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Effects of natural fermentation on the rice slurry properties related to rice paper production

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ABSTRACT

The objective of this work was to study the microbiological, chemical and physicochemical changes during the natural fermentation process of rice slurry. Slurry for rice paper processing was prepared from rice grain which contained at least 27% (w/w) amylose. Rice (Surin1) was soaked in water for 12 h, wet-milled into flour, and then fermented for 1, 2 and 3 days. During this fermentation period, clear upper phase of water was changed every day to control microbial load. The obtained rice slurry was then mixed with 1% NaCl before steaming and drying (rice paper) in sunlight. The result of total viable count, yeast mold and lactic acid bacteria count (from 5.39-9.39, 3.85-7.21, and 4.08-9.14 log CFU/g, respectively) in the slurry increased with the increasing fermentation duration. This caused an increase in total acidity (from 0.81-1.44% w/v as lactic acid) of the slurry but reduction of pH (from 4.56-3.53) and amylose content (from 36.49–35.72% w/w) through microorganism activity. The enthalpy level (ΔH_{gel}) (from 1.87-2.17 J/g) of the slurry consistently increased with the fermentation time. Scanning electron microscopy revealed that starch granule surface porosity increased with the fermentation time due to the starch hydrolysis and the corrosion caused by enzymes and organic acids during fermentation. These resulted in the slurry-viscosity reduction (from 52.16-32.42 cP), which produced the rice paper of better texture. With these results, the fermentation time during rice paper production could possibly be shortened to modify rice paper properties in conventional settings.

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INTRODUCTION

Rice paper is used for making spring rolls and Nham Neung in Vietnamese cuisine. It is very popular food among Thai people. The raw material is rice flour which contains not less than 27% amylose. Generally, rice is soaked in water for 12 hrs before wet-milling into flour and fermenting for 3 days. During this fermentation period, clear upper phase of water is changed every day. The resulting slurry is mixed with salt steamed on a cloth, dried in sunlight and then held for 3 hrs at ambient temperature. The characteristics of good rice paper are slim, transparent white, with high elasticity. The variety of rice affects the quality of the final product; however, microorganisms cause changes in the composition of rice flour during natural fermentation. The fermentation process is linked with the growth of microorganisms and an increase in lactic acid; therefore, it needs to be well controlled. Jiang and Liu (2002) noted that the lactic acid content increased during fermentation. Majzoobi and Beparva (2013) concluded that the two common organic acids in the food products; lactic acid and acetic acid, can affect the physicochemical properties of native wheat starch and cross-linked wheat starches. From the results of previous studies structural changes of the starch granules caused by the acids are important since they are probably the reasons for the physicochemical changes of the starches treated by the acids including interactions with water, thermal and gelling properties. Lu *et al*. (2002) noted that influence of natural fermentation on whole milled rice granules and on the physicochemical characteristics of rice starch and the rheological properties of rice noodles. From the results of previous studies it is concluded that organic acids can affect chemical and functional properties of starch. Phothiset and Charoenrein (2007) noted the morphological and physicochemical changes in rice flour (Chai Nat1) during rice paper production. The starch granule surface porosity increased with fermentation time. This was due to starch hydrolysis and corrosion caused by enzymes and organic acids during fermentation.

The natural fermentation process affects the rice slurry properties and elasticity of the paper. The production process and the rice variety used in each different locality affects the quality of the rice paper produced there. The purpose of this research was to study the effect of natural fermentation on the rice slurry properties during rice paper production in Ubon Ratchathani.

MATERIALS AND METHODS

Materials

Polished rice (variety Surin1) was obtained from the Indochina restaurant in Ubon Ratchathani. It contains at least 27% (w/w) amylose and is more than 3 months old.

