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Research article

Nutritional and functional properties changes during facultative submerged fermentation of gadung (*Dioscorea hispida* Dennst) tuber flour using *Lactobacillus plantarum*

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ABSTRACT

This work aims to examine the influence of flour concentrations (5%–25% w/v), inoculum loading (2.5%–15% v/v), and fermentation time (0–144 h) on the nutritional and functional properties of gadung (Dioscorea hispida Dennst) tuber flour. The flour was microbiologically treated through facultative submerged fermentation using Lactobacillus plantarum. The carbohydrate, lipid, protein, fiber and ash contents were reduced by fermentation, while moisture content was increased. In general, the swelling power and the solubility of fermented flour were below those of the native flour. Carboxyl group content increased with fermentation time, whereas no clear trend was found for carbonyl group. The amylose content of the fermented flour was larger than that of the native flour, which most probably was due to the depolymerization of amylopectin branches to form new amylose-like molecules. The best fermentation conditions were flour concentration of 10% (w/v), inoculum loading of 5% (v/v), and fermentation for 48 h.

1. Introduction

Dioscorea hispida Dennst or locally known as gadung is a twiner affiliated to the Dioscorea genus of the Dioscoreaceae family. This vegetation possesses a thorny stem twinning to the left, which may achieve up to 20 m in height with plumy trifoliate leaves and bears small pale-yellow flowers (Nashriyah et al., 2010). This vegetation is facilely discovered in the shade or the vicinity streams of secondary forests of Southeast Asia and its neighboring areas. Being commonly planted at the commencement of wet season (October–November), a large mature tuber with white-to-yellow flesh is generally harvested in the dry season (April–September). Unfortunately, this tuber contains antinutrients such as cyanogens, alkaloids (dioscorin), tannins, and saponins, which may be attributed to the bitterness and toxicity of the tuber (Ashri et al., 2014). Concerning its high carbohydrate content, this tuber has long been utilized as staple food for some ethnic groups in the countryside areas, especially during World War II. In Indonesia, the Philippines, Malaysia, Vietnam and Thailand, gadung tubers are commonly eaten boiled, fried or steamed following a complicated cyanogen-reduction process for detoxification purposes. Being gluten free and rich in resistant starch content, gadung tuber can be a promising food source for individuals to reduce the risk of obesity, diabetes, wheat allergy, and the incidence of celiac diseases. Today, most of the people in several Western communities are applying a gluten-free diet, dodging the wheat, rye, and barley (Lundin, 2014). The demands for gluten-free products have been escalating rapidly, extending the chances for the development of new techniques to develop new products using gluten-free raw materials as a replacement for conventional manufacturing of bakery products (Schober, 2009). Considering these motivating situations and advantages, an effort for the processing of gadung tuber into functional food materials was performed.

Gadung tuber starch gelatinizes at a high-temperature range, which is almost equal to the pasting temperature of cereal starches. This property indicates the potential application of gadung tuber starch as a thickening agent in retort foods or foods that require heat-stable viscosity. Starches with restricted swelling and stabilized granular structure are highly desired in the food and pharmaceutical industry (Ratnayake and Jackson, 2008). With a swelling power of 15.6 g/g of dry starch at 90 °C (Tattiyakul et al., 2006), gadung tuber starch falls in the restricted swelling category (Shimelis et al., 2006). Based on this characteristic, gadung tuber starch is desirable for the production of value-added products such as noodles. However, due to its low setback viscosity, gadung tuber

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starch has a low retrogradation tendency and, consequently, fails to form strong gels (Tattiyakul et al., 2006). Therefore, gadung tuber starch may not be suitable for application in foods that need firm texture. Commonly, breeders and the food industry use amylose content as the most important determinant in food texture development. In general, stronger starch gels can be well correlated with higher amylose content (Ishiguro et al., 2000). Starches with low amylose content show a higher degree of crystallinity (Tukomane et al., 2007). Based on this point of view, one of the suitable efforts to increase the gel strength of gadung tuber flour is by increasing the amylose content, which finally increases the retrogradation tendency. Fermentation has shown its capability to increase the breakdown viscosity value, which is a measure of the retrogradation tendency of the starch (Kaur and Singh, 2005).

Fermentation is one of the oldest biotechnological procedures of food preservation and processing that is widely applied in the world. Fermentation is a metabolic process involving bacteria, yeast, molds, or their combination in which carbohydrates and related compounds are oxidized with the release of energy in the absence of any external electron acceptors under anaerobic or facultative condition. Over millenniums, the demands for fermented foods have greatly increased due to the increased demand for nutritious, safe, natural, additive-free, and well-preserved foods. Therefore, these foods become an important part of human diet worldwide (Elyas et al., 2015). During the fermentation processes, microorganisms produce specific metabolites such as enzymes, acids, alcohols, antibiotics, carbohydrates, and inhibitory compounds that contribute to the safety and nutritional quality of fermented foods. The lactic acid bacteria (LAB) that are generally regarded as safe (GRAS), play an essential role in the majority of food fermentation and preservation. These properties are important for the use of LAB as starters or adjuncts to maintain and improve the nutritional, sensory, and safety qualities of the final products. In this regard, various strains of LAB have been routinely applied as starter cultures in the production of dairy, meat, vegetables, and bakery products (Saeed et al., 2014).

