Profile of Modified Sorghum Flour Fermented by 
Lactobacillus Brevis

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Abstract. Lactobacillus brevis is an excellent species of lactic acid bacteria for improving 
functional and physicochemical properties of flour. The study aimed to characterize sorghum 
flour profiles modified using Lactobacillus brevis. Sorghum grains of a local variety KD4 was 
polished using a mini polisher. The flour was fermented with L.brevis at different concentrations. 
The experiment was designed in a completely randomized, two factors and three replications. 
The first factor was microbial concentrations (0.5; 0.10; 0.15; 0.20%) an 
d the second factor was 
fermentation time (4, 8, and 12 hours). The results showed that the microbial concentrations and 
fermentation time affected the physicochemical properties of the flour such as starch content, 
amylose, bulk density, and whiteness degree of flour. Increasing the fermentation temperatures 
increased the swelling power of starch and resulted in high solubility of the granules. Sorghum 
starch solubility was influenced by amylose content. The longer the 
time of incubation, the 
lower the tannin content. Flour water absorption ranged from 11.58 to 21.81%, whereas oil absorption 
had no significant differences. The study suggests that L.brevis in a 
concentration of 0.20% and 
12 hour of fermentation can be used in the fermentation of sorghum flour.

1. Introduction
Sorghum is a potential cereal crops to be cultivated in Indonesia as alternative staple foods. 
Carbohydrate content in sorghum is almost equivalent to carbohydrate from other staple foods like 
brown rice, corn, and wheat [1]. Other nutrients contain in the sorghum were fat (3.1%), protein 
(10.4%), crude fiber (2%), and energy (329 kcal), vitamins, including vitamin A, E, thiamine, riboflavin, 
nicotinic acid, pantothenic, vitamin B6, vitamin B12, and chlorine [2]. However, there are some 
obstacles in utilization sorghum for food products. Mostly, protein in the endosperm of sorghum consists 
of 77-82% kafirins, a part of prolamin proteins group, and the rest is non-prolamin poteins (30%), 
including albumins, globulins, and glutelins [3]. Due to the presence of kafirins that have too short and 
tightly cross-linked chains of a polypeptide, sorghum dough has poor viscoelasticity and low density. 
Low rheology properties of the dough resulted in a low quality of bread [4]. Sorghum grains also contain 
tannin which has polyphenolic compound and antinutrient component. Tannin and phytate cause 
indigestion on human, such as absorption of mineral, especially iron [5].

Fermentation is a potential method for eliminating antinutrient and improving functional and 
physicochemical properties of sorghum starch. Fermentation increases the solubility of the protein of 
sorghum flour, oil-binding capacity, emulsifying capacity and stability and decreases water-binding 
capacity [6]. Fermentation of sorghum has also increased in vitro accessibility of iron [5]. Fermentation 
improves the functional properties of sorghum [7]. During the fermentation process, tannin content of
several local varieties of south-west Saudi Arabia reduces until 35% [8]. The recommended method for fermenting sorghum is using lactic acid bacteria because of its capability to produce desirable flavors [9]. Additionally, the use of lactic acid bacteria modified the compositions of carbohydrate resulted in organic acids and carbon dioxide anaerobically [10].

*Lactobacillus brevis* is a lactic acid bacterium that has been used for modifying characteristics of functional and physicochemical properties of flour. Proteolytic activities of the bacterium during fermentation breaks down the protein of sorghum that would be easily hydrolyzed [11]. When *L. brevis* is growing on casein, it degrades casein to essential nutrition for stimulating their growth [12]. The study aimed to analyse the functionally and physicochemical properties of sorghum flour profile after modifying with *L. brevis*.

2. **Materials and methods**

2.1 **Materials**
Sorghum starch used in the study was a local variety of KD 4 from Lamongan, East Java. An isolate of *L. brevis* originated from the microbial culture collection of Indonesian Center for Agricultural Postharvest Research and Development. Isolate of *L. brevis* was cultured on MRS deMann, Rogosa and Sharpe broth (Merck, Germany) for 24 hours at 37°C.

2.2 **Methods**
Sorghum grains was polished using a mini polisher (Satake Grain Testing Mill, Japan), capacity about 300 grams, for 2 minutes to remove sorghum hull. The polished sorghum was fermented in one litre baker glass containing water (pH 7) and different concentrations of *L. brevis* culture (1:1 ratio) at 37°C room temperature. After soaking process, the fermented sorghum was dried for 8 hours in an oven blower with far infrared (FIR), 50°C. The dried sorghum grains were milled using a milling device (Milcent Magnetic 1-HP) and refined using a sieving machine (Retsch type AS200 basic, 2002) of 100 mesh. The experiment was designed in a completely randomized, two factors, and three replications. The first factor was microbial concentrations (0.5; 0.10; 0.15; 0.20%) and second factor was fermentation times (4, 8, and 12 hours). The control of treatment was sorghum flour without microbial fermentation.

