

Application of modified sorghum flour for improving bread properties and nutritional values

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Abstract

This research was to improve the nutritional values and bread properties by adding proportion of fermented sorghum flour to wheat flour. The optimization of the fermentation was conducted at the mix of dry baker yeasts and pure *Lactobacillus plantarum* with 30% sorghum flour concentration (w/w) for 24 h. The fermented sorghum flour has a lower content of moisture (3.3% w/w) and fat (2.8% w/w), and also a higher content of protein (23.4% w/w), ash (9.52% w/w), and total phenolic contents (1.8% w/w). Another, It was also found that amylose content of fermented sorghum flour decrease by 64%. Furthermore, it was shown that fermented sorghum flour has ability to increase volume expand of bread dough, to decrease bread crumb hardness and to produce darker bread color. Moreover, its nutritional value was higher than those of bread with 100% wheat flour and bread with 30% non-fermented sorghum flour.

Keywords

Bread properties
Fermented sorghum flour
Fermentation
Lactobacillus plantarum

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Introduction

Sorghum (*Sorghum-bicolor* (L) Moech) is a kind of cereals that has great potential to be cultivated and developed commercially because of its wide adaptability, high productivity, a little requirement for grow, resistance to pests and plant diseases, and more tolerance of marginal conditions, such as drought, salinity and land sour (Sirappa, 2003). Sorghum has long been recognized to be cultivated by farmers in Indonesia especially in Java, Nusa Tenggara Timur (NTT), and Nusa Tenggara Barat (NTB). Indonesia has sorghum production at around 13,000 ton/year. In Java, sorghum is known as “candel” that usually is planted by farmers as an intercropping plants. It has an enough value of nutrition as the source of food. It contained 83% carbohydrate, 3.50% fat and 10% protein. Sorghum seeds which contain high carbohydrate are used as animal feed and a raw material in various industries such as beer, starch, sugar, liquid (syrup), jaggery (a kind of brown sugar), and ethanol. They are also used as a food with traditional cooking (steaming and served with coconut)

Bread is one of the common foods that also becomes a stuff food in several countries. Wheat flour is the dominant composition of bread, however

it is the source of gluten which may promote celiac disease (CD). The lifetime obedience to the gluten-free diet is the only treatment for this disease. The finding of a new material in order to obtain gluten-free product is an important topic.

Sorghum is a kind of grain that is free of gluten and high level of antioxidant. Ivana *et al.* (2011) reported that crackers made from sorghum flours can be regarded as health promoting functional foods, especially for celiac disease patients. Besides, sorghum is rich in amino acid content particularly leucine, glutamic acid and alanine. Kamath *et al.* (2008) confirmed that the cereal grain sorghum (white) serves as a significant source of natural antioxidant compounds that may give health benefits.

Fermentation was used to improve nutritional content of sorghum flour (Abd *et al.*, 2005) and cassava flour (Gunawan *et al.*, 2015), resulting modified sorghum flour (mosof) and modified cassava flour (mocaf), respectively. Abd *et al.* (2005) and Pranoto *et al.* (2013) reported that mosof had a better properties than non-fermented sorghum, e.g. higher solubility of its starch, and lower water binding capacity. Furthermore, mosof has many benefits in the bread products, such as enriching protein content as lysine, leucine, isoleucina and methionine (Abd *et al.*, 2005), improving texture (Gelinis, 2007),

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reducing the acrylamide content (Arwa *et al.*, 2009), and mouth-feel, acceptability and shelf-life of bread (Angioloni, 2012).

The previous works of sorghum flour fermentation only reported the traditional method by involving the indigenous microbe inside. However, the application of mosof on the bread properties has not been studied. Therefore, the objective of this work was to produce mosof in optimum conditions and to evaluate its bread properties. The effects of fermentation parameters, such as microbe types, sorghum flour concentrations, and fermentation time, on the nutritional values and bread properties were systematically investigated.

