of amylaceous raw material, such as wheat (Naveena, 2004), potato (Chatterjee *et al*., 1997), or cassava (Mestres *et al*., 1997) and different starchy substrates. Some strains of *Lactobacillus* spp. produce extracellular amylase and ferment starch directly to lactic acid.

The degradation of starch granule by lactic acid and ultraviolet (UV) irradiation during fermentation could change the porosity and surface area of the granule and would determine their properties eg thermal conductivity, thermal diffusivity and mass diffusion coefficient (Vatanasuchart *et al*., 2005). The activity of lactic acid bacteria used as starter cultures could influence the magnitude of change on chemical and physical properties of starch. This study was to examine the properties of sour cassava starch produced through lactic acid fermentation compared with lactic acidified and raw cassava starch, with a view to determine their application in food industry.

Material and Methods

Microorganism

The microorganism used were *Lactobacillus plantarum* subsp. *plantarum* AA11, AA2 and UA3 isolated from soaking cassava during *growol* fermentation (Putri *et al*., 2010) and *Lactobacillus amylophyllus* NBRC 15881 (NITE Biological Resources Center, Chiba, Japan). All of these strains had a different ability to grow in starch base medium and to degrade raw starch (Putri *et al*., 2010). *L.*

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amylophylus NBRC 15881 used as an amylolytic reference strain in this study.

Starch extraction and fermentation

Cassava starch extraction was carried out using a method described by Demiate *et al*. (2005). The fresh cassava roots were washed, peeled, and homogenized with sterile water (1: 2) to release starch using a waring blender. The mixture was stirred with a stirring rod for 2 min and filtered using a triple cheese (muslin) cloth. The filtrate was allowed to stand for 3 hours to facilitate starch sedimentation and the top liquid was decanted and discarded.

Native cassava starch was made by drying the starch in a cabinet dryer at 60°C for 18 hours after irradiated with ultraviolet radiation UVA (315 – 400 nm) for 9 h. Fermentation of raw starch (fermented starch) was carried out in capped 250 ml erlenmeyer flasks with 150 ml starch medium (50 g of sterile raw cassava starch suspended in 150 ml sterile water). For all the strains, the medium was inoculated with 10% (v/v) culture grown on MRS medium and incubated at 30°C for 48 h. Lactic acidified starch was prepared by soaking raw starch in 1% lactic acid solution for 30 minutes. Then, fermented starch and lactic acidified starches were washed three times with sterile water before irradiated with UVA (315 – 400 nm) for 9 h and dried at 60°C for 18 hours.

Amylose content

The amylose contents were measured by iodine affinity method (Knutson, 1986).

Swelling power and solubility

Swelling power and solubility of the starch granules was determined according to Abera *et al*. (2003) by dispersing 0.5 of starch samples in 15 ml of distilled water in a pre-weighed centrifuged tube and the suspension was heated at 85°C for 30 min with continous stirring to avoid precipitate formation. The cool paste was centrifuged at 2250 g for 20 min and the supernatant was recovered. The supernatant was evaporated overnight at 100°C for 8 h and weighed. The relationship between the dried supernatant weight and the initial starch dry weight was used to calculate the solubility. The sediment was weighed and the swelling power was determined as the ratio of weight of sediment to dry weight of starch solidified by swelling (g/g).

Thermal properties

Thermal properties of starch sample were determined using a Differential Scanning calorimeter (DSC-7, Universal V4.2E, TA Instruments). 5 mg of starch samples were weighed in aluminum DSC pan and added with 15 µl deionized water. The cover was carefully put on and sealed hermetically using sealing tools. To check for water leakage due to improper sealing, the weights of the sealed pans were recorded before and after determination. The pans were kept overnight before determination. Following equilibration at room temperature overnight, the samples were heated from 10 to 100°C at a rate of 10°C/min. An empty pan was used as reference and the instrument was calibrated using indium. Exothermal curves showing gelatinization onset (T_o), peak (T_p), and end (T_e) temperatures and transition enthalpy (J/g sample weight) of the starch sample were recorded. The gelatinization temperature range (R) and peak height index (PHI), was calculated as $2(T_p - T_o)$ and $\Delta H/(T_p - T_o)$, as described by Krueger *et al*. (1987).

