Modification of sorghum flour (Sorghum bicolor l. Moench) using lactic acid bacteria from wikau maombo

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Abstract. The purpose of this study was to determine the effect of LAB (Lactic Acid Bacteria) isolate types and LAB concentrations on sorghum fermentation on the physicochemical characteristics of sorghum flour. The sorghum flour was modified using a fermentation process with two types of LAB derived from wikau maombo (traditional fermented cassava used as local staple food), namely isolates UM1.3A and UM1.4A. Three levels of cell concentration measured by optical density (OD), each 0.50, 0.75, 1.00, at a wavelength of 600 nm with fermentation time 24, 48, and 72 hours. The best fermentation treatment using LAB UM1.3A isolates, with OD 0.75. The flour characteristic from the best treatment has a viscosity value of 16.18 cP, swelling power 8.17 (g / g), water solubility index (IKA) 16.35%, and the degree of acidity (pH) 6.92. The flour has proximate content in a row: water, ash, fat, protein, crude fiber, and total carbohydrates of 7.27% bb, 1.05% bk, 3.42% bb, 9.29% bk, 1.23% bk, and 87.43% bk. The results showed that the interaction of the types of LAB isolates and the concentrations of LAB isolates influenced the improvement in the physicochemical properties of modified sorghum flour.

1. Introduction
The development of alternative food based on tubers, tree crops, or grains is one of the efforts to improve national food security in overcoming the problem of community dependence on wheat-based food use. This is in line with the leading national policies that prioritize food security and adequacy [1]. It also aims to create food diversification, which is an effort to increase the availability of diverse foods and based on potential local resources [2]. The development of sorghum (Sorghum bicolor (L.) Moench) is one type of cereal plant that can be used as an alternative to wheat flour in the manufacture of various processed food products. Processing sorghum into semi-finished products is recommended because it has a longer shelf life, is easily substituted and fortified, and is faster processed into processed food products such as crackers, white bread, cakes, analog rice, biscuits, cookies, dry...
noodles [3] In addition, sorghum is known to have no gluten, so consumers can consume sorghum flour with gluten allergies (sufferers of celiac disease) [4].

The main problem in processing sorghum is the presence of tannin in the aleurone, which is antinutrient and gives a sense of soupy to the processed product. According to Armanda et al. [5], to overcome some weaknesses in the properties of sorghum flour starch. It is necessary to do the method of starch modification by fermentation. Sorghum fermentation is known to improve the functional properties of sorghum flour [6], increase the digestibility of protein and starch, the availability of amino acids lysine, leucine, isoleucine and methionine to improve the LABance of amino acids as well as the availability of carbohydrates and other nutritional content [7]. Sorghum fermentation with LAB is also known to reduce antinutrients, such as phytate [7] and tannins [8]. Besides natural fermentation of cereals using LAB is known to inhibit enteropathogenic bacteria so that it can improve food safety guarantees under unhygienic environmental conditions [9].

LAB can be found naturally in high carbohydrate foods such as cassava. One of the processed food products of Southeast Sulawesi, which is made from cassava, is wikau maombo. This product is processed by soaking cassava with seawater and continued with the fermentation process. The results of screening of LAB isolates from the fermentation process of Maombo during three days obtained 11 isolates of LAB, which were divided into three isolates from bitter cassava and eight isolates from cinnamon cassava [10]. LAB origin of maombo with code isolates UM1.3A and UM1.4A has amylase enzyme content. That can destroy starch cell walls, so the release of starch granules is appropriately used to modify starch through fermentation [11].

Based on the description, the results of the sorghum modification study were published to produce modified sorghum flour using lactic acid bacterial isolates from maombo with UM1.3A and UM1.4A isolates which were expected to produce modified sorghum flour with better physicochemical properties and could support flour substitution local food program for health purposes.

