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Influence of *Lactobacillus plantarum* Fermentation on Functional Properties of Flour from Jackfruit (*Artocarpus heterophyllus* Lamk.) Seeds

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ABSTRACT

Effort to improve the functional properties of jackfruit seed flour has been made by introducing *Lactobacillus plantarum* fermentation on the jackfruit chips, which are latter processed into flour. The jackfruit chips were fermented using *L. plantarum* for up to 32 hours, and air dried before they were grounded to pass through an 80 mesh sifter. The results of the experiment showed water holding capacity (WHC) of fermented flour was higher compared with that of the unfermented. The longer fermentation time, the higher the WHC of the flour. In contrast, the flour oil holding capacity (OHC) decreased during longer incubation time, indicating that some shorter amyloses were released during fermentation. Earlier studies on pasting characteristics of jackfruit seed showed that the peak, setback and breakdown viscosity of fermented jackfruit seed flour were higher compared with that of unfermented flour, indicating that fermented flour will have a higher thickening power and is more susceptible to heat and mechanical shear. This finding indicates *L. plantarum* fermentation will increase the potency of jackfruit seed flour for food industry application either as a thickener and or biodegradable film. Furthermore, HPLC analysis of the soluble saccharide of fermented flour showed the differences of oligosaccharides content such as raffinose, stachiose and verbaschose, indicating the capability of the enzymes released by *L. plantarum* during fermentation to hydrolyse the starch to become shorter oligosaccharide but without monosaccharide.

Keywords: Oil holding capacity, pasting properties, raffinose, stachiose, water holding capacity

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INTRODUCTION

The demand for healthy foods has increased in tandem with health awareness. Consumers are also becoming concerned about how their diet contributes to obesity, cardiovascular disease, and hypertension (Bruhn, Cotter, & Diaz-Knauf, 1992). There have been attempts to lower the fat content of fat-rich food by promoting several types of flour as a source of fibre without damaging the physicochemical and sensory attributes of food, such as low fat high fibre meat *kofta* formulated using oat flour and carrageenan (Modi, Yashoda, & Naveen, 2009). Cassava has been used in the food industry to supplement wheat flour in the production of confectionaries (Gyedu-Akoto & Laryea, 2013; Kulchan, Boonsupthip, & Suppakul, 2010; Shittu, Dixon, Awonorin, Sanni, & Maziya-Dixon, 2008). Based on this evidence, several types of potential flour from different sources have been investigated to be developed as functional foods. However, many flours are reported to be deficient in some functional properties, which limits their industrial use. Therefore, several techniques have been proposed to resolve this problem including fermentation technique using microorganism, such as lactic acid bacteria, to find a simpler and cheaper method to improve their sensory characteristics, nutritional value, and technical-functional qualities (Blandino, Al-Aseeri, Pandiella, Cantero, & Webb, 2003). Fermentation-based flour process could be a potential technique not only to improve physicochemical characteristic of the flour (Chowdhury, Bhattacharyya, & Chattopadhyay, 2012), but also to increase the functional nutritional compounds, which include dietary fibre, minerals, antioxidants, prebiotics and vitamins (Coda, Rizzello, Trani, & Gobbetti, 2011). The application of fermentation method for flour production has been established to produce various food forms which include bread, beverages, and porridge from the raw materials of several cereals origin including rice, wheat, corn and sorghum (Helland, Wicklund, & Narvhus, 2004); tubers and legumes (Noorfarahzilah, Lee, Sharifudin, & Hasmadi, 2014), where these fermentation techniques were mainly used to produce a physico-chemically and functionally better flour. Noorfarahzilah et al. (2014) reviewed studies that looked at formulation of composite flour from different local source, such as tubers, legumes, cereals, and even from underutilised fruit and vegetables to substitute wheat flour to produce variety of a higher quality of many local food products. It was found that other than cereals, tubers and legumes, flour from fruit by-product such as mango kernel (Menon, Majumdar, & Ravi, 2014) and seed (Kittiphoom, 2012), rambutan seed (Eiamwat, Wanlapa, & Kampruengdet, 2016), pumpkin seed (Białek, Rutkowska, Adamska, & Bajdalow, 2016) and jackfruit seed (Retnowati, Ratnawati, & Purbasari, 2015) have also been explored as composite flour. However, the functional properties of those native seed-based flour need to be improved since many did not fulfil the technical-functional properties for food uses. Fermentation method to produce, especially jackfruit seed as the composite flour, has been understudied. Therefore, this research was undertaken to study the effect of *L. plantarum* fermentation on jackfruit seed characteristic changes and the functional properties of its flour.

