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Effect of Lactic Acid Fermentation on Physico-chemical Properties of Starch Derived from Cassava, Sweet Potato and Rice

Kanjana Chinsamran[1,2], Kuakoon Piyachomkwan[3], Vilai Santisopasri[4] and Klanarong Sriroth[1,5]

ABSTRACT

Instead of natural fermentation, the microbial starters prepared from natural fermentation of mung beans were applied to cassava, sweet potato and rice during extraction and the starch was collected without or with prolonged fermentation [lactic acid fermented starch (LAF-starch)]. Their structural and physico-chemical properties of each starch types were evaluated and then compared with the natural fermented starch (NF-starch) and the control (i.e. freshly extracted starch). No great significant change in chemical composition (i.e. starch, lipid and fiber) of LAF-starch was observed, but the protein and ash content increased. When compared to the control starch, both NF- and LAF-starch had lower paste viscosity, but the latter was more pronounced due to starch depolymerization of a higher acid concentration. Prolonged fermentation did not significantly cause more change to starch properties. In addition, the lactic acid fermentation caused an alteration in the gel texture, which was starch-dependent. The endothermic peak of LAF-starch gelatinization, as analyzed by the Differential Scanning Calorimeter, was slightly shifted to a higher temperature, suggesting the modification in starch granules. This was evident by Scanning Electron Microscopy as all fermented (NF and LAF) starch granules had lost their surface smoothness. The addition of the microbial starter led to more irregular surface due to external corrosion. The results suggested a similar trend in property modification by the natural fermentation and by the application of microbial starter, which provided a greater extent of starch modification within a shorter period of time. The use of the microbial starter, therefore, can minimize the fermentation time and starch properties can be further diversified by varying the quantity of starter used.

Key words: lactic acid fermentation, cassava, sweet potato, rice, starch

INTRODUCTION

Cassava (*Manihot esculenta* Crantz), sweet potato (*Ipomea batatus* (L.) Lam.) and rice (*Oryza sativa* L.) are important industrial crops for starch production in many countries. The starch industry of cassava, sweet potato, and rice have ranged from small-scale, simply-mechanized factories to large-scale, well-mechanized factories. In practice, a small-scale production of starch involves wet milling with a rasper, sedimenting the slurry in open tanks, and sun drying. During sedimentation,

1 Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand.
2 Rajamangala University of Technology Krungthep, Bangkok, Thailand.
3 National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand.
4 Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.
5 Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, Bangkok 10900, Thailand.

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a natural fermentation of starch by some microorganisms, predominantly lactic acid bacteria occurs and affects starch processing and properties. In a traditional sweet potato starch processing, some processors have intentionally applied lactic acid (referred as sour liquid, an aqueous acidic fermented extract obtained from a natural fermentation of dried beans) during starch extraction, in order to improve the sedimentation efficiency as well as the quality of produced starch (Timmins *et al*., 1992).

In some starches, the natural fermentation has been implemented to produce some starch-based products such as Gari, a sour cassava starch produced by 30-day fermentation (Cardenas and Buckle, 1980) and Khanom-jeen, the Thai noodle made from 3-day fermented rice (Nittaya, 1989). Although natural fermentation is widely utilized in starch industry, the economical development is still limited owing to regional specificity and small-scale processing. Improving the process, mainly of the fermentation stage by using the microbial starter, it would allow standardization (i.e. quality control of fermentation process) and scaling-up (i.e. reduction of fermentation time) of the production. Furthermore, transferring the fermentation technology to other starchy crops would contribute to add values and diversify the use of indigenous raw materials. The objectives of this work were, therefore, to evaluate the effect of lactic acid fermentation on physico-chemical properties of starch derived from cassava, sweet potato and rice by using microbial starter previously prepared by the natural fermentation of bean.

MATERIALS AND METHODS

Cassava roots, the variety "Kasetsart-50", were obtained from the Field Crop Research Center at Nakornratchasima. Rice grains of the " Suphanburi 1" were obtained from The Rice Research Institute, Patumthani, Thailand. Sweet potato roots, the variety "Kaset" and mungbeans were purchased from the local market.