2. Methods

2.1. Material
The main ingredient is Sorghum (Sorghum bicolor (L.) Moench). Lactic acid bacterial isolates (LAB) UM1.3A and UM1.4A, which are obtained from fermentation products when maombo, man rosgosa and sharpe) Broth and MRS-Agar received from HiMedia, India. NaOH chemicals, 37% HCl, C6H14, C3H2OH, obtained from Merck, Germany. CuSO4 • 5H2O, H2SO4, HgO, NaHCO3, Na2SO4, Na2HAsO4 • 7H2O, KNaC4H4O6 • 4H2O obtained from Sigma – Aldrich, Singapore.

2.2. Sorghum fermentation using isolates LAB UM1.3A and UM1.4A
The first thing to do is a rejuvenation of LAB isolates UM1.3A and UM1.4A. After that measured the cell concentration reached OD 0.75. (LAB isolates were suspended in liquid distilled water to reach OD concentration of 0.75 at a wavelength of 600 nm). The first thing to do is a rejuvenation of LAB isolates UM1.3A and UM1.4A. After that measured the cell concentration reached OD 0.75. (LAB isolates were suspended in liquid distilled water to reach OD concentration of 0.75 at a wavelength of 600 nm). Next, prepare the sorghum seeds to be fermented. Sorghum seeds that have been washed, drained until there are no drops of water. Then put in a glass container that has been sterilized and added to the colony LAB OD = 0.75 as much as 10% of the total material. The fermentation treatment is carried out at several levels, 24 hours, 48 hours, and 72 hours in an incubator with a temperature of 35 °C. The method used is dry fermentation (Solid-state fermentation) until the optimum fermentation process is obtained. The fermentation process is carried out under sterile conditions to prevent contamination.
2.3. Making modified sorghum flour
Fermented sorghum seeds are washed thoroughly using sterile water, then drained, then heated using an oven to dry at 60 °C for 24 hours. After drying, sorghum is mashed using a blender and then sieved using a 100 mesh size sieve to get uniform sized sorghum flour.

2.4. Characterization of physicochemical of modified sorghum flour

2.4.1. Viscosity analysis. Viscosity analysis was performed using the Oswald viscometer (Oswald method). This test is done by calculating the time needed by the sorghum flour solution, which has reached the maximum point to flow from the Oswald viscometer until it reaches the stop point. Calculation of time using a stopwatch and as a comparison test sample solution used flour, which was treated the same as the sample [12].

2.4.2. Swelling power analysis and water solubility index. Starch suspension solution with a concentration of 1% or 0.5 g is heated in a water bath shaker with a temperature of 90°C for 30 minutes, then centrifuged at 3000 rpm for 15 minutes. The sample is cooled to room temperature. Then the samples were centrifuged at 3000 rpm for 15 minutes. The supernatant is separated from the precipitate, then poured into a petri dish and evaporated overnight at 70°C, and the sediment is weighed. Swelling power is the ratio between the weight of sludge left in the centrifuge with the sample dry weight. The solubility index water (solubility) is the percentage weight of starch that dissolves in water [13].

2.4.3. Analysis of pH Value. The pH value is measured using a Jenway 3505 pH meter. Before use, the pH meter is calibrated using a buffer of pH 4 and 7. After being calibrated, a sample is measured by making a sample suspension of 10% in a water solvent.

2.4.4. Morphological analysis SEM. The sample is placed on an aluminum plate that has two sides. The sample is then coated with a gold layer with a vacuum to make a conductive sample. Starch morphology was observed using a scanning electron microscope (SEM Philips XL30), and images were taken at a potential acceleration of 20 kV [14].

2.4.5. FTIR analysis. Sorghum flour was analyzed using Fourier Transform Infrared Spectroscopy (FTIR). Sago starch was made pelleted with KBr using manual felt equipment (Shimadzu, Tokyo, Japan). The sample spectrum was read by FTIR ABB MB3000 (Clakuadeset Scientific, Northampton, UK) with DTGS detectors in Shimano, Tokyo, Japan. Middle infrared area (4000 - 400 cm⁻¹) with a resolution of 4 cm⁻¹.

