

# Turnitin-Effect of Addition Fermented Cabbage Extract on Antioxidant Activity and Antinutritional Compounds of Foxtail Millet Flour

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## Effect of Addition Fermented Cabbage Extract on Antioxidant Activity and Antinutritional Compounds of Foxtail Millet Flour

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### ABSTRACT

Fermented cabbage extract contains lactic acid bacteria *Lactobacillus plantarum* which produces tannase enzymes and *Lactobacillus casei* which produces phytase enzymes, which are very effective in reducing antinutrient compounds and increasing the antioxidant compound foxtail millet flour. This study aimed to determine the optimal fermentation time (0, 12, 24, 36, and 48 hours) to decrease antinutrient compounds and increase antioxidant compounds of foxtail millet flour using fermented cabbage extract. The results showed that increase in fermentation time from 0 to 48 hours reduced levels of tannins and phytic acid in foxtail millet flour by 68.64 % (2.71 - 0.85 mg/g) and 66.60 % (5.33 - 1.78 mg/g) respectively. Afterwards there is an increase in antioxidant activity by 83.10 % (42.25 - 77.26 %) and a decrease in total phenolic content by 18.22 % (7.41 - 6, 06 mg GAE/g). In conclusion, the optimal fermentation time to produce FMF with the best characteristics (low antinutrients and high antioxidant activity) is 48 hours.

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## 1. INTRODUCTION

Foxtail millet (*Setaria italica* L.) is a cereal in the *Poaceae* family, and one of the oldest cereal crops. Foxtail millet has good adaptability and thrives on dry soil (He *et al.*, 2015). Utilization of foxtail millet so far has not optimal yet, just for bird feed. Foxtail millet are source of calories, protein, fiber, minerals, essential micronutrients, vitamins, and antioxidant components that are very beneficial for human body (Amadou *et al.*, 2014; Sharma & Niranjana., 2018).

Besides being rich in nutritional benefits, foxtail millet also contains tannins and phytic acid compounds which have anti-nutritional effects (Saleh *et al.*, 2013). Tannins and phytic acid are distributed unevenly throughout the millet seed, these compounds cause inhibition in absorption and digestion of carbohydrates, proteins, minerals, and vitamins B (Devisetti *et al.*, 2014; Lestienne *et al.*, 2007).

Fermentation process is known can be very effective in reducing tannin and phytic acid compounds in millet significantly. The decrease in tannin and phytic acid levels was related to the activity of the tannase and phytase enzymes during the fermentation process (Onyango *et al.*, 2013). Xiong *et al.* (2012) reported that there are two types of lactic acid bacteria (LAB) that grow in the fermented cabbage extract (FCE), namely *Lactobacillus plantarum* which produces tannase enzymes, and *Lactobacillus casei* which produces phytase enzymes.

*Lactobacillus plantarum* has had tannase enzyme activity of 11.4 U/g protein (Rodríguez *et al.*, 2008), while the phytase enzyme activity of *Lactobacillus casei* reaches 12.06 U/g protein (García-Mantrana *et al.*, 2016). Determination of precise fermentation time will result in high enzyme activity. Yoon *et al.* (2006) have reported that the optimal fermentation time of *Lactobacillus plantarum* and *Lactobacillus casei* to produce high enzyme activity ranges from 12 - 48 hours, depending on the availability of the substrate. In addition to playing an important role in reducing anti-nutritional compounds, LAB in FCE was also reported contribute to increasing antioxidant activity (Hunaefi *et al.*, 2013).

Application of FCE fermentation to reduce tannin and phytic acid levels in foxtail millet flour (FMF) has never been done before. FCE uses as a starter because it can produce tannase and phytase enzymes with high activity, is environmentally friendly, economical, and very applicable (Ananda *et al.*, 2008). Determination of precise fermentation time will reduce anti-nutritional compounds in FMF optimally, also followed by an increase of antioxidant compounds as a result of its potential to become a source of functional food. This study aims to determine the optimal time of FMF fermentation using FCE, with the parameters including analysis of tannin content, phytic acid content, total phenolic, and antioxidant activity. In addition, this study also observed total LAB and pH FCE.

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## 2. MATERIALS AND METHODS

### 2.1. Materials

Main ingredients in this study included Majene variety foxtail millet seeds obtained from Majene – West Sulawesi, white cabbage obtained from Sumowono – Semarang, salt, and distilled water. Chemical materials used were methanol, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) reagent, Folin-Ciocalteu reagent, Na<sub>2</sub>CO<sub>3</sub>, NaNO<sub>2</sub>, AlCl<sub>3</sub>, NaOH, Folin Dennis reagent, KSCN, and FeCl<sub>3</sub>. All reagents are pro-analysis from Sigma-Aldrich.

