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Effect of *Saccharomyces cerevisiae* fermentation on the quality of Samurai 2, a mutant sorghum flour

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Abstract

Sorghum is a remarkably nutritious cereal plant primarily found in Africa. It was introduced to Indonesia in 1989. Joining the effort to improve the quality of local flour as part of the Indonesian government's food security program, we applied the yeast *Saccharomyces cerevisiae* during sorghum flour fermentation with different parameters to yield better final flour products. Since low digestibility of proteins and high levels of tannins that are present in grains are considered as obstacles to favourable sorghum flour production, we aimed at formulating a method for flour production by using the mutant sorghum variety called Samurai 2, and employing various fermentation periods and concentrations of *S. cerevisiae* as starter culture. The quality controls were performed on the basis of chemical and physical properties. The present work employed a complete factorial randomised design, by varying the durations of fermentation (20, 40, and 60 h) and starter culture concentrations of 10^7 CFU/mL (0, 2, 4, and 6%, w/v). The results showed that the longer the fermentation time and the higher the starter concentration, the lower the water, ash, and in-flour tannin contents, whiteness intensity, and viscosity ($p < 0.05$). On the other hand, this treatment increased the levels of dissolved protein ($p < 0.05$), and the microstructure of starch granules became coarser.

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is a cereal plant commonly used for livestock feed in developed countries such as the United States of America. However, this cereal plant also has a remarkable place for thousands of years as an outstanding staple food in Africa (Belton and Taylor, 2004). In Indonesia, sorghum was introduced at the beginning of 1989 in the dryland farming area of East Nusa Tenggara. In 2015, sorghum became an object of keen interest for Indonesia after the Centre for the Application of Isotope and Radiation Technology, National Nuclear Energy Agency (Pusat Aplikasi Teknologi Isotop dan Radiasi, Badan Tenaga Nuklir Nasional; PATIR BATAN), Indonesia, with the support of joint FAO/IAEA (Food and Agriculture Organization/International Atomic Energy Agency) Division, introduced new varieties of mutant sorghum grain such as Samurai 1 and Samurai 2; the name Samurai is abbreviated from *sorghum mutan radiasi* (radiation mutant sorghum). Such an action is one of the government's food security programs to reduce rice dependency and a massive import of wheat. Besides, sorghum is performing relatively well under various environmental stress from marginal land to drought-prone areas (Human and Indriatama, 2020). Sorghum also has relatively higher carbohydrate, protein, vitamin, and mineral contents than rice and corn, so it has potential for becoming a part of food diversification. In addition, sorghum grain also has an inhibitory effect on chronic disease related to functional activity due to the presence of indigenous polyphenol.

The critical challenge in sorghum processing for food consumption is the relatively low protein digestibility caused by kafirin resistance and tannin content in sorghum (Duodu *et al.,* 2003; Dykes and Rooney, 2006). Low digestibility of protein is due to the interaction of protein bodies in the endosperm with starch granules (Wong *et al.,* 2009). The utilisation of microorganisms during the fermentation of sorghum can improve the quality and protein digestibility of its flour product. Fermentation is also known to increase the nutrient content, improve ingredient texture, and reduce antinutrient contents in cereals. Previous studies reported that *Saccharomyces cerevisiae* (SC) as starter structure could produce

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α-amylase, which has a role in the breakdown of amylose into simple sugars (Wahono *et al.,* 2015).

Very few studies have examined the characteristics of mutant sorghum (Zhu *et al.,* 2019; Wahyuni *et al.,* 2019). In addition, there are also limited studies that investigated the effect of fermentation on the characteristics of mutant sorghum flour. To the best of our knowledge, there is no study related to SC usage to improve the characteristics of mutant sorghum flour. Hence, the objective of the present work was to evaluate the effect of fermentation period and concentration of SC starter culture on the physicochemical properties of flour made of mutant sorghum grain (Samurai 2).