2.4.6. X-Ray Diffraction (XRD) analysis. Sorghum flour is characterized using X-ray diffraction techniques (XDR JEOL JDX-3530 X-ray diffractometer) to determine the crystal phase and crystallinity. A sample of 1 gram is placed in cell diffraction (the sample area) and then irradiated with CuKα radiation at wavelength λ = 1.541 Å, the voltage at 40 kV and current at 30 mA with an angular range of 2θ = 5-50°.

2.4.7. Chemical composition analysis. AOAC standards [14] are used to analyze water, ash, protein, fat, fiber, and carbohydrate content.

2.5. Data analysis
Data were analyzed using Analysis of Variants, the results of which had a significant effect on the observed variables, followed by Duncan's Multiple Range Test (DMRT) at a 95% confidence level (α = 0.05).
3. Results and discussion

3.1. Physical characterization of fermented flour with an isolate of lactic acid bacteria from wikau maombo

The best results on the physicochemical characteristics of the modified sorghum flour were obtained at 48 hours fermentation using LAB UM1.3A isolates. The addition of fermentation time will cause nutrients in the LAB growth media to decrease, thereby reducing the ability of LAB to hydrolyze starch. The best results on the physicochemical characteristics of the modified sorghum flour were obtained at 48 hours fermentation using LAB UM1.3A isolates. The addition of too long fermentation time will cause nutrients in the LAB growth media to decrease. It is thereby reducing the ability of LAB to hydrolyze starch. The number of LAB population can influence the reduced hydrolysis ability of LAB due to nutrient deficiencies, which too much in a medium, and it causes competition in obtaining nutrition. Similar results, according to the research of Armanda et al. [5], on whole brown sorghum fermented flour yeast tape has increased on the physicochemical properties of flour after fermentation using LAB.

Table 1. Physicochemical characteristics of modified sorghum flour.

| Lab type          | Old fermentation (hours) | Viscosity (cP) | Swelling Power (g/g) | Solubility index (%) | pH      |
|-------------------|--------------------------|----------------|----------------------|----------------------|---------|
| Sorghum flour     |                          |                |                      |                      |         |
| control           | 0                        | 12.80±0.26     | 5.96±0.19            | 12.16±0.26           | 7.41±0.05 |
| UM1.3A            | 24                       | 15.15±0.19     | 6.74±0.33            | 13.93±0.12           | 7.23±0.03 |
| UM1.4A            | 24                       | 15.04±0.22     | 6.49±0.12            | 13.57±0.18           | 7.28±0.04 |
| UM1.3A            | 48                       | 16.11±0.12     | 8.17±0.09            | 16.35±0.14           | 6.92±0.02 |
| UM1.4A            | 48                       | 15.76±0.15     | 7.83±0.08            | 15.72±0.22           | 6.97±0.02 |
| UM1.3A            | 72                       | 15.33±0.25     | 7.55±0.09            | 15.48±0.11           | 6.79±0.03 |
| UM1.4A            | 72                       | 15.25±0.26     | 7.38±0.06            | 15.26±0.15           | 6.86±0.03 |

3.2. Physicochemical of modified sorghum flour

Table 2. Effect of type and concentration of LAB on the value of viscosity, swelling power, solubility, and pH of modified sorghum flour.

| Treatment       | Viscosity (cP) | Swelling Power (g/g) | Solubility (%) | pH      |
|-----------------|----------------|----------------------|----------------|---------|
| B1K1 (LAB UM1.3A OD : 0.50) | 14.97±0.09 | 6.51±0.11 | 14.80±0.17 | 7.25±0.03 |
| B2K1 (LAB UM1.4A OD : 0.50) | 14.67±0.10 | 6.93±0.12 | 14.28±0.26 | 7.20±0.03 |
| B3K1 (LAB Combination OD : 0.50) | 14.82±0.08 | 6.48±0.08 | 14.18±0.09 | 7.23±0.02 |
| B1K2 (LAB UM1.3A OD : 0.75) | 16.11±0.12 | 8.17±0.09 | 16.35±0.09 | 6.92±0.02 |
| B2K2 (LAB UM1.4A OD : 0.75) | 15.76±0.15 | 7.83±0.08 | 15.72±0.22 | 6.97±0.02 |
| B3K2 (LAB Kombinasi OD : 0.75) | 15.42±0.14 | 7.49±0.16 | 15.16±0.07 | 7.05±0.02 |
| B1K3 (LAB UM1.3A OD : 1.00) | 15.54±0.10 | 7.62±0.26 | 15.38±0.13 | 7.12±0.06 |
| B2K3 (LAB UM1.4A OD : 1.00) | 15.29±0.18 | 7.15±0.11 | 15.23±0.13 | 7.09±0.02 |
| B3K3 (LAB Combination OD : 1.00) | 15.12±0.10 | 6.96±0.08 | 14.51±0.20 | 7.13±0.02 |