MATERIALS AND METHODS

Preparation of Jackfruit Seed for *L. plantarum* Fermentation Processes

Jackfruit seed was used for this experimentation, and the inoculums used for chips fermentation were prepared using the method used by Jayus, Setiawan and Giyarto (2016). The *L. plantarum* were sub-cultured using medium containing 0.03% (w/v) refined sugar, 0.05% (w/v) raw jackfruit seed flour, and 0.02% (w/v) commercial skim milk to produce a stock culture. These sub-culturing processes were maintained to provide a starter culture with a minimum population of *L. plantarum* at 10^8 cfu/ml.

Fermentation of Jackfruit Seed Chips

The jackfruit chips were fermented in a submerged culture using the starter culture as previously reported by Jayus et al. (2016) for 8, 16, 24, and 32-hour incubation time. Fifty grammes of approximately 2cm thickness of jackfruit chips were UV radiated for 15 minutes before fermentation process. The fermented chips were rinsed 3 times to eliminate any salt residue. The chips were then sun dried, and dry-milled to achieve 80-mesh size of flour particles.

Measurement of Jackfruit Seed Flour Water Holding Capacity (WHC)

Water Holding Capacity (WHC) of jackfruit seed flour was calculated using the method described previously by Traynham, Myers, Carriquiry and Johnson (2007) with some modification. The sample of 0.5 g flour and 3.5 mL distilled water suspension was stirred for three minutes. The 10-min centrifugation of the samples were carried out at 6000×g using a refrigerated centrifuge (Himac compact RXII series, Hitachi). Decantation was used to separate the water. The WHC values were calculated using the following equation:

$$WHC = \frac{[(\text{weight of bottle and flour after decanting - dry bottle weight}) - \text{weight of flour}]}{\text{weight of flour}} \times 100\%$$

Measurement of Oil Holding Capacity (OHC)

The OHC values of jackfruit seed flour were determined using the modification of method described previously by Mirhosseini and Amid (2012). The sample of 0.5 g flour and 10 mL refined palm oil suspension were mixed for three minutes. The centrifugation of the samples was carried out at 6000×g for 10 minutes using a refrigerated centrifuge (Himac compact RXII series, Hitachi). The OHC values were calculated using the following equation:

$$OHC = \frac{[(weight\ of\ bottle\ and\ flour\ after\ decanting\ -\ dry\ bottle\ weight)\ -\ weight\ of\ flour]}{weight\ of\ flour} \times 100\%$$

Studies on Pasting Characteristics of Jackfruit Seed Flour

The pasting properties or characteristics of the flour samples were determined using RVA (Tec Master, Australia). Jackfruit seed flour samples (3 g, 10% moisture) were suspended into 25g distilled water homogenised under constant shear rate at 160×g for 13 min, controlled using heating and cooling cycle system, heated from 50 to 95°C. Initially, the slurry was heated to 50°C and agitated at 960×g for 10 seconds and then the mixing speed was lowered to 160×g. After 4 minutes and 42 second mixing time, the slurry was heated up to 95°C for 11 minutes. The flour temperature was then lowered to 50°C for 2 min. The parameters obtained were the *peak viscosity* (PV), breakdown (B), *minimum viscosity* (MV), *final viscosity* (FV), set back (SB), *pasting temperature* (PT), and *time to peak* (Ptime).

