Study of sorghum (*Sorghum bicolor* (L.) Moench) grains fermentation with *Lactobacillus plantarum* ATCC 14977 on tannin content

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Abstract. Sorghum is one of the most important sources of carbohydrate and protein, but least utilized staple crops because of low nutrient bioavailability. The research aimed to study the fermentation of sorghum grains with *Lactobacillus plantarum* ATCC 14977 (*L. plantarum*) on tannin content. The study employed two factors experiment of *L. plantarum* starter cultures concentrations (2, 4, 6, 8, and 10 %) and duration of fermentation. Samples were taken every 12 h during sorghum fermentation (72 h). The viability of microbes, pH, titratable acidity (TA) of the fermentation medium and tannin content of the fermented sorghum grains were measured. The results showed that at the end of fermentation (72 h), the pH decreased from 6.4 to 5.8-6.0, TA increased to 0.59, 0.63, 0.8, 0.95 and 0.88 % at a starter cultures concentrations of 2, 4, 6, 8, and 10 %, respectively. Tannin content decreased significantly after fermentation for 48 h for all treatments. However, prolonged fermentation from 48 to 72 h did not reduce tannin.

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the staple foods and is mostly consumed by poor world countries [1]. The crop is suitable to be cultivated in dry and sub-tropical lands [2]. Sorghum is regarded as inferior grains because of its low nutritional value, bitter taste and sandy textures [3]. However, the consumption of sorghum is relatively high in countries or areas where the climate does not allow to produce other cereals and rice [4]. Sorghum contains anti-nutritional compounds of phytates and tannins which cause low digestibility of protein and starch. Tannin and other polyphenols give a bitter and astringent tastes [5]. Tannin sorghum can bind proteins or protein enzymes forming complexes tannin-protein, which are the primary factor affecting digestibility of protein [6]. Complexes tannin-protein can influence digestive enzymes involving in starch, lipid and protein digestion [7][8]. Phytic acid has a potent binding capacity, forming complexes with multivalent cations and proteins [8].

Some researchers have been carried to reduce the antinutritional compounds in sorghum such as fermentation [9], germination [3] and chemical treatments. Fermentation of sorghum flour has been
studied to reduce the content of antinutritional compounds such as phytic acid and thus increase the bioavailability of minerals and in vitro digestibility of protein [10],[11],[1],[8],[12],[3]. Fermentation of brown sorghum flour using starter cultures of *Lactobacillus plantarum* resulted in decreasing tannin by 77% [9],[3]. Fermentation of sorghum can degrade disulfide bond in sorghum protein resulting increasing protein digestibility [9]. *L. plantarum* used as starter cultures could be applicable to the improvement of the nutritional quality of traditional fermented cereal [13]. Moreover, *L. plantarum* produces tannase which can degrade tannin resulting digestible simple molecules [14].

Most previous research focused on sorghum flour fermentation which limits the flour’s application for further uses. Fermented sorghum grains can be further processed into a derived variety products such as boiled or steamed grains as staple foods or sorghum flour for further diversification of sorghum-based food product. The objective of the research was to study the fermentation of sorghum grains with *Lactobacillus plantarum* ATCC 14977 on tannin content.

2. Material and methods

2.1. Materials and chemicals

Brown sorghum grains used for this study were a local variety cultivated in Jombang East Java Indonesia. The isolate of *Lactobacillus plantarum* ATCC 14977 (*L. plantarum*) was obtained from Laboratory of Microbiology Universitas Brawijaya Malang Indonesia. Peptone, de Man, Rogosa and Sharp (MRS) agar, MRS broth and Vanillin were purchased from Merck distributor in Malang. All chemicals used in this study were analytical grade.

2.2. Methods

2.2.1. Starter cultures preparation *L. plantarum*. Starter cultures of *L. plantarum* was prepared by transferring a loopful of the isolate from MRS broth into MRS broth and incubated at 37 °C for 24 h. A 24-h starter cultures of 1.5 mL (3% v/w) was added to a mixture of 50 g sorghum grains and 100 mL of demineralized sterile water.