Preparation of microbial starter from mung beans

The microbial starter was prepared according to Timmins *et al.* (1992) with a slight modification. Mungbeans were initially ground with water (1:3, w/v) and sieved through 100-mesh screen. The filtrate was allowed to sediment and the aqueous layer was collected for subsequent fermentation at 25-30°C for a week to obtain the first fermented liquor. On the third day of fermentation, another fresh aqueous slurry was reprepared using new mungbeans with the addition of the first fermented liquor to obtain the second fermented liquor and this liquor was then mixed with the fresh aqueous slurry reprepared at day 5. The liquid was subsequently fermented for another 3 days and then collected as a starter for further study. The quality of starter including pH, titratable acidity (TA, % w/v for lactic acid equivalent by titrating with 0.1 M NaOH), and lactic acid bacterial count (by spread-plate technique on MRS agar at 25-30°C for 48 hr) was determined before uses.

Preparation of NF-starch and LAF-starch from cassava, sweet potato and rice

Freshly harvested roots of cassava and sweet potato were washed, peeled and ground with water (1:2 w/v). The mash was then sieved through 170-mesh screen and allowed to sediment. The wet cake was then reslurried with distilled water to10 Be′ and used to prepare the natural-fermented (NF) samples. The starch slurry was kept at 25-30° C for a period of time until the pH of the slurry was constant. Then, starch was collected, washed with water (1:2 w/v) and dried at 50°C in a hot air oven. The LAF-starch was prepared by adjusting the concentration and pH of starch slurry to 10 Be′ and 4.0 by adding the starter with the ratio of starch : starter about 1:2 (w/v). One portion of acidified slurry was allowed to settle for 3 hr (Non-incubated LAF-starch) and the wet starch was collected

whereas the other portion was incubated at room temperature for the same period as the natural fermented sample (Incubated LAF-starch). Subsequently, the starch was collected and prepared similarly to the natural-fermented sample. The starch collected immediately after wet extraction was used as the control sample. In case of rice, the steeped grains were wet-milled with water (1:2 w/v) and the slurry was sieved through 106-mesh screen. Rice flour was then collected for making rice starch according to the method of Sawai and Morita (1968) using 5 % sodium chloride, 85 % ethanol and 0.35 % sodium hydroxide. The wet cake of rice starch was then treated with the starter as previously described for cassava and sweet potato.

Analysis of starch properties

Chemical composition

The content of moisture, protein, fiber, fat and ash were determined by the standard methods of AOAC (1990). The content of starch was determined by a polarimetric method according to the procedure of Thai Industrial Standard Institute (1978).

Solubility and swelling power

Solubility and swelling power of starch samples were determined according to the procedure described by Schoch (1964) at 65, 75, 85 and 95°C. The solubility was reported as the percentage of the weight of total soluble starch to the weight of dried sample. The swollen starch paste was estimated as the ratio of the swollen gel weight to the total dried starch excluding the soluble one in aqueous solution.

Pasting properties

Pasting properties of starch samples (11% concentration) were determined by using a Rapid Visco-Analyzer (RVA series 4, Newport Scientific Instruments, Australia) according to the method of Collado and Corke (1997). A programmed-heating and cooling cycle were used at a constant shear rate, where the sample was held at 50°C for 1 min, heated from 50°C to 95°C at 6 °C/min, held at 95° C for 5 min, cooled to 50°C at 6°C/min and held at 50°C for 5 min. The observed parameters were pasting temperature (P_{temp}), peak time (P_{time}), peak viscosity (PV), trough or hot paste viscosity (HPV), final or cold paste viscosity (CPV), breakdown (PV-HPV) and setback (CPV-HPV).

Gel textural properties

The slurry (30 % w/w) of various starch types was prepared and then cooked in a water bath at 95°C for 30 min. After cooking, the samples were settled at the room temperature for 3 hr and the texture profile analysis (TPA) of starch gel (1.5 $\times$ 1.5 cm) was then evaluated by the texture analyzer (TA-XT2I, Texture Technologies Corp., NY.) according to Bourne (1978) using the two-cycle compression test (a speed of pretest 2.0 mm/sec, test speed 1.0 mm/sec, and posttest 5.0 mm/sec, with 3.0 g trigger force to a distance of 2.0 mm with a 50-mm diameter aluminum plate probe). The gel texture including hardness, cohesiveness, gumminess and springiness were recorded.

Thermal properties

Thermal properties of starch sample were determined using a Differential Scanning Calorimeter (DSC-7, Perkin-Elmer, USA) by the method of Sriroth *et al.* (1999). The starch samples were hydrated to 70% moisture content. Each starch suspension was then transferred to aluminium pan and hermetically sealed. Following equilibration at room temperature overnight, the samples were heated from 0 to 110°C at a rate of 10°C/min.