Identification of Dissolved Oligosaccharide in the Flour of Jackfruit Seed. HPLC analyses were undertaken to identify the dissolved oligosaccharide content of the flour, preparing 1 g of flour sample to be dissolved in mili-Q water (25 ml) and heated at 70°C for 60 minutes. The mixture was then filtered using 0.45 µm filters, before injected to Metacharb 87C column eluted using water at 0.6 mL/min flow rate using RID detector.

Reproducibility of the Data

Data obtained here are the means from at least triplicates. The variations of the means represent standard deviation.

RESULTS AND DISCUSSION

Influence of Fermentation on the WHC and OHC of Jackfruit Seed Flour

The WHC of fermented jackfruit seed flour was slightly higher compared with that of the unfermented one (0 h incubation time, see Figure 1), indicating that only a small amount of macromolecule was degraded during fermentation. *L. plantarum* enzyme produced during fermentation may only be able to degrade a few macromolecules to a shorter oligosaccharide (Jayus et al., 2016). This finding is in contrast to what had been observed by Kee and Saw (2010) on the jackfruit flour produced by fermentation of its raw flour using *Lactobacillus sp*., where the WHC of this fermented flour decreased. This could be due to differences of the *Lactobacillus* strain used. The enzymes released by the various strains may differ in terms of their mode of action, which could be either exo- or endo-action, releasing different oligosaccharides as the hydrolysis product. Different mode of action of enzyme secreted by microorganism had been reported by Talamond, Noirot and de Kochko (2006), and Jayus, McDougall and Seviour (2001; 2004). Afoakwa, Aidoo and Adjonu (2010) also pointed out fermentation can lower the capacity of cowpea-fortified nixtamalised maize to absorb water because of the reductions in the availability and action of the hydrophilic groups which bind water. Even though the fermentation of the cowpea was spontaneous, but some amylase-rich flour added to the composite will increase the activity of the micro-flora during fermentation and the amylolytic enzymes will degrade the starch.

Meanwhile, the OHC of fermented jackfruit seed flour is lower compared with that of the unfermented one (Figure 2). The reduction in OHC occurs when the degradation of some poly- and oligosaccharide by enzyme of *L. plantarum* may reduce the hydrophilic side of the molecules and increases the presence of non-polar side in the flour (Adebowale, Afolabi, & Lawal, 2002). Many of OHC of the flour are affected by the method of process, even just a peeling process has been reported to lower the OHC of pumpkin (Aziah & Komathi, 2009).

Pasting Properties Studies

The pasting temperature of unfermented flour is similar (88.90°C) to that of the fermented one (88.00°C). This finding is

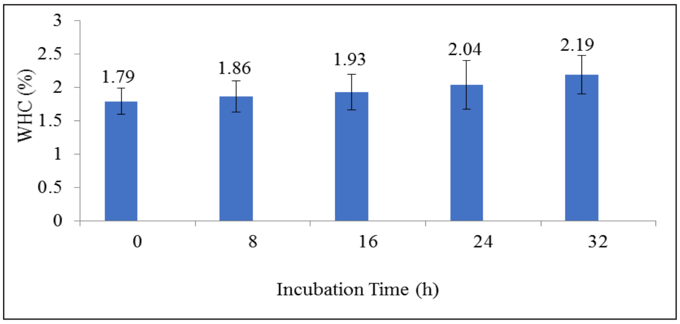

Figure 1. The WHC value of unfermented (0 h incubation time) and *L. plantarum* fermented jackfruit seed flour