The current work aimed to study the influence of flour concentration (5%–25% w/v), inoculum loading (2.5%–15% v/v), and fermentation time (0–144 h) on the swelling power, solubility, carbonyl and carboxyl group content, and amylose content during fermentation of gadung tuber flour using *Lactobacillus plantarum*.

2. Materials and methods

2.1. Microorganism and materials

Gadung flour was obtained by milling of dried detoxified gadung tuber chips of matured gadung tuber (nine months age) harvested from Gunungpati-Semarang, Indonesia. The detoxification process was the same as previously developed by Widiyanti and Kumoro (2017). The *Mucor racemosus* strain used for the detoxification process was obtained from Microbiology Laboratory, Departement of Chemical Engineering, Faculty of Industrial Technology, Institut Teknologi Bandung, Indonesia. The L. *plantarum sp* CCRC 12251 was procured from Food and Nutrition Inter-University Center, Universitas Gadjah Mada, Yogyakarta-Indonesia and maintained in de Man Rogosa Sharpe (MRS) agar slant at 4 °C. Multipurpose wheat flour was the product of PT. Indofood Sukses Makmur Bogasari Flour Mills Tbk. Jakarta – Indonesia and used as a control sample. All of the chemicals and reagents used in this study were of analytical grade with a purity of ≥99.5% (w/w) and manufactured by Sigma-Aldrich. They were purchased from authorized chemicals distributors in Semarang, Indonesia, and directly used without prior treatments.

2.2. Methods

The general diagram describing the global methodology used to conduct the research is depicted in Figure 1.

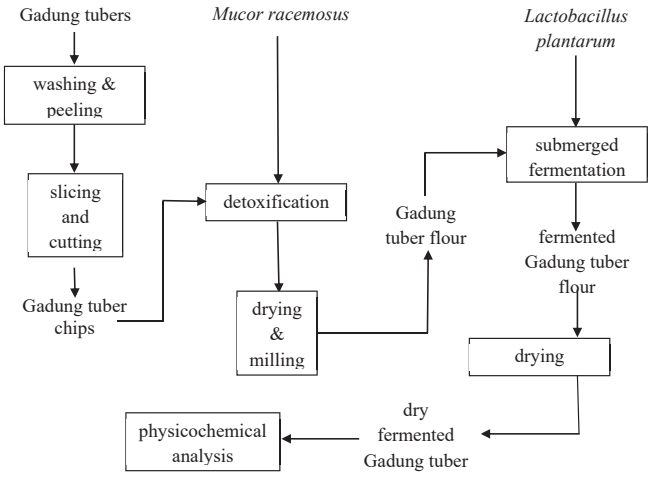

Figure 1. General methodology diagram of the research.

2.3. Detoxification of gadung tuber

Before detoxification, gadung tubers were sliced into chips with ±5 mm thickness and 5 cm´5 cm square dimension. They were then washed with flowing tap water and drained to remove the adhering water. Detoxification process was carried out by fermentation of approximately 450 g gadung tuber chips using 3.4×10^6 CFU/g Mucor racemosus inoculums at room temperature (28 ± 1.0 °C) under constant light intensity for 120 h. The fungi were previously cultivated in potato dextrose agar (PDA) for 7 days as suggested by Jakovljevic *et al.* (2014). The fermented gadung tuber chips were then washed with distilled water to remove the fungi and dried in an electric oven at ± 60 °C for 8 h. The dried fermented gadung tuber chips were then ground into grits and followed by milling them into powder in a laboratory hammer mill (Glen Creston, England). The powder was sifted with an 80 mesh sieve (British Standard) to obtain detoxified gadung tuber flour.

2.4. Inoculum preparation

The inoculum of *L. plantarum* was prepared in a 250 mL Erlenmeyer flask containing 100 mL of modified de Man Rogosa Sharpe (MRS) liquid medium (a mixture of peptone, 10 g; beef extract, 10 g; yeast extract, 5 g; glucose, 20 g; polyoxyethylene sorbitan mono-oleate (Tween 80), 1 mL; Na_2HPO_4, 2.0 g; $CH_3COONa.3H_2O$, 5 g; triammonium citrate, 2 g; $MgSO_4.7H_2O$, 0.2 g; $MnSO_4.4H_2O$, 0.05 g; and distilled water, 1000 mL at pH 6.8) by transferring a loop full of microorganisms (*L. plantarum*) from a stock culture and incubated at 35 °C and 120 rpm for 48 h in an orbital incubator-cum-shaker. The number of viable bacteria was counted by the total plate count (TPC) method as also used by previous researchers (Rizzello et al., 2010). The inoculums were found to contain 3×10^7 CFU/mL.