Materials and methods

Materials

The variety of mutant sorghum grain called Samurai 2 was obtained from the Research Division for Natural Product Technology, Indonesian Institute of Sciences (Balai Penelitian Teknologi Bahan Alam, Lembaga Ilmu Pengetahuan Indonesia; BPTBA LIPI), Wonosari, Indonesia. Samurai 2 was developed from the sorghum grain variety referred to as Pahat via irradiation with 300 Gy doses of gamma rays. It has several superior qualities such as higher content in biomass, drought tolerance, and resistance to plant disease. The yeast culture *Saccharomyces cerevisiae* FNCC 3012 was obtained from the Food and Nutrition Culture Collection (FNCC), Centre for Food and Nutrition Study, Gadjah Mada University, Daerah Istimewa Yogyakarta, Indonesia. Yeast extract, peptone, and dextrose (collectively called YEPD), and bacteriological agar were purchased from Sigma Aldrich, USA. Analytical-grade chemical materials including tannic acid, Folin-Ciocalteu reagent, Lowry A and B reagents, Nelson's arsenomolybdate reagent, glucose, and sodium carbonate were purchased from Merck, USA.

Sorghum flour preparation

Sorghum flour was produced in accordance with a previous study (Pranoto *et al.,* 2013). Sorghum seeds were carefully cleaned to remove foreign materials such as twigs, stones, and other particles. Then, the seeds were detached from their chaff by using a husking machine for 5 min. The remaining sorghum was subsequently milled, and sifted through 60-mesh sieves.

Starter culture preparation

The lyophilised culture of SC was aseptically rehydrated into vials containing 0.5 mL of yeast extract, peptone, and dextrose (YEPD) broth. The culture was then streaked onto YEPD agar plates, and grown at 30°C for 24 h. The plates were refrigerated at 4°C, and used to propagate the inoculation culture. For the experiment, one dose of 24-h pure culture was aseptically transferred into 100 mL of YEPD broth in a 500 mL flask. Subsequently, it was incubated at 30°C for 48 h at 200 rpm. The high-density cultures containing 10^7 CFU/mL of SC were then centrifuged at 4,500 rpm for 15 min, and washed by using sterile *aqua destilata*. The remaining pellet was then dissolved in 100 mL of sterile distilled water for further experiments.

Sorghum flour fermentation

Sorghum flour (55 g) was placed in a glass jar followed by sterile water at a ratio of 1:1. Subsequently, the SC starter culture (10^7 CFU/mL) was added into the glass jar at the concentration of 0% (for spontaneous fermentation), with the process repeated for concentrations of 2, 4, and 6% (w/v). Each glass jar was then tightly closed and incubated at 37°C for 20, 40, and 60 h. Afterwards, the resulting fermented sorghum flour was dried in the drying cabinet at 60°C for 16 h (Memmert). The dried fermented flour was then ground and sifted through a 60-mesh sieve, and refrigerated in polyethylene bags at 4°C prior to analysis.

Chemical analysis

The moisture and ash contents of fermented sorghum flour were determined according to the official method of analysis of Association of Official Analytical Chemists (AOAC, 2006), by using the thermogravimetric and combustion methods, respectively.

Dissolved protein content

The dissolved protein was determined according to the Lowry method (AOAC, 2005). Briefly, 1 g of sample was diluted with 60 mL of distilled water. The dilution was incubated in an orbital shaker for 2 h at 30°C and 150 rpm. It was then filtered through a filter paper, and the supernatant was collected. Next, 1 mL of supernatant and 8 mL of Lowry B reagent were added in a test tube, and incubated for 10 min at 30°C. Then, 1 mL of Lowry A reagent was added into the solution, and incubated for another 20 min. The absorbance of the sample was then measured at 600 nm. Various Bovine Serum Albumin (BSA) concentrations (30 - 300 µg/mL) were used to construct a calibration curve.

Reducing sugar content

The reducing sugar content was determined according to the Nelson-Somogyi method (AOAC, 2005). Briefly, 5 g of the sample were diluted with distilled water in 100 mL volumetric flasks. Pb-acetate was added to clear the dilution. The resulting solution was filtered through a filter paper, and the supernatant was collected. Na-oxalate of the same quantity as the Pb-acetate was added to the supernatant. Later, 50 mL of the solution was added with distilled water in a 100 mL volumetric flask. Then, 1 mL of the solution was added to 1 mL of Nelson's reagent, and heated to its boiling point at 20 min. The sample was cooled to 25°C, and then added to 1 mL of arsenomolybdate and 7 mL of distilled water. The absorbance of the sample was measured at 540 nm. Various glucose concentrations (2 - 10 mg/100 mL) were used to construct a calibration curve.

