Effect of Fermentation using *Lactobacillus casei* on the Physicochemical and Functional Properties of Sorghum Flour

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Abstract. Sorghum is one of the high carbohydrate food sources that is still underutilized for processed food products due to its anti-nutritional content and functional properties. Fermentation is one of the processes that can reduce anti-nutritional content and improve the functional properties of sorghum flour. The purpose of this study was to determine the effect of *Lactobacillus casei* fermentation on the physicochemical and functional properties of sorghum flour. Sorghum used in this study was a local variety of KD-4 from Lamongan District, East Java. The experimental design used was Completely Randomized Design with two factors and three replication. The first factor was the concentration of bacteria in soaking water (0.05%; 0.10%; 0.15%; and 0.20% of the volume of water) and the second factor was the fermentation period (4, 8 and 12 hours). The results showed that concentration of bacteria and fermentation period generally affected the physicochemical and functional properties of sorghum flour. Increasing concentrations of bacteria and fermentation period tended to decrease tannin content, starch, amylopectin, and water and oil absorption but increased the value of whiteness, dietary fiber, amylose, and viscosity of sorghum flour.

1. Introduction

Sorghum is a type of cereal, contains high carbohydrate that can be used as food, but it is mostly used only as animal feed. Although its nutritional value is not inferior compared to rice, sorghum is rarely used as ingredient for processed food products. The chemical composition of sorghum includes high protein (8-12%), which is equivalent to wheat and superior to rice with only 6-10% of protein. Sorghum is also high in fat (2-6%), four times higher than rice (0.5-1.5%).

One of the obstacles in the utilization of sorghum as food ingredient is its high tannin content (40.79 mg/100 g). Tannin, in addition to affecting the taste (bitter) when contained in food in large amount, has anti-nutritional property, i.e. interfere with the mineral absorption in human body. Application of sorghum in processed food is also limited due to its functional properties. Therefore, a certain processing technology is needed to be developed.

Sorghum starch content range from 56-73% with an average of 69.5% composed of amylose (20-30%) and amylopectin (70-80%), depending on genetic factors and environment [1]. Starch is the main ingredient in various food processing systems, such as it is the main energy source, and also has function as a determinant of the structure, texture, consistency, and appearance of food [2].

Fermentation is a process that can reduce the content of anti-nutrients in grains such as tannin and phytic acid, and it can improve the digestibility of starch and protein, and increases the nutritional content. The fermentation process is also known to be able to increase the protein content and amino acid balance [3]. Fermentation can improve the protein digestibility and functional properties of...
sorghum. Fermentation decreases albumin and globulin fraction, and increases kafirin content although those changes do not show a clear trend. The fermentation process also can improve the gelling properties of sorghum flour which is very important for making pudding products. Fermentation can decrease water holding capacity but can increase the oil holding capacity by approximately 7%. Flour with low water holding capacity is very suitable for gruels thinner products, while flour with high oil holding capacity is suitable for products that retain oil. Fermentation during 16 hours caused emulsification capacity of sorghum flour increased by 7 % and emulsion stability increased by 9% [4].

Modification of sorghum starch with lactic acid bacteria (Lactobacillus acidophilus) produced sorghum flour with tannin content 0.062%, whiteness 22.93%, soluble protein 1.365%, reduced sugar 1.278% and viscosity 39.38 cP [5]. Fermented sorghum using Lactobacillus plantarum for 28 hours resulted sorghum flour with optimal levels of starch digestibility 84.59%, the level of protein digestibility 76.88%, brightness of 82.49, gelatinization temperature of 79°C and a peak viscosity of 689 cP with the appearance of microstructures that were not compact than unfermented sorghum flour [6]. The fermentation process by adding Lactobacillus plantarum starter S4512 on sorghum flour media affected on the appearance of the microstructure of sorghum flour as indicated by the release of the protein matrix and starch granules after fermented sorghum flour [7].

Modification of sorghum flour through the fermentation process has been developed, but information about the influence of microbial fermentation on the physicochemical and functional properties of sorghum flour is still very limited, so the study of it is very important to do. The aim of this study was to determine the effect of Lactobacillus casei fermentation on the physicochemical and functional properties of sorghum flour.