2.5. Fermentation

Slurries of detoxified gadung flour with various flour concentrations (5%–25% w/v) were prepared in 250 mL Erlenmeyer flasks containing 100 mL distilled water. To prevent starch gelatinization, no thermal sterilization was done. Then, the slurries were inoculated with various amounts (2.5%–15% v/v) of freshly prepared inoculums and covered with aluminum foil. Attention should be taken that although traditionally 1% (v/v) inoculum refers to the addition of 1mL inoculum to 99 mL medium, but this procedure does not assure the same initial optical density (OD) of the inoculated medium as a result of imprescriptible OD variation of the inoculum prepared from batch to batch. Therefore, to ensure the initial starting OD and reproducibility in the fermentation

time, more volume of inoculum from a single bacterial colony on agar medium of the logarithm growth phase of bacteria, which provides an OD of about 0.1 was transferred to obtain a carefully prepared 100 mL inoculated medium. This principle would not change the final medium volume after inoculation significantly. The fermentation was carried out at room temperature (28 ± 1.0 °C) and let to proceed till 144 h with continuous shaking at approximately 120 rpm on a horizontal incubator shaker. Samples were withdrawn from the fermentation system at 6 and 12 h, followed by every 24 h. The samples were filtered under vacuum to obtain the fermented flour. The fermented flour was dried in an electric tray dryer at 60 °C for 24 h to dryness and stored in an airtight container or directly subjected to assessment of swelling power, solubility, amylose, carbonyl group and carboxyl group contents. All fermentations were carried out in triplicate, and hence, the reported values are the average of three experiments.

2.6. Analytical methods

The proximate composition (protein, lipid, ash, fiber and moisture) of all flour samples was determined following the standard methods of the AOAC (Latimer Jr, 2016). Crude protein was determined by multiplying crude nitrogen content by 6.25 for gadung flour and 5.70 for wheat flour. Accordingly, the carbohydrate content was then calculated by difference.

$$\text{Carbohydrate content} = 100 - (\text{protein} + \text{lipid} + \text{ash} + \text{fiber} + \text{moisture contents}) \quad (1)$$

The swelling power (*SP*) and the solubility (*WS*) of the flours were measured as described elsewhere (Tattiyakul et al., 2012) with slight modification. Briefly, 0.5 g (W_{ds}) of dried flour sample was dispersed in 15 mL of distilled water. The flour dispersion was heated under gentle stirring at 60 °C for 30 min. The gelatinized flour dispersion was then centrifuged at 16,000 ×g for 15 min using a high-speed refrigerated centrifuge (CR-21G; Hitachi). The supernatant was separated from the swollen granules and dried at 105 °C in an electric drying oven to the achievement of a constant weight (W_{dsup}). The sediment of the swollen granules was weighed (W_{sed}) after being carefully drained. The solubility was expressed as the percentage of the weight of total soluble fraction to the weight of dried sample. The swollen flour paste was calculated as the percentage of ratio of the swollen gel weight to the total dried flour excluding the soluble fraction in aqueous solution. The swelling power and water solubility were estimated using the following equations (Chung et al., 2010):

$$WS\ (\%) = \frac{W_{dsup}}{W_{ds}} \times 100 \quad (2)$$

$$SP\ (\%) = \frac{W_{sed}}{W_{ds} \times \left(1 - \frac{WS}{100}\right)} \times 100 \quad (3)$$

The carboxyl content of the flour was determined by titration as previously described (Chattopadhyay et al., 1997). Carefully weighed dry flour (5 g) was dispersed in 25 mL of 0.1 N HCl solution by mild stirring for 30 min. The flour slurry was filtered through a fritted glass crucible (G 4) and the residue was washed with distilled water until it was free from chlorine. The residue was then redispersed in a 500 mL beaker glass by addition of 300 mL of distilled water. The flour residue dispersion was boiled with continuous agitation for 30 min to the achievement of complete gelatinization. The gelatinized samples were then titrated to pH 8.2 with 0.01 mol/L NaOH solution employing phenolphthalein as indicator. A blank test using native flour sample was also performed. Carboxyl content was expressed as the quantity of carboxyl groups per 100 glucose units (COOH/100 GU), as calculated as follows:

$$\frac{COOH}{100\ GU} = \frac{(V_s - V_b) \times M \times 0.045 \times 100}{W_{ds}} \quad (4)$$

where V_s is the volume of NaOH solution required for the titration of sample (mL), V_b is the volume of NaOH solution used to titrate the blank (mL), M is the molarity of NaOH solution and W_{ds} is the sample weight (d.b.).

