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Physical and Chemical Characteristics of Jackfruit (*Artocarpus heterophyllus* Lamk.) Seeds Flour Produced Under Fermentation Process by *Lactobacillus plantarum*

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Abstract

Jackfruit seed is a potential local food source, which can be derived as flour. However, this seed contains several oligosaccharides such as raffinose and stachyose, which can cause flatulence for humans, and these substances will also create a darker color during flour processing. Effort to reduce this type of oligosaccharides is reported here by introducing fermentation technique using *Lactobacillus plantarum*. The aim of this study is to determine the physical and chemical characteristics of jackfruit seed flour fermented by fermentation process for 32 hours incubation time. The results showed that the jackfruit seed flour after fermentation processes have different characteristics compare to that of non-fermented one. The degree of whiteness of jackfruit flour produced under longer time of incubation during fermentation were higher compare to that of shorter duration. Based on the result of FTIR analysis, starch structure differences could not be seen between flour produced from fermentation process and without fermentation. It means that the fermentation process by *L. plantarum* were not able to change the structure or the degree of polymerization of the starch molecules. The only changes observed were the differences of the oligosaccharides content. The more incubation time assigned, the higher the amount of raffinose detected in the flour, followed by the decrease of the bigger oligosaccharides content such as stachyose and verbascose, as has been shown by HPLC chromatogram, which indicate that enzyme produced by *L. plantarum* during fermentation process is able to degrade bigger oligosaccharides rather than raffinose.

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Keywords: jackfruit seed, oligosaccharides, flatulence, raffinose, *Lactobacillus plantarum*

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1. Introduction

Jackfruit seeds are potential local food source which can be processed into flour. According to BPS (2012), production of jackfruit in Indonesia was 720,208 tons a year, produced seeds between 57,600 to 108,000 tons. Anneahira (2010) stated that the jackfruit seeds are known to contain a lot of carbohydrates, protein, and minerals (calcium and phosphorus). However, to process it further, it requires a treatment such as blanching. since this kind of seed is generally susceptible to browning during processing. Other problems in processing jackfruit seed to become food raw material, is the oligosaccharides content (Wichienchot et al., 2010). These oligosaccharide types which include raffinose, stachyose and verbascose are difficult to be digested because the small intestine of mammals do not have the enzymes which can degrade this kind of oligosaccharide. When these oligosaccharides passed into large intestine, after fermented by intestine microflora, it will produce gas and cause flatulence. Flatulence is considered to be a serious problem although it is not toxic. An increase of gas in the rectum will cause pathological symptoms including headache, dizziness, and even mental disorder. Therefore, it is necessary to eliminate those kind of oligosaccharides from the jack bean seed before use it as food.

An alternative technique to reduce oligosaccharides from the bean is fermentation process (Gu et al., 2013). Several microbes have been widely used in the fermentation of foodstuffs are lactic acid bacteria (LAB), suh as *L. plantarum* SMN 25, *L. plantarum* pentosus SMN 01 and *L. plantarum* pentosus FNCC 235 isolated from the fermentation of traditional foods, showing the ability to produce α-galactosidase which is able to degrade oligosaccharides (Sumarna, 2008). According to Lee et al. (2011), LAB can ferment various types of carbohydrates include raffinose and stachyiose, as these oligosaccharides are found in many plants, especially in grains. Surono (2004) explained that LABs able to grow on some non-dairy products were also *L. plantarum* species members. So far, implementation of LAB on production of jackfruit seed flour has not yet investigated. Therefore, this study will discuss the characteristics of jackfruit seed flour produced using *L. plantarum as* its starter-culture.

2. Material and Methods

2.1. Materials

Jackfruit seed used in this study were obtained from Tanjung Market located in Jember district, Indonesia. *L. plantarum* were provided by culture collection of Gajah Mada University, skim milk, MRS agar (Merck), MRS broth (Merck), and NaOH, which all are p.a. grade.

2.2. Peparation of L. plantarum as starter-culture

The method of Ouwehand et al. (2001) with some modifications were used to prepare the starter culture. The stock culture of *L. plantarum* were grown in the media of MRSB 10 mL at 37 °C for 24 hours. This MRSB culture were grown further in 50 mL of 10% w/v sterile medium containing 2.5 g jackfruit seed flour, refined sugar of 1.5 g, and 1 g of skim milk, incubated at 37 °C for 24 hours. This culture was cultured again at the same condition. The amount of starter culture used for jackfruit seed fermentation was set at the concentration of 25-250 cfu *L. plantarum* per ml of working media.