2.2.2. Fermentation of sorghum grain. Sorghum grains of 50 g was cleaned from dust and foreign matter and then washed with running water. Then, sorghum was soaked in 1000 mL water and inoculated with a 24-h starter cultures of *L. plantarum* at various concentrations of 2, 4, 6, 8, and 10% (v/v). Fermentation was carry-out for 72 h at room temperature. Sampling of liquid (for pH measurement and titratable acidity) and grains (for tannin analysis) was conducted every 12 h.

2.2.3. Enumeration of viable lactic acid bacterial (LAB) during fermentation. Starter cultures of *L. plantarum* of 1 mL was added into a sterile test tube containing 9 mL sterile peptone diluted in distilled water. The mixture was then vortexed. A ten-fold serial dilution of the sample was prepared. Bacterial suspensions of 0.1 mL were surface plated on MRS agar for lactic acid bacterial counts. Plates were then incubated at 37 °C for 48 h. After incubation, viable microbial counts were determined. The colonies that appeared on the selected plates (50–300 colonies per plate) were counted as colony-forming units (CFU) per mL of the sample.

2.2.4. Determination of pH and titratable acidity [15]. pH was determined using a pH meter and titratable acidity was determined by titrating mixture of the liquid fermentation [15]. Total titratable acidity was measured by titration of the fermentation liquid with a standard solution of 0.1 N NaOH. End of titration was indicated by the formation of the pink color of phenolphthalein indicator.

2.2.5. Determination of tannin content [16]. Sorghum flour (80 mesh) of 1 g was extracted with 10 mL 1% HCl in methanol for 24 h at room temperature. After that, the mixture was centrifuged at 5000 rpm for 5 min. The supernatant of 1 mL was added 5 mL of Vanillin HCl and incubated for 20 min. The
absorbance of the sample was read at wavelength of 500 nm on a spectrophotometer UV-Vis. Catechin was used as a standard curve.

2.2.6. Statistical analysis. All experiments were repeated three times. Data were subjected to analysis of variance (ANOVA) using Minitab 17. Comparison tests were performed using the T test at significance level p<0.05.

3. Results and discussion

3.1. LAB growth curve

Figure 1 shows the growth kinetics of LAB during fermentation of sorghum grain inoculated with L. plantarum starter cultures. After 24 h of fermentation, the number of viable cells was 6.5 log CFU/mL. From 0 to 24 h, bacterial growth showed an adaptation phase of the cells to the starter cultures conditions. LAB need a time to induce of specific messenger RNA (mRNA) and protein synthesis [18]. On 48 h of fermentation, the cell number increased very significantly about 7.2 log CFU/mL. Fermentation period from 24 to 48 h showed log phase of LAB growth as an indication that the cells begin dividing frequently by the process of binary fission [17]. Carbohydrate and protein in the sorghum were used as source of carbon and nitrogen for bacterial growth. At log phase, the medium of fermentation provides all nutrients required for LAB growth and the environmental parameters were optimal. Afterwards, the number of viable LAB began to decrease insignificantly, and the cell population reached 7.1 log CFU/mL after 60 h fermentation. At this point, the cells came to a stationary phase where the number of cells going through division appears to be equal to that being dead due to the limited of nutrients [17]. At this period, carbon and energy source or other essential nutrients became completely consumed for cells growth. In addition, the accumulation of waste product or toxic metabolites inhibited the cell growth [18]. Finally, from 60 to 72 h, bacteria fail the ability to divide, and the number of dead cells surpasses that of live cells.

![Figure 1](image1)

**Figure 1.** Growth kinetic of LAB during fermentation of sorghum grains inoculated with 3 % of starter cultures of L. plantarum.