Scanning Electron Microscope (SEM) observation

Starch granule characteristics were obtained with Scanning Electron Microscope Hitachi Model S-800. Dry samples (from fermented cassava starch, lactic acidified and oven dried cassava starch) were sprinkled onto double-sided cellophane tape attached to aluminum stubs. These samples were then coated with 25 nm of gold-palladium (60:40) at 10 milliamps for 3 minutes (Hummer Sputter Coater, Techincs EMS, Inc, VA). Samples were examined at 10.0 kV.

Baking property of starches

Baking property was measured by weighing 24 g of starch sample and partially cooking by addition of 30 ml of boiling de-ionized water over this starch mass. This partially cooked starch was homogenized to produce dough that was molded to three small balls and baked on an electric oven at 200°C for 20 min. After baking, the doughs were weighed, and made impermeable by using paraffin and their volumes determined on graduated cylinders as the volume of water displaced. The expansion was obtained by dividing volume by weight and was expressed as specific volume (ml/g). The methodology is adequate to show differences between very high, high and low expansion values but it is not very sensitive. For the scope of this work, the measurements were not influenced by this low sensitivity and it was possible to clearly differentiate the samples (Demiate *et al*., 2000).

Results and Discussion

Amylose content, swelling power and solubility

The amylose content, swelling power and solubility of starches are presented in Table 1.

Table 1. Amylose content, swelling power and solubility of cassava starches

Treatments	Amylose (%)	Swelling power (g/g)	solubility (g/100g)
Native starch	33.21±0,09 b	22.28±0,39 a	10.61±0,67 b
Acidified starch	32.66±6,99 b	20.29±0,01 b	11.88±0,10 b
Fermented starch 15881[1]	34.02±1,82 a	21.40±0,10 a	13.95±0,15 a
Fermented starch UA3[2]	32.18±6,54 b	21.74±0,03 a	12.18±1.17 a
Fermented starch AA2[3]	32.60±0,01 b	20.88±0,17 b	11.96±1,94 b
Fermented starch AA11[4]	33.24±0,88 b	21.52±0,04 a	13.84±0,61 a

[1]Fermented starch by *L. amylophyllus* 15881; [2]Fermented starch by *L. plantarum* UA3; [3]Fermented starch by *L. plantarum* AA2; [4]Fermented starch by *L. plantarum* AA11
All values are averages of triplicates + standard deviation
Means of each properties followed by same letter are not significantly different p<0,05)

Fermentation by different strains of *Lactobacillus plantarum* and acidified cassava starches did not give significant differences among each others. Native starch as a control treatment tend to have the highest values of swelling power (22.28 g/g) whereas fermented starch by *L. amylophyllus* gave the highest on amylose content (34.02%). Swelling power of all starches showed a similar pattern as the solubility. The differences in swelling and solubility behavior indicate differences in the structure of starches. Lactic acid fermentation by *Lactobacillus amylophyllus* NBRC 15881, an amylolytic LAB strain, affected these molecular structures change, whereas fermentation by the other strains of lactic acid bacteria and soaking of starch in 1% lactic acid solution did not much influence and had similar amylose content with native starch (Table 1). Swelling power of starch depends on the capacity of starch molecules to hold water through hydrogen bonding (Takizawa *et al*., 2004). Tomoko and Junko (1998) reported that swelling has a negative correlation with amylose content. In this study the swelling power had a positive correlation with amylose content which is in line with the results were expressed by Mweta *et al*. (2008). The swelling power and solubility of starch granules showed a great evidence of interaction on the starch chains between the amorphous and crystalline regions. When starch is submitted to heat in excess of water, there is a relaxation of the crystalline structure and the groups of amylose and amylopectin associate with water molecules through hydrogen bondings. This causes an increase in the swelling power and in the solubility of the granules (Hoover, 2000). However, the amylose contents in this study were slightly higher (32.20 - 34.02%) and swelling power were less (20.88 - 22.28 g/g) than that reported by Abera and Rakhsit (2003) and Mweta *et al*. (2008). Cassava varieties with amylose contents exceeding 20% are likely to have lower swelling power (Tomoko and Junko, 1998).