Tannin content

The tannin content was determined by using the method described by Chanwitheesuk *et al.* (2005). Sample (0.5 g) was extracted with 10 mL of diethyl ether for 20 h, filtered, and the residue was collected. The residue and 100 mL of distilled water were boiled for 2 h, and then cooled and filtered. The supernatant was transferred into a volumetric flask, and distilled water was added until the total volume was 100 mL. Then, 0.1 mL of extract, 0.1 mL of Folin-Ciocalteu reagent, and 2 mL of Na_2CO_3 were mixed, and incubated for 30 min at room temperature. The absorbance of the mixture was read at 760 nm. Tannic acid was used as a standard. The total tannin content was expressed in mg tannic acid/kg extract.

Surface colour analysis

The surface colour of sorghum flour resulting from both the fermentation which was spontaneous, and that with SC was measured by using Chromameter CR 410 (Konica Minolta, Sensing Inc., Tokyo, Japan). Triplicate measurements of L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness) were made. The degree of whiteness (whiteness index) was calculated using Eq. 1 (Tong *et al.,* 2015):

$$\text{Whiteness (\%W)}: 100 - [(100 - L^*)^2 + (a^{*2} + b^{*2})]^{1/2} \quad \text{(Eq. 1)}$$

Pasting analysis

The pasting properties of fermented sorghum flour were measured by using a Brookfield digital viscometer (DV-E, Massachusetts, USA). The flour (20 g) was mixed with 100 mL of distilled water, heated for 5 min, and subsequently cooled down to 26°C prior to the viscosity test. The measurement was conducted by using spindle no. Q3 rotating at 100 rpm, and the cold slurry paste was deducted in centipoise (cP).

Scanning electron microscopy (SEM)

The microscopic structure of 2 mg of dried fermented sorghum sample was then mounted on a sample holder by using double-sided scotch tape, and was coated by using a thin layer of gold-palladium (60:40%) in a sputter coating equipment. All measurements were conducted by using an accelerating voltage of 5000 Kv, and a magnification of 2000× through a SU3500 scanning electron microscope (Hitachi, Co., Ltd., Tokyo, Japan).

Statistical analysis

The present work employed a complete factorial randomised design by varying the fermentation periods (20, 40, and 60 h) and starter culture concentrations (0, 2, 4, and 6%). There were 12 treatment groups, and one group as a control, which was sorghum flour without fermentation. Three fermentation batches were conducted, and all the measurement was conducted in triplicate. The effect of the treatment on the quality attributes of fermented sorghum flour was statistically analysed by using a two-way ANOVA followed by the Duncan Multiple Range Test at a 0.05 significance level.

Results and discussion

Effect of the fermentation period and the starter culture concentration on the physical properties of fermented sorghum flour

The chemical compositions of sorghum flour samples fermented at different periods and SC concentrations are shown in Table 1. Moisture is an essential constituent in a flour product, which also acts as an indicator of shelf stability. Our result showed that the fermentation period significantly affected the moisture content of fermented sorghum flour with or without SC. Spontaneous fermentation affected the degradation of a starch granule of sorghum. However, SC gave significant effect (as compared to control) when its concentration was higher than 2%. This result is consistent with our previous study (Kurniadi *et al.,* 2019). The effectiveness of starch degradation elevates with the increasing fermentation period and addition of SC.

Table 1. Effect of fermentation periods on chemical composition of fermented sorghum flour.