3.2. pH and titratable acidity (TA)

As shown in Figure 2, a pH value of 6.4 was detected in samples at the initial sampling time. With the progress of fermentation, the pH values in all treatments decreased gradually to 6.3-6.4 after 24 h of fermentation and this coincided with the increasing of TA. From 24 to 36 h, all treatments show a significant decreased of pH. At this time (from 24 to 36 h), the pH decreased concomitantly to the significant growth of L. plantarum (Figure 1) which leads to a significant increase of organic acid levels. At the end of fermentation (72 h), the pH decreased to 6.0 and 5.9 on fermentation added 2 and
4% of starter cultures and to pH of 5.8 for those 6, 8 and 10% of starter cultures. The TA values were 0.59, 0.63, 0.8, 0.95 and 0.88% on fermentation added with 2, 4, 6, 8 and 10% of starter cultures, respectively. During incubation from 0 to 48 h, fermentation with starter cultures 6, 8, and 10% gave the same changes of TA (shown as overlapped lines in the Figure 2). The results agree with the previous studies [8],[9],[11] that during fermentation of sorghum flour the pH decreased along with the increase of the TA resulting from organic acid accumulation of microbial activity. *L. plantarum* A6 isolated from fermented cereal in Africa produce amylase can hydrolyze amylase, amyllopectin, and fermentable maltose from which the lactic acid is produced [13]. The organic acid produced during fermentation of African traditional cereal-based were lactate, succinate, pyruvate, DL-pyroglutamate, formate, citrate and uric acid [19]. During the fermentation of sorghum, *L. plantarum* also produced proteolytic enzymes that were able to degrade protein to produce free amino acids or peptides that serve as a growth-limiting factor of LAB in cereal fermentation [20]. The amino acids produced are used by *L. plantarum* to increase their growth and to produce more amylase.

![Figure 2. Changes in pH and titratable acidity (TA) during fermentation of sorghum inoculated with different concentration of starter cultures of L. plantarum for 72 h at room temperature. Each values represents mean of three replications.](image)

3.3. Tannin content

The changes in tannin content of sorghum during fermentation are presented in Figure 3. Sorghum grain for this experiment contains tannin 179.88 mg/100 g. After fermentation for 24 h, tannin decreased insignificantly for all treatments. Tannin content reduced significantly (p<0.05) after fermentation for 36 h with the addition of starter cultures of 4, 6, 8 and 10%, the tannin contents of those treatments were 111.3, 99.9, 100.1 and 105.3 mg/100 g, respectively. The reducing tannin continued until 48 h of fermentation. From 36 to 48 h, tannin in sorghum decreased very significantly (p< 0.05) and it is simultaneous with the log phase of bacterial growth (Figure 1). From 48 to 72 h, tannin content did not decrease. The tannin content of sorghum after fermented for 48 h with starter cultures of 2, 4, 6, 8, and 10% were 112.4, 94.0, 78.7, 78.9 and 65.0 mg/100 g, respectively. It has been suggested that the loss of tannin during fermentation could be a result of the activity of tannase resulted from bacterial activity. Some studies showed *L. plantarum* produces tannase that has specific substrate for gallotannins and can break protein and tannin complexes [14]. Tannase produced by *L. plantarum* can hydrolyze tannin resulting in gallic acid and glucose [21]. Furthermore, glucose can be used as a substrate for bacteria growth. The hydrolyzing of tannin by *L. plantarum* involves the action of a tannase and a gallate decarboxylase to decarboxylate the gallic acid produced by tannase activity [14]. The increasing of in vitro protein digestibility of spontaneous fermented sorghum was also an indication the presence of tannase produced by microflora during fermentation [22]. Fermentation of sorghum flour for 48 h reduced tannin from 3.1 mg/g (whole sorghum flour) to 0.1 mg/g [3]. Process of fermentation of sorghum resulted in lower tannin content compared to the malting process [3].
Figure 3. Changes in tannin content during fermentation of sorghum inoculated with different concentration of starter cultures of *L. plantarum* for 72 h at room temperature. Each values represents mean of three replications.

4. Conclusions
Fermentation of sorghum grains for 72 h, the pH decreased from 6.4 to 5.8-6.0, TA increased to 0.59, 0.63, 0.8, 0.95, and 0.88% at starter cultures concentration of 2, 4, 6, 8, and 10%, respectively. The tannin content in sorghum decreased significantly after fermentation for 48 h for all treatments. However, prolonged fermentation from 48 to 72 h did not reduce the tannin.

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