2. Material and Method

2.1. Material
The study was conducted in the Agricultural Postharvest Laboratory Installation, Karawang and Indonesian Center for Agricultural Postharvest Research and Development, Bogor. The main material used in this study was local variety of sorghum KD-4 from Lamongan District and Lactobacillus casei isolate from Microbiology Laboratory of Indonesian Center for Agricultural Postharvest Research and Development in Bogor. The equipment used included soaking tub, dryer (oven blower dryer type), polisher, sieve and milling machine.

2.2. Fermentation
Firstly, grain sorghum was polished for 2 minutes to remove the husk. Furthermore, polished sorghum was fermented using Lactobacillus casei. Fermentation process of sorghum flour included polishing of sorghum grain, the addition of bacteria in soaking water, fermentation, drying, milling, and sieving. Ratio of polished sorghum and water used in the fermentation process was 1:2. Fermentation was done by soaking of polished sorghum in soaking water at room temperature with a neutral pH conditions for 4 hours, 8 hours and 12 hours. After fermentation, sorghum dried in oven until the moisture content reached 12%, then it was milled and sieved using 100 mesh sieve.

2.3. Experimental Design
The experimental design used was Completely Randomized Design Factorial with two factors and three replicates, i.e. the concentration of bacteria in soaking water and fermentation duration. The number of bacteria colonies used was 9.65 x 10^{15} and the concentration of bacteria used consisted of four levels i.e. 0.05%; 0.10%; 0.15%, and 0.20% volume of soaking water. The fermentation period was consisted of three levels, i.e. 4 hours, 8 hours, and 12 hours. Experimental data were analyzed using ANOVA and continued with Duncan Multiple Range Test.
2.4. Analysis Method

The parameters which were analyzed on the fermented sorghum flour included the physicochemical and functional properties. The physicochemical properties of modified sorghum flour that were observed namely density, whiteness, protein content, tannin content, starch content, amyllose/amylumpectin, and dietary fiber. Functional properties of modified sorghum flour were observed namely water and oil absorption capacity, viscosity and gel consistency.

3. Results And Discussion

3.1. Physicochemical properties of fermented sorghum flour using Lactobacillus casei

The measurement on the physicochemical properties of fermented sorghum flour was conducted to determine the effect of Lactobacillus casei during fermentation on the physical and chemical properties of sorghum flour. Overall fermentation using Lactobacillus casei for a certain period affected on the physicochemical properties of sorghum flour, which were included the content of proximate, density, whiteness, tannin content, starch content, amyllose/amylumpectin and dietary fiber. Increased of concentrations of bacteria and fermentation period tended to increase the value of whiteness, density, dietary fiber, amyllose and amylopectin in sorghum flour, and the other hand it tended to decrease the tannin content of sorghum flour. Physicochemical properties of fermented sorghum flour using Lactobacillus casei are shown in Table 1.

The protein content of sorghum flour after fermentation was quite high between 7-11%, which after fermentation of 12 hours the decreasing of protein content about 2% on the same concentration of bacteria. Table 1 shows that fermentation period significantly affected on the protein content of sorghum flour, where the increased of fermentation period tend to increase the protein content of sorghum flour. In vitro protein digestibility of sorghum increased with fermentation period [8]. Sorghum proteins are classified, according to solubility, as albumins, globulins, prolamins and glutelins. Similar to protein of sorghum flour, carbohydrate and energy content of sorghum flour after fermentation showed significant differences among the treatments. Increased concentration of bacteria and period fermentation tended to decrease carbohydrate and energy of sorghum flour. It was possibly decreased carbohydrate content due to its degradation during fermentation. Carbohydrate of fermented sorghum flour was quite high above 81%, as well as its energy value which was above 371 kcal.

Density of sorghum flour after fermentation using Lactobacillus casei showed quite high value above 481 g/lt, however this value tended to decrease with increasing concentration of bacteria and fermentation period. This is similar with research reported that bulk density of fermented sorghum decreased during fermentation. Fermentation of sorghum flour for 24 h decreased the bulk density of the sorghum flour by about 10%. The decrease in bulk density of fermented flour would be an advantage for the preparation of infant foods [3].

Bulk density and particle density is an important parameter of design, model and optimization of food processing because it affects directly to the thermo physical properties of raw material [9]. The low bulk density in a material can be associated with the high levels of protein and lipid of ingredients [10]. Bulk density is one of the physical quality parameter of flour which is closely related to the purposes of flour packaging, materials handling, and particle size.