2.2.1. Fermentation of jackfruit seed

Jackfruit seeds were peeled and sliced into ± 2 cm thickness form as a chip shape, and soaked in water briefly to avoid browning. After drained, the chips were then UV irradiated for 15 minutes. The fermentations of the seed chips were carried out using submerged system (approximately 50 g of chips in 500 mL of sterile water), incubated for 8, 16, 24, and 32 hours. At the end of fermentation, the jackfruit seeds were washed using clean water and then soaked in 500 mL 10% salt solution for 15 minutes. To remove the salt residue, the chips were washed 3 times using clean water followed by sun drying processed for 3 days. The dried chips were then dry-milled using a blender and sieved to 80-mesh size (Diah, 2011 with minor modifications).

2.3. Characterization of Jackfruit Seed Flour

The degree of whiteness of the jackfruit seed flour was measured using color reader, the method of Subagio (2003). The changes of functional groups of polysaccharides of the flour were identified using Fourier Transform Infra-Red Spectrophotometry (FTIR) (assigned by Chemistry Laboratory of Pharmacy Faculty, the University of Jember, Indonesia). Spectra were recorded in the 500–4,000 cm^{-1} using spectrophotometer at ambient temperature.

The oligosaccharide content analyses of the flour were performed using High Performance Liquid Chromatography (HPLC) using 87°C meta-carb column conducted by the Laboratory of Research and Analyses Centre, Gajah Mada University, Indonesia.

3. **Results and Discusiion**

3.1. The Degree of Whiteness of Fermented Jackfruit Seed Flour

The degree of whiteness of the flour produced is likely to increase when the incubation time of fermentation process was longer. The more incubation time implemented, the higher the level of whiteness as can be seen in Figure 1. This possibly because of the degradation yellow pigment of jackfruit seed as occurred during fermentation. High acidity in culture broth may also prevent Maillard reaction during incubation. This is consistent to the observation of Porres et al. (2003) which found that during fermentation will also leads to an increase of phytate compounds degradation and reduced its ash content, so that the flour color becomes brighter. Long fermentation is done can reduce levels of the protein in the flour jackfruit seeds. Non-enzymatic browning reactions can occur when reducing sugars react with compounds having NH_2 groups (protein, amino acids, peptides, and ammonium). Reduced levels of the protein in the flour will prevent browning upon heating or drying process (Agustawa, 2012). Furthermore, immersion of jackfruit seeds in saline solution may inactivate enzymes capable for supporting browning reaction (Hudaida, 2004). According to Agustawa (2012), soaking in a salt solution resulting in color closer to white. This is because Na ions from the salt bind to the phenol -OH group, avoiding the formation of the brownies color of *quinones.*

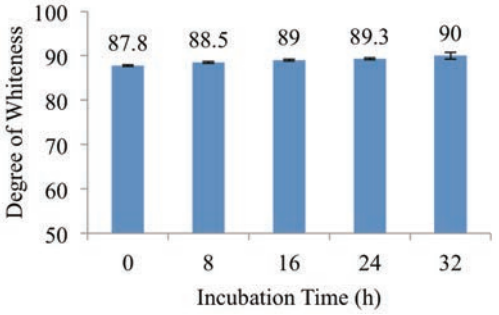

Fig. 1. The degree of whiteness of jackfruit seed flour produced from different incubation time of *L. plantarum* fermentation

3.2. Identification of Functional Groups of Jackfruit Flour Using FTIR

From the FTIR spectral analyses, it can be seen that the polysaccharides of unfermented and fermented jackfruit seed had typical carbohydrate patterns (Figure 2), and there is not a major different peak of spectrum between the two spectral of the flour produced. The same characteristic strong broad bands near 3,400 cm^{-1} of the spectrum of both flour type, indicated the presence of OH, hydroxyl stretching in hydrogen bonds which was indicative of strong inter- and intramolecular interaction of the polysaccharide chains. A weaker band at around 2,900 cm^{-1} was also appears in both spectral as attributed to the C–H stretching and bending vibrations. Both symmetrical stretching band around 1,700 cm^{-1} and a weaker symmetric one near 1,600 cm^{-1} suggested for carboxylate groups (C=O). A stronger band appear near 1000-1200 cm^{-1} indicated the presence of a β-pyran linkage. The two flour

polysaccharides were also exhibiting peak absorptions about 800-900 cm^{-1}, indicating the present β-glycopyranosidic linkages and α-configurations. Overall, both fermented and unfermented processed flour contain polysaccharides displayed both β-D-pyran-type and α-D-pyran-type sugar rings, similar to polysaccharides reported by Wang et al. (2014) and Wu et al. (2015).