Materials and Methods

Materials

Sorghum (*Sorghum bicolor* L.Moench) grain was harvested from Pemalang, Indonesia. It had a median diameter of 0.8 cm and its color is brownish white. The black husk was removed from the sorghum grain to remove its cellulose. Pericarp was still intact because it covers sorghum starch inside. Then, it was ground sieved using 60-mesh screen. Dry baker's yeast was purchased from Saf-instant (PT. Saf Indonusa) and *Lactobacillus plantarum* was obtained from Biotechnology Lab, (AIT, Thailand). Wheat flour (White Swan), water, dry baker's yeast, salt (Prung Thip), sugar (Mita Phol), shortening (Best Food), and skim milk were obtained from commercial market.

Fermentation

Dry baker's yeast and *L. plantarum* either single or mixed culture were used. It was 10^{10} CFU in the single and 10^7 CFU in the mixed culture. *L. plantarum* was prepared by incubating the culture in MRS medium at 37°C for 18 h. While, the incubation of dry baker's yeast was not necessary. Briefly, sorghum flour concentration (10-50 % w/w) was transferred into a flask. Total volume of fermentation was 350 mL. Starter was prepared by putting the microbe at 10% of total volume. The mixture was fermented at 37°C, pH 5.5, and 200 rpm of agitation speed under anaerobic condition. The fermentation were operated in a bio-reactor type 884101/1 no. 02002/96; product of B. Braun Bio-tech International. The fermentation time was varied between 24 and 72 h. After fermentation, solid particle (flour) and liquid phase was separated by sedimentation for 3 h. The filtrate was discarded. Furthermore, the residue was dried using hot air oven at 60°C for 12 h. It was dried and sieved by 60-mesh sieve. Finally, the flour was ready for further analyses.

Moisture content determination

The sample (5 g) was transferred into a Petri-dish of known weight. The weighed sample was put into an oven at 110°C until constant weight was obtained (AOAC, 2004). The difference between the initial and final weight of the sample was recorded as the moisture content.

Ash content determination

The sample (5 g) was transferred into a previously ignited and cooled silica dish. It was heated at 600°C for 3h in a muffle furnace. The dish and its content was cooled in a dessicator. The residue was recorded as ash content.

Crude fat determination

Crude fat was determined by the method of AOAC (2004). This was determined using a Soxtec System HT2 fat extractor. Crude fat was extracted from the sample (2 g) with 40 mL diethyl ether. The solvent was evaporated. The residue was recorded as crude fat content.

Total starch determination

Acid method (AOAC,1990) was used to determine the starch content. The amount of 2 g of crushed sample was put in a 250 mL beaker glass, added with 50 mL distilled water and stirred for 1 h. The suspension was filtered using a Whatman 42 filter paper and washed with distilled water until reached 250 mL of filtrate. The filtrate containing soluble carbohydrates was discarded. Furthermore, the residue on the filter paper was washed with 10 mL diethyl ether. After allowing the ether to evaporate, it was washed again with 150 mL of 10% ethanol for further release of soluble carbohydrates. The residue was removed from the filter paper, moved into a 200 mL Erlenmeyer flask by washing with distilled water, added with 20 mL of 25% HCl and heated above the boiling temperature in a water bath for 2.5 h. After cooling at room temperature, it was neutralized with 45% NaOH solution, diluted to 500 mL, and filtered using a Whatman 42 filter paper. Furthermore, sugar content of the filtrate was analyzed using the Nelson-Somogyi method. It is expressed as the glucose content and the percentage of starch was determined by multiplying the glucose content by a factor number of 0.9.

Crude protein determination

Crude protein was determined by Kjeldahl method using Kjeltec TM model 2300, as described in Foss Analytical manual, AB, (2003). The method involved digestion of the sample at 420°C for 1 h to

liberate the organically bound nitrogen in the form of ammonium sulphate. The ammonia in the digest ammonium sulphate was then distilled off into a boric and receiver solution, and then titrated with standard hydrochloric acid. A conversion factor of 6.25 was used to convert total nitrogen to percentage crude protein (AOAC, 2004).