Density and whiteness are important factor for application of sorghum flour in processed food products because it will affect the appearance and physical properties of final products. Whiteness is a power reflects light on the surface level of starch whiteness compared to the standard. Sorghum flour fermented with Lactobacillus casei showed whiteness values between 90.59 to 98.58% with the highest level was indicated in the fermentation with bacterial concentration of 0.20% and fermentation period of 12 hours. Generally, whiteness of fermented sorghum flour increased due to increasing of bacterial concentration and fermentation period. Whiteness of fermented sorghum flour increased as the fermentation period and starter concentration used [5].
Table 1. Physicochemical properties of fermented sorghum flour using *Lactobacillus casei*

| Concentration of bacteria (%) | Fermentation period (hour) | Density (g/l) | Whiteness (%) | Protein content (% | Tannin content (mg/100 g) | Starch content (%) | Amylopectin (%) | Amylose (%) | Dietary fiber (%) |
|------------------------------|----------------------------|---------------|---------------|-----------------|------------------------|------------------|-----------------|--------------|------------------|
| Control*                    | 4                          | 660±2.83      | 89.77±0.14    | 10.10±0.06      | 35.17±0.14            | 73.37±0.10       | 71.10±0.07     | 71.10±0.07   | 3.94±0.20        |
| 0.05                        | 4                          | 586±1.41      | 90.08±0.14    | 7.78±0.04       | 27.83±0.14            | 73.97±0.10       | 72.37±0.10     | 72.37±0.10   | 4.12±0.06        |
| 0.10                        | 4                          | 558±2.83      | 90.59±0.14    | 7.63±0.04       | 23.31±0.28            | 74.82±0.03       | 31.48±0.03     | 31.48±0.03   | 3.85±0.07        |
| 0.15                        | 4                          | 561±1.41      | 90.83±0.14    | 8.89±0.13       | 19.75±0.14            | 73.28±0.11       | 35.17±0.10     | 35.17±0.10   | 3.85±0.07        |
| 0.20                        | 4                          | 563±1.41      | 95.41±0.14    | 8.50±0.14       | 15.98±0.11            | 72.37±0.10       | 38.91±0.08     | 38.91±0.08   | 4.42±0.06        |
| Control*                    | 8                          | 608±2.83      | 90.60±0.28    | 10.83±0.04      | 32.60±0.21            | 74.87±0.10       | 31.36±0.08     | 31.36±0.08   | 3.58±0.11        |
| 0.05                        | 8                          | 574±1.41      | 95.29±0.14    | 9.31±0.08       | 25.27±0.14            | 75.08±0.11       | 36.72±0.08     | 36.72±0.08   | 3.72±0.03        |
| 0.10                        | 8                          | 571±1.41      | 95.60±0.64    | 10.17±0.03      | 22.26±0.14            | 70.99±0.13       | 33.58±0.07     | 33.58±0.07   | 3.91±0.08        |
| 0.15                        | 8                          | 561±1.41      | 97.79±0.14    | 10.45±0.02      | 19.26±0.14            | 73.28±0.11       | 37.24±0.14     | 37.24±0.14   | 4.08±0.11        |
| 0.20                        | 8                          | 540±1.41      | 90.10±0.07    | 10.20±0.07      | 14.87±0.14            | 73.50±0.07       | 37.89±0.01     | 37.89±0.01   | 3.98±0.11        |
| Control*                    | 12                         | 600±2.83      | 98.98±0.11    | 9.49±0.07       | 28.69±0.14            | 75.70±0.07       | 34.26±0.14     | 34.26±0.14   | 4.02±0.03        |
| 0.05                        | 12                         | 514±1.41      | 96.12±0.14    | 11.16±0.11      | 24.86±0.14            | 75.27±0.10       | 35.36±0.07     | 35.36±0.07   | 4.12±0.06        |
| 0.10                        | 12                         | 413±1.41      | 97.35±0.14    | 11.80±0.07      | 21.61±0.14            | 73.98±0.11       | 40.01±0.15     | 40.01±0.15   | 3.95±0.07        |
| 0.15                        | 12                         | 496±2.83      | 98.38±0.14    | 11.36±0.04      | 16.77±0.14            | 72.99±0.13       | 40.58±0.14     | 40.58±0.14   | 4.31±0.07        |
| 0.20                        | 12                         | 481±1.41      | 98.58±0.14    | 10.83±0.04      | 11.45±0.14            | 71.86±0.08       | 42.01±0.14     | 42.01±0.14   | 4.55±0.07        |