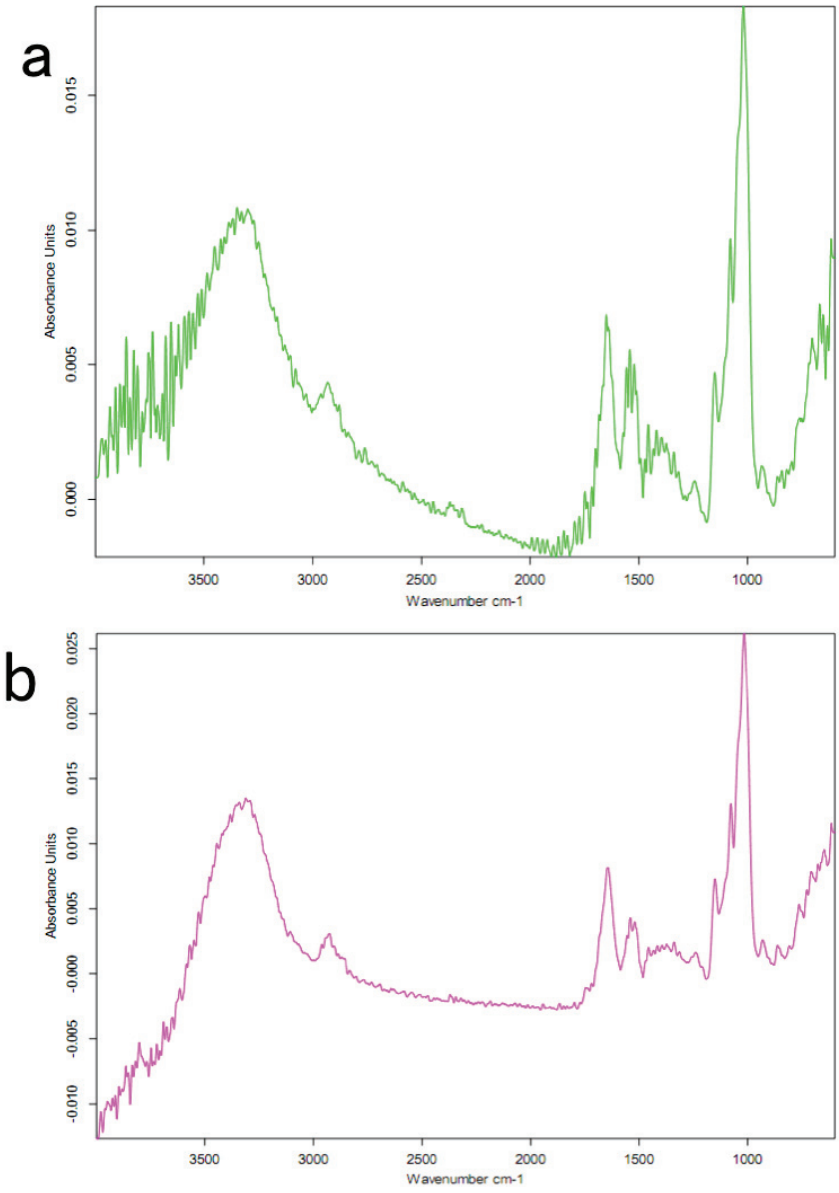

Figure 2. Infra-red spectra of jackfruit seed flour prepared without fermentation (a) and 32 hours of incubation time (b)

3.3. Measurement of Oligosaccharide Content of Jackfruit Seed Flour

The HPLC data analyses showed that raffinosa content of jackfruit seed flour fermented by *L. plantarum* at longer incubation time was higher compare to that of shorter fermentation time, as indicated by elution time 7.58; 7.39 and 7.35 minutes (Fig. 3). The raffinose content of jackfruit seed flour was varied between 390.55 to 514.02

mg/g as seen in Table 1. The increment of raffinose content of jackfruit seed flour was observed from the flour prepared by longer incubation time. Presumably, the more enzyme produced during fermentation, the more raffinose released from the degradation of the bigger oligosaccharide such as stachyose [elution time 6.13 minutes in Fig. 3 (a)] and verbascose as appear after 24 h incubation time [elution time 6.82 minutes as shown in Fig. 3 (b)]. Since raffinose content were found higher in a flour prepared from longer time of incubation, the enzyme produce during fermentation might have higher hydrolytic activity on bigger oligosaccharide such as stachyose and verbascose (pentasaccharide) rather than that on trisaccharide (raffinose). Stachyose is tetrasaccharide having two α-D-galactose, α-D-glucose, and β-D-fructose. It has been reported that α-galaktosidases from *L. plantarum* SMN 25, *L. plantarum pentosus* SMN 01 dan *L. plantarum pentosus* FNCC 235 were able to degrade raffinose and stachyose for more than 60% (Sumarna, 2008; Lambui, 2013).

