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Influence of Natural Fermentation on the Morphology and Physicochemical Properties of Indonesian Rice Flour and Their Effect on Rice Paper

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Abstract: Rice paper is one of the most popular traditional derivative rice products in Asia region, especially in Thailand and Vietnam. It is called as Bahn Da Nem in Vietnam. The important factor in Rice Paper production is fermentation. Fermentation can change physicochemical and morphological properties of rice flour. It also can influence rice paper products. Fermentation can reduce the moisture, starch, and lipid content whereas protein increased. Because of lactic acid bacteria and microorganisms activities, the total acid, lactic acid bacteria increased and pH decreased. Scanning electron microscopy analysis showed degradation in fermented rice flour characterized by the presence of pores. Fermentation also influence FTIR analysis and pasting properties. Rice papers from fermented rice flour have higher tensile strength and lower elongation capacity than the non fermented rice flour. This research took two steps experiments. There were fermented broken rice flour and Rice Paper production. This research was conducted in single factorial randomized complete design with 5 levels (3, 12, 24, 36, and 48 hours time of fermentation).
Key words: natural fermentation, fermented rice flour, rice paper.

Introduction

Rice is a staple food in Indonesia and Asia region. Indonesia is a country that is known as producer and consumer of rice in the world. Food and Agricultural Organization data also showed that the largest agricultural production in Indonesia is paddy or rice. It is 65.740.900 Metric Ton[1]. In Indonesia, normally rice is used for rice flour and vermicelli, besides consumed as cooked rice[2].

In the other Asian countries such as Thailand, Vietnam, and China, rice is used for making Rice Paper. It is used as spring roll papers and known as Bahn trang, Bahn da in Vietnam and Thailand[3]. Production of Rice paper and rice noodles almost the same. It is started by natural fermentation[4,5]. The aims of natural fermentation are to change morphology and physicochemical of fermented rice flour and Rice Paper. Fermented rice flour has lower viscosity than nonfermented, so it can be spreaded easily[6,7].

Natural fermentation relies on the role of natural microorganism from rice, water, and environment. Most of the microorganisms in the rice supernatant are lactic acid bacteria such as *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Aerococcus, Streptococcus,* and *Enterococcus*[8,9,10,11].

Natural microorganisms have amylolytic, proteolytic, and lipolytic activities so it can change amylose, starch, protein, and lipid qualitatively or quantitatively. The purpose of this research was to investigate the effect of natural fermentation to Indonesian rice flour and their influence on Rice Paper quality. It also to

diversify a derivative product of rice in Indonesia, most of Indonesian rice has medium amylose content. It is 20-24%[12].

Materials and Methods

Materials

IR-64 rice variety which is commonly harvested in Indonesia was used to make fermented rice flour. It has medium amylose content, 24,43% (dry basis). Then Rice Paper was produced using fermented rice flour. Natural fermentation was conducted using tap water. All chemicals used were of analytical grade and distilled water for analyzing.

Preparation of fermented rice flour

Fermented rice flour was produced by natural fermentation. Rice without washing was soaked in water in the ratio of 1:2 and added with 5% NaCl (w/w rice). Fermentation was carried out at room temperature. There were 5 treatments of fermentation base on the period of fermentation: 3, 12, 24, 36, and 48 hours. Three hours of fermentation represented non fermented sample (control).

After fermentation, the rice was washed with water, and then milled using dry blender (Philips) to get rough rice flour. Then it was dried in cabinet dryer for 5 h and sieved through an 80 mesh sieve (ASTM E-11).

Preparation of Rice Paper

Fifty percent (w/v water concentration) of fermented rice flour were mixed using mixer (National MK-H100N) in medium speed for 3 minutes. Ten milliliters homogenous batter was taken and spreaded it into flat frying (20 cm diameter). This was steamed for 2 minutes and then dried at ambient temperature for 5 h.