The carbonyl content was determined according to the titrimetric hydroxylamine method (Yi et al., 2014). Carefully weighed dry flour (4 g) was suspended in 100 mL distilled water. The flour slurry was boiled for 30 min with steady stirring to the achievement of complete gelatinization. It was then stored at 40 °C. The pH was adjusted to 3.2 by addition of 0.1 mol/L HCl solution. Then 15 mL hydroxylamine chloride solution slurry (prepared by dissolving 25 g of reagent grade hydroxylamine chloride in water, adding 100 mL of 0.5 mol/L NaOH solution and making up the final volume to 500 mL) was then added to the flour. The flour slurry samples were covered with plastic film and heated them at 38 °C in an electric oven for 4 h and quickly titrated to pH 3.2 with 0.1 mol/L HCl solution. Carbonyl content was calculated as the quantity of carbonyl groups per 100 glucose units (CO/100 GU), according to the following formula:

$$\frac{CO}{100\ GU} = \frac{(V_b - V_s) \times M \times 0.028 \times 100}{W_{ds}} \quad (5)$$

where *Vb* is the volume of HCl solution used for the titration of blank (mL), *Vs* is the volume of HCl solution required for the titration of the sample (mL), *M* is the molarity of HCl solution and W_{ds} is the sample weight (d.b.).

The amylose content of the flour samples was determined following the iodine-NH_4I method previously developed by Juliano et al. (2012). Undefatted milled gadung flour (100 mg) was dispersed with 1.0 mL of 95% ethanol and swirled gently to disperse the clumps. Then, this ethanol – wetted flour was transferred to a 100 mL volumetric flask and was dispersed in 9.0 mL of 1 N NaOH solution and let stand overnight. The solution was made up to 100 mL by addition of distilled water and was mixed carefully. A 5 mL aliquot (0.09 N NaOH solution) was placed in a 100 mL volumetric flask with approximately 50 mL of distilled water. After dispersion of the alkaline solution of gadung flour, 2 mL of 0.2% iodine in 3.5% NH_4I was added to the 5 mL aliquot of the 0.09 N NaOH gadung flour dispersion without prior acid neutralization and made up to 100 mL with distilled water. The color absorbance was read at 620 nm 20 min after mixing. Calibration was performed using both potato amylose alone and potato amylose – gadung flour as standards.

2.7. Statistical analysis

All experiments were performed in triplicate and the data were analyzed statistically using Statistica 10 software (StatSoft Inc., USA). For multiple comparisons, one-way analysis of variance (ANOVA) was performed. The significance of differences between the mean values was judged based on Tukey's test at the significance $p = 0.05$.

3. Results and discussion

3.1. Proximate composition of native and fermented gadung flours

The results of the proximate analysis of gadung flour samples shown in Table 1 reveal that the moisture contents of the flour samples were 11.50%,12.32%, and 12.88%, respectively, for the native, M. *racemosus* detoxified and L. plantarum fermented gadung flour. The significant increase ($p < 0.05$) in the moisture content could mainly be due to the addition of water to the flours before the fermentation. It should be kept in mind that when the flours are left to equilibrate for a long period with ambient air at 60% relative humidity and at room temperature (25–30 °C), the moisture content might increase accordingly. The moisture content of food samples is an indicator of shelf life and the quality of solid foods. Foods with a moisture content higher than 14% are prone to bacterial attacks and mold growth, which produce undesirable changes

Table 1. Proximate composition of gadung tuber flour per 100 g (dry basis).

Type of Flour	Components (g)					
	Moisture	Protein	Lipid	Ash	Fiber	Carbohydrate
NF	11.55 ± 0.09^{a}	1.60 ± 0.02^{a}	1.60 ± 0.02^{a}	0.70 ± 0.02^{a}	2.40 ± 0.05^{a}	81.94 ± 0.11^{a}
M. racemosus DF	12.32 ± 0.10^{b}	1.71 ± 0.03^{b}	1.40 ± 0.01^{b}	0.70 ± 0.04^{a}	2.33 ± 0.03^{a}	81.54 ± 0.09^{ab}
L. plantarum FF	12.88 ± 0.08^{c}	1.58 ± 0.04^{c}	1.36 ± 0.02^{b}	0.43 ± 0.03^{b}	2.27 ± 0.03^{b}	81.48 ± 0.08^{b}
Wheat flour	12.74 ± 0.09^{c}	13.60 ± 0.21^{d}	1.18 ± 0.08^{c}	0.42 ± 0.05^{b}	0.13 ± 0.04^{c}	78.80 ± 1.27^{c}

All reported values in the table are means ± standard deviation. Means with the same letter are not significantly different from each other (Turkey test, $P < 0.05$). NF = Native Flour, DF = detoxified flour, FF = fermented flour.