### 2.2. Production of Fermented Cabbage Extract (Yonata *et al.*, 2021)

Cabbage was cleaned under running water, then cut into small pieces. The cabbage pieces were placed in a glass jar, then molasses (6.7% v/v) and salt (3% w/v) were added, stirred until smooth and the bottle was tightly closed using aluminium foil. Fermentation process was carried out for 8 hours at room temperature (23-25°C). The liquid extract obtained is FCE which is used as a starter in FMF modification stage.

### 2.3. Modified Foxtail Millet Flour (Yonata *et al.*, 2021)

Foxtail millet seeds (FMS) amount of 400 g were placed in a glass jar and 600 ml of FCE was added. Then, the jar was covered with aluminium foil and fermented according to treatment (0, 12, 24, 36, and 48 hours) at 37°C. Then, fermented FMS was dried in an oven for 8 hours at a drying temperature of 60°C. Dried FMS was ground using a disk mill and filtered with a 100 mesh sieve to obtain FMF.

#### 2.4. Phytic Acid (Gull et al., 2016)

An amount of 2 g of FMF was put into a measuring glass, then 100 ml of 23% HCl was added and allowed to stand for 5 hours at room temperature (23-25°C), and filtered using Whatman paper grade 1. Afterward, 25 ml of filtrate was put into an Erlenmeyer and 5 ml of 0.3% KSCN solution was added, then titrated with a standard FeCl<sub>3</sub> solution. Persistence of brownish-yellow colour for 5 minutes, indicates the endpoint. Concentration of FeCl<sub>3</sub> is 1.04 %w/v and mole ratio of Fe to phytate = 1:1.

$$\text{Phytate phosphorus concentration} = \frac{\text{Titration volume} \times 0.064}{100 \times \text{sample weight}} \quad (1)$$

#### 2.5. Tannin (Gull et al., 2016)

Tannin analysis used Folin-Denis reagent to make a standard curve for pure tannic acid. A total of 1 g of FMF was extracted with 40 ml of 10% methanol and then centrifuged (3000 g for 15 minutes) to obtain the filtrate. Filtrate obtained was diluted with methanol to a volume of 50 ml. After that 1 ml of the extract was pipetted into a measuring flask, added 10 ml of 35% Na<sub>2</sub>CO<sub>3</sub> reagent, adjusting the volume to 100 ml. Absorbance was read at a wavelength of 760 nm after 45 min. Tannin content is expressed as the tannic acid equivalent of the standard curve.

#### 2.6. Total Phenolic (Tian et al., 2020)

Amount of 200 mg of FMF was extracted using 4 ml of acidified methanol (HCl: methanol: water; 1:8:10; v/v/v) at room temperature for 2 hours. An aliquot of FMF extract (0.36 ml) was added to 1.5 ml of Folin-Ciocalteu reagent which has been diluted 10 times. The mixture was allowed to stand for 15 minutes (23-25°C) and then mixed with 1.5 ml of Na<sub>2</sub>CO<sub>3</sub> solution (60 g/L). After being incubated for 90 min at room temperature (23-25°C), absorbance of the mixture was read at a wavelength of 725 nm. Blank solution used was acidified methanol. Gallic acid in methanol with a concentration of 2-10 ppm is used as a standard solution. Total phenolic is expressed as mg of gallic acid equivalent per gram of FMF (mg GAE/g).

#### 2.7. Antioxidant Activity (Sharma & Gujral, 2011)

Amount of 100 mg of sample was extracted with 1 ml of methanol for 2 hours, then centrifuged at 3000 g for 10 minutes. Supernatant (0.1 ml) was then reacted with 3.9 ml of 1 mM DPPH solution for 30 minutes at a temperature of 23-25°C in a dark room. The absorbance was measured using a spectrophotometer at a wavelength of 517 nm.

$$\% \text{ Antioxidant activity} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100 \quad (2)$$

#### 2.8. Research Design

This study used a one-factor completely randomized design, fermentation time which consisted of five levels (0, 12, 24, 36, and 48 hours), each level was repeated 5 times so that 25 experimental units were obtained.

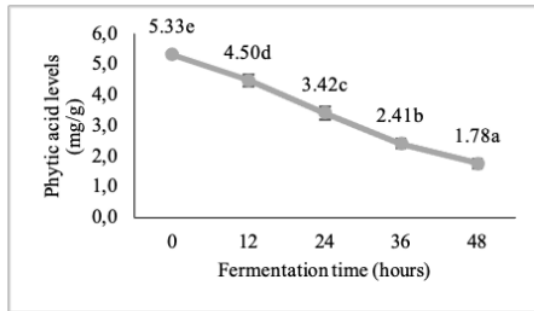
#### 2.9. Statistical Analysis

All data obtained were analyzed using a one-way ANOVA test and followed by a post hoc LSD test to determine significant differences between the mean variables for the selected parameters. The significance level of the differences was defined at p<0.05. Statistical analyzes were performed using SPSS 22.0 software.