Scanning electron microscopy (SEM)

Starch samples were vacuum-dried, mounted on a circular specimen stub with the adhesive tape and coated with gold using an ion sputter-coater according to Valetudie *et al.* (1993). The SEM of starch samples were then performed using a scanning electron microscope (JSM-5600, JEOL, Japan) at an accelerator potential of 10 kV.

Statistical analysis

All experiments were done in duplicate with a randomized complete block design and the data were analyzed by the analysis of variance (ANOVA) and the Least-Significant Difference (LSD) method at $p<0.05$.

RESULTS AND DISCUSSION

The quality of microbial starter

The microbial starter prepared by the natural fermentation of mung beans was dominated by lactic acid bacteria (LAB) with counts of 10^{10} - 10^{12} CFU/ml after 7 days of fermentation. The pH decreased to 4.0 and titratable acidity (TA, % w/v for lactic acid equivalent) reached 1.0 % . This was in agreement with Salminen and Wright (1993) who reported that a natural fermentation, was principally caused by mesophilic lactic acid bacteria and the main product was lactic acid.

Physico-chemical properties of NF-starch and LAF-starch from cassava, sweet potato and rice

In this work, when the freshly wet-extracted starch of cassava, sweet potato and rice starch was left at the ambient temperature, the pH of starch changed to pH 3.7 72 hr after fermentation, indicating the acid production by some bacteria, mainly the lactic acid bacteria as assayed by MRS agar at 25-30°C (lactic acid bacteria count about 10^9, 10^{11} and 10^5 CFU/ml for cassava, sweet potato and rice starch, respectively). 72-hr of fermentation, the starch slurry was acidified with the total acidity of 0.1 % for cassava and sweet potato and 0.01% for rice. To shorten the processing time, the microbial starter was applied to freshly wet-extracted starch. The mixture was settled for 3 hr and the cake was collected as the non-incubated lactic acid fermented starch (Non-incubated LAF-starch). The other acidified slurry with the microbial starter was incubated for a same period of time as the natural fermentation, i.e. 72 hr (Incubated LAF-starch). Physico-chemical properties of all starch samples were included.

Chemical composition

Table 1 displays the chemical composition of all starches. No significant difference in chemical composition, i.e, starch, lipid and fiber content of NF and LAF-starches was observed. This was in agreement with Cardenas and Buckle (1980) who demonstrated that chemical composition of sweet (non-fermented) starch and sour cassava starch (the product of natural fermentation) was similar. Yet a remarkable increase in protein content, determined as the total nitrogen content of all LAF-starches was observed, which were incubation-time independent. In addition, a slight increase in the ash content was also detected in LAF-rice starch.

Solubility and swelling power

Changes in solubility and swelling power of cassava, sweet potato and rice starches during heating are shown in Figure 1. In general, starch could be solubilized and swollen when heated with excess water. The higher the temperature, the higher the solubility and swelling power of starch. Cassava could be hydrated more readily than sweet potato and rice as evident by the swollen granules produced at 65°C whereas the starch granules of sweet potato and rice were negligible changed (data not shown). At 75°C, sweet potato and rice starch became swollen and solubilized, but at a lower extent than cassava. The addition of lactic acid bacteria starter did not cause any significant change in solubility and swelling power of cassava and rice starches. Furthermore, the swelling power and solubility of LAF-starch were not affected by the incubation time. However, the swelling power and solubility of LAF-sweet potato starch decreased slightly when cooked at 85 and 95°C. This could presumably be caused by the effect of acid during starch incubation. Numfor *et al.* (1995) also reported that solubility and swelling power of

Table 1 Chemical composition of control, natural fermented (NF) starch, non-incubated lactic acid fermented (LAF) and incubated LAF-starches derived from cassava, sweet potato and rice.

Starch Source	Treatment	Chemical composition (% dry basis)[1/]				
		Starch	Protein	Ash	Lipid	Fiber
Cassava	Control[2/]	98.26 [a]	0.07 [b]	0.06 [a]	0.05 [a]	0.16 [a]
	NF-starch	99.73 [a]	0.04 [b]	0.06 [a]	0.08 [a]	0.13 [a]
	Non-incubated LAF-starch	98.97 [a]	0.17 [a]	0.13 [a]	0.09 [a]	0.08 [a]
	Incubated LAF-starch	99.98 [a]	0.20 [a]	0.13 [a]	0.06 [a]	0.11 [a]
Sweet potato	Control[2/]	99.04 [a]	0.07 [b]	0.28 [a]	0.18 [a]	0.26 [a]
	NF-starch	99.11 [a]	0.08 [b]	0.18 [a]	0.17 [a]	0.23 [a]
	Non-incubated LAF-starch	98.91 [a]	0.31 [a]	0.28 [a]	0.17 [a]	0.23 [a]
	Incubated LAF-starch	98.93 [a]	0.29 [a]	0.25 [a]	0.15 [a]	0.17 [a]
Rice	Control[2/]	95.38 [a]	0.81 [b]	0.08 [b]	0.10 [a]	0.18 [a]
	NF-starch	95.52 [a]	0.79 [b]	0.09 [b]	0.09 [a]	0.26 [a]
	Non-incubated LAF-starch	93.95 [a]	1.38 [a]	0.52[a]	0.14 [a]	0.21 [a]
	Incubated LAF-starch	93.56 [a]	1.39 [a]	0.54[a]	0.10 [a]	0.23 [a]