(Ihekoronye and Ngoddy, 1985). The moisture contents of gadung flours obtained from detoxification using M. racemosus and fermentation using L. plantarum were comparable with the moisture content of commercial multipurpose wheat flour. Therefore, the moisture contents of gadung flours obtained in this study were within the acceptable values for dried foods.

As shown in Table 1, fermentation of detoxified gadung flour using L. plantarum significantly reduced the fiber content. In contrast, no significant ($p > 0.05$) reduction of carbohydrate content was observed. Detoxification of gadung tuber chips using M. racemosus did not significantly reduce both fiber and carbohydrate contents. The loss of some nutrients can be associated with leaching out or due to utilization of the nutrients by the microflora. Most microorganisms can decompose starches, proteins, and lipids (Lu et al., 2003). During fermentation, LAB performs an important role in the breakdown and acidification of polysaccharides and fiber, and utilizes them for growth, energy and other metabolic activities (Ojokoh and Bello, 2014). The microorganisms will produce amylase and glucoamylase to degrade starches and other types of polysaccharides (Phothiset and Charoenrein, 2007).

In addition to the loss of carbohydrate and fiber, the protein contents of all of the fermented gadung flours also decreased significantly ($p < 0.05$) primarily due to leaching and activity of microorganisms. Besides, fermentation of gadung chips using M. racemosus also caused significant ($p < 0.05$) reduction of lipid content. However, there was no significant reduction of lipid content when gadung flour was fermented using L. plantarum. Most of the LAB also can digest proteins and lipids into simpler molecules through the production of enzymes such as protease and lipase (García-Cano et al., 2019). Lipid was shown to depolymerize into free fatty acids and glycerol during fermentation with the help of lipolytic enzymes (lipase) as biocatalyst (Uvere et al., 2010), while protein was decomposed to amino acids by proteases (Lu et al., 2003). For certain food product preparations, the protein content of wheat flour can be too high and need to be diluted with other flours of lower protein content. For example, the protein content of the flour composite required for sweet biscuits is around 7.0–8.5 %; while for biscuit sponge is between 8.4 – 10 % (Snow and O'Dea, 1981). Hence, the fermented gadung flour shows its capacity to become a partial replacer of wheat flour.

The ash content of gadung flour fermented using L. plantarum, which is a measure of the presence of minerals, was significantly ($p < 0.05$) lower than that of the native flour. However, there was no significant reduction of ash content when gadung chips were detoxified using M. racemosus. The reduction in ash content may be caused by either leaching of soluble minerals into fermentation medium or consumed by microorganisms due to their affinity to some nutrients and minerals needed for their growth and development during fermentation (Talaro and Chess, 2012). Besides, the heat used during oven drying of the fermented flour could also decrease specific minerals including calcium, phosphorus, and iron, which can be unfavorably influenced by heat (Ajayi et al., 2016). The heat may induce oxidative degradation of iron, which finally resulted in the liberation and decline of the iron in the dried fermented flour (Danso-Boateng, 2013). This finding is in good agreement with ash contents of the spontaneously fermented and the Lactobacillus sp. starter culture fermented yellow fleshed - sweet potato flour, which was lower than those of the unfermented yellow fleshed - sweet potato flour (Ajayi et al., 2016).

3.2. Effect of flour slurry concentration

The production level of degrading enzymes used for flour granules modification is maximum during the exponential phase of LAB growth. Since the exponential phase of *L. plantarum* occurs between 32 h and 72 h, the study of the effect of flour concentrations was conducted at 48 h fermentation time (Chookietwattana, 2014). The flour concentrations were varied from 5% w/v to 25% w/v using an inoculum loading of 5% v/v. The results of this investigation are presented in Table 2.

Table 2 shows that the solubility of the fermented gadung flour obtained in this study were significantly ($p < 0.05$) lower than those of native and detoxified gadung flour. Similarly, the values of swelling power of the fermented gadung flours obtained in this study were significantly ($p < 0.05$) lower than that of detoxified gadung flour. Detoxification using M. *racemosus* was found to significantly ($p < 0.05$) increase both swelling power and solubility of gadung flour. In most cases, flour concentration exhibited significant ($p < 0.05$) effect on swelling power and solubility of fermented gadung flour. An earlier study (Numfor et al., 1995) had described a similar phenomenon regarding the reduction of swelling power of cassava starch to the extent of 12.1% under natural fermentation and 15.5% under inoculum-provided fermentations. The lower value of swelling power can be explained based on the weakening of associative forces in the granules, specifically in the amorphous regions. The side branches function to prevent the intermolecular association of carbohydrate polymers. However, when some branches are hydrolyzed, as occurring during fermentation, there will be possible intermolecular hydrogen bonding of the fragments. As a result, the number of free hydroxyl groups where the water molecules would usually hydrogen-bond decreases significantly. This situation would lead to a smaller extent water uptake and less swelling during thermal treatment (Numfor et al., 1995).