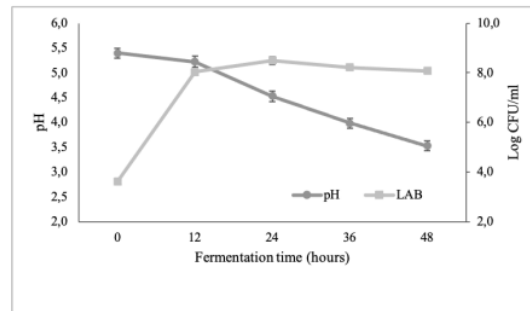
### 3. RESULTS AND DISCUSSION

#### 3.1. Phytic Acid

FMF phytic acid level in this study was 5.33 mg/g (Figure 1), lower than reported by Kumar *et al.* (2016) of 8.9 mg/g, and Gull *et al.* (2016) which reached 6.1 to 9.2 mg/g. These differences are caused by several things, such as genetics, environmental conditions, planting locations, irrigation conditions, soil types, and fertilization factors (Wu *et al.*, 2009). Fermentation caused the FMF phytic acid content decreases significantly from 5.33 to 1.78 mg/g (Figure 1). Similar results have previously been reported by Osman (2011) and Simwaka *et al.* (2017). During fermentation, the phytase enzyme hydrolyzes phytic acid into inositol and orthophosphate, a few phytic acids are hydrolyzed to inorganic phosphate (Azeke *et al.*, 2011; Li *et al.*, 2010).



**Figure 1.** FMF phytic acid levels based on fermentation time with FCE (Values followed by different letter are significantly different at 95% confidence level)



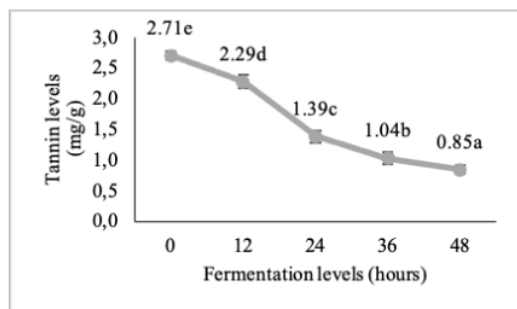
**Figure 2.** pH value and total LAB of FCE during FMF fermentation time

The pH value has an important role in reducing FMF phytic acid. A lower pH value of a liquid makes an optimal activity of the phytase enzyme in hydrolyzing phytic acid into inorganic phosphate and inositol. In this study, FCE pH was seen to decrease significantly (5.40-3.53) when the fermentation time was increased (Figure 2). The decrease in FCE pH generally afterward an increase in total LAB from 3.61 to 8.50 log CFU/ml (Figure 2). The decrease in FCE pH generally afterward an increase in total LAB from 3.61 to 8.50 log CFU/ml (Figure 2). However, total LAB looks sloping and tends to decrease after 24 hours of fermentation. Han *et al.* (2014) previously reported that LAB growth will be inhibited and die if liquid pH is too acidic. It was revealed that after 24

hours of fermentation, LAB growth in FCE entered the death phase, which produced various metabolites causing the liquid condition to become more acidic with a result FCE pH continued to decrease when the fermentation time was increased (Du et al., 2018). During fermentation, the LAB of the *Lactobacillus* sp group produces more acetic acid which causes a decrease in the pH value of the liquid (Hutkins, 2006; Xiong et al., 2012).

### 3.2. Tannin Level

Tannins are polyphenolic compounds with unique characteristics. Tannins have higher antioxidant properties than vitamins E and C but are also anti-nutritional because they can be lowering the digestibility of carbohydrates and proteins (Ojo, 2022). Tannin levels in FMF without fermentation reached 2.71 mg/g (Figure 3), lower than the report of Shejawale et al. (2016) which ranged from 3.24 to 4.13 mg/g. The tannin content of FMF was seen to decrease significantly (2.71 to 0.85 mg/g) as the fermentation time increased (Figure 3). FCE generally consists of several types of LAB, one of them *Lactobacillus plantarum* (Utama et al., 2018), is known to produce metabolites such as enzyme tannase (Rodríguez et al., 2008) which is confirmed to hydrolyze tannins during fermentation by catalyzing the hydrolysis reaction of their esters (Aguilar-Zarate et al., 2014). Fermentation process also causes proteolytic enzyme activity increases resulting from the secondary metabolite *Lactobacillus plantarum* will hydrolyze the protein-tannin complex so that the tannin levels decrease which in turn will increase the bioaccessibility of minerals such as iron and zinc (Onweluzo & Nwabugwu, 2009).