[1/] Each value represents the mean of two replicates.

[2/] Starch was collected immediately during wet extraction.

For each starch, the values followed by the same letter within the same column are not significantly different at $p < 0.05$.

cassava starch at 60 and 85°C could be lower by the natural fermentation.

Pasting properties

During heating in water, starch began to gelatinize as the granules became swollen and partially solubilized, contributing to a viscous starch paste. Figure 2 demonstrates the viscosity profile of different starch. Similar to the swelling of starch granules, cassava starch was more readily to paste; the pasting temperature was around 67°C whereas sweet potato and rice became pasting at a very high temperature of 77 to 80°C (Table 2). Moreover, the paste properties of starch was starch-type dependent (Figure 2). Cassava provided starch paste with the highest viscosity (Peak viscosity of the control starch = 427 RVU) while rice provided the lowest paste viscosity (Peak viscostiy of the control starch = 180 RVU). However, the hot paste viscosity of rice starch was higher than that of cassava starch, suggesting a more stable of paste viscosity during heating and shearing, i.e. low paste breakdown (breakdown of cassava and rice starch = 300 and 33 RVU, respectively). Besides, rice starch produced the paste with a higher cold paste viscosity than cassava starch (Table 2). The paste properties of starch were not only depending on starch type, but were also affected by starch processing. By natural fermentation, all starches exhibited a reduction in paste viscosity as a peak viscosity reduction of cassava was highest (13%) and rice was lowest (4%), probably was due to the lower microbial and acid content of control rice starch. This results were in agreement with other studies (Numfor *et al.*, 1995; Plata-Oviedo and Camargo, 1998). A decrease of viscosity was presumably contributed to the hydrolysis of acid produced by lactic acid bacteria. For this purpose, one may expect that the addition of lactic acid to starch can be used to substitute the natural fermentation. However, these studies demonstrated some different properties in lactic acid-treated

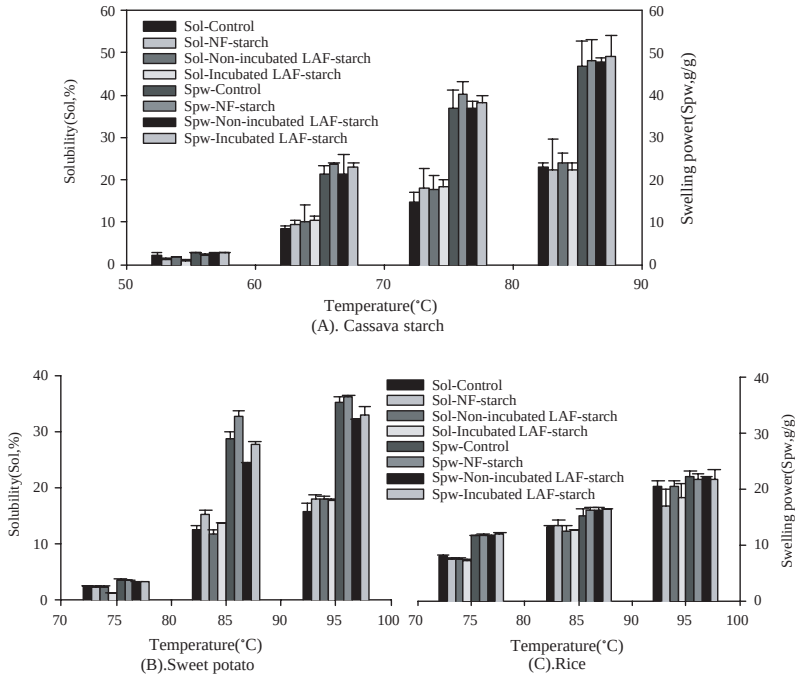

Figure 1 Solubility (Sol) and swelling power (Spw) of control,natural fermented (NF) starch and lactic acid fermented (LAF) starches (non-incubated and incubated) derived from different sources; (A) Cassava (B) Sweet potato and (C) Rice (Bars represent the standard deviation of two replicates).