Amylose content determination

Colorimetric method was used to analyze amylose content. The sample (100 mg) was mixed with ethanol (95%, 1 mL) and NaOH (1 N, 9.2 mL) for overnight. Then, acetic acid (1 N, 1 mL) and iodine solution (0.2% I₂ in 2% KI, 2 mL) were added into an aliquot (5 mL of mixed sample). The volume made up to 100 mL with distilled water and mixed for 20 min. A blank was made by 5 mL 0.09 N NaOH, to which acetic acid (1 mL) and iodine solution (2 mL) were added in 100 mL total volume. The absorbance of the sample and blank was measured at 620 nm.

Rheological properties determination

Rheological properties was determined with a Rapid Visco Analyzer (RVA) (Newport Scientific RVA Super 3). An aliquot sample (3 g) was transferred into a vessel. 25 mL of distilled water was dispensed into a new test canister. The sample was then transferred into the water surface in the canister. The paddle was placed into the canister and the blade was vigorously jogged through the sample up and down ten times. The slurry was heated from 50 to 95°C and cooled back to 50°C within 12 min. Peak viscosity, setback viscosity, final viscosity, trough, breakdown value, pasting temperature and time to reach peak viscosity were recorded as rheological properties.

Phenolic content determination

The analysis of total phenolic of the samples was performed according to the spectrophotometric method of Folin-Ciocalteu using gallic acid as standard. A mixture of 0.5 mL of the sample, 2.5 mL of Folin-Ciocalteu reagent, and 27 mL water was mixed for 5 min. Then, 2.0 mL Na₂CO₃ solution (4 g/100 mL) was added into the mixture and mixed for 2 h in the absence of light. The results were analyzed on a spectrophotometer at 740 nm.

Mosof bread production

Breads were made by sponge and dough method that was adopted from "Sari Roti" (the most popular bakery industry in Indonesia). The composition of bread ingredients are 200 g flour, 7 g yeast, 12 g sugar, 0.5 g malt, 3 g salt, 6 g shortening, and 120 mL water. Fermented sorghum flour (60 g) was blended

with wheat flour for mosof bread production. Then, it was compared to control 1 (wheat flour 100%) and control 2 (non-fermented sorghum flour 30%). In this method, there were two times of dough mixing processes. First mixing involved 95% of the flour, 90% of water and dry baker's yeast. It was mixed and fermented for 3 h. In the second mixing, other ingredients were mixed to get an elastic dough. Then, the dough was left for 5 min and divided to desired size. The prepared dough was allowed to the intermediate proofing for 17 min. The next step was moulding and panning. The proofed dough was flattened using bread roll to avoid the big holes inside of bread crumb. After that, the flat dough was formed to be a bread formation. It was allowed to the last proofing in temperature 28°C. This temperature was adjusted in a closed room with air conditioner. The dough was baked in oven at 195°C for 33 min.

Volume expand determination

Volume was determined by starch pearls replacement in a box. A portion of bread dough was placed into the volumetric cylinder then the initial volume was recorded. Afterward, the bread dough (loaf) was allowed to expand for the higher level. It was recorded as the final volume. The volume expand was calculated as the difference of the initial and final volumes of loaf.

Bread texture determination

The principal of this analysis was back extrusion (LLOYD Instruments). Bread was placed into a small cylinder. Then, it was forced and extrude outside. Force and length of bread were measured to determine the texture.

Color determination

The color was determined using the Color and Color Difference meter (Model 45/0, BYK-Gerdner, Germany). A sample was prepared (about 10 -20 g) and put it into a clean Petri dish. It was cover and the spillage was wiped out. The color was determined in terms of L* (lightness), a* (redness), and b* (yellowness) values.