Enumeration of lactic acid bacteria

The total lactic acid bacteria were enumerated on a selective media MRS agar. Lactic acid bacteria were grown using pour plate method[13]. Petri dish were incubated at 37^{o}C for 48 h. After 48 h, lactic acid bacteria were enumerated by SPC (Standart Plate Count).

Determination of pH and total acidity

The supernatant from fermented rice flour were analyzed for total acidity using AOAC (2000)[14]. The total acidity was calculated as lactic acid. pH was measured directly using pH meter (rex model pHS-3C).

Chemical analysis of fermented rice flour

The moisture and lipid content of rice flour were determined using AOAC (2000). Total starch content (glucose x 0.9) was analyzed by direct acid hydrolysis method[14]. Crude protein (N x 5.95) was determined by macro kjeldahl method[14]. The amylose content was determined based on the blue colour reaction with iodine[15]. All of results were expressed on a dry basis.

Colour measurement of fermented rice flour

Colour of fermented rice flour were measured by colour reader. Colour reader showed *L* (Lightness), *a* (redness), and *b* (yellowness). The whiteness (W) was calculated as:

$$W = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}$$

Scanning electron microscopy (SEM)

Scanning electron microscopy (Hitachi TM-3000) was used to see the morphology or microstructure of the control, 24 h fermented, 48 h fermented rice flour. Flour samples were placed on scanning electron microscope stubs using double sided adhesive tape. It observed at 15 kV accelerating voltage.

FTIR Analysis

The shimadzu Fourier Transform Infrared Spectrophotometer was used for FTIR experiments. Samples were weight and homogenized with KBr anhydrous using mortar. The mixtures were pressed using vacuum hydrolic to obtain transparent pellet and then placed into FTIR instrument.

Pasting Properties

Rapid Visco Analyzer (TecMaster from Perten) was used to study pasting properties of 3 flour samples, the control, 24 h fermented, and 48 h fermented rice flour. This analysis was supported by PT. Indofood Sukses Makmur Bogasari Flour Mills Indonesia.

Physical properties of Rice Paper

Mechanical tests were carried out using Imada Force Measurement type ZP-200N. Rice Paper samples were cut with sharp scissors, 3 cm x 1 cm in dimensions. Before tested, samples were conditioned at humid room for 5 h. Tensile strength and elongation capacity were determined using ASTM standard method D882.

Statistical analysis

All tests were in duplicate at least. Analysis of variance (ANOVA) was determined using Duncan's multiple range test ($P<0.05$) using Microsoft excel.

Results and Discussion

Total Lactic Acid Bacteria

Analysis of total lactic acid bacteria (LAB) performed on rice flour supernatant. It showed that total LAB in fermented supernatants varied between 2.87 – 4.25 log cfu ml^{-1}. The data are showed in Table 1.

Table 1 Total LAB (log cfu ml-1) of the fermented supernatant

Fermentation time (h)	Total lactic acid bacteria (LAB)
3 (control)	0.91 ± 0.09^{a}
12	2.87 ± 0.19^{b}
24	3.94 ± 0.10^{c}
36	4.04 ± 0.01^{d}
48	4.25 ± 0.19^{e}

Values in columns with different letters are significantly different ($P < 0.05$). Results given as means ± standart deviation of four times repetition.

Table 1 shows that total LAB have significantly effect during fermentation ($P < 0.05$). Lactic acid bacteria are dominant microorganisms in fermented rice flour supernantant[8]. The largest increase of total LAB occurred during the first 12 h and 24 h fermentation. Further incubation gave a little increase to total LAB. Generally, microorganisms which identified in natural fermentation are *Lactobacillus brevis, Lactobacillus farciminis, Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus sakei, Pediococcus acidilactici, Pediococcus pentosaceus, W. Cibaria,* and *W. confuse*[16,17,18]. *Lactobacillus plantarum* was one of LAB that can survive from the beginning to the end of fermentation[10,19,20,21].