starch and LAF-starch (data not shown) that might be due to starter impurities and/or microbial metabolism. Further investigation should be performed to understand more about the role of microorganisms in starch fermentation. A similar trend of viscosity reduction was also observed in LAF-starch of all starch types, yet the degree of modification was more pronounced (peak viscosity reduction of 20, 11 and 10% for cassava, sweet potato and rice, respectively). A greater reduction of viscosity could be a result of a higher acid concentration in lactic acid fermentation (1.0 and 0.1% for LAF and NF). It was interesting to note that prolonged incubation time for LAF-starch did not significantly alter paste properties. The paste viscosity properties of non-incubated and incubated LAF-starch were still comparable, implying unnecessity of incubation time.

Gel texture properties

After heating, the solubilized molecules of gelatinized starch could rearrange themselves to form the gel network. The texture properties of starch gel varied, depending on starch source. Cassava starch formed very soft gel while sweet potato and rice formed hard gel (Figure 3). Furthermore, the influence of fermentation on gel texture of starch also varied by starch-type. By natural fermentation, the gel texture of cassava did not significantly change (Table 3), whereas Numfor *et al.* (1995) reported that the gels prepared from cassava flour after 7 days of fermentation had lower hardness values than native flour. A significant decrease in gel hardness of sweet potato starch was noticeable after the natural fermentation. By using the microbial starter, the gel hardness of sweet potato starch was also minimized. In rice, a

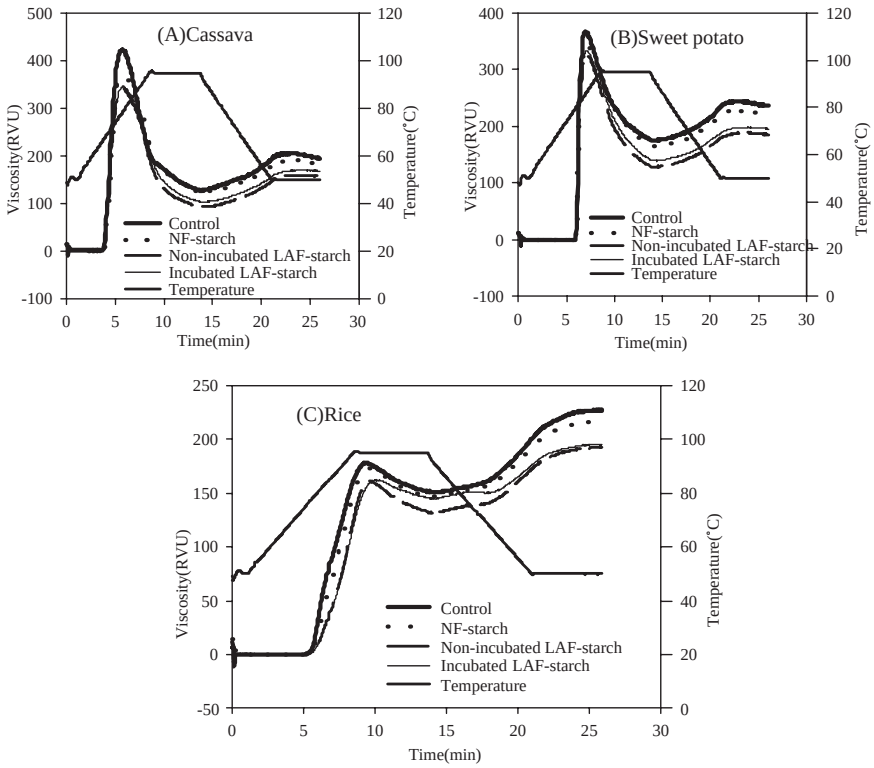

Figure 2 Paste viscosity profile, as determined by a Rapid Visco Analyzer (RVA, using 11% (dry basis) starch, of natural fermented (NF) starch and lactic acid fermented (LAF) starches (non-incubated and incubated) derived from cassava, sweet potato and rice.

slight reduction in gel hardness was observed when subjected to natural fermentation. In contrast, the use of microbial starter resulted in an increase of gel hardness as well as gumminess. Similar to the pasting characteristics, prolonged incubation time for LAF-starch did not significantly alter gel texture properties of cassava and rice, but in the case of sweet potato starch did.