Based on the results of the study, it was found that the best treatment on modified sorghum flour was by using LAB UM1.3A isolate at OD concentration = 0.75 and 48 hours fermentation time.

3.2.1. Viscosity. An increase in the viscosity value of modified sorghum flour (Table 2) is due to the breakdown of hydrogen bonds when heated at a specific temperature and interference with the
crystalline structure of the starch, and the amylose and amyllopectin hydroxyl groups will be exposed so that the water molecule can bind to the hydroxyl starch group. Similarly, according to Ratnayanke et al. [15], the breakdown of hydrogen bonds and the separation of hydrogen molecules from the amylose and amyllopectin hydroxyl groups can cause increased granular development and solubility.

The gelatinization temperature of the modified sorghum flour ranged from 73° C-85 ° C and within 23-30 minutes. The amylose content in the modified sorghum flour is low. This causes the gelatinization temperature in the modified sorghum flour to be low. The modified viscosity of sorghum flour in this study ranged from 14.67-16.11 cP. These results are following the results of Armanda et al. [5] that the value of the viscosity of whole brown sorghum flour fermented yeast tape with a concentration of 6% and 18 hours fermentation time ranged from 18.40 cP.

3.2.2. Swelling power. The swelling power of sorghum flour is influenced by the interaction between the types of LAB isolates and the concentration of OD (optical density) of LAB used in making modified sorghum flour. The increase in swelling power is due to LAB secreting enzymes that can degrade starch granules. This results in more porous starch granules that can absorb water. Following Elvira's research [9] that LAB UM1.3A isolates have stronger starch hydrolysis ability compared to UM1.4A LAB isolates shown by the more full clear zone diameter on the test media.

According to Armanda et al. [5], during the fermentation process, growing microbes can degrade starch, thus forming holes in the starch granules. These starch granules with holes and uneven make it easier for water from entering so that the starch granules swell. Besides that, the increase in swelling power has influenced the size of the binding strength of the micellar tissue, amylose molecular structure, and amylose content. Swelling power will increase with increasing branched-chain amyllopectin content and decreasing amylose content [16]. The ratio of amylose and amyllopectin influences the properties of starch during gelatinization. Amylopectin plays a role in the development and properties of starch dough, while amylose inhibits the growth of starch dough [17,18].

3.2.3. Water Solubility Index. Pukkahuta et al. [18] reported that amylose was one of the factors that influenced the solubility value. The presence of microbial activity produces enzymes that can destroy sorghum cell walls and degrade starch, resulting in the liberation of starch granules, which causes starch granules to perforate and become damaged so that they have increased solubility. The water solubility index value of modified sorghum flour in this study ranged from 14.18-16.35%. This is similar to what was reported by Suprijadi [19] that the solubility value of sorghum flour soaking with Na₂CO₃ for 8 hours ranged from 11.93–19.59%.

3.2.4. pH. The fermentation process that uses LAB isolates will cause the pH value to decrease. LAB can produce acidic compounds during the fermentation process so that the atmosphere of the material or fermentation media becomes more acidic [20]. The use of higher OD LAB concentrations tends to make the pH value decrease. This is due to the longer fermentation, the pH value of the modified flour will decrease. Besides, it is caused by organic acids produced due to microbial metabolism, including lactic acid. Lactic acid produced by LAB will be excreted out of cells and will accumulate in the fermentation media so that it will increase acidity.