pH and total acidity of fermented rice flour supernatant

Table 2 pH and total acidity of fermented rice flour supernatant

Fermentation time (h)	pH	Total acidity (%)
3 (control)	5.81 ± 0.26^{d}	0.04 ± 0.01^{a}
12	5.27 ± 0.05^{c}	0.07 ± 0.01^{b}
24	5.21 ± 0.05^{bc}	0.08 ± 0.01^{c}
36	5.17 ± 0.07^{b}	0.08 ± 0.01^{c}
48	5.02 ± 0.14^{a}	0.10 ± 0.01^{d}

Values in columns with different letters are significantly different ($P < 0.05$). Results given as means ± standart deviation of four times repetition.

during fermentation, the pH of fermented rice flour supernatant was decrease (Table 2). The pH obviously decrease in 12 h fermentation and then remained steady until 48 h fermentation. Different from the pH, total acidity was increased. pH and total acidity are influenced by the growth of microorganisms.

Chemical analysis of fermented rice flour

Table 3 Chemical composition of fermented rice flour

Fermentation time (h)	% Moisture	% Starch	% Amylose	% Lipid	% Protein
3 (control)	14.18 ± 0.14^{e}	64.38 ± 0.31^{b}	23.44 ± 0.54^{c}	3.31 ± 0.34^{e}	6.72 ± 0.05^{a}
12	13.88 ± 0.24^{d}	67.71 ± 1.15^{d}	22.62 ± 0.11^{a}	2.11 ± 0.37^{d}	6.80 ± 0.04^{b}
24	13.57 ± 0.37^{c}	67.42 ± 0.79^{d}	22.54 ± 0.19^{a}	1.46 ± 0.47^{c}	6.82 ± 0.04^{b}
36	13.15 ± 0.30^{b}	65.02 ± 0.88^{c}	22.52 ± 0.13^{a}	0.64 ± 0.07^{b}	6.90 ± 0.03^{c}
48	12.38 ± 0.36^{a}	62.54 ± 1.51^{a}	23.11 ± 0.31^{b}	0.41 ± 0.06^{a}	7.00 ± 0.07^{d}

Values in columns with different letters are significantly different ($P < 0.05$). Results given as means ± standart deviation of four times repetition.

The chemical components of control were decrease along with the increase of fermentation time (Table 3). The results indicated that the moisture, starch, and lipid were decreased whereas the protein was increased.

Decreasing of moisture content is caused by the decrease of bonds strength. When the time of fermentation increase, polymers which were originally in a compact form become simpler, so it difficult to bind the water. The water evaporates easily when drying.

Beside there was a loss of lipid and starch due to leaching, there was also activity of microorganism. Microorganisms can degrade these molecule into simpler than the first one. Most of these microorganisms have the ability to digest starches, proteins, and lipids[8]. The present of microorganisms will produce amylase to degrade starches. Lipid was depolymerized to free fatty acid during fermentation[22].

The higher of starch hydrolysis normally comparable with increasing of total acid. It was caused by the production of glucosidase enzyme by microorganism. This enzyme were reported can hydrolyze starch to glucose and change glucose to lactic acid through Embden-Meyerhof cyclic[10].

Amylose data showed that amylose content decreased from 3 h – 36 h fermentation and then increased again at 48 h of fermentation. It might be due to amylose depolymerized into short chain and lost into water in the beginning, whereas amylopectin depolymerized into amylose in the end of fermentation. The most common enzymes of microbial origin produced during fermentation of starches are α-amylase and glucoamylase[5]. We presume that α-amylase worked in the beginning of fermentation to degrade amylose into short chain. In the last fermentation, glucoamylase degraded amylopectin into amylose[23].

Protein in fermented rice flour showed increase. This increase was influenced by decreasing of moisture content. Protein content in this research was measured as crude protein (N x 5.95). The longer the time of fermentation, the total microorganism and enzyme production were increase. Protein content increase quantitatively but possibility it decrease qualitatively.