A lower solubility of fermented flour than that of native flour has been observed earlier for sweet potato flour (Yuliana et al., 2014). It has also been mentioned that the solubility of cassava starch obtained from

Table 2. Effect of flour concentration.

Concentration (% w/v)	Swelling Power (%)	Solubility (%)	Carbonyl Group (%)	Carboxyl Group (%)
5	4.90 ± 0.05^{a}	3.50 ± 0.02^{a}	0.22 ± 0.01^{a}	0.66 ± 0.02^{a}
10	5.10 ± 0.02^{a}	5.50 ± 0.03^{b}	0.17 ± 0.01^{a}	1.07 ± 0.08^{b}
15	4.80 ± 0.10^{ae}	5.00 ± 0.07^{ce}	0.20 ± 0.01^{a}	0.38 ± 0.01^{c}
20	4.35 ± 0.03^{b}	4.50 ± 0.03^{d}	0.33 ± 0.02^{b}	0.30 ± 0.04^{c}
25	4.16 ± 0.01^{c}	5.00 ± 0.06^{ce}	0.29 ± 0.02^{b}	0.26 ± 0.02^{c}
DF	5.90 ± 0.20^{d}	7.00 ± 0.10^{f}	0.54 ± 0.02^{c}	0.24 ± 0.01^{c}
NF	4.67 ± 0.18^{ae}	6.53 ± 0.15^{g}	0.03 ± 0.00^{d}	-

All reported values in the table are means ± standard deviation. Means with the same letter are not significantly different from each other (Turkey test, $P < 0.05$). DF = detoxified flour, NF = native flour.

natural and mixed culture fermentation was reduced by 26.5% and 37.8%, respectively (Numfor et al., 1995). The distribution of chain lengths in the starch molecules may also cause differences in solubility. In this case, the decline in solubility has been associated with changes in the internal structure of the starch granule as the result of enzyme/acid activity (Numfor et al., 1995). The starch in the flour particles was decomposed into shorter polymer chains due to the activity of the enzymes secreted by LAB. These shorter polysaccharides, which are primarily simple sugars, are more soluble and probably dissolved in the fermentation media resulting in longer chain polysaccharides retained in the flour. In addition, the granular size also affects the solubility of the starches, where smaller starch granule's diameter will exhibit a higher solubility. A lower solubility value means that there is only a slight degradation of starch and leads to fewer numbers of soluble molecules in food. Fermented gadung flour with a lower value of solubility and swelling power would be useful in the manufacture of baked products, such as biscuits, cakes, and breads (Mepba et al., 2009).

The carbonyl group content of all the fermented flours was significantly ($p < 0.05$) higher than that of the native flour. However, no clear trend was observed in the increase of carbonyl group content as a function of gadung flour slurry concentration. As expected, the carboxyl group content of the L. plantarum fermented gadung flours was higher than that of the M. *racemosus* detoxified one. However, there was no significant difference between carboxyl group content of fermented gadung flour obtained from fermentation using 15% w/v, 20% w/v and 25% w/v flour concentration with that of detoxified gadung flour. The increase in carboxyl group content of fermented flour compared to that of detoxified flour was probably due to the effect of fermentation and acidification. A similar result was reported about the fermentation of cassava starch (Putri et al., 2011). The highest carboxyl group content was obtained when gadung flour slurry with 10% w/v concentration was used. However, the carboxyl group content declined when the flour concentration was increased further, which was most probably due to inhibitions triggered by the high substrate concentration. The other reason of decreasing the utilization of starch beyond 10% (w/v) c concentration might be due to the increase in osmotic effects or due to hydrolysis of starch to reducing sugars or the microorganisms were incapable to hydrolyze the starch present in flour at high concentration because they generally grow and being productive at higher water activity (Ray et al., 2009). Besides, a higher flour concentration will lead to an increase in the viscosity of the culture medium, which could lead to decreased water activity, as the process might have shifted from submerged dispersed solid to semi-solid-state fermentation (Ray et al., 2009).

According to the European Union Scientific Committee for Food (EU SCF), the safe level of carboxyl group in food material is a maximum of 1.1% (Commission of the European Communities, 1976bib_citation_to_be_resolved). Therefore, gadung flour slurry with 10% w/v consistency is selected as the best flour consistency for fermentation using L. *plantarum*.