**Statistical analysis**
Experimental data were subjected to one-way analysis of variance (ANOVA), using the SPSS 16 (SPSS Inc., USA). Treatment means were tested by Duncan’s Multiple Range Test (DMRT).

3. **Results and Discussion**

3.1 **Physical properties of sorghum flour**
The physical properties of sorghum flour were affected by *L. brevis* (Table 1). The study showed that microbial concentrations and fermentation time significantly influenced the whiteness values of the flour. The longer the fermentation time, the increase the whiteness values of modified sorghum flour. Microbial concentrations inconsistently affected the whiteness value. However, the fermentation time did not increase the whiteness (L value) of sorghum flour. Whiteness value describes the degree of brightness of sorghum flour compare to BaSO₄ (83.40%). Increasing of whiteness wheat flour because of decreasing of b value which expresses yellowish parameter of flour [13].

The bulk density of the modified sorghum flour was lower than the control, however, amongst the treatments the bulk density significantly different. The bulk density is parameter that describes the level of compactness of flour. The longer the fermentation time and the bigger of the microbial concentration, the smaller the bulk density of modified sorghum flour. The level of microbial concentrations and fermentation time periods caused the structure of sorghum flour to become more compact. Previous study showed that the longer the fermentation time, the smaller the bulk density of
modified sorghum flour [6]. Apparently, protein and fat content in the sorghum flour contributed to the decreasing of bulk density [14,15].

The longer the fermentation time, the higher the water absorption capacity (WAC) of sorghum flour. However, oil absorption capacity (OAC) of the modified sorghum flour was similar amongst the treatments. WAC value of sorghum flour was greater than the value of OAC, therefore the modified sorghum flour to be more hydrophilic. WAC is an important parameter that describes the ability of flour to absorb water, while OAC describes the ability of flour to bind the oil element. Increase in WAC decreased the fat content. De-fatted treatment of wheat flour increased in water absorption capacity [16].

Tannin content of the modified sorghum flour decreased despite increasing of microbial concentrations. The increase in fermentation time, the decrease of tannins contents in the modified sorghum flour. It indicated the hydrolysis process of polyphenolic compounds of the sorghum flour. Microbial activity in the fermentation process brook down phenol compounds due to the presence of the polymerization process and interaction with protein component [17]. Previous study showed that by natural fermentation, tannins content reduced about 31-35% [18]. In addition, milling and immersion could also reduce tannin levels [19].

The viscosity and gel consistency of the modified sorghum flour did not affect by treatments of microbial concentration and fermentation time. Fat content determines the viscosity level [20]. However, the gel consistency was affected by amylose content; the higher amylose content, the harder gel consistency of starch [21]. In the absence of fat content in starch, the peak viscosity and fermentation time increased, therefore, the energy required for the gelatinization process became greater [16]. The starch viscosity was influenced by the ratio of amylose and amylpectin contents; the higher amylose content, the higher the viscosity of starch. On the other hands, the higher amylpectin, the lower viscosity of starch [22].

3.2 Swelling power and solubility of sorghum flour

The analysis results of swelling power and solubility of modified sorghum flour and control were presented in Figures 1 and 2. Generally, modification starch using different microbial concentrations and fermentation times cause a different change of granule starch structure. Figure 1 showed that raising of heating temperature causes swelling of granules of fermented sorghum starch. Incubation process using different concentrations of L.brevis and increasing of heating temperature increased the swelling power of starch granules. However, fluctuate trend of swelling power shown on treatment using 0.10% of microbial concentration during 4 hours of the fermentation.

Variety swelling power value of modified sorghum flour influenced by differences of structure and composition of sorghum. Amylose and amylpectin contents affect the swelling ability of starch granules. In addition, swelling power is also influenced by characteristics (shape and length of chain) of amylose and amylpectin molecules [23]. On a study about characterization of taro tuber starch showed that increasing of heating temperature causes the starch granules are swollen because of water particle penetration into the structure of starch. Penetration of water particles occurs because of disruption of energy equilibrium in starch molecules so bonds intermolecular become unstable [24]. The same thermodynamic phenomenon also described on the study about waxy rice starch characteristics [25].

The solubility of modified sorghum flour is a quality parameter of function that indicates flour soluble capabilities in water. Flour solubility is influenced by amylose content and gelatinization temperature. The high gelatinization temperature and amylose content cause starch to have low ability for absorbing water and swelling its structure. It has been proven in a research about sago starch with high amylose which has the ability to absorb less water [26]. In general, solubility of modified sorghum flour increase linear to increase of swelling power of the flour. This trend showed at 12-hour fermentation treatment, the flour has a solubility value increases along with increasing heating temperature.
**Figure 1.** The swelling power of modified sorghum flour at different fermenting time. Fermented for 4 hours (a), 8 hours (b), and 12 hours (c).