Thermal properties

Thermal properties or gelatinization of starches from three treatment methods, fermented starch by four strains of lactic acid bacteria, lactic acidified starch and native starch are summarized in Table 2 and the thermogram showed in Figure 1. Starting temperature of these cassava starch gelatinization were around 60°C (Figure 1), with peak gelatinization temperatures ranged 68.19 - 69.26°C. Native starch have the highest values of enthalphy (12.20 J/g) and peak temperature (T_p), although T_o and T_c were noted similar. Gelatinization of fermented starches exhibited smooth and narrow peaks of endotherms similar to those of the acidified starch. Table 2 showed peak height index of starches, a measure of uniformity in gelatinization, was found to be the lowest for native starch (1.55). On the contrary, it was found to be the highest for fermented starch AA2 (1.66). The gelatinization range was found to be the lowest for acidified starch (14.27°C) and the highest for native starch (15.80°C). Modified cassava starches exhibited patterns of DSC thermograms similar to that of the native starch (Figure 2). However a slight decrease in peak temperature of starches were showed by acidified starch and fermented starches using *L. plantarum* UA3, *L. plantarum* AA11 and *L. amylophyllus* NBRC 15881. The differences of amylose content did not much affect transition enthalpy among

Table 2. Gelatinization properties of cassava starches from different treatments

Starch samples	Enthalphy (J/g)	Gelatinization temperature (°C)			Gelatinization Range (°C)	Peak Height Index
		Onset	Peak	End		
Native starch	12.20±0,03	61.35±0,51	69.26±0,17	81.77±0,03	15.80±0,35	1.55±0,00
Acidified starch	11.54±0,18	61.06±0,21	68.19±0,01	80.99±0,56	14.27±0,56	1.62±0,01
Fermented starch 15881[1]	11.82±0,05	60.97±0,03	68.32±0,01	81.02±0,63	14.69±0,02	1.61±0,00
Fermented starch UA3[2]	11.67±0,02	61.18±0,42	68.33±0,03	81.20±0,77	14.30±0,54	1.63±0,01
Fermented starch AA2[3]	11.93±0,18	61.07±0,42	68.29±0,00	81.01±0,61	14.44±1,48	1.66±0,02
Fermented starch AA11[4]	11.57±0,13	61.00±0,21	68.44±0,07	81.02±0,77	14.88±0,16	1.56±0,00

[1]Fermented starch by *L. amylophyllus* 15881; [2]Fermented starch by *L. plantarum* UA3; [3]Fermented starch by *L. plantarum* AA2; [4]Fermented starch by *L. plantarum* AA11
All values are average of triplicate ± standard deviation

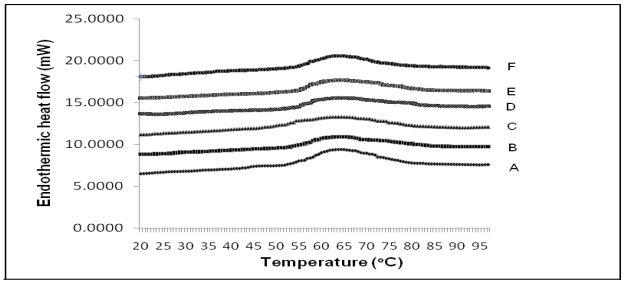

Figure 1. DSC Thermographs of gelatinization of starches from native starch (A), acidified starch (B), fermented starch by *L. amylophyllus* NBRC 15881 (C), fermented starch by *L. plantarum* UA3 (D), fermented starch by *L. plantarum* AA2 (E), fermented starch by *L. plantarum* AA11 (F).