Note: Mean values in each column with same letter are not significantly different (p = 5%).
*Sample was not fermented using *Lactobacillus Casei*.
Generally, the result of tannin analysis of fermented sorghum flour using *Lactobacillus casei* showed that the bacterial concentration and fermentation period influenced to the decreasing levels of tannins content. Tannin is anti-nutrient compounds that can affect proteins, carbohydrates and minerals metabolism [11]. The content of polyphenols (tannins) and phytates in food products are known affected to the availability of minerals such as iron which significantly contributes to anemia due to iron deficiency [12]. Besides that, the high amount of tannins in the grain can cause astringent and bitter taste and cause a dark color. Therefore, decreasing tannin content of sorghum besides to improve reception also aims to reduce negative effects of tannins as protein binding, enzyme, and the availability of minerals in the body. Sorghum genotypes with high phenolic and tannin levels are associated to enzyme inhibition and starch molecule interaction. These facts impair starch digestibility, increase resistant starch and decrease glycemic index of foods [13]. Tannins content may increase resistant starch content by binding to amylase and reducing α-amylase activity.

The analysis results on the tannins level of sorghum flour by microbial fermentation using *Lactobacillus casei* showed that the tannin content decreased after fermentation of 8 hours, and its level was lower after fermentation of 12 hours. This result similar to the other research stated that the tannin levels decreased with increased concentrations of starter and fermentation period [5]. The tannin levels decreased of 31-35% over a 24 hour fermentation process [14]. Sorghum fermentation using lactic acid bacteria was known can reduce the anti-nutrient tannin from 0.09 to 4.5 (g/100 g db) become 0.06 to 3.93 (g/100 g db) [15]. Soaking process was known can reduce the tannin content of sorghum because it was water soluble. Codex Standard requires maximum tannin content in sorghum is 0.3% [16].

Starch and amylopectin of sorghum flour showed lower value after fermentation of 12 hours. Overall fermentation period and concentration of bacteria affected on the starch and amylopectin of fermented sorghum flour. Increasing of them affected on the decreasing of starch and amylopectin content. Fermentation period and concentration of bacteria also affected on the amylase content. Reverse with starch and amylopectin, amylase content of sorghum flour increasing in line with increasing of concentration of bacteria and fermentation period. Although generally its percentage was lower than amylopectin percentage in the end of fermentation, but amylase content of sorghum flour showed higher value than its value in the beginning of fermentation. The high value of amylopectin sorghum flour indicated that dominant structure of its starch after fermentation was a straight chain structure.

Generally, sorghum flour have a fairly high starch content of over 70% at the end of fermentation with *Lactobacillus casei* where its value was lower compared with control sorghum flour (Table 2). The starch content of sorghum flour after fermentation using *Lactobacillus casei* was lower than starch content of unfermented control flour. The same result on the amylopectin content of fermented sorghum flour which has lower value than amylopectin of control sorghum flour, but otherwise the amylose of fermented sorghum flour tended to be higher than amylose of unfermented sorghum flour. This happened due to breaking of long chains into shorter branched chain during fermentation thus affecting the amylose content of fermented sorghum flour.

Measurement of dietary fiber of sorghum flour showed that concentration of bacteria and fermentation period influenced dietary fiber of sorghum flour, where its value tend to increase with increasing concentration of bacteria and fermentation period. Dietary fiber is the edible cell wall, polysaccharides, lignins and other compounds that are resistant to human digestive enzymes [17]. Dietary fiber of fermented sorghum flour was quite high above 3%, and its value was higher than control sorghum flour. The highest dietary fiber of sorghum flour was 4.55±0.07 where reached after fermentation of 12 hours using *Lactobacillus casei* of 0.20%. Dietary fiber in sorghum only 2.5-9%, with soluble fraction was about 0.5%. Dietary fiber in sorghum flour consisted mainly of glucans and pentosan (arabinose and xylose) [18].