Thermal properties

The DSC thermograms of starch samples are shown in Figure 4. There was a slight shift of an endothermic peak of LAF-starch derived from rice and cassava to a higher temperature, indicating a higher gelatinization temperature of LAF-starch, compared to the control starch. As a result of starch granule architecture, it was assumed that the less ordered packing of starch molecules, i.e. amylose and amylopectin in the amorphous phase should be more susceptible to acid hydrolysis. Consequently, the crystallites are decoupled from and no longer destabilize by the amorphous parts and the remaining starch, therefore, melts at a higher temperature (Hoover, 2000).

Scanning electron microscopy (SEM)

Changes in physico-chemical properties of natural fermented (NF) and lactic acid fermented (LAF) starches implied changes in starch structure. As evidenced by scanning electron micrographs (Figure 5), starch granules, when subjected to fermentation, still retained their whole figures but their surface extremely changed. Typically, the fermented starch lost their surface smoothness.

Table 2 Paste viscosity profiles, as determined by a Rapid Visco Analyzer (RVA, using 11% (dry basis) starch), of natural fermented (NF) starch and lactic acid fermented (LAF) starches (non-incubated and incubated) derived from cassava, sweet potato and rice.

Starch source	Treatment	Pasting temperature (°C)	Pasting properties (RVU)[1/] Peak viscosity	Trough (Hot paste viscosity, HPV)	Break down	Cold paste viscosity (CPV)
Cassava	Control[2/]	67.4 [a]	427 [a]	126 [a]	301 [a]	195 [a]
	NF-starch	67.8 [a]	372 [ab]	117 [a]	255 [a]	184 [ab]
	Non-incubated LAF-starch	67.7 [a]	346 [b]	92 [b]	254 [a]	156 [b]
	Incubated LAF-starch	67.5 [a]	348 [b]	102 [b]	246 [a]	168 [b]
Sweet potato	Control[2/]	79.6 [a]	368 [a]	175 [a]	193 [a]	235 [a]
	NF-starch	79.3 [a]	348 [b]	164 [b]	196 [a]	220 [b]
	Non-incubated LAF-starch	79.7 [a]	328 [b]	128 [c]	200 [a]	186 [c]
	Incubated LAF-starch	79.5 [a]	334 [b]	139 [c]	195 [a]	195 [c]
Rice	Control[2/]	77.4 [a]	180 [a]	151 [a]	33 [a]	226 [a]
	NF-starch	78.2 [a]	174 [a]	146 [a]	28 [ab]	218 [a]
	Non-incubated LAF-starch	79.2 [a]	162 [b]	131 [b]	31 [ab]	192 [b]
	Incubated LAF-starch	76.5 [a]	163 [b]	144 [ab]	19 [a]	195 [b]

[1/] Each value represents the mean of two replicates.

[2/] Starch was collected immediately during wet extraction.

For each starch, the values followed by the same letters within the same column are not significantly different at $p < 0.05$.

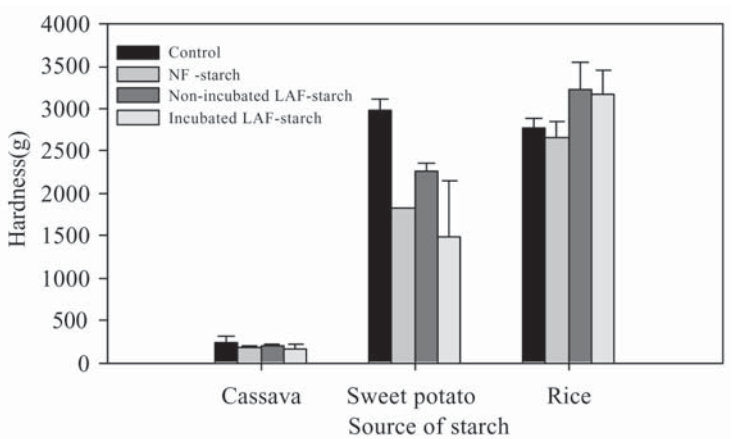

Figure 3 Texture properties, as determined by a Texture Analyzer, of natural fermented (NF) starch and lactic acid fermented (LAF) starches (non-incubated and incubated) derived from cassava, sweet potato and rice.

Table 3 Gel texture, as determined by a Texture Analyzer, of natural fermented (NF) starch and lactic acid fermented (LAF) starches (non-incubated and incubated) derived from cassava, sweet potato and rice.