the cassava starches. Wang *et al*. (2003) found that molecules of both amylose and amylopectin were reduced simultaneously by acid hydrolysis of different starches in contrast to the present study, but their result indicated that the molecular size of the amylose portion increased due to the hydrolysis of amylopectin molecules. Similarly, Camargo *et al*. (1988) and Mestres *et al*. (1997) found no significant change in the enthalpy of the sun-dried fermented cassava starches compared with the native starch. The high gelatinization range temperature values of native starch suggests the presence of crystallites of varying stability within the crystalline domains of its granule (Cavallini and Franco, 2010; Sandhu and Singh, 2007).

Microscopy of cassava starch granules

SEM observation showed that after fermentation and acidified treatment of the starch granules had a different morphological appearance compare to native starch granules as determined in Figure 2. Cassava starch granules of diameter ranging from 3 to 10 µm had small to large rounded irregular with oval and truncated ellipsoidal shapes, with a one side containing a conical pit which extended to a very deep well for some starches. Native starch granules have smooth surfaces with some portions being irregular, whereas a partly broken granule and rougher surfaces are exhibited by fermented and acidified starches. As Sotomayor *et al*. (1999) reported that lentil starch granule keeps the internal integrity, but a cavity in the middle might be an evidence of structural changes during fermentation. SEM observation of this study were in line with Giraud *et al*. (1994) results, they showed that enzymatic hydrolysis also revealed the lamellar organization of the starch granules. However, the progress of degradation was not homogeneous. Smooth granules and entirely digested granules were observed in the same sample. In addition, the proportion of granules displaying enzymatic attack appeared to be fairly small, whatever the stage of

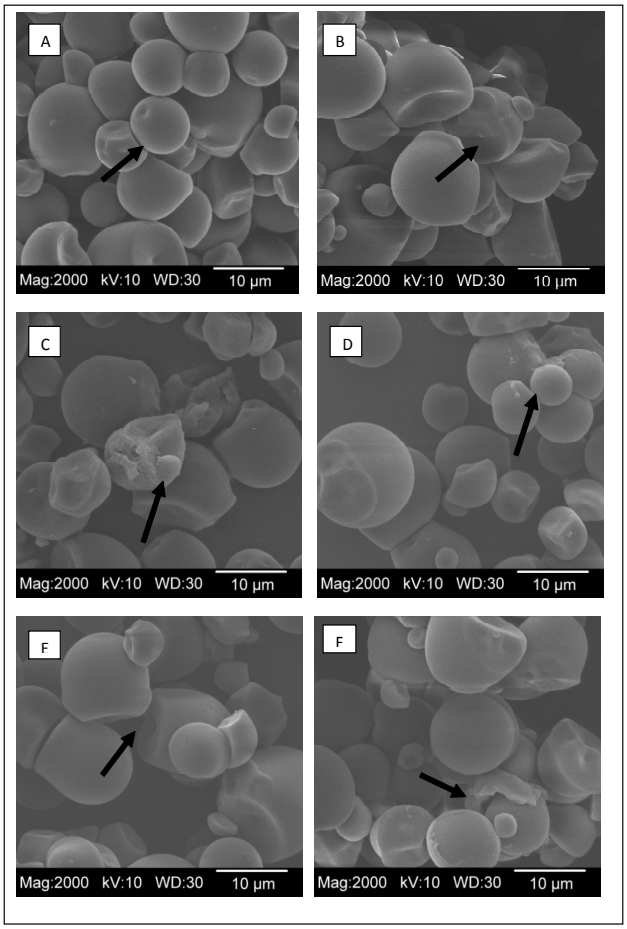

Figure 2. SEM photograph of cassava starch granule native starch (A), lactic acidified starch (B), fermented starch by *L. amylophyllus* NBRC 15881 (C), fermented starch by *L. plantarum* UA3 (D), fermented starch by *L. plantarum* AA2 (E), fermented starch by *L. plantarum* AA11 (F). Arrows indicate surface granules differences between starches.

fermentation. A decrease in the number of granules was observed as fermentation progressed, with total disappearance occurring after 3 days. Although, they did not examine the effect of starch granule changes on thermal properties and baking expansion characteristics of cassava starch.