Statistical analysis

This experiment was designed by using response surface methodology (RSM) of Design Expert 8.1. Three variables; type of microbe (pure dry baker's yeasts, pure *L. plantarum*, and mix of dry baker's yeasts and *L. plantarum*), sorghum concentration (10-50% w/v) and duration of fermentation (24-72 h) were used to find the optimum condition of fermentation. They were considered with same level

of variable. The optimum condition was given by the software by selecting the protein content as the key parameter.

Results and Discussion

Characterization of sorghum flour

Sorghum has a high content of carbohydrate that was considered as a source of staple food. It could be converted to the flour in the using for industry. In this research, it was very important to characterize the raw material, sorghum flour, of fermentation. Proximate chemical and physical properties were obtained. It was presented in the Table 1.

The major composition of sorghum flour is starch. Sorghum flour has total starch 40.85%. Starch has a main role in the forming of baking product. It depends on the comparison of amylose and amylopectin content. The amylose content of sorghum flour is 25.66% and the amylopectin is 15.19%. This affected pasting properties of sorghum flour. Pasting properties involve the viscosity and also pasting temperature. It was presented in the Table 1. Moreover, other carbohydrates compounds, such as monosaccharide and disaccharide, were not measured in this study. Therefore, the chemical composition of the flours is less than 100%.

Optimum condition for sorghum flour fermentation

Fermentation with different microbe types, sorghum flour concentrations, and fermentation time were applied in this study. The experimental design was run automatically using Design Expert 8.1 software. Protein content was used as the key parameter. The optimum conditions for this fermentation can be seen on the Figure 1.

The optimization of sorghum flour fermentation was arranged for increasing the protein content based on three factors; microbes (pure dry baker's yeasts, pure *L. plantarum*, and mix of dry baker's yeasts and *L. plantarum*), sorghum flour concentrations (10-50% w/v), and the duration of fermentation (24-72 h). Based on Figure 1, it could be informed that RSM provide the optimum condition of fermentation of sorghum flour is fermentation by the mix of dry baker's yeasts and pure *L. plantarum* with 37% sorghum flour (w/w) concentration for 24 h. It was predicted that total protein content in this condition is about 18.90% w/w. It was selected to be used for the next study. It was compared to the non-fermented sorghum flour and wheat flour.

Basically, fermentation has ability to increase the protein contents. The activities of microorganisms allowed protease synthesise amino acids. It supports

Table 1. Comparison of different flour propertiesa

Difference	Wheat flour	Non-fermented Sorghum flour	Fermented Sorghum flour
Proximate			
Moisture (%)	7.02 ± 0.56	6.78 ± 0.31	3.30 ± 0.29
Total starch (%)	39.67 ± 1.77	40.58 ± 0.29	60.73 ± 1.68
Total protein (%)	12.20 ± 0.29	12.43 ± 0.18	23.43 ± 0.53
Fat (%)	0.97 ± 0.0	3.70 ± 0.0	2.83 ± 0.01
Ash (%)	0.56 ± 0.01	1.64 ± 0.0	9.52 ± 1.93
Rheological properties			
Viscosity (RVU)	125.33 ± 2.18	197.53 ± 7.19	109.53 ± 4.10
Pasting time (min)	5.66 ± 0.08	5.51 ± 0.08	5.73 ± 0.04
Pasting temperature (°C)	87.32 ± 1.36	87.55 ± 0.44	90.27 ± 0.49