### 3.2. Functional properties of fermented sorghum flour using *Lactobacillus casei*

Measurement on the functional properties of fermented sorghum flour using *Lactobacillus casei* included analysis on the water absorption, oil absorption, viscosity, gel consistency of sorghum flour completely are shown in Table 2. Water absorption of sorghum flour fermented using *Lactobacillus*...
casei showed lower values than control sorghum flour. Water absorption is used to measure the flour's ability to absorb water and determine the amount of water available for gelatinization process. Starch granules can become wet and spontaneously dispersed in water. Water absorbed due to absorption by granule-bound physically or intermolecular on amorphous part. Based on Table 2 it can be concluded that water absorption tended to decrease with increasing concentration of bacteria and fermentation period. Water absorption of sorghum flour after fermentation for 12 hours and Lactobacillus casei concentration of 0.15% showed the smallest value that was 11.79±12:14%.

| Concentration of bacteria (%) | Fermentation period (hour) | Water absorption (%) | Oil absorption (%) | Viscosity (cP) | Gel Consistency Long (mm) | Description |
|-------------------------------|---------------------------|---------------------|-------------------|---------------|---------------------------|-------------|
| Control*                      | 4                         | 20.46 ±0.14i        | 11.42 ±0.14f      | 12.00±0.21i   | 81±2.83s                 | Soft        |
| 0.05                          | 4                         | 15.02 ±0.14i        | 12.21 ±0.14g      | 12.20±0.17a   | 102±2.83y                | Soft        |
| 0.10                          | 4                         | 24.27 ±0.14i        | 11.22 ±0.14d,e,f  | 12.00±0.23d   | 89±2.83c,f,g             | Soft        |
| 0.15                          | 4                         | 12.79 ±0.14b        | 10.05 ±0.28c,d,e  | 12.00±0.14a   | 67±2.83a                 | Soft        |
| 0.20                          | 4                         | 14.74 ±0.28c,d,e    | 10.84 ±0.28b,c,d  | 12.00±0.14a   | 92±2.83g,h               | Soft        |
| Control*                      | 8                         | 17.24 ±0.28b        | 11.37 ±0.14c      | 13.00±0.23b   | 76±2.83b                 | Soft        |
| 0.05                          | 8                         | 13.95 ±0.21d        | 11.39 ±0.14c      | 13.20±0.24b   | 86±2.83d,e               | Soft        |
| 0.10                          | 8                         | 17.34 ±0.14b        | 10.52 ±0.14b,c,e  | 13.00±0.15b   | 92±2.83g,h               | Soft        |
| 0.15                          | 8                         | 16.22 ±0.14g        | 10.17 ±0.14b,c    | 13.20±0.22b   | 91±2.83f,g               | Soft        |
| 0.20                          | 8                         | 14.87 ±0.14f        | 13.44 ±0.14c      | 13.00±0.22b   | 91±2.83g,h               | Soft        |
| Control*                      | 12                        | 16.24 ±0.14g        | 11.95 ±0.07f,g    | 13.20±0.16b   | 76±2.83b                 | Soft        |
| 0.05                          | 12                        | 14.43 ±0.14e        | 10.34 ±0.14b,c,e  | 13.40±0.17c   | 87±2.83d,e,f             | Soft        |
| 0.10                          | 12                        | 13.21 ±0.14c        | 10.82 ±0.14b,c,e,d| 13.40±0.11c   | 64±2.83a                 | Soft        |
| 0.15                          | 12                        | 11.79 ±0.14a        | 9.83 ±0.14a       | 13.40±0.13c   | 96±2.83b                 | Soft        |
| 0.20                          | 12                        | 14.03 ±0.08d        | 10.22 ±0.14b      | 13.20±0.22b   | 84±2.83d                 | Soft        |

Note: Mean values in each column with same letter are not significantly different (p = 5%)
*samples was not fermented using Lactobacillus Casei

The high water absorption is related to amylose content of flour where it has low amylose content, it will show a higher value of water absorption [19]. It is similar to the results of this study where water absorption inversely proportional to amylose content due to increasing concentrations of bacteria and fermentation period. This is not similar to previous research reported that the higher the amylose content, impact on the higher of starch's ability to absorb water and swell because amylose has the ability to form hydrogen bonds greater than the amylopectin [20]. The difference of water absorption is expected because of strong granular structure due to incorporation of amyllose molecules. Water absorption is affected by starch damage, where it is able to absorb water 4-5 times more than intact starch [21].