**Figure 2.** Solubility of sorghum flour (a) fermented for 4 hours, (b) fermented for 8 hours, and (c) fermented for 12 hours.
3.3 Starch composition of sorghum flour

The high starch content of sorghum is a consideration factor in the utilization of sorghum to compete with other starch sources. In general, sorghum starch content is relatively high (Table 2). Compared to controls, starch content of modified sorghum flour tends to decrease. Reduction of dissolved sugar could be used as an indicator of performance bacteria to degrade amylase in starch which occurs because of reduction of starch content [11]. Starch consists of two components, i.e. amylose that is soluble in water and amylopectin which are poorly soluble in water. The existence of them affects to functional properties of starch. Modified sorghum flour and control have amylose content lower than amylopectin content. It indicates that dominant structure of sorghum starch is a branched-chain structure rather than a straight chain. Generally, ratio of amylose/amylopectin starch in a row in range of 25-28% and 72-75% [27]. Submersion treatments using microbial or not be able to modify starch chain from branched-chain into a linear chain. The longer of fermentation time, the higher amylose content of the flour, whereas the height of microbial concentration does not correlated to the height of amylose content.

The experiment factors do not cause significant changes to dietary fiber and fat of modified sorghum flour compared to controls. The increase of dietary fiber content about 10-20% be able to decrease swelling power value. Fat content effects to gelatinization temperature and viscosity of starch. Increased gelatinization temperature is closely related to the formation of a fatty bond with amylose component [28]. Protein and fat content also affect functional properties of flour and determine the utilization of starch as a raw material of food products [29]. Combination treatments between microbial concentrations and fermentation time have influenced to increase protein content. It occurs because of hydrolysis activity of nitrogen by protease bacteria thereby increasing levels of soluble protein and amino acids [12]. Amino acids that are formed will be a source of additional nutrients that support the performance of L. brevis activity to degrade of starch granules [30]. Performance protease bacteria in degrading peptide in protein chains facilitate amylase bacteria in degrading starch [31].

3.4 Microstructure of sorghum starch

Due to fermentation process, there is a change in shape of the starch granules from the intact structure into smaller structures (Figure 3). In fermentation using L. brevis at a concentration of 0.05% for 4 hours does not indicate a breakdown process where starch granule-bound in a larger structure resemble the structure of unmodified sorghum starch. Change of starch structure is shown on modified sorghum starch using L. brevis at 0.20% that has been fermented for 12 hours where the structure are not intact, and mostly the starch granules are already separated one another. Compared to the commercial wheat flour, the structure of starch granules tend to be elliptical, intact, and the most of the starch granules bound one another. Starch granules of modified sorghum flour have round form, incomplete and mostly granular starch are already bound/joined one another because of the treatment effect of microbial fermentation. In the picture with the same magnification showed that sorghum starch granules modified with L. brevis have a smaller size than the commercial wheat flour.
4. Conclusion
Fermentation time and microbial concentration of \textit{L. brevis} improved the functional and physicochemical properties of sorghum flour. The whiteness, protein content, and swelled granules increased, but the bulk density and tannin content decreased. Solubility of sorghum starch was influenced by amylose content. The study suggests that \textit{L. brevis} in microbial concentration 0.20\% and 12 hour fermentation time can be used in the fermentation of sorghum flour.

5. References
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\textbf{Figure 3.} Microstructure of sorghum starch (a) native sorghum starch, (b) modified sorghum starch at 0.05\% of \textit{L. brevis} for 4 hours, (c) modified sorghum starch at 0.20\% of \textit{L. brevis} for 12 hours, (d) wheat starch
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Table 1. Physical of sorghum flour treated with different microbial concentrations of L. brevis and fermentation time

| Microbial conc. (%) | Time (hours) | Whiteness (%) | Density (g/l) | WAC (%) | OAC (%) | Tannin (%) | Viscosity (Cp) |
|---------------------|--------------|---------------|---------------|---------|---------|------------|---------------|
| Control             | 4            | 89.56±0.03    | 630.0±0.57    | 10.31±0.29 | 10.65±0.28 | 0.023±0.001 | 13.00±0.28    |
| 0.05                | 4            | 97.67±0.42    | 580.0±0.49    | 18.92±0.31 | 10.54±0.46 | 0.016±0.0004 | 12.60±0.12    |
| 0.10                | 4            | 95.02±0.14    | 559.5±0.71    | 17.78±0.02 | 11.01±0.31 | 0.021±0.0006 | 12.80±0.04    |
| 0.15                | 4            | 97.19±0.28    | 556.0±0.16    | 20.81±0.03 | 11.42±0.44 | 0.024±0.0003 | 12.40±0.42    |
| 0.20                | 4            | 91.66±0.13    | 572.0±0.42    | 11.42±0.35 | 10.50±0.45 | 0.017±0.0014 | 13.20±0.42    |