Chemical composition	Fermentation period (h)	*S. cerevisiae* concentration				
		0%	2%	4%	6%	Average
Moisture (%)	20	9.629 ± 0.021	9.576 ± 0.046	9.440 ± 0.037	9.195 ± 0.074	9.460 ± 0.014^{a}
	40	7.481 ± 0.067	7.450 ± 0.009	7.252 ± 0.015	7.092 ± 0.007	7.319 ± 0.014^{b}
	60	5.707 ± 0.053	5.648 ± 0.031	5.256 ± 0.070	5.088 ± 0.080	5.425 ± 0.014^{c}
	Average	7.605 ± 0.017^{A}	7.558 ± 0.017^{A}	7.316 ± 0.017^{B}	7.125 ± 0.017^{C}	
Ash (%)	20	0.413 ± 0.032	0.492 ± 0.018	0.593 ± 0.016	0.661 ± 0.012	0.540 ± 0.009^{a}
	40	0.410 ± 0.014	0.516 ± 0.044	0.582 ± 0.023	0.672 ± 0.078	0.545 ± 0.009^{a}
	60	0.421 ± 0.002	0.508 ± 0.026	0.562 ± 0.019	0.663 ± 0.016	0.539 ± 0.009^{a}
	Average	0.415 ± 0.011^{A}	0.505 ± 0.011^{B}	0.579 ± 0.011^{C}	0.666 ± 0.010^{D}	
Dissolved protein (%)	20	0.609 ± 0.043	0.626 ± 0.036	0.564 ± 0.002	0.543 ± 0.005	0.586 ± 0.007^{a}
	40	0.625 ± 0.006	0.694 ± 0.007	0.705 ± 0.033	0.739 ± 0.009	0.691 ± 0.007^{b}
	60	0.631 ± 0.019	0.749 ± 0.040	0.798 ± 0.005	0.841 ± 0.016	0.755 ± 0.007^{c}
	Average	0.622 ± 0.008^{A}	0.690 ± 0.008^{B}	0.689 ± 0.008^{B}	0.708 ± 0.008^{B}	
Reducing sugar (%)	20	1.670 ± 0.023	1.624 ± 0.019	1.400 ± 0.048	1.129 ± 0.261	1.456 ± 0.023^{a}
	40	1.592 ± 0.044	1.651 ± 0.016	1.467 ± 0.008	1.354 ± 0.028	1.516 ± 0.023^{a}
	60	1.516 ± 0.027	1.661 ± 0.008	1.761 ± 0.003	1.601 ± 0.008	1.635 ± 0.023^{b}
	Average	1.593 ± 0.026^{AB}	1.645 ± 0.026^{A}	1.543 ± 0.026^{B}	1.361 ± 0.026^{C}	
Tannin (%)	20	0.017 ± 0.0002	0.009 ± 0.0006	0.008 ± 0.0020	0.011 ± 0.0011	0.012 ± 0.000^{a}
	40	0.015 ± 0.0001	0.013 ± 0.0003	0.010 ± 0.0002	0.015 ± 0.0001	0.014 ± 0.000^{b}
	60	0.009 ± 0.0032	0.008 ± 0.0005	0.007 ± 0.0002	0.013 ± 0.0003	0.009 ± 0.000^{c}
	Average	0.014 ± 0.000^{A}	0.010 ± 0.000^{B}	0.009 ± 0.000^{C}	0.013 ± 0.000^{A}	

Values were expressed mean ± SD (n = 6). Different lowercase superscripts indicated significant difference at 95% confidence level (Duncan Multiple Range Test) between fermentation periods of each parameter. Different uppercase superscripts indicated significant difference at 95% confidence level (Duncan Multiple Range Test) between *S. cerevisiae* concentrations of each parameter.

The increasing number of microorganisms would be followed by more starch degradation to form a porous and soft granule. Consequently, the bonded water in the material will turn into free water that is quickly lost during the drying process. The higher the starch content, the greater the ability of a material to retain water. The maximum acceptable moisture content for flour product is 14% (Okuda *et al.,* 2016). Results showed that both fermented and unfermented sorghum flour samples had low moisture contents (5.088 - 9.629%). Lower moisture of a flour product correlates with the length of shelf life since water in flour could enhance enzymatic deterioration and spoilage (Kong and Singh, 2016). Overall, the moisture content in the flour samples was better than the Codex Standard 173-1989 for sorghum flour (max. 15%).