aobtained from three independent experiments

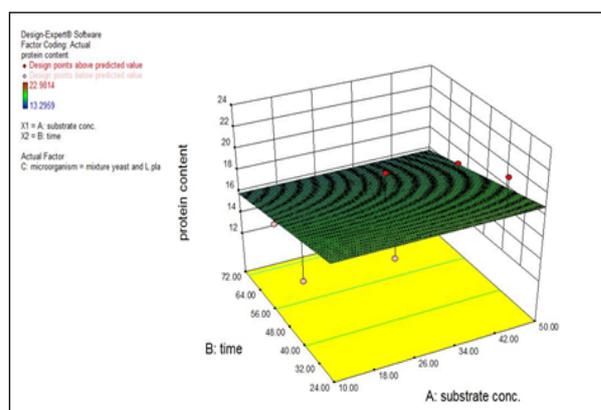


Figure 1. Predicted optimum conditions of sorghum flour fermentation

the report presented by Ge'linas (2006) which mentioned that yeast fermentation of starch-based materials enrich the protein contents until 8% from the initial protein (yeast protein). This protein content was not only increase nutritional value of fermented sorghum flour, but also affect aromatic compounds. In term of health, protein played functional roles such as provide structural rigidity to the cell, control the flow of materials across the body and cellular membranes, and keep the immune system.

Proximate analysis of fermented sorghum flour

Proximate composition of different flour is shown in Table 1. It consists of moisture, total protein, fat and ash content. Wheat flour and non-fermented sorghum flour were used as the control.

Basically, native dried sorghum starch has a composition of 10.73% moisture, 0.30% ash, 1.06% protein and 1.07% fat (Aviara et al., 2010). In this research, proximate analysis of Mosof was compared to non-fermented sorghum flour and commercial

wheat flour (Table 1). Moisture content of Mosof was lower than that of non-fermented sorghum flour. That is good for flour properties because a lower moisture gives higher protection from microorganism that cause spoilage. Microorganisms and many enzymes cannot be functioned without water. However, it is usually necessary to the lower moisture content below 5 wt% in food to maintain the nutrition and flavor (Geankolis, 1993). Moreover, in order to reduce moisture content of commercial flour, manufactures often use drying process. However, drying needs a high number of energy. Therefore, this lower moisture content contribute to reduce the energy for the flour drying since it is absolutely needed for preservation.

Sorghum flour fermentation could increase total protein content (23.43%) significantly ($p < 0.05$). It was also reported by Gunawan *et al.* (2015) that protein content of mocaf increased as the result of fermentation. Fermentation allowed microorganism to produce metabolism that synthesized protein (Hu *et al.*, 2012). Both of residue and filtrate has protein content. Filtrate has less protein content than residue (flour). It means that water soluble protein was founded less than water non-soluble protein. Water soluble protein include arginine, asparagine, glycine, histidine, lysine, tyrosine, threonine, serine, aspartic acid, and glutamic acid and water non-soluble protein are alanine, isoleucine, leucine, methionine, proline, valine, phenylalanine, tryptophan, and tyrosine. However, the types of amino acids produced by this fermentation are needed to be analyzed further.

In addition, mosof has different aroma from wheat flour and non-fermented sorghum flour. Its odor is like a banana essence that is isoamyl acetate. Procopio (2013) explained that more valine, leucine and isoleucine (hydrophobic amino acid) concentration in the yeast fermentation strongly produced higher isobutyl acetate, isoamyl acetate and ethyl acetate, respectively. This may in turn lead to enhanced ester production. Therefore, it was confirmed that fermentation of sorghum flour could increase hydrophobic amino acid that promoted the formation of aromatic compounds. Moreover, Ehrlich and anabolic pathways were adopted by Chen (1978) to determine the reaction of isobutyl acetate, isoamyl acetate and ethyl acetate forming.

Other influence of fermentation on the sorghum flour is fat content decreasing. This is very useful for low fat bakery product. The lower fat content also reduces oxidation of bread during storage. Microorganism activity might able to use water in the media of fermentation to hydrolyze lipid into fatty acids and glycerol. Furthermore, the unsaturated fatty acids was potentially to be oxidized. This reaction is

one kind of food degradations that produce numerous volatile and nonvolatile compounds. Some of the volatiles are exceptionally odorous compounds. It contributes to the stale flavor and unpleasant aroma. Lipid oxidation was taken place by present of lipid and oxygen but the latter was only occurred in the presence of the enzyme (Belitz *et al.*, 2009).