Sujka and Jamroz (2007) stated, in general, native starch granules are practically inert toward chemical reactions unless they are pretreated to activate them, for example by enzymatic hydrolysis. It has been showed that the starch granules porosity significantly influences starch chemical reactivity. Since starch molecules in the amorphous regions were depolymerized partially by enzymatic and lactic acid hydrolysis, as SEM observation shown in Figure 3, the size-reduced starch molecules were responsible for baking expansion. Fermentation of starches by *L. amylophyllus* exhibited uneven surfaces having a number of thorough pits with large diameters of starch granules damage.

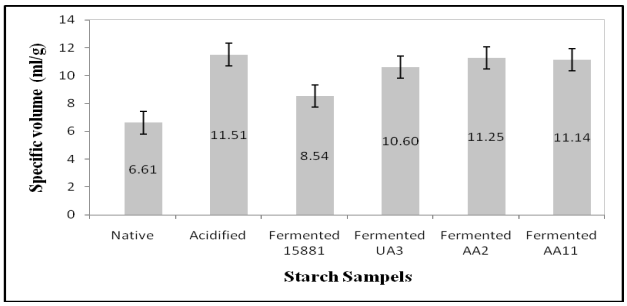

Figure 3. Baking expansion ability of cassava starch samples. Bar indicated standard deviation of values

Baking expansion properties

An expansion occurred during the baking of cassava starch-based dough. Both the fermentation and acidified treatment significantly influenced the expansion ability of cassava starches and showed high specific volumes (8.54 - 11.51 ml/g), in contrast with the native starch sample which gave a lower loaf specific volume (data showed in Figure 3). Both fermented starch by *L. plantarum* AA2 and lactic acidified starch gave high expansion on baking, 11.25 ml/g and 11.51 ml/g respectively, when associated with oxidation treatment (UV irradiation). Bertolini *et al*. (2001) found that the expansion of sour cassava starch during baking can be attributed to the pressure increase by water evaporation, then the low melt viscosity due to starch depolymerization would reduce the resistance force to expansion. Baking expansion was produced by forming amorphous matrix structure with hydrogen bonds. When degradation was too extensive the bubble walls lost their integrity earlier, they ruptured at lower temperatures causing no expansion of the starches treated with high intensity energy. This could be correlated to thermal properties and morphological structure of starch granules, whereas the baking expansion increased with starch disintegration and degradation. Cassava starches with lower solubility and swelling values had less expansion ability during baking. Furthermore, these samples had a higher gelatinization temperature that could also influence the expansion ability. Thus, a low in amylose molecules of the acidified cassava starches provided small linear fragments and facilitated formation of an amorphous matrix structure of starch dough during baking. Consequently, a good baking expansion of the cassava starch was achieved. In contrast, higher amylose content did not provide an effective starch structure for baking expansion. Likewise, Demiate *et al*. (2000) found that cassava starch immersed in 1% lactic acid solution for 4 h and sun dried for 8 h had a high specific volume.

It has been proven that lactic acid fermentation and acidified treatments influence the structure and starch granules porosity. Although their gelatinization properties did not much affected, the physical characteristic of starch granules could modify the dough's thermo mechanical properties which cause the change of baking properties of starches.

Conclusion

Results of this study have revealed differences on physical characteristic of starches with different treatments. The ability of lactic acid bacteria as starter cultures to degrade and produce lactic acid during fermentation influenced peak gelatinization temperature and peak height index within strains. Lactic acid immersion of cassava starch had better baking properties than fermented cassava starches and native starch. Fermentation caused extensive damage of starch granules which reduced the baking expansion ability. These changes could be also attributed to different chemical compositions and structures of starch.

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