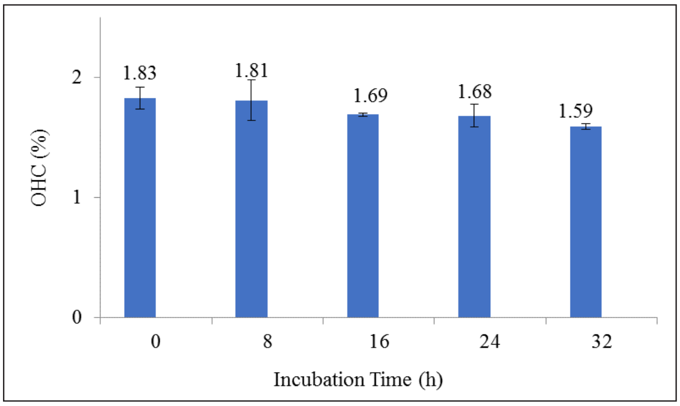

Figure 2. The OHC value of unfermented (0 h incubation time) and *L. plantarum* fermented jackfruit seed flour

consistent with the milled prepared jackfruit seed flour measured by Mukprasirt and Sajjaanantakul (2004), though jackfruit seed starch had higher pasting temperature (81.58 °C) as reported by Rengsutthi and Charoenrein (2011). Jackfruit seed flour pasting temperatures are higher compared with that of cocoyam (Oke & Bolarinwa, 2012) and cassava (Nwokocha, Aviara, Senan, & Williams, 2009), indicating a weaker granular structure of the latter compared with jackfruit seed flour. The differences of this granular structure may occur because of the major contribution of the enzyme work released by controlled *L. plantarum* only during fermentation of the seed. Meanwhile, other researchers used spontaneous fermentation techniques where the growth of microorganisms on the seed were uncontrolled, which might produce different and inconsistent properties of the flour. The detailed effects of fermentation on the pasting properties of jackfruit seed flour described shown in Table 1.

Table 1
Unfermented and fermented jackfruit seed flour pasting profile

Treatment	Peak viscosity (cP)	Minimum viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Set back (cP)	Pasting temperature (^oC)	Peak time (min)
Unfermented	1757	1071	686	1394	323	88.90	5.13
Fermented	2340	1590	750	2412	822	88.00	5.40

The peak viscosity of fermented jackfruit seed flour was higher (2340 cP) compared with that of unfermented flour (1757 cP) as seen in Figure 3. Likewise, a similar trend was observed for setback and breakdown viscosity. This may indicate that fermented flour will have a higher thickening power and more susceptible to heat and mechanical shear. Higher viscosity profile of flour has been reported to have a higher potency as a material for biodegradable film. This can increase the Young's modulus and tensile strength of the film (Retnowati et al., 2015).

Controlled fermentation using *L. plantarum* as the only inoculum on jackfruit seed produce the higher pasting properties of the flour, in contrast with the spontaneous fermentation work on other seed as what have been observed by Oke and Bolarinwa (2012) in cocoyam flour, where the fermented flour appeared to have lower peak viscosity. The reason for this contradictory phenomenon is still unclear. It could be due the different type of amylase released by the microorganism used for fermentation. In the case of cocoyam flour, the fermentation processes increase the amylose content of the flour and lower the peak and the final viscosity observed. Meanwhile, similar enzyme of *L. plantarum* fermentation on jackfruit seed may not be able to degrade the starch into a higher proportion of amylose and increase the amylopectin proportion instead. Hence, the