Rheological properties of fermented sorghum flour

One of important properties of flour is rheological properties. In this research, Rapid Visco Analyze (RVA) was used to measure the viscosity and pasting properties of fermented sorghum flour. It was found that mosof has different viscosity and pasting properties with non-fermented sorghum flour and also wheat flour. The results of RVA was presented in Table 1.

Rheological properties of mosof was indentified by its viscosity. The higher viscosity promote the lower pasting temperature and longer pasting time as shown in Table 1. It was found that fermentation causes decreasing in viscosity but increasing pasting time and pasting temperature. These properties have effects on bread making. Higher pasting temperature need a higher temperature for baking. This lower viscosity consequently indicated the higher pasting temperature of flour (Richard, 1990). In the RVA, flour was heated until reach peak viscosity. In this point, starch molecule was degraded and released amylose to the water. Then, the amylose make a pasta in which temperature reached is called pasting temperature. Amylose has α -1,4 linked that is simpler to be degraded than amylopectin (α -1,6). Degree of polymer (n) also affect the pasting temperature. Higher n-value give higher Δ HR kl so that have a higher pasting temperature.

Moreover, lower viscosity indicates a lower amount of amylose. The difference of amylose and amylopectin content of wheat flour, non-fermented sorghum flour and Mosof was significant ($p < 0.05$). It was found that amylose content of mosof decrease by 64%. This is because microorganisms produce enzyme that convert amylose to amylopectin by changing the chemical bond between one glucose and another. Link α -1,4 of amylose was changed to α -1,6 of amylopectin or combine small amylose by form α -1,6 glucosidic bond. Therefore, amylopectin of mosof was increased in a high number (115%) by the fermentation. These results are supported by Detchewa (2012) who reported that rice flour with 33.36% amylose has higher final viscosity (503.69 relative viscous unit, RVU) than rice flour with amylose content 4.42% (256.69 RVU).

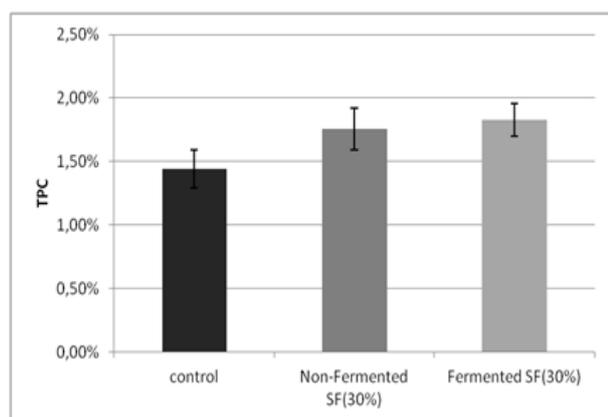


Figure 2. Total phenolic compound (TPC) of flours

Phenolic content

Phenolic compound is an important composition of sorghum flour that has a role as an antioxidant agent. Total phenolic compound (TPC) was measured by DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. The DPPH method can be used for solid or liquid samples. It was not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample (Prakash *et al.* 2001). Spectrophotometer was used to read the absorbance of solution at 740 nm that represent the TPC. Results of Mosof phenolic content was described as Figure 2.

Generally, an antioxidant has ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases.

Figure 2 shows that the fermentation cause increasing the TPC of sorghum flour. TPC of fermented sorghum flour was significantly higher than those of commercial wheat and non-fermented sorghum flours ($p < 0.05$). This result is supported by Rebeca *et al.* (2009) which mentioned that fermentation by *L. plantarum* increases total phenolic compound. Therefore, fermentation could improve antioxidant content of sorghum from its origin high level content. Kamath *et al.* (2010) also confirmed that the cereal grain sorghum (white) serve a significant source of natural antioxidant compounds that may give health benefits.