Generally oil absorption of fermented sorghum flour using Lactobacillus casei had lower values than oil absorption of control sorghum flour, which its values tended to decrease due to higher concentrations of bacteria and fermentation period. Overall oil absorption of sorghum flour ranged from 9.83 ± 0.14 until 13.44 ± 0.14. Oil absorption of flour is also affected by its chemical content, one of them is amylopectin. Oil absorption of flour is also affected by its chemical content, one of them is amylopectin. A high amylopectin of flour likely have a high oil absorption as expected oil trapped into the branch chain of amylopectin, so more and more branches of the chain will impact on the higher capability of oil absorption [22].

The results of viscosity measurement showed that viscosity of fermented sorghum flour had a higher value than viscosity of control sorghum flour. Table 2 showed that fermentation period
significantly affected viscosity of fermented sorghum flour, but the difference in concentration of bacteria did not give effect to the viscosity of fermented sorghum flour. After fermentation for 4, 8 and 12 hours viscosity of sorghum flour increased successively into 12.20 cP, 13.20 cP and 13.40 cP. The longer of fermentation process impact on the activity of bacteria degrading starch more optimized so the structure of starch becomes more porous and absorbs more water. This causes increasing of viscosity value. During spontaneous fermentation of sorghum, microbes that grow during fermentation will produce the amylase enzymes and degraded starch which resulting many hole in starch granules. Perforated starch granules and uneven causes water more easily enter inside, penetrate into the starch granules and causes the starch granules swell which is reflected in the increasing of viscosity [23]. This is similar with previous research reported that fermentation hydrolyzed proteins and form starch granules that can swell so increasing viscosity proportionally with the length of fermentation, so that viscosity derived from fermentation of 48 hours produced a higher viscosity and significantly different with viscosity derived from fermentation of 0, 24, and 36 hours [5].

Gel consistency is one of functional properties that effect on texture of processed products based on flour. Generally gel consistency of fermented sorghum flour had a soft texture (Table 2). Gel consistency of fermented sorghum flour has a length ranges between 67-96 mm and generally its value of flour fermented using Lactobacillus casei was higher than control flour. Fermentation for 4 hours using 0.05% of Lactobacillus casei had the longest gel (102 mm) and it was longer than gel length of control sorghum flour (81 mm). This happened for fermentation of 8 hours and 12 hours where fermented sorghum flour generally has longer gel length than gel length of control sorghum flour.

4. Conclusion
Concentration of bacteria and fermentation period significantly affected the physicochemical and functional properties of sorghum flour. Increasing concentrations of bacteria and fermentation duration tended to decrease tannin content, starch, amylopectin, water absorption, and oil absorption. On the other hand, it increased whiteness, dietary fiber, amylase, and viscosity of sorghum flour. Fermentation using 0.20% of Lactobacillus casei for 12 hours gave the best sorghum flour based on its physicochemical and functional properties.