The presence of ash in flour products is correlated with essential mineral levels. Results showed that the concentration of SC significantly affected the ash content of the flour product (Table 1). The trend is in line with a previous report showing that the addition of SC caused an increase in Ca, Mg, and Fe in tapioca fermentation (Kustyawati *et al.,* 2013). Our previous study also showed that fermentation of cassava flour elevated the ash level (Frediansyah *et al.,* 2012; Frediansyah and Kurniadi, 2016). The abundant ash content in the present work might be due to the cellular component of SC incorporated in the flour (Li *et al.,* 2001). The higher concentration of starter culture could increase the number of cells present in the flour, thereby increasing ash content therein. However, there was no significant difference in ash content between flour

products of different fermentation periods. This contradicts with some of the previous studies which observed higher ash levels after fermentation of fruit pulp flour (Makawi *et al.,* 2019), maize (Gernah *et al.,* 2011), and mung bean flour (Onwurafor *et al.,* 2014). Meanwhile, reduced ash content has been reported in fufu fermentation (Sobowale *et al.,* 2007), and fermentation of mahogany bean flour (Igbabul *et al.,* 2014). However, our finding is in line with Aini *et al.* (2016). The steady-state level of ash might occur due to the regulation of mineral balance in SC (Cyert and Philpott, 2013). During the fermentation, there was no addition of external nutrients and minerals so that ash content elevation was not externally affected.

The present work also revealed that fermentation by SC increased the dissolved protein content (Table 1). It was found that long fermentation period significantly contributed to the rate of dissolved protein of sorghum flour ($p < 0.05$). However, the elevation of SC concentration from 2 to 6% did not significantly affect the dissolved protein content. A similar trend also occurred in our previous study using *L. plantarum* (Kurniadi *et al.,* 2019). Various other fermentation studies also reported the same finding; in corn flour (Amankwah *et al.,* 2009; Gernah *et al.,* 2011; Aini *et al.,* 2016); in cassava fermentation (Frediansyah *et al.,* 2012; Gunawan *et al.,* 2015; Frediansyah and Kurniadi, 2016); in foxtail millet fermentation (Amadou *et al.,* 2014), and in mung bean bioprocess (Onwurafor *et al.,* 2014). The activity of microorganisms through fermentation could improve the functionality and protein content of the flour product (Olukomaiya *et al.,* 2020), and the increase in dissolved protein content in sorghum flour was probably caused by proteolytic enzyme activity of SC (Grbavac *et al.,* 2017). However, the protein content of fermented sorghum flour obtained in the present work was lower than that of wheat flour (Akubor and Badifu, 2014). Thus, fermented sorghum flour cannot be considered as an essential protein source.

The level of reducing sugar in fermented sorghum flour samples with the addition of 2 and 4% of SC concentration was not significantly different as compared to that in control (Table 1). In the spontaneous fermentation group, reducing sugar level decreased as fermentation period increased. This finding might be due to indigenous microorganisms in sorghum which were able to digest polysaccharide for growth. Torres-Cortes *et al.* (2019) reported that cereal seeds play an important role as reservoirs for plant microbiome. In addition, Utami *et al.* (2015) observed that indigenous microorganisms showed excellent growth since the beginning of spontaneous fermentation. Meanwhile, in the group with the addition of starter culture concentrations of 2, 4, and 6%, the reducing sugar decreased as the SC concentration increased. Such a phenomenon could possibly be due to the utilisation of starch by SC using α-amylase (Liu *et al.,* 2014). The presence of its enzyme could break the 1,4-glycosidic bonds of starch to produce a mixture of dextrin, glucose, and maltose. Overall, high amount of reducing sugar could be used as a good source of energy.

Table 1 further shows that the tannin content in fermented sorghum flour samples with the addition of SC was significantly different as compared to that in the group without SC ($p < 0.05$). The tannin content in SC groups increased as the fermentation period increased. Inversely, in the group without SC, the tannin content decreased with the increase in fermentation period. This is in line with previous study which observed that gallic acid decarboxylase in yeast was involved in the degradation of tannic acid (Meier *et al.,* 2017). Kanpiengjai *et al.* (2016) also found various tannin-tolerant yeasts from the tea leaf. This occurred without any addition of SC, and might be due to the presence of indigenous lactic acid bacteria in sorghum flour. *Lactobacillus* has also been reported to be able to produce tannase, which breaks the ester bond of tannins into gallic acid and glucose (Aguilar-Zarate *et al.,* 2014). In addition, the presence of SC could induce the growth and interaction of indigenous lactic acid bacteria. Thus, the increase in fermentation period and SC concentration would, respectively, increase hydrolysis time and elevate the production of tannase (Kurniadi *et al.,* 2019). Overall, the tannin content of the final flour product was better than the minimum requirement of the Codex Standard for sorghum flour (max. 0.3%).