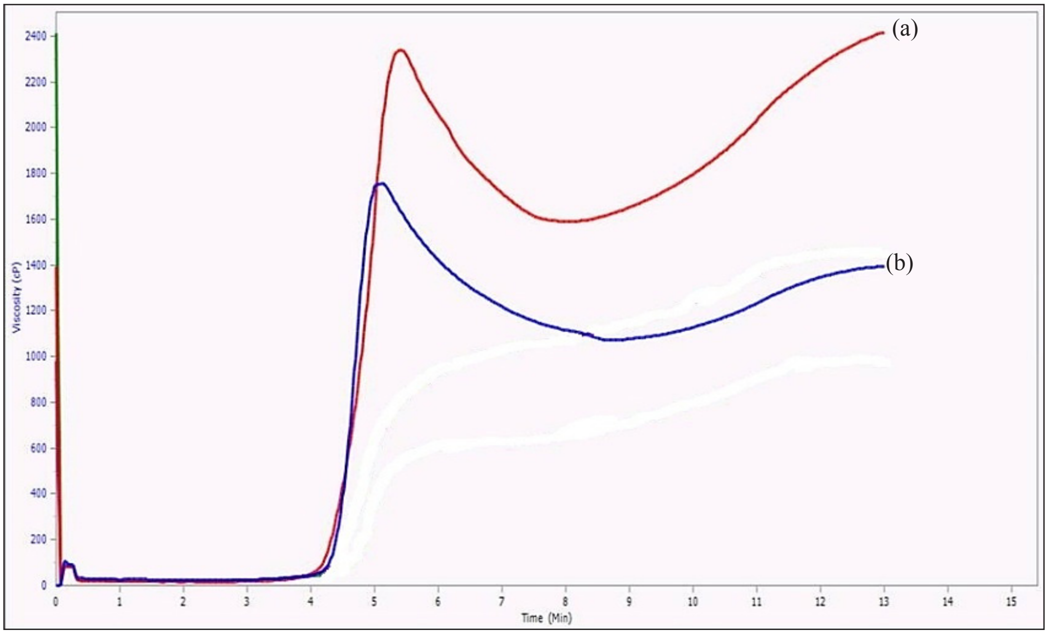

Figure 3. The viscosity profile of fermented (a), and unfermented (b) jackfruit seed flour.

final viscosity of the flour may increase. This reason cannot be confirmed until the content of amylose in jackfruit seed flour is determined. In this study, the amount of the compound was not measured. However, *L. plantarum* fermented jackfruit seed flour has been reported to contain higher water-dissolved shorter oligosaccharides, such as raffinose (Jayus et al., 2016), but not glucose, as the hydrolysis product of enzyme released by *L. plantarum* to degrade α-1,4- and α-1,6- glyosidic linkages (Olympia, Fukuda, Ono, Kaneko, & Takano, 1995). The presence of raffinose and slightly higher molecular weight of oligosaccharides including stachiose and verbaschose (as reported in this study) may change the viscosity profile of the flour as shown in Figure 3.

Differences of oligosaccharide content of unfermented and fermented seed flour were detected in the HPLC chromatogram (Figure 4). The raffinoses content were slightly increased over the 24-hour fermentation time (0.36 to 0.44 µg/g of flour), but the stachiose and verbaschose content noticeably increased. In the unfermented seed flour, stachiose and verbaschose content were almost undetected, 0.44 and 0.42 µg/g of flour respectively, but in 24 h fermented seed flour, verbaschose was increased sharply (4.95 µg/g of flour) and no apparent stachiose was detected at this time. Interestingly, the stachiose was noticeable in the 32 h fermented seed flour (5.21 µg/g of flour) followed by the reduction of the amount of verbaschose, indicating that enzymes released by *L. plantarum* were most probably endolytic action enzyme capable to degrade polysaccharide of the flour randomly to produce shorter oligosaccharide.