Control 8 91.02±0.57 601.0±0.17 12.16±0.13 11.03±0.17 0.019±0.0002 13.00±0.14
0.05 90.98±0.14 580.5±0.57 14.63±0.03 10.84±0.27 0.014±0.0007 13.00±0.11
0.10 96.60±0.31 551.0±0.35 19.97±0.31 10.25±0.16 0.019±0.0005 13.80±0.42
0.15 90.79±0.47 581.0±0.48 13.47±0.51 10.83±0.25 0.019±0.0004 13.20±0.47
0.20 91.89±0.20 538.5±0.22 16.27±0.16 11.20±0.45 0.018±0.0005 12.60±0.39

Control 12 96.64±0.28 528.5±0.42 18.14±0.25 11.95±0.25 0.017±0.0004 12.80±0.28
0.05 90.91±0.32 641.0±0.47 11.58±0.17 10.90±0.34 0.007±0.0002 13.00±0.16
0.10 97.94±0.58 551.0±0.98 20.77±0.44 10.72±0.37 0.018±0.0013 13.80±0.44
0.15 96.63±0.31 614.0±0.59 15.76±0.24 11.24±0.27 0.012±0.0005 13.40±0.27
0.20 98.18±0.12 517.5±0.19 19.82±0.19 12.82±0.24 0.009±0.0007 13.20±0.25

Note: Mean values in each column with the same letter are not significantly different (p = 5%)

Table 2. Starch composition of sorghum flour

| Microbial conc. (%) | Time (hours) | Starch (%) | Amylose (%) | Amylopectin (%) | Dietary fiber (%) | Protein (%) | Fat (%) | Carbohydrate (%) |
|---------------------|--------------|------------|-------------|-----------------|-----------------|-------------|---------|------------------|
| Control             | 4            | 74.37±0.16  | 34.87±0.48  | 65.13±0.19     | 3.91±0.16       | 10.44±0.16  | 10.44±0.01 | 83.74±0.28       |
| 0.05                | 4            | 76.00±0.39  | 39.55±0.49  | 60.45±0.46     | 4.23±0.18       | 10.11±0.15  | 10.11±0.02 | 85.07±0.43       |
| 0.10                | 4            | 70.42±0.35  | 38.50±0.28  | 61.50±0.29     | 4.45±0.32       | 10.51±0.14  | 10.51±0.03 | 83.98±0.44       |
| 0.15                | 4            | 72.97±0.32  | 39.80±0.43  | 60.22±0.47     | 4.32±0.18       | 10.73±0.08  | 10.73±0.02 | 84.76±0.47       |
| 0.20                | 4            | 71.93±0.55  | 31.75±0.32  | 68.26±0.43     | 3.99±0.14       | 10.22±0.10  | 10.22±0.27 | 83.35±0.41       |

Control 8 73.91±0.37 37.18±0.31 62.82±0.34 3.97±0.45 10.15±0.07 10.15±0.38 84.08±0.55
0.05 72.47±0.33 33.19±0.45 66.81±0.85 4.23±0.31 10.82±0.04 10.82±0.42 83.08±0.39
0.10 73.52±0.49 38.40±0.06 61.60±0.48 4.19±0.49 10.72±0.03 10.72±0.21 84.95±0.02
0.15 75.01±0.14 28.08±0.29 71.94±0.35 4.42±0.34 10.67±0.11 10.67±0.27 83.08±0.17
0.20 70.91±0.44 34.21±0.46 65.79±0.49 3.84±0.21 10.79±0.03 10.79±0.24 83.22±0.17

Control 12 76.11±0.48 42.41±0.43 56.94±0.47 3.93±0.44 10.75±0.13 10.75±0.33 83.54±0.18
0.05 73.08±0.40 39.56±0.31 60.44±0.48 3.42±0.23 10.52±0.08 10.52±0.28 83.83±0.19
0.10 73.74±0.39 26.80±0.45 73.20±0.32 3.84±0.49 10.75±0.07 10.75±0.48 85.83±0.21
0.15 74.03±0.46 37.42±0.48 62.60±0.32 3.78±0.44 11.40±0.00 11.40±0.64 84.31±0.24
0.20 74.72±0.50 40.08±0.27 59.92±0.27 4.11±0.16 8.39±0.03 8.39±0.31 86.76±0.25

Note: Mean values in each column with the same letter are not significantly different (p = 5%)