Colour measurement of fermented rice flour

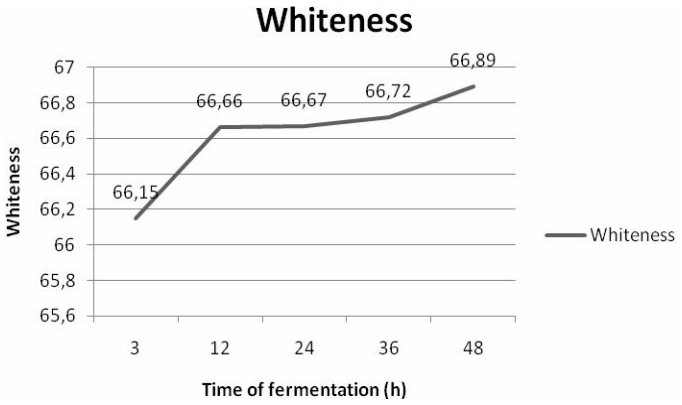

Figure 1 Whiteness of fermented rice flour

Fermentation can improve the whiteness of fermented rice flour (Figure 1). It was caused by the decrease of ash content and total lipid. Ash content is a critical factor affecting the whiteness of rice flour[4]. Fermentation can purify starch.

SEM Analysis

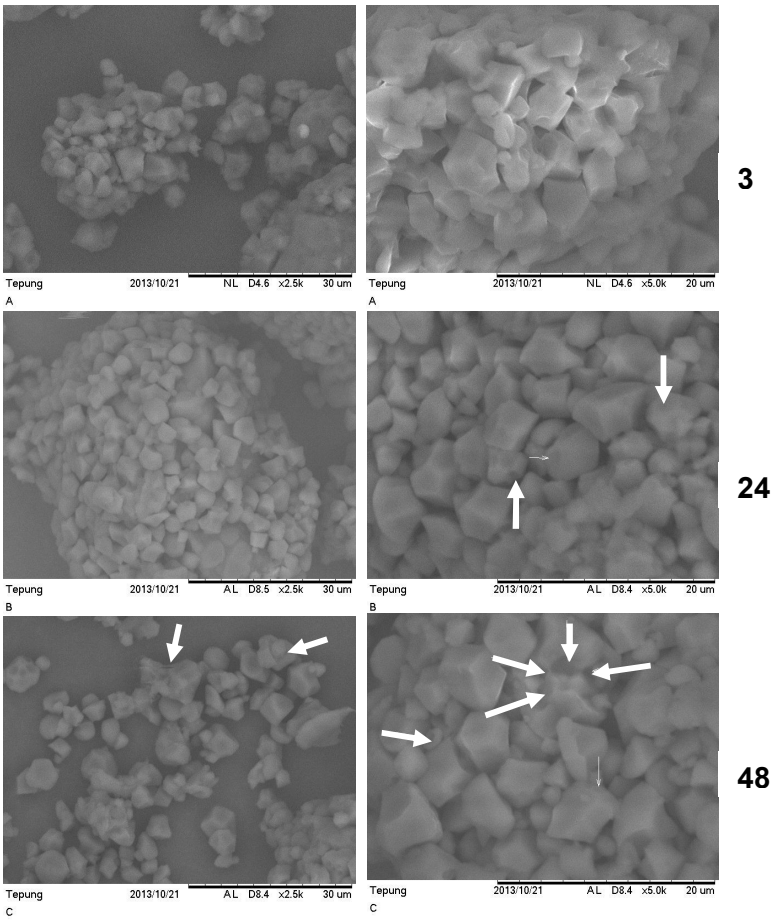

Figure 2 SEM micrographs of 3, 24, and 48 h fermented rice flour: left – 2500x; right – 5000x. The arrows show pores and changes on the starch granules.

Rice flour were photographed under a scanning electron microscope at magnification of 2500X and 5000X (Figure 2). The surface of origin starch granule was smooth with no pores. However, after 24 h fermentation, there were little pores in the surface of starch granule and the more pores in the 48 h fermentation.