3.3. Effect of inoculum loading

In industrial applications, the inoculum loading range for lactic acid fermentation is usually between 3% and 10% (v/v) of the fermentation broth volume (Clark and Blanch, 1997). Suitable inoculum loading would eliminate the probable phenomenon of lag phase or shorten the lag phase period. The influence of inoculum loading on the swelling power, solubility, and carbonyl and carboxyl group contents during fermentation of gadung flour using 10% w/v flour concentration for 48 h is presented in Table 3.

As shown in Table 3, the swelling power, solubility, and carboxyl group content of fermented gadung flours obtained in this study were significantly ($p < 0.05$) lower than those of detoxified gadung flour. In contrast, the carboxyl group content significantly ($p < 0.05$) increased as the inoculum loading caused gradual reductions in carbonyl group content of the fermented gadung flour. Therefore, 5.0% v/v was chosen as the optimum L. *plantarum* inoculum loading for fermentation of gadung flour. In their study on the lactic acid production from paneer whey by L. *delbrueckii* under submerged fermentation process, Tripathi et al. (2015) reported that the lactic acid production increased substantially from 2.8 to 5.6 g/L, when the inoculum loading was increased from 3% to 8% v/v. However, no significant effect on lactic acid production was observed when the inoculum loading was further increased beyond 8% v/v. A long lag phase is unexpected in the fermentation process because it is time-wasting and more medium culture is used to support a viable culture before the growth. Therefore, 5% v/v inoculum loading exhibited better performance than 10% v/v because the lag phase of 5% v/v inoculum loading was a little shorter than that of 10% v/v during fermentation of whey using L. bulgaricus for lactic acid production (Taleghani et al., 2016). In submerged liquid fermentation, an appropriate loading of inoculum is an utmost important parameter for obtaining high product yield and productivities. At a low value of inoculum loading, the substrate is slowly utilized by microorganisms and prolongs the incubation time. On the other hand, a high value of inoculum loading will lead to the competition of the growth of microorganisms over the limited substrate supply.

Table 3. Effect of inoculums loading.

Loading (% v/v)	Swelling Power (%)	Solubility (%)	Carbonyl Group (%)	Carboxyl Group (%)
2.5	5.10 ± 0.20^{a}	5.00 ± 0.10^{a}	0.15 ± 0.02^{a}	0.48 ± 0.03^{a}
5.0	5.10 ± 0.02^{a}	5.50 ± 0.03^{b}	0.17 ± 0.01^{a}	1.07 ± 0.08^{b}
10	5.70 ± 0.14^{b}	5.00 ± 0.08^{a}	0.16 ± 0.01^{a}	0.95 ± 0.05^{c}
15	6.40 ± 0.11^{c}	5.50 ± 0.20^{b}	0.12 ± 0.01^{a}	0.81 ± 0.02^{d}
DF	5.90 ± 0.20^{b}	7.00 ± 0.10^{c}	0.54 ± 0.02^{b}	0.24 ± 0.01^{e}
NF	4.67 ± 0.18^{d}	6.53 ± 0.15^{d}	0.03 ± 0.00^{c}	-

All reported values in the table are means ± standard deviation. Means with the same letter are not significantly different from each other (Turkey test, $P < 0.05$). DF = detoxified flour, N = native flour.

3.4. Effect of fermentation time

During the exponential phase of growth, LAB produces the highest amount of degrading enzymes that play important roles in flour modification (Tavea et al., 2016). Therefore, an optimum fermentation time should exist for the microbiological modification of gadung flour through fermentation using *L. plantarum*. Table 4 presents the effect of fermentation time on the swelling power, solubility, and carbonyl group, carboxyl group, and amylose contents.

As shown in Table 4, the swelling power of fermented gadung flour decreased significantly ($p < 0.05$) at the beginning of fermentation. Then, the swelling power values started to increase when the fermentation reached 6 h and achieved a maximum value of 5.10 (%) at 48 h of fermentation. This pattern was repeated as the fermentation was further prolonged until 144 h. In most cases, fermentation time significantly ($p < 0.05$) affected the carbonyl and carboxyl content of gadung flour. However, no clear trend was observed for solubility, carbonyl and carboxyl group contents of fermented gadung flour as a function of fermentation time.

Based on the swelling power value, gadung tuber flour has already met the requirement for its applications in food and pharmaceutical industries (Ratnayake and Jackson, 2008). The facultative-submerged fermentation of gadung flour using L. plantarum is expected to change the molecular structure of its starch granules into a more stable form that leads to improvement in the retrogradation tendency of the starch granules. An indirect method to ensure the retrogradation tendency is by observation of the change in the amylose content (Tukomane et al., 2007). Therefore, one of the criteria for the selection of the best operating condition is the amylose content of the fermented gadung flour. The amylose content of fermented gadung flour increased significantly ($p <$

Table 4. Effect of fermentation time.