5. References
[1] Suarni dan Firmansyah I.U. 2013. Struktur, komposisi nutrisi dan teknologi pengolahan sorgum (Jakarta: IASRD Press) available in www:balitseral.litbang.pertania.go.id/buku sorgum accessed on August, 18th 2018
[2] Caransa, A. and W.G.M. Bakker. 1987. Modern Process for the Production of Sorghum Starch. Stark/Strake 39 (11) 381−385
[3] Alka, S., Neelam, Y., and Shrutti, S. 2012. Effect of fermentation on physicochemical properties &in vitro starch and protein digestibility of selected cereals. International Journal of Agriculture and Food Science, 2(3) 66-70.
[4] Elkhahalifa, A. E. O., Schiffler, B. and Bernhardt, R. 2004. Effect of fermentation on the starch digestibility, resistant starch and some physicochemical properties of sorghum Flour. Nahrung/Food. 48 91 – 94
[5] Kurniadi, M., Andriani, M., Faturohman, F. and Damayanti, E. 2013. Karakteristik fisikokimia tepung biji sorghum (sorghum bicolor L.) terfermentasi bakteri asam laktat Lactobacillus acidophilus. Agritech. 33 (3) 288-295
[6] Efendi, Z. 2011. Perubahan kecernaan pati, protein dan karakteristik tepung sorgum (Sorghum bicolor, L. Moench) selama fermentasi. Tesis. Program studi Ilmu dan teknologi pangan, Pascasarjana UGM
[7] Utami, T., Nurhayati, R. and Rahayu, ES. 2015. The effect of addition of lactobacillus plantarum microbiological and chemical characteristics during sorghum (Sorghum bicolor l. Moench) fermentation. Agritech 35 (4) 449-455
[8] Youssif, N. E., and El Tinay, A. H. 2001. Effect of fermentation on sorghum protein fractions and in vitro protein digestibility. Plant food for human nutrition. 56 175-182
[9] Rahman, I.E.A. dan Osman, M.A.W. 2011. Effect of sorghum type (Sorghum bicolor) and traditional fermentation on tannins and phytic acid contents and trypsin inhibitor activity. *Journal of Food, Agriculture and Environment* 9 163 – 166
[10] Oladunmoye, O.O., Akinoso, R., Olapade, A.A. 2010. Evaluation of some physical–chemical properties of wheat, cassava, maize and cowpea flours for bread making. *Journal of Food Quality*. 33 693–708
[11] Mohammed, A.N., Ahmed, I.A.M. dan Babiker, E.E. 2011. Nutritional evaluation of sorghum flour (Sorghum bicolor L. Moench) during processing of Injera. *World Academy of Science, Engineering and Technology*. 51 72 – 76
[12] Towo, E., Matuschek, E. dan Svanberg, U. 2006. Fermentation and enzyme treatment of tannin sorghum gruels: effects on phenolic compounds, phytate and vitronectin accessible iron. *Food Chemistry*. 94 369 – 376
[13] Moraes, E.A., Marineli, R.S., Lenquiste, S.A., Steel, C.J., de Menezes, C.B., Queiroz, V.A.V. and Júnior, M.R.M. 2015. Sorghum flour fractions: Correlations among polysaccharides, phenolic compounds, antioxidant activity and glycemic index. *Food Chemistry*. 180 116–123
[14] Osman, M.A. 2004. Changes, in sorghum enzyme inhibitors, phytic acid, tannins, and in vitro protein digestibility occurring during Khamir (local bread) fermentation. *Food Chem*. 88 129-134.
[15] AwadElkareem, A.M. dan Taylor, J.R.N. 2011. Protein quality and physical characteristics of Kisra (fermented sorghum pancake like flatbread) made from tannin and non-tannin sorghum cultivars. *Cereal ChemISTRY*. 88 (4) 344 – 348
[16] Codex Alimentarius Commision. Codex Standard for Sorghum Flour 173-1989. Available in http://codex_stan_173-1989.cac.co.us accessed on 21 Juli 2015
[17] Porsky, L. 2000. What is dietary fiber. *Journal of AOAC International*. 83 (4): 985-987
[18] Nyman, M., Siljestrom, M., Pedersen, B., Bachknudsen, K.E., Asp, N.G., Johansson, C.G., and Eggum, B.O. 1984. Dietary Fiber and Composition in Six Cereals at Different Extraction Rates. *Cereal Che.* 61(1) 14-19
[19] Suarni and M. Hamdani. 2009. *Karakterisasi nutrisi dan sifat fisikokimia beberapa galur dan varietas unggul gandum*. Makalah disampaikan dalam Simposium Teknologi Inovatif Pascapanen II: 2009. Bogor
[20] Juliano, B.O. 1994. *Production and utilization of rice*. In: B.O. Juliano, ed. Rice-Chemistry and technology. St. Paul, M.N: American Society of Cereal Chemistry, pp. 1-14
[21] Hatcher, D. W., G. G. Bello, and M. J. Anderson. 2009. Flour particle size, starch damage, and alkali reagent: impact on uniaxial stress relaxation parameters of yellow alkaline noodles. *Cereal Che*. 86 361–368
[22] Nur Alam dan Nurhaeni. 2008. Komposisi kimia dan sifat fungsional pati jagung berbagai varietas yang diekstrak dengan pelarut natrium bikarbonat. Jurnal Agroland 15(2):89-94 [41] Armanda, Y., dan Putri,
[23] Armanda, Y., dan Putri, W.D.R. 2015. Tepung Sorgum Coklat Utuh Terfermentasi Ragi Tape. *Jurnal Pangan dan Agroindustri*. 4 (2) 438-46