Effect of the fermentation period and the starter culture concentration on the surface colour of fermented sorghum flour

The surface colours of sorghum flour samples fermented at different periods and SC concentrations are shown in Table 2. The colour analysis revealed significant differences in lightness (L^*), redness/greenness (a^*), and yellowness/blueness (b^*) in different fermentation periods (20, 40, and 60 h). The degree of whiteness of fermented sorghum flour samples increased with the increase in fermentation period, and showed a real inter-treatment difference ($p > 0.05$). The degree of

Table 2. Effect of fermentation periods on the surface colour of fermented sorghum flour.

Surface colour	Fermentation period (h)	*S. cerevisiae* concentration				
		0%	2%	4%	6%	Average
*L**	20	81.29 ± 0.227	80.43 ± 0.279	81.00 ± 0.063	81.17 ± 0.037	80.97 ± 0.151[a]
	40	81.78 ± 0.099	81.23 ± 0.053	81.89 ± 0.099	81.90 ± 0.038	81.70 ± 0.072[c]
	60	81.90 ± 0.039	81.10 ± 0.060	80.99 ± 0.038	80.87 ± 0.018	81.20 ± 0.038[b]
	Average	81.90 ± 0.053[A]	80.92 ± 0.130[B]	81.29 ± 0.067[A]	81.31 ± 0.031[A]	
*a**	20	7.13 ± 0.019	7.16 ± 0.020	6.98 ± 0.234	6.99 ± 0.013	7.07 ± 0.071[a]
	40	6.92 ± 0.052	6.91 ± 0.044	6.90 ± 0.035	6.77 ± 0.028	6.88 ± 0.039[c]
	60	6.90 ± 0.032	6.92 ± 0.087	6.97 ± 0.117	7.04 ± 0.073	6.96 ± 0.075[b]
	Average	6.98 ± 0.034[A]	6.99 ± 0.050[A]	6.95 ± 0.128[A]	6.93 ± 0.038[B]	
*b**	20	4.61 ± 0.071	5.00 ± 0.084	5.01 ± 0.062	4.45 ± 0.076	4.76 ± 0.073[a]
	40	4.11 ± 0.099	4.42 ± 0.077	4.36 ± 0.023	4.51 ± 0.037	4.35 ± 0.059[c]
	60	4.24 ± 0.051	4.29 ± 0.098	4.30 ± 0.032	4.51 ± 0.050	4.47 ± 0.058[b]
	Average	4.32 ± 0.073[A]	4.57 ± 0.086[B]	4.56 ± 0.039[B]	4.49 ± 0.054[B]	
Whiteness (%)	20	79.47 ± 0.058	78.78 ± 0.037	79.19 ± 0.057	79.33 ± 0.064	79.19 ± 0.054[a]
	40	79.78 ± 0.078	80.07 ± 0.120	79.54 ± 0.019	80.19 ± 0.095	79.89 ± 0.078[c]
	60	80.33 ± 0.078	79.57 ± 0.079	79.45 ± 0.072	79.21 ± 0.149	79.64 ± 0.094[b]
	Average	79.86 ± 0.071[A]	79.47 ± 0.078[B]	79.39 ± 0.049[B]	79.57 ± 0.102[B]	

Values were expressed mean ± SD (n = 6). Different lowercase superscripts indicated significant difference at 95% confidence level (Duncan Multiple Range Test) between fermentation periods of each parameter. Different uppercase superscripts indicated significant difference at 95% confidence level (Duncan Multiple Range Test) between *S. cerevisiae* concentrations of each parameter.

whiteness is calculated on the basis of L^*, a^*, and b^* of flour powder. In addition, the degree of whiteness increased along with time-dependent fermentation in the 0% group (the group without SC) and showed better results as compared to SC groups (the flour samples produced with SC were darker in colour). This could be due to the contribution of indigenous microorganisms. A previous study found that lactic acid bacteria were dominant during spontaneous fermentation of sorghum (Yousif *et al.,* 2010). Another study showed that *L. plantarum* together with other lactic acid bacteria were found in natural sorghum fermentation (Rao *et al.,* 2015). Our previous finding showed that fermentation with *L. plantarum* could improve the degree of whiteness in flour products (Frediansyah *et al.,* 2012; Frediansyah and Kurniadi, 2016) significantly. The darker colour produced by SC groups might be due to the activity of cellulase secreted by SC (Lee *et al.,* 2017) which made granules more porous and consequently caused the light reflection by sorghum granules to lower in intensity.