Starch source	Treatment	Gel texture1/			
		Hardness	Cohesiveness	Gumminess	Springiness
Cassava	Control2/	236.13^{a}	0.91^{a}	259.18^{a}	0.90^{a}
	NF-starch	172.46^{a}	0.89^{a}	195.70^{a}	0.90^{a}
	Non-incubated LAF-starch	193.28^{a}	0.90^{a}	216.02^{a}	0.90^{a}
	Incubated LAF-starch	168.37^{a}	0.90^{a}	186.90^{a}	0.92^{a}
Sweet potato	Control2/	2985.98^{a}	0.86^{a}	3496.44^{ab}	0.93^{a}
	NF-starch	1818.65^{b}	0.83^{a}	2212.74^{bc}	0.88^{a}
	Non-incubated LAF-starch	2265.37^{ab}	0.82^{a}	4028.43^{a}	0.81^{b}
	Incubated LAF-starch	1483.05^{b}	0.79^{a}	1947.66c	0.88^{a}
Rice	Control2/	2771.36^{b}	0.86^{a}	3250.16^{ab}	0.95^{a}
	NF-starch	2661.99^{b}	0.85^{a}	3145.30^{b}	0.93^{a}
	Non-incubated LAF-starch	3233.76^{a}	0.86^{a}	3799.93^{a}	0.93^{a}
	Incubated LAF-starch	3170.17^{ab}	0.82^{a}	3884.53^{a}	0.93^{a}

1/Each value represents the mean of two replicates.

2/ Starch was collected immediately during wet extraction.

For each starch, the values followed by the same letters within the same column are not significantly different at $p< 0.05$.

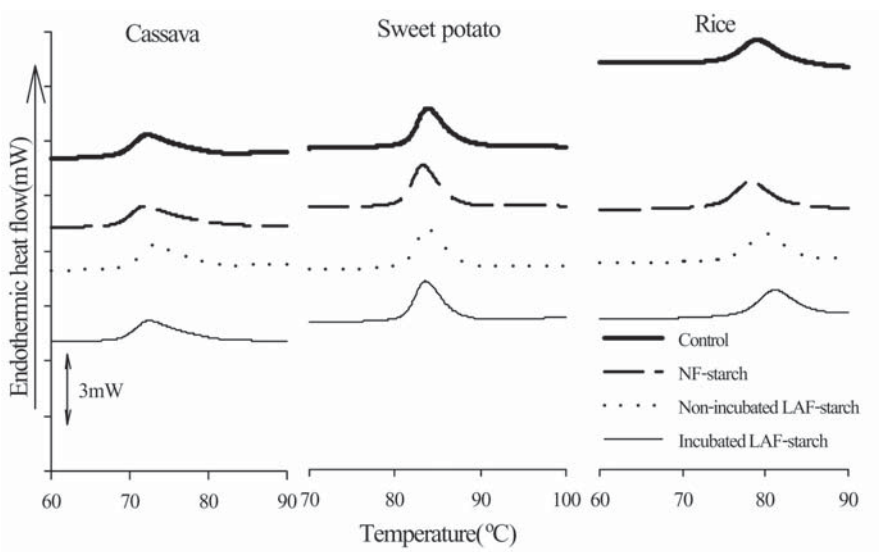

Figure 4 Thermal analysis, as determined by a Differential Scanning Calorimeter, of natural fermented (NF) starch and lactic acid fermented (LAF) starches (non-incubated and incubated) derived from cassava, sweet potato and rice.