Preparation of rice slurry and rice paper

One kilogram of polished rice was immersed soaked in 2 L of water (in jar) for 12 hrs. Then it was wet-milled into flour and fermented for 3 days at 37 °C in an incubator. During this fermentation period the clear upper phase of water was changed every day and 1%NaCl (w/w) was added on the 3rd day (D3+1%NaCl) (as it is done in Indochina restaurant in Ubon Ratchathani). Treatment D3+1%NaCl was made into rice paper by spreading the rice slurry on a cloth and steaming for 15 s. Rice paper was dried in sunlight and then held for 3 hrs at ambient temperature (RP). All rice slurry treatments (D0, D1, D2, D3, D3+1%NaCl and RP) were then analyzed.

Chemical analysis

All slurry treatments (D0, D1, D2, D3 and D3+1%NaCl) were centrifuged at 3,000xg for 3 min to remove excess water before drying in convection oven at 45 °C for 24 hrs. The sample was hammer milled to a granule size that would pass through a 100-mesh sieve. The moisture, lipid and protein content of the rice flour was determined on Day0 (D0) using AOAC method (2000) and amylose content from Day0 to D3+1%NaCl (D0, D1, D2, D3 and D3+1%NaCl) was determined according to the method of Juliano *et al.* (1981).

pH and total acidity

The pH value of the slurry was measured directly using a pH meter and total titratable acidity as lactic acid was determined using AOAC method (2000).

Microbiological analysis

On each sampling day (D0, D1, D2, D3 and D3+1%NaCl), slurry samples were diluted into sterile 0.1% peptone water by 10-fold dilution. Total plate counts were determined on plate count agar (PCA) pour plates an enumerated after an incubation period of 24 hrs at 37 °C. Lactic acid bacteria were counted on de Man Rogosa and Sharpe agar (MRS) containing 0.004% Bromcresol purple pour plate an enumerated after an incubation period of 48 hrs at 37 °C. Yeast and mold populations were determined by surface plating sample on Potato dextrose agar (PDA) of 48 hrs at 37 °C.

Gelatinization

The thermal behavior of rice slurry was analyzed by Differential scanning calorimeter (DSC; METTLER TOLEDO, DSC1/400W, JAPAN) equipped with a liquid nitrogen cooling system. Sampling of rice slurry having a moisture content of 75% were put into a crucible that was hermetically sealed using an aluminium o-ring and the reference was an empty crucible. The sample was scanned from 25 °C to 125 °C at a rate of 10 °C/min. The gelatinization temperature onset temperature (T_0), peak temperature (T_p), conclusion temperature (T_c) and enthalpy of gelatinization (ΔH_{gel}) were determined using the software provided with the instrument.

Viscosity

Viscosity of rice slurry (slurry samples of all treatments) was measured using a Brookfield viscometer (Brookfiled RTV, spindle no. 1) at 37 °C.

Morphology changes

The change in microstructure of starch granules during fermentation was observed using scanning electron microscopy (SEM; JSM-6010PLUS/LV InTouchScope™, USA). Samples of slurry from all treatments were freeze-dried to a moisture content less than 5%. The outer surface of starch granules was observed. Samples were attached to and SEM stub using double sided adhesive tape and were coated with gold, SEM was set to 5,000 diameters.

Statistical analysis

All tests were performed at least in duplicate. Analysis of variance (ANOVA) was performed and the mean separations were determined using Duncan's multiple range test (*p<0.05*) using SPSS version 15.0

RESULTS AND DISCUSSION

Effect of microbiological activity on pH and total acidity

Total viable count, yeast and mold count and lactic acid bacteria all increased with fermentation time (Table 1). This microbial proliferation caused an increase in total acidity of slurry with reduction in pH as in Table 2. The common fermenting bacteria are species of *Leuconostoc, Lactobacillus, Streptococcus, Pediococcus, Micrococcus* and *Bacillus* in cereal (Blandino *et al.*, 2003). Blandino *et al.* (2003) found that some of the lactic acid bacteria are homofermentative, and produce lactic acid as the main product of glucose fermentation, while others are heterofermentative and produce carbon dioxide and ethanol in addition to lactic acid. According to Moorthy and Methew (1998) organic acids are produced during fermentation.