Effect of the fermentation period and the starter culture concentration on the pasting properties of fermented sorghum flour

The pasting property results are shown in Figure 1 which shows the significant effect of fermentation. The viscosity value was lower in accordance with the length of fermentation period (Pranoto *et al,.* 2013). Meanwhile, the viscosity value rapidly increased at SC concentration of 2%. The increased pasting/viscosity property is presumably due to the liberation of granules from the complex matrix through the presence of α-amylase secreted by SC. Once the granules mixed with water, the viscosity of the flour also increased.

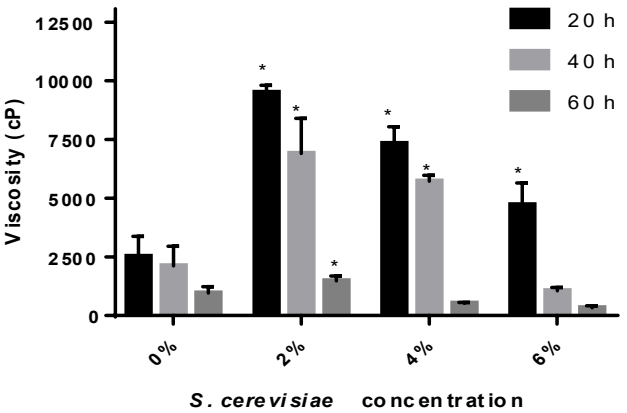
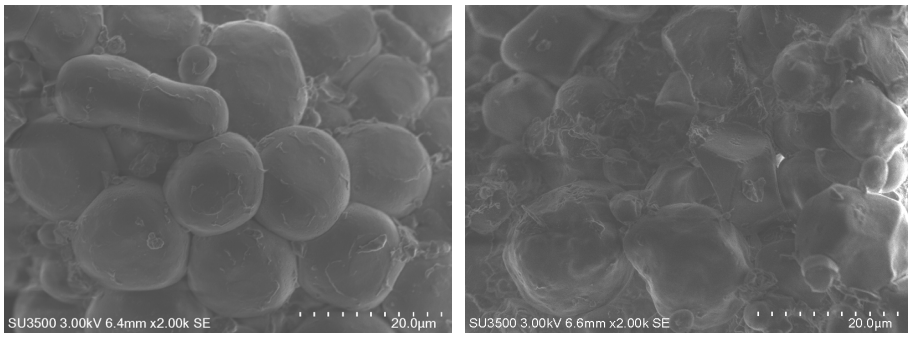


Figure 1. Effect of fermentation periods on the pasting properties of fermented sorghum flour.



(A) **(B)**

Figure 2. Sorghum starch granules at 2000× magnification; (A) without fermentation, and (B) with fermentation (60 h) and addition of starter culture *S. cerevisiae* (6%).

The effect of fermentation duration and culture concentration on the microstructure of fermented sorghum flour

The microstructures of sorghum starch granules from unfermented sorghum flour and sorghum flour fermented with 6% of SC concentration for 6 h were observed by using Scanning Electron Microscope (SEM) with 2000× magnification (Figure 2). Sorghum starch granules without fermentation (Figure 2A) ranged between 14 and 17 µm in size, and had smooth surface. The sorghum starch granules were polygonal in shape, and coated with a thin protein layer. The size of the sorghum starch granules after fermentation with 6% for 6 h ranged between 10 and 19 µm in size, and had coarse surface (Figure 2B). The sorghum starch granules with fermentation had an irregular form, and the protein layer appeared thick. The fermentation with SC could cause an erosion of the starch granule surface of sorghum (Lee *et al.,* 2017).

Conclusion

The present work demonstrated that when SC was applied in the fermentation of the mutant sorghum Samurai 2, it could improve the quality attributed to the final flour product. Moreover, the method applied in the present work could decrease moisture content and reducing sugar, and increase dissolved protein. However, further investigation still needs to be conducted, especially to reveal the interaction between *S. cerevisiae* and indigenous microorganisms in the sorghum flour.

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