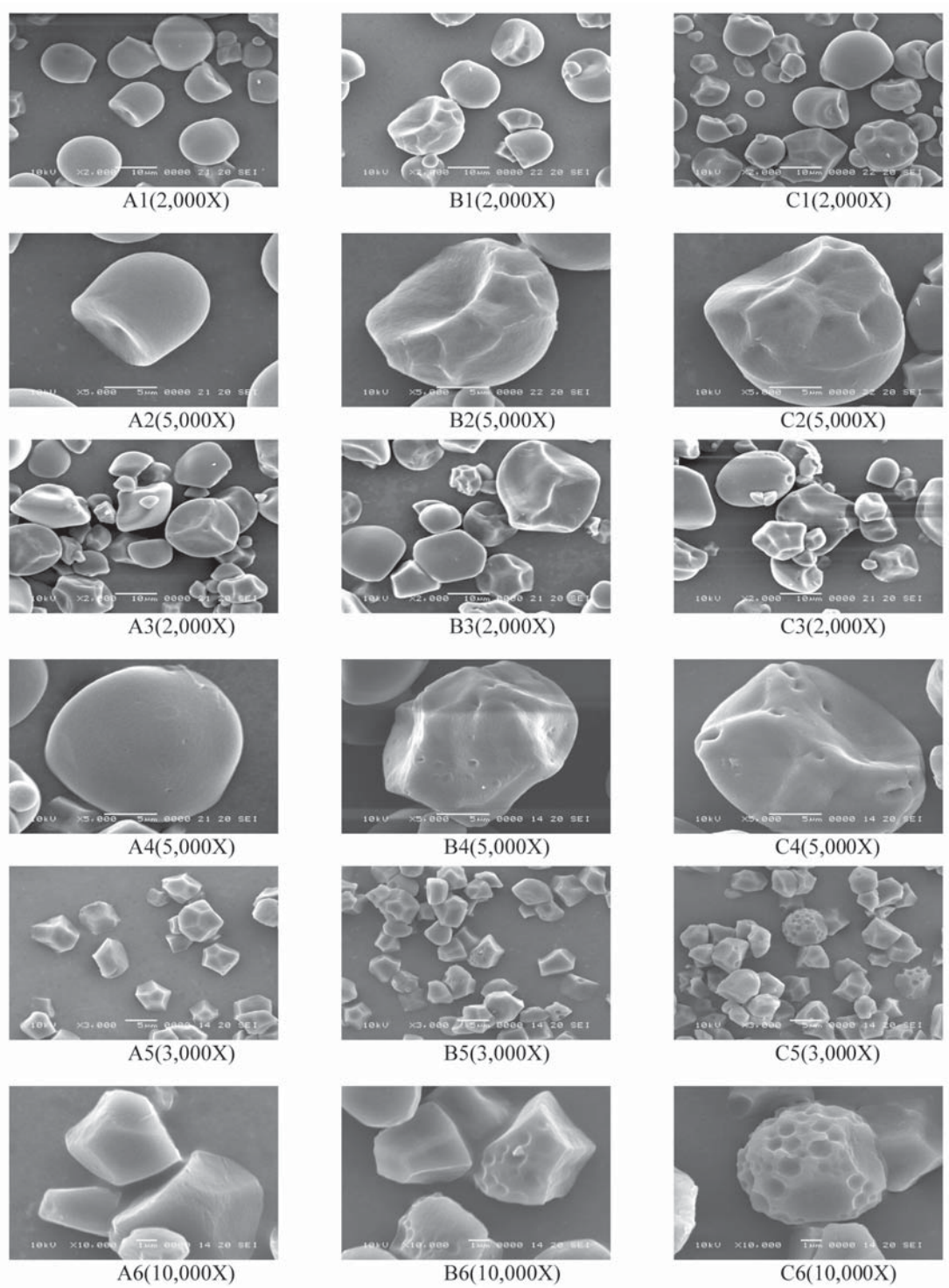

Figure 5 Scanning electron micrographs of control (A), natural fermented starch (B) and lactic acid fermented starches (C) derived from cassava (1,2), sweet potato (3,4) and rice (5,6).

The addition of microbial starter in all starch samples led to more irregular surface due to external corrosion with granule pitting, but in a slightly different pattern (Figure 5). Fermented cassava starch granules exhibited uneven surfaces having a number of shallow pits with large diameters in accordance with SEM observations of organic acid-hydrolysed (Plata-Oviedo and Camargo, 1998) and enzyme-hydrolysed cassava starch (Franco *et al.*, 1988). In contrast, sweet potato starch granules showed a number of pinned-holes on the surface while granule-peeling mechanism of sweet potato starch was reported in samples being hydrolyzed by pancreatic a-amylase (Zhang and Oates, 1999). The fermentation of rice starch induced a "Swiss cheese" pattern with numerous big and deep holes on starch granules as reported by Sarikaya *et al*. (2000).

CONCLUSION

Natural fermentation, predominantly caused by mesophilic lactic acid bacteria, of starch can induce an alteration in structural and physico-chemical properties of starch and expand more starch application. However, the natural process is typically time-consuming and the process, which dependent on the indigenous microbial present in the raw material, is hardly controlled. In this study, the lactic acid starter prepared by natural fermentation was introduced during starch preparation. The results suggested that the microbial starter could cause a modification in starch properties similar to the natural process, but with a greater extent within a very short period of time. The use of the microbial starter, therefore, could minimize the fermentation time. Besides, some starch properties could be divesified by varying the quantity of starter used.

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