Time (hr)	Swelling Power (%	Solubility (%)	Carbonyl Group (%)	Carboxyl Group (%)	Amylose (%)
4	4.75 ± 0.01^{a}	3.00 ± 0.02^{a}	0.25 ± 0.01^{a}	0.47 ± 0.02^{a}	38.41 ± 0.15^{a}
6	4.99 ± 0.03^{ab}	2.50 ± 0.01^{b}	0.52 ± 0.01^{b}	0.52 ± 0.02^{a}	40.93 ± 0.21^{b}
12	4.30 ± 0.02^{c}	3.50 ± 0.00^{c}	0.48 ± 0.04^{b}	0.53 ± 0.16^{a}	41.59 ± 0.25^{b}
24	4.56 ± 0.07^{a}	3.00 ± 0.04^{a}	0.16 ± 0.01^{c}	0.65 ± 0.10^{b}	41.92 ± 0.18^{b}
48	5.10 ± 0.02^{b}	5.50 ± 0.03^{d}	0.17 ± 0.01^{c}	1.07 ± 0.08^{c}	43.05 ± 0.20^{c}
72	4.31 ± 0.01^{e}	2.50 ± 0.01^{b}	0.21 ± 0.01^{a}	0.76 ± 0.03^{d}	43.89 ± 0.14^{d}
96	4.45 ± 0.01^{f}	2.00 ± 0.01^{e}	0.44 ± 0.02^{b}	0.86 ± 0.04^{e}	41.63 ± 0.09^{b}
120	4.60 ± 0.08^{a}	1.50 ± 0.01^{f}	0.41 ± 0.01^{b}	0.92 ± 0.02^{e}	41.35 ± 0.05^{b}
144	4.94 ± 0.10^{ab}	3.50 ± 0.11^{c}	0.30 ± 0.01^{a}	1.05 ± 0.12^{c}	39.52 ± 0.08^{a}
DF	5.90 ± 0.20^{g}	7.00 ± 0.10^{g}	0.54 ± 0.02^{b}	0.24 ± 0.01^{f}	34.72 ± 0.13^{e}
NF	4.67 ± 0.18^{a}	6.53 ± 0.15^{h}	0.03 ± 0.00^{d}	-	34.75 ± 0.20^{e}

All reported values in the table are means ± standard deviation. Means with the same letter are not significantly different from each other (Turkey test, $P < 0.05$). DF = detoxified flour, NF = native flourGadung tubers.

0.05) with fermentation time to a maximum value of 43.89% at 73 h and then leveled off. As expected, the amylose contents of the fermented gadung flours in this study were higher than those of native and detoxified gadung flours. Based on carboxyl group and amylose contents of the fermented flour, 48 h is selected as the optimum fermentation time of gadung flour using L. plantarum. The incubation period of 48 h has been generally used for lactic acid production using different lactobacilli (Kumar et al., 2001). The shorter fermentation time is additionally advantageous in increasing the economics of the process.

The development of amylose-like material resulting from enzyme/acid hydrolysis of amylopectin in the amorphous regions of starch granules during fermentation may be the cause of the apparent increase in amylose content of starch and flour after fermentation (Numfor et al., 1995). In this case, during fermentation, the glucoamylase degrades amylopectin into amylose (Van der Maarel et al., 2002). On the other hand, the organic acids synthesized during fermentation may form complex molecules with the soluble amylose fraction, thereby leading to a visible reduction in soluble amylose content. This complexation leads to improve the gelatinization temperature and qualities of the fermented flour, as the stickiness caused by soluble amylose has been reduced. In addition, amylose may be depolymerized by α-amylase into short chains and lost into water (Van der Maarel et al., 2002). Due to their high amylose content, fermented gadung flour can be a potential substitute for wheat flour in snack food formulations to obtain food products with a crunchy texture (Huang et al., 2006).

4. Conclusion

A study on the microbiological treatment of gadung tuber flour through facultative-submerged fermentation using L. *plantarum* was successfully conducted. The flour slurry concentration, inoculum loading, and fermentation time significantly ($p < 0.05$) influenced the nutritional and functional properties of the flour. The swelling power and the solubility of the fermented flours were significantly ($p < 0.05$) lower than those of the detoxified flour. The best fermentation conditions were flour concentration of 10% (w/v), inoculums loading of 5% (v/v), and fermentation time for 48 h. Based on its physicochemical properties, the fermented gadung flour obtained in this study is suitable for the manufacture of value-added products such as crunchy texture snacks, cakes, and bakeries.

Declarations

Author contribution statement

Andri Cahyo Kumoro: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ratnawati Ratnawati: Conceived and designed the experiments; Wrote the paper.

Marissa Widiyanti: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Diah Susetyo Retnowati: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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