Table 1. Raffinose Content of Jackfruit Seed Flour Prepared by Different Fermentation Time of *L. Plantarum*

Incubation Time (h)	Raffinose content (µg/g)
0	390.55
24	417.58
32	514.02

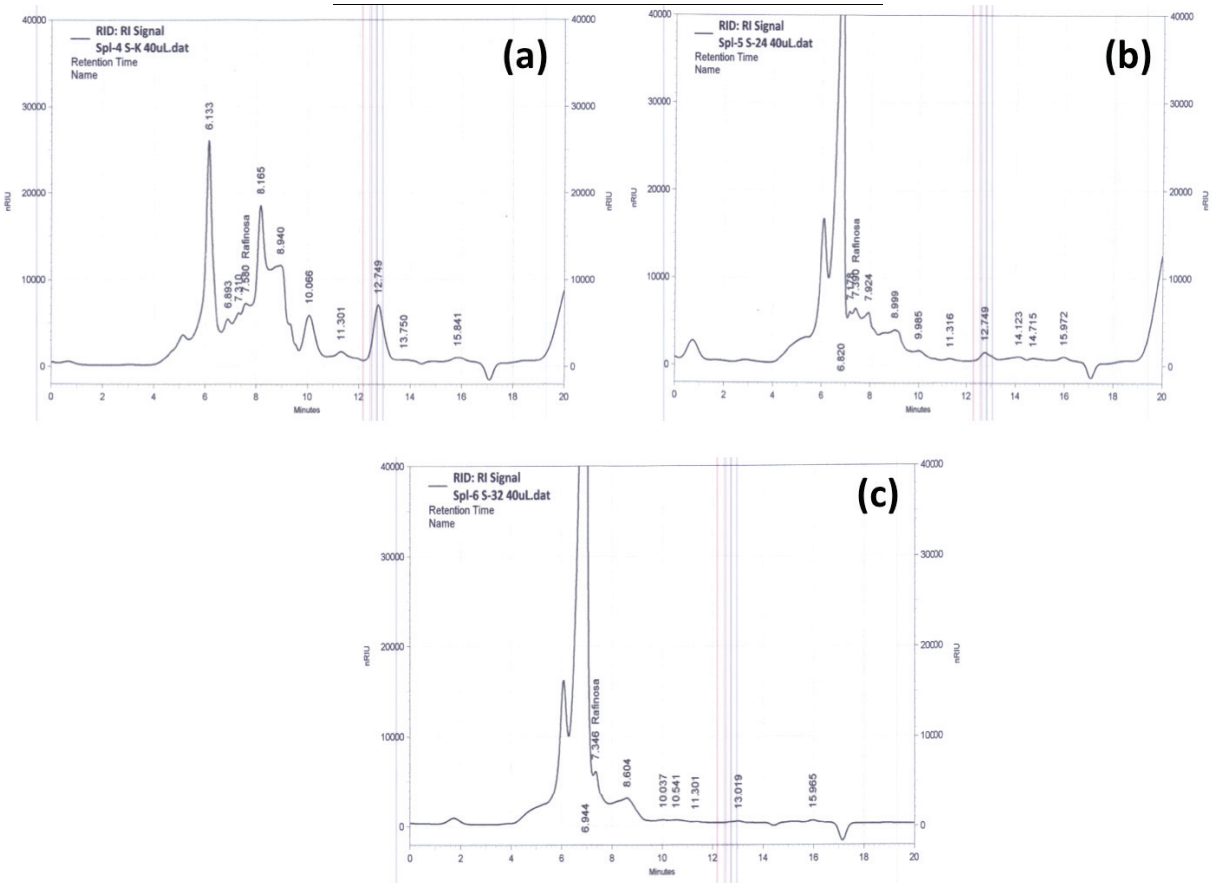

Fig. 3 (a) unfermented jackfruit seed flour; (b) 24 hours fermented jackfruit seed flour; (c) 32 hours fermented jackfruit seed flour

4. Conclusion

Treatment of *L. plantarum* fermentation time to the characteristic of flour produced can affect the value of flour whiteness, and increase the levels of raffinose content of flour. Fermentation of jackfruit seed chips by *L. plantarum* did not alter the polysaccharides profile of the flour, but the oligosaccharides types and content were changed in response to incubation time alteration. The α-galactosidase produced by *L. plantarum* is only able to degrade oligosaccharide from the jackfruit seed.

Acknowledgements

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