Antioxidant has a great contribution to reduce lipid oxidation (Dziki *et al.*, 2013). It was mentioned that modified bread with the addition of onion skin increase inhibition of lipid peroxidation of bread. Lipid peroxidation is avoided in term of bread

Table 2. Volume expand of different bread dough composition^a

Proportion of Mosof to wheat flour (% w/w)	Volume expand (mL/min)
0	0.25 ± 0.09
10	0.70 ± 0.05
20	0.75 ± 0.09
30	0.90 ± 0.18

^aobtained from three independent experiments

quality maintenance. This lipid peroxidation release some undesired aromatic compounds and flavours. There are many techniques for reducing staling and deterioration of bread evenly modify bread packaging. This experiment result, higher antioxidant, might provide one of them because antioxidant has ability to reduce the reaction of lipid oxidation.

The reaction of lipid peroxidation involve enzymes, water activity, lipid content and presence of reactive oxygen (Belitz *et al.*, 2009). The generation of reactive oxygen species (ROS) such as hydroxyl, peroxy or superoxide radicals as well as other oxidant species is inevitable in aerobic metabolism of the human body. On the other hands, excess of ROS production in the body, oxidative stress takes place leading to the damage of tissues and to cell death (Marazza *et al.*, 2012). Thus, regular consumption of antioxidant-rich-foods may help to reduce the deleterious action of ROS and free radicals, and to balance the oxidative stress related to aging process and serious illnesses. Another, Marazza *et al.* (2012) also mentioned that antioxidants could be produced by fermentation using lactic acid bacteria (LAB).

Bread properties (color, texture, and volume expand)

Mosof was blended with wheat flour at 30% sorghum flour for bread production. Then, the bread properties were compared to control 1 (wheat flour 100%) and control 2 (non-fermented sorghum flour 30%). Volume expand, color, and texture are presented in Table 2, and Figure 3, respectively.

Volume expand of different bread dough composition is shown in Table 2. It can be seen that volume expand of bread dough increases by increasing proportion of mosof to wheat flour. This is because of the presence of CO₂. The bread appearance of wheat flour was significantly brighter than those obtained from fermented sorghum flour (FSF) and non-fermented sorghum flour (NSF). However, color meter was more accurate in the determination of color difference. The bread color of mosof was darker than those obtained from fermented and non-fermented

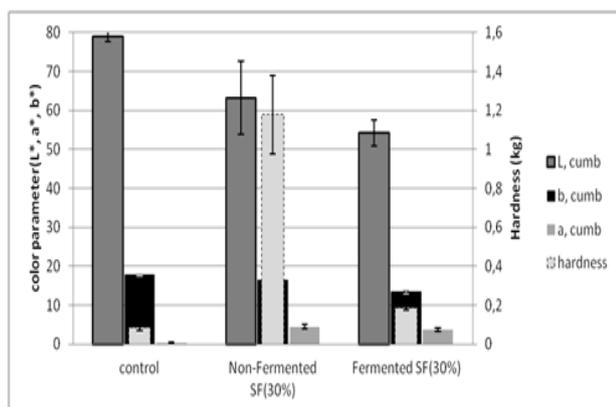


Figure 3. Color and hardness of different breads

sorghum flour. The value of L^* (lightness) and b^* (yellowness) decreased from the wheat flour, non-fermented sorghum flour and mosof, respectively. Conversely, the value of redness (a^*) increased as shown in Figure 3.

In other hand, the hardest bread texture was obtained in bread with substitution of 30% non-fermented sorghum flour. This is because of the absence of gluten in the non-fermented sorghum flour. Therefore, it was difficult to make an elastic dough with strong water binding inside. However, fermentation could reduce hardness of bread crumb and make it softer. Another, it is possible that lower amylose content of fermented sorghum flour make it softer within the role of gluten.

Conclusion

Fermentation of sorghum flour by mix of dry baker's yeast and *L. Plantarum* at the optimum condition provided higher nutritional value than non-fermented sorghum flour and wheat flour. And more, it has influence to the rheological properties of flour. Whereas, its application increased volume expand, decreased bread crumb hardness and produced darker bread color. Since the higher protein content of fermented sorghum flour has some advantages to the bread, it is necessary to study the characterization of the amino acids in the fermented flour in the future.

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