The presence of the pores in starch granules indicated some breakdown in the starch. It might be caused by the digestion of some starch by lactic acid bacteria. The surface of starch granule is composed from amylose and amylopectin[7].

FTIR Analysis

The FTIR spectroscopic features of 3, 24, and 48 h fermented rice flours were similar (Figure 3). It indicated that some degree of modification rice starch occurred but no derivatives or new groups were produced during fermentation[4]. FTIR spectra showed that there are some differences between native and fermented (24 h and 48 h) rice flour but 24 h and 48 h fermented rice flours are not different.

It can be observed that –O-H stretching band was exhibited at 3200-3700 cm^{-1}, C-H at 2930 cm^{-1}, and C-O-H at 1080 cm^{-1}. Native starch has 3380 cm^{-1}, 2930 cm^{-1}, 1081 cm^{-1}, and 1022 cm^{-1} wavelength whereas 24 h fermented starch has 3381 cm^{-1}, 2929 cm^{-1}, 1080 cm^{-1}, and 1021 cm^{-1}. FTIR wavelength of 48 h fermented starch almost same with 24 h fermented starch, 3358 cm^{-1}, 2929 cm^{-1}, 1080 cm^{-1}, and 1021 cm^{-1}. It indicated that there was a shift in the wave number after fermentation.

C-O-H at 1080 cm^{-1} showed retrogradation phenomenon[24]. Compared the peaks in native starch with wavelength 1081 cm^{-1}, that was observed shift to 1080 cm^{-1} after fermentation. It indicated modification in there[25].

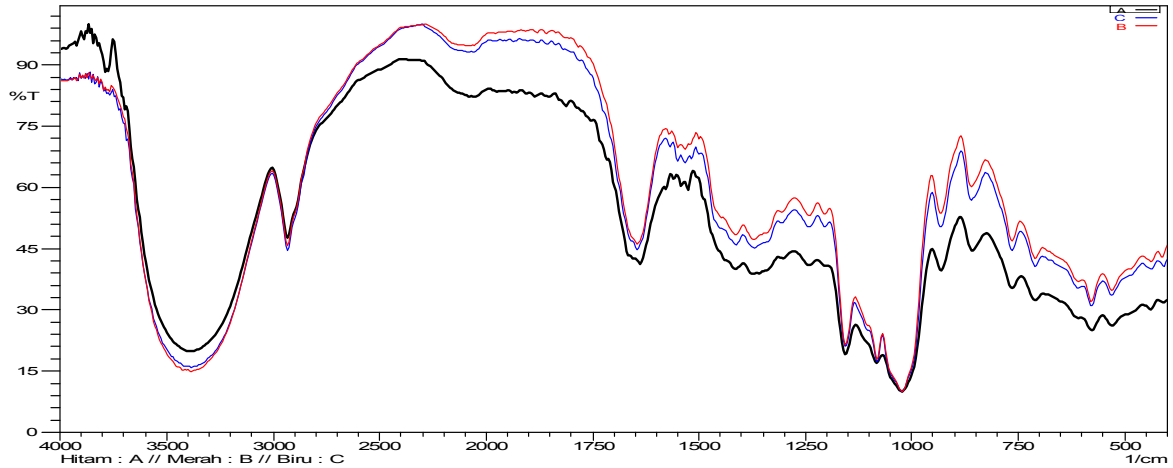

Figure 3. FTIR Spectroscopy of 3, 24, and 48 h fermented rice flour:

Native rice flour
24 h fermented rice flour
48 h fermented rice flour

Pasting properties

Table 4 Pasting properties of 3, 24, and 48 h fermented rice flour

No	Parameters	Pasting Properties		
		Native rice flour	24 h fermented rice flour	48 h fermented rice flour
1.	*Peak* 1 (cP)	4662,00 cP	4607,00 cP	4587,00 cP
2.	*Trough* 1 (cP)	2624,00 cP	2594,00 cP	2960,00 cP
3.	*Breakdown (cP)*	2038,00 cP	2013,00 cP	1627,00 cP
4.	*Final Viscosity (cP)*	4470,00 cP	5227,00 cP	5908,00 cP
5.	*Setback (cP)*	1846,00 cP	2633,00 cP	2948,00 cP
6.	*Peak Time (minute)*	6,13	6,13	6,33
7.	*Pasting Temperature (°C)*	81,5 °C	81,45 °C	82,30