3.3. Morphological analysis of starch with SEM (Scanning Electron Microscope). SEM characterization was carried out to determine the morphology of sorghum flour (starch) at a micro-scale. The morphology of sorghum flour starch in Figure 1 shows around the irregular polygonal shape with a smooth surface with a starch granule size of 9.329-18.76 μm [21]. Modified sorghum flour starch structure shows that fermentation with LAB UM 1.3A OD concentration = 0.75 with 48 hours fermentation time, makes granules on starch more decompose (Figure 1b) than negative control sorghum flour (without fermentation) (Figure 1a) and modified sorghum flour combined with LAB UM1.3A and LAB UM1.4A with concentrations of OD = 0.75 (Figure 1c). Negative control
sorghum flour (without fermentation) has starch granules not yet fully decomposed and is still bound by fibers.

![Figure 1. SEM structure morphology of flour at 1500 times magnification. (a) Control sorghum flour (b) Selected modified sorghum flour (c) LAB combination sorghum flour starch [22].](image)

### 3.4. FTIR analyze

![Figure 2. FTIR spectrum (a) Control sorghum flour (b) Selected modified sorghum flour (c) combination sorghum flour.](image)

The FTIR spectrum results obtained are a comparison of absorption control sorghum flour and modified sorghum flour. The sample spectrum shows the same absorption at wavelengths of 2924 cm⁻¹ (aliphatic -CH₂ groups) which is strengthened by the presence of C-H bonds at wavelengths of 1419 cm⁻¹ and 1381 cm⁻¹ (control); 1373 cm⁻¹ (selected); 1334 cm⁻¹ (combination), 1651 cm⁻¹ (stretching COO group), 1157 cm⁻¹ (C-C stretching), and 1018 cm⁻¹ (glycosidic C-O-H group). While the wavelength differences occur in the stretching O-H group with a wavelength of 3448 cm⁻¹ (control); 3387 cm⁻¹ (selected); 3410 cm⁻¹ (combination). Based on this identification, it can be seen that the modification of functional groups occurs due to the interaction of LAB in flour [23]. Also, fermentation time can increase amylolytic activity to hydrolyze starch, which decreases the number of bonds in the sample molecule.

### 3.5. X-Ray Diffraction Analysis (XRD)

The purpose of XRD analysis on sorghum flour is to determine the XRD pattern that characterizes the crystalline starch granules. This can be seen from the peak position with strong intensity at 15° and 23° (2θ), and double peaks at 17°-18° (2θ) [24]. The results obtained in this study are by the literature on some starches reported by Acevedo et al. [25]. The pattern of crystals shows that there are double helical sequential bonds in which amylopectin is the main contributor to the order of crystals in the granules [26]. In contrast to fermentation with a long time, peak intensity decreases during fermentation. The length of time of fermentation increases the amylolytic activity to hydrolyze amylopectin starch, which reduces the levels of the compound in the sample. This shows that sorghum flour has been modified.
Figure 3. Flour XRD pattern (a) Control sorghum flour (b) Selected modified sorghum flour (c) combination sorghum flour.

3.6. Proximate content of selected sorghum flour

The amount of water content in the modified sorghum flour product will affect its durability, appearance, texture, and taste. This water content was produced from LAB isolate UM1.3A with OD concentration = 0.75 at 48 hours fermentation time of 7.27% bb. while the water content in the control sorghum flour was 9.26% bb. Both sorghum flour has a moisture content according to Codex Standard 173-1989 with a maximum quality of sorghum flour 15% bk. This is consistent with the study of Armanda et al. [5], who reported that the water content of whole brown sorghum flour was fermented with each increase in tape yeast concentration and fermentation time decreased from 9.79% to 6.10% bb. Water content in flour can also be affected by the fermentation process, where fermented flour has a lower water content than flour without the fermentation process. The decreased water content of modified sorghum flour is caused by microbial activity that degrades starch resulting in reduced starch chains and tends to be shorter. So the bound water can become free water due to the loss of hydroxyl groups [27,28].