Table 1 Microbial count of rice slurry at different stages of fermentation.

Time (Day)	Total viable count (logCFU/g)	Yeast & mold count (logCFU/g)	Lactic acid bacterial count (logCFU/g)
D0	5.38^{d}±0.13	3.85^{d}±0.17	4.08^{d}±0.28
D1	7.44^{c}±0.23	5.11^{c}±0.09	7.58^{c}±0.26
D2	8.99^{b}±0.38	6.39^{b}±0.02	8.30^{b}±0.11
D3	10.26^{a}±0.75	7.39^{a}±0.05	9.21^{a}±0.17
D3+1%NaCl	9.39^{b}±0.03	7.21^{a}±0.17	9.14^{a}±0.15

Means and standard deviations of duplicate sample based on three measurements for each samples.
Different letters in the same column indicate statistical differences (*p≤0.05*).

Effect of microbiological activity on amylose content

Table 2 shows a decrease in amylose content with increasing duration of fermentation from the beginning. According to Aggarwal and Dollimore (1998), the enzymes of microbial origin produced organic acids including lactic acid in starch fermentation. These enzymes are glucoamylase and alpha-amylase. Glucoamylase can act on both α-1,4 and α-1,6 links. Alpha-amylase breaks the α-1,4 linkages but cannot act on the α-1,6 linkages. The amylose content, which is main factor that determines the functional properties of rice flour, declined during fermentation. This may be due to the soluble amylose being lost into the water, of which the clear upper phase of water was changed every day. The loss of soluble amylose during fermentation of starches can also be due to conversion of amylose to soluble oligosaccharides and α-limit dextrin (Jiang and Lui, 2002)

Table 2 Chemical properties of rice slurry and rice flour at different stages of fermentation.

Time (Day)	pH	Total acidity (as g lactic acid/100 ml)	Amylose content (% w/w)
D0	4.56^{a}±0.00	0.81^{d}±0.09	36.49^{a}±0.09
D1	3.52^{b}±0.01	1.20^{c}±0.05	35.48^{b}±0.05
D2	3.19^{c}±0.11	1.47^{b}±0.05	30.59^{c}±0.09
D3	2.35^{d}±0.00	1.59^{a}±0.05	30.45^{c}±0.37
D3+1%NaCl	3.53^{b}±.0.01	1.44^{b}±0.00	35.72^{b}±0.22

Means and standard deviations of duplicate sample based on three measurements for each samples.
Different letters in the same column indicate statistical differences (*p<0.05*).

Effect of pH and total acidity on gelatinization and viscosity

This study was conducted to observe the gelatinization processes in slurries as measured by DSC. The results show the onset, peak and end temperature as well as enthalpy of gelatinization of slurry during rice paper production (all treatments) in Table 3. The results indicate an increase in enthalpy gelatinization temperature due to hydrolysis by organic acids and enzymes in the rice slurry during fermentation. Amorphous regions of the granules were rapidly hydrolyzed, so that the crystallites were decoupled and no longer stabilized by the amorphous parts and thus the cooperative melting between amorphous and crystalline regions was destroyed (Phothiset and Charoenrein, 2006). Moreover water adsorption and swelling of the starch granules decreased (data not shown). The reduction of the amorphous fraction, leading to an increase in the crystallinity of the residual starch caused an increase in the enthalpy of gelatinization with increase duration of fermentation.

Table 3 Gelatinization properties of D0, D1, D2, D3 and D3+1%NaCl at different stages of fermentation.