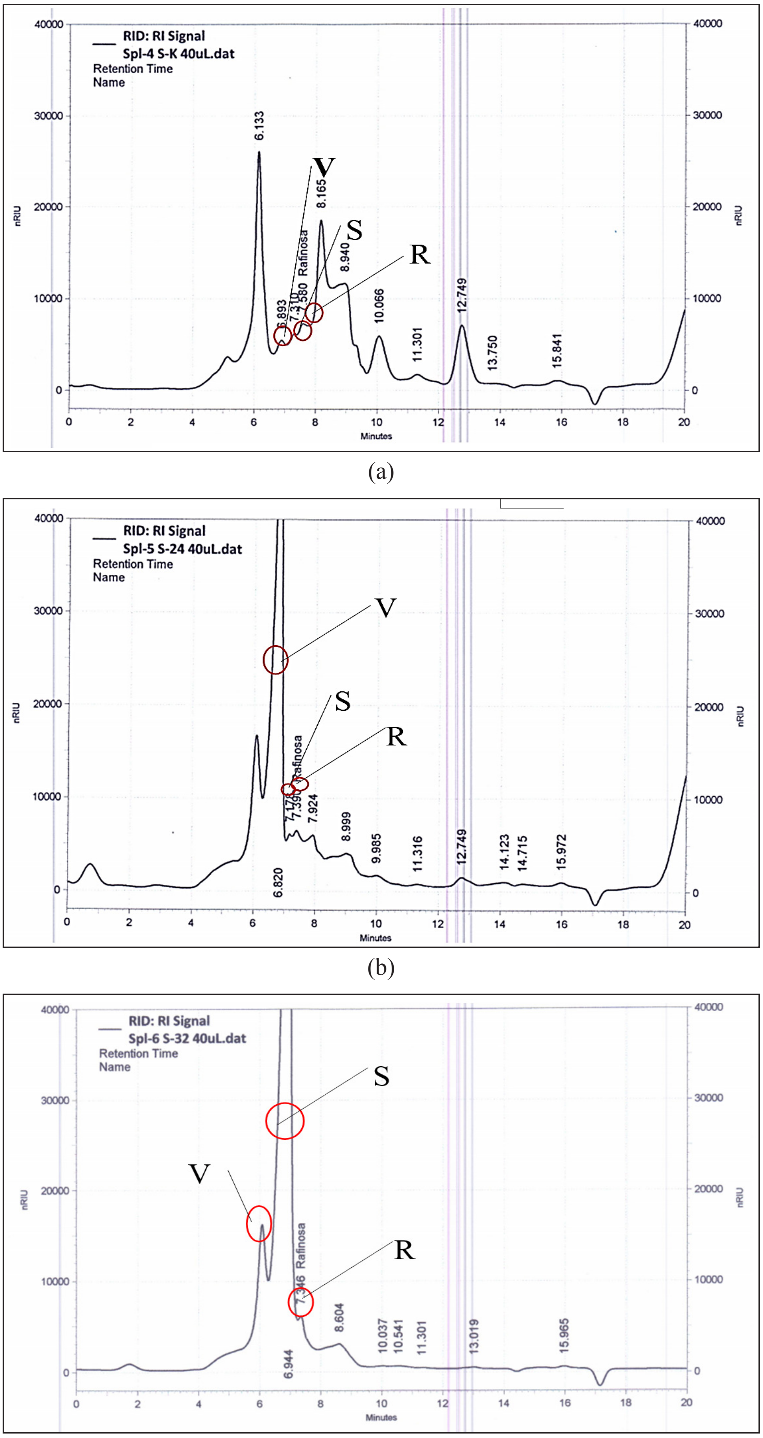

Figure 4. The HPLC chromatogram of: (a) unfermented; (b) 24 h fermented; and and 32 h fermented (c) jackfruit seed flour. V = Verbaschose, S = Stachiose and R = Raffinose

CONCLUSION

The *L. plantarum* fermentation of jackfruit seed appeared to influence WHC and OHC of the flour obtained, indicating that shorter amyloses were released during fermentation. The peak viscosity, setback and breakdown of fermented jackfruit seed flour were higher than that of the unfermented one, indicating that fermented flour would have a higher thickening power and possibly be more susceptible to mechanical shear and heat, and hence has a potential as a thickening agent. The enzymic activity of *L. plantarum* on the preparation of the flour contributed to changes of the soluble saccharide, and the differences in oligosaccharides content, which include stachiose and verbaschose, indicate enzymes secreted by *L. plantarum* during fermentation capable to hydrolyse the starch randomly, releasing shorter oligosaccharide but not monosaccharide, in which this enzyme character will produce the flour containing more oligosaccharides which may have a potential as a material for biodegradable film.

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