Table 3. Proximate content of sorghum flour.

| Component            | Negative Control (Sorghum) | Selected Flour (Sorghum) | Positive Control (Wheat) |
|----------------------|-----------------------------|--------------------------|--------------------------|
| Moisture content (% bb) | 9.26±0.40                  | 7.27±0.07                | 14.50±0.51              |
| Ash content (% bk)    | 1.67±0.20                   | 1.05±0.05                | 0.70±0.18               |
| Fat content (% bk)    | 2.27±0.44                   | 3.42±0.56                | 1.50±0.36               |
| Protein content (% bk) | 8.46±0.31                   | 9.29±0.43                | 11.00±0.36              |
| Crude fiber content (% bk) | 1.38±0.50                  | 1.23±0.28                | 2.50±0.73               |
| Carbohydrate content (% bk) | 79.41±0.70                 | 87.43±0.70               | 85.02±0.46              |

Note: Numbers followed by different letter notations show real differences based on the 0.05 DMRT test the 95% confidence level

Ash content in the modified sorghum flour products produced by 1.05% bk has met the Codex Standard 173-1989 quality, which is a maximum of 8.5% bk. This shows that the modified sorghum flour has a higher inorganic mineral content than wheat flour. The ash content in the modified sorghum flour product was lower than the ash content in the control sorghum flour of 1.67% bk. Mineral degradation occurs because bacteria utilize minerals as their nutritional sources, albeit in small amounts.

Fat content in the modified sorghum flour produced 3.42% according to Codex Standard 173-1989 sorghum flour, a maximum of 4.7%. The fermentation treatment causes the fat content to increase because LAB produces microbial oil during the fermentation process [29]. These results are following the research of Lestari et al. [30] that there was an increase in the fat content of sorghum flour by spontaneous fermentation for five days by 4.41% compared to sorghum flour without treatment by 4.19%.
The protein content in the modified sorghum flour produced was 9.29%. The protein content of modified sorghum flour was higher than that of control sorghum flour. An increase in protein levels of modified sorghum flour is caused by an increase in the number of microbes that can raise protein and produce amino acids. Aini [31] states that during fermentation, the bacterium Lactobacillus Plantarum produces the enzyme protease. Protease causes complex proteins to undergo proteolysis into proteins with shorter chains. Furthermore, microbes are also able to synthesize the joining of several peptides to form proteins, thereby increasing protein levels. These results are consistent with the research of Fadlallah et al. [32] that the protein content in sorghum flour, which is processed for fermentation for 0, 8, 16, and 24 hours, continues to increase.

The results showed the levels of crude fiber in the modified sorghum flour produced was 1.23%. The level of crude fiber modified sorghum flour was higher than the control of sorghum flour by 1.38%. Decreased levels of crude fiber in modified sorghum flour because LAB utilizes fiber for cell metabolism and hydrolyzes it into simple compounds. Whereas Kurniati et al. [33], stated that the decrease in crude fiber levels was due to lactic acid bacteria being able to hydrolyze fibers into simpler compounds, namely monosaccharides (glucose). Therefore, the fiber content in modified sorghum flour is lower than in the control of sorghum flour.

Based on the results obtained, it can be concluded that the fermentation process affects the carbohydrate content of food, which is to increase the carbohydrate content. The fermentation process causes the increased carbohydrate content of modified sorghum flour. These results are the same as reported by Paiki [34] that sorghum fermentation for 72 hours can increase sorghum flour carbohydrates from 74.68% to 75.36%. Carbohydrate content is calculated by a difference influenced by other nutritional components such as protein, fat, water, and ash. The higher the other nutritional component, the lower the carbohydrate content and vice versa if the other nutritional components are smaller, the higher the carbohydrate content.

4. Conclusion
The best fermentation time for making modified sorghum flour was 48 hours using LAB UM1.3 isolate with OD = 0.75. The interaction of LAB types UM1.3 and the concentration of LAB OD = 0.75 has a very significant effect on the increasing physicochemical characteristics of the modified sorghum flour, which increases proximate content.

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