Time (Day)	Onset temperature (°C)	Peak temperature (°C)	Conclusion temperature (°C)	Enthalpy (J/g)
D0	68.51^{d}±0.36	75.84^{c} ±0.15	80.52^{c}±0.02	1.87^{d}±0.02
D1	69.39^{cd}±0.52	76.11^{c} ±0.81	80.72^{ab}±1.07	2.09^{c}±0.03
D2	70.39^{c}±0.12	77.27^{b} ±0.05	81.57^{b}±0.43	2.18^{b}±0.03
D3	73.92^{a}±1.45	78.23^{a}±0.19	84.52^{a}±0.01	2.33^{a}±0.02
D3+1%NaCl	72.6^{b}±0.25	77.89^{ab}±0.07	81.47^{ab}±0.03	2.17^{b}±0.01

Means and standard deviations of duplicate sample based on three measurements for each samples.
Different letters in the same column indicate statistical differences (*p<0.05*).

The viscosity of rice slurry (all treatments) was reduced with increase duration of fermentation (Figure 1). This was due to starch granules being attacking by organic acids (mostly lactic acid). According to Moorthy and Methew (1998), the greater solubility of fermented starch in hot water; may possibly be due to the presence of the shorter starch chains produced during fermentation. Decreased viscosity is beneficial for spreading the rice slurry on a cloth as well giving a suitable texture to rice paper

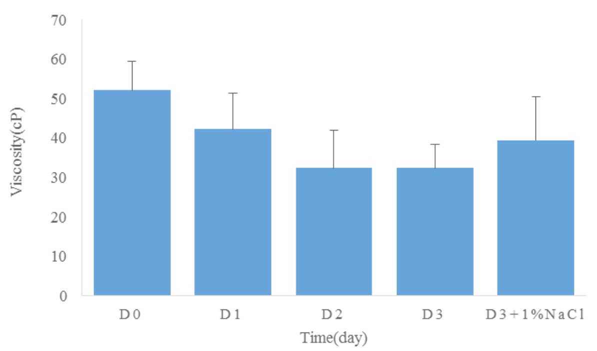

Figure 1 Viscosity of rice slurry at different stages of fermentation.

Morphological changes

Scanning electron micrographs of rice flour at 5,000x are shows in Figure 2. The surface of starch granules was smooth with D0 but after D1 of fermentation, starch granules developed a slightly rough and porous surface due to exo-corrosion. This occurs during starch hydrolysis by enzymes attacking the surface and forming pores and after this the enzymes cause further alteration and degradation of the exterior of the granule. As fermentation continued, many of the granules adhered to each other (Moorthy and Methew, 1998).

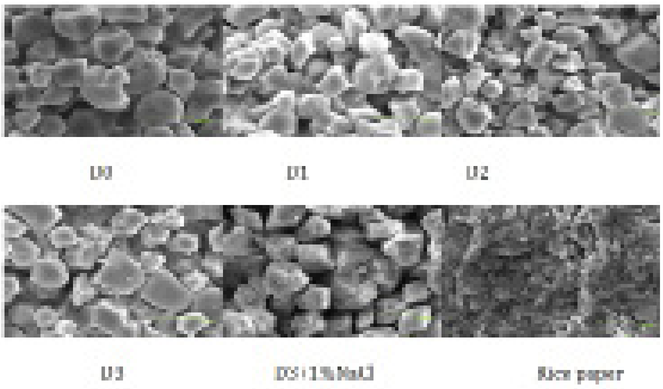

Figure 2 SEM micrographs of rice flour at different stages of rice paper production: 5000X.

CONCLUSIONS

Each fermentation period shows different microbiological, chemical and physicochemical properties. With increasing duration of fermentation, total viable count, yeast mold and lactic acid bacteria increased, with reduction in pH and corresponding increases in total acidity. This affected the gelatinization and viscosity properties of the slurry. Morphological changes of flour at each step also increased with duration of fermentation. This result was caused by enzymes and organic acids (lactic acid) during fermentation. The fermentation of slurry is the middle step of rice paper production which affects the final product. Besides the quality of the final product was found to be determined by the activity of microorganisms, involving lactic acid bacteria.

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