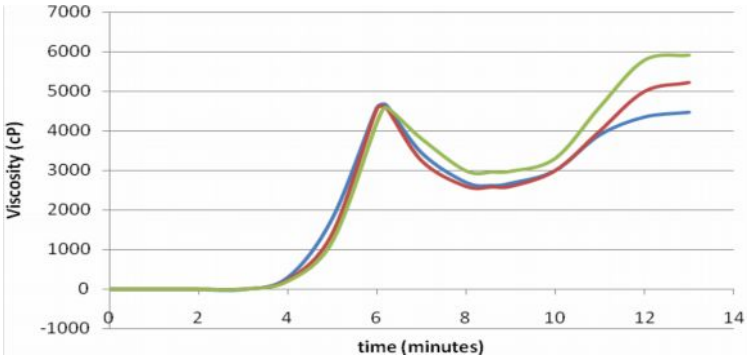

Figure 4. RVA analysis of 3, 24, and 48 h fermented rice flour:

Native rice flour
24 h fermented rice flour
48 h fermented rice flour

The pasting properties of control, 24 h, and 48 h fermented rice flour are shown in Figure 4 and Table 4. When the time of fermentation increase, peak viscosity decrease whereas setback viscosity increase. Decreased in peak viscosity was due to the presence of the shorter starch chains produced during fermentation[5,7]. Fermented rice flour had greater solubility than the native one. The lower peak viscosity is beneficial for spreading the batter rice.

The increased in setback viscosity indicated that fermented rice flour was retrogradated easily. Retrogradation was influenced by amylose. Based on amylose data, amylose content increase in the last fermentation, so it can be retrogradated easily.

Physical properties

Table 5 Tensile strength and elongation capacity of Rice Paper

Fermentation time (h)	Tensile strength (MPa)	Elongation at break (%)
3 (control)	6.1 ± 0.5^{d}	4.0 ± 0.6^{e}
12	7.0 ± 0.7^{c}	3.3 ± 0.2^{d}
24	9.5 ± 0.5^{bc}	2.5 ± 0.2^{c}
36	11.4 ± 0.7^{b}	2.2 ± 0.1^{b}
48	13.7 ± 0.9^{a}	1.7 ± 0.4^{a}

Values in columns with different letters are significantly different ($P < 0.05$). Results given as means ± standart deviation of four times repetition.

The longer time of fermentation, elongation capacity of rice paper decrease and their tensile strength increase (Table 5). Before mechanical test was conducted, samples were put on humid room for rehydration for 5 h. It was showed that the longer the time of fermentation, the equilibrium of moisture rice paper decrease. It was due to retrogradation inhibit the absorption of water. Water is a powerful plasticizer in starchy and protein rich materials, rice papers were also plasticized by water before mechanical test. So that rice paper with higher moisture explaining their lower tensile strength and their higher elongation capacity.

Conclusion

The length of fermentation time influence the physicochemical properties of fermented rice flour. It also influenced Rice Paper production. Total lactic acid bacteria and total acidity of fermented rice flour supernatant increased as long as time of fermentation increased but pH decreased.

As fermentation time increased, moisture, starch, and lipid content decreased whereas protein content increased. Amylose content initially decreased at the first and middle fermentation then increased again at the end of fermentation. Morphological analysis of starch granule at control, 24, and 48 h fermented rice flour showed greater degradation with longer fermentation time.

The fermentation time also obviously affected the FTIR spectra and pasting properties. Variation of fermented rice flour also influenced mechanical properties from Rice Paper. Rice paper can be made by medium amylose Indonesian rice but it need more researches to improve their physical properties.

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