**Figure 3.** FMF tannin levels based on fermentation time with FCE (Values followed by different letter are significantly different at 95% confidence level)

Tannins are polyphenolic compounds with unique characteristics. Tannins have higher antioxidant properties than vitamins E and C but are also anti-nutritional because they can reduce the digestibility of carbohydrates and proteins (Ojo, 2022). Tannin levels in FMF without fermentation reached 2.71 mg/g (Figure 3), lower than the report of Shejawale et al. (2016) which ranged from 3.24 to 4.13 mg/g. Tannin content of FMF was seen to decrease significantly (2.71 to 0.85 mg/g) along with the increase in fermentation time used. (Figure 3). FCE generally consists of several types of LAB, one of them *Lactobacillus plantarum* (Utama et al., 2018), is known to produce metabolites such as enzyme tannase (Rodríguez et al., 2008) which is confirmed to hydrolyze tannins during fermentation by catalyzing the hydrolysis reaction of their esters (Aguilar-Zarate et al., 2014). Fermentation also increases proteolytic enzyme

activity caused by the secondary metabolite *Lactobacillus plantarum* will hydrolyze the protein-tannin complex so that the tannin levels decrease afterward increasing the bioaccessibility of minerals such as iron and zinc (Onweluzo & Nwabugwu, 2009).

### 3.3. Total Phenolic

Foxtail millet of phenolic compounds exist in the form of free phenolic, bound phenolic and total phenolic. Phenolic compounds discussed in this study is total phenolic expressed as mg ferulic acid per gram of FMF. Total phenolic FMF can be seen in Table 1, where Unfermented FMF contained a total phenolic of 7.41 mg GAE/g, and it was seen to decrease by 6.24 mg GAE/g after 12 hours of fermentation. When fermentation time was increased, total phenolic FMF tended to decrease even though the 24-hour fermentation increased slightly but not significantly. In general, total phenolic FMF decreased up to 18.22% (7.41 to 6.06 mg GAE/g) after being fermented with FCE for 48 hours (Table 1).

**Table 1.** Total phenolic and antioxidant activity of FMF based on fermentation time with FCE

31 Fermentation Time (Hours)	Total Phenolic (mg GAE/g)	Antioxidant Activity (%)
0	7.41 ± 0.08 <sup>d</sup>	42.25 ± 0.80 <sup>a</sup>
12	6.24 ± 0.11 <sup>bc</sup>	56.48 ± 0.65 <sup>b</sup>
24	6.33 ± 0.10 <sup>c</sup>	67.19 ± 0.96 <sup>c</sup>
36	6.20 ± 0.10 <sup>bc</sup>	73.22 ± 0.98 <sup>d</sup>
48	6.06 ± 0.12 <sup>a</sup>	77.36 ± 0.68 <sup>e</sup>

Note: All values are mean ± standard deviation 5 times repetition. Different superscript values showed significant differences with a 95% confidence level based on the ANOVA difference test and the LSD post hoc test.

Hydrolysis of tannin compounds during fermentation process leads to degradation of polyphenolic compounds, decreasing total phenolic content (Taylor & Duodu, 2014). There is a linkage between a reduction of tannins and a decrease in total phenolics, with the correlation value reaching 85% (Sharma & Gupta, 2019). Total phenolic decreases during fermentation were also caused by glucosidase, phenolic acid reductase, and phenolic acid decarboxylase, which are secondary metabolites of *Lactobacillus sp.* (Svensson *et al.*, 2010). According to Curie *et al.* (2009), tannase and feruloyl esterase activities were identified in *Lactobacillus sp* which can further contribute to release of phenolic acids from the wall, especially protocatechuic acid and p-hydroxybenzoic acid. This mechanism is associated with a decrease in total phenolic FMF with increasing fermentation time.

### 3.4. Antioxidant Activity

Antioxidant activity of FMF without fermentation reached 42.25% (Table 1). This is following the statement in his research reported by Siroha *et al.* (2016) where millet flour generally contains antioxidant activity of around 31.8 – 46.7%. The fermentation process causes the antioxidant activity of FMF to increase significantly by 83.10% (42.25 to 77.36%). These study results are to the report of Amadou *et al.* (2013), that Foxtail millet fermentation using *Lactobacillus sp* strain showed a large effect on increasing the antioxidant activity of FMF. The fermentation process has long been known to increase the antioxidant activity of foodstuffs. A β-glucosidase enzyme from

LAB can hydrolyze phenolic glycosides and release free phenolic bioactive compounds. Phenolic compound changes are responsible for the increase in antioxidant activity after fermentation (Pyo *et al.*, 2005; Wang *et al.*, 2011). Foxtail millet has been recognized as a potential food ingredient for health and reduces the risk of various diseases (Anand *et al.*, 2008). The health benefits of millet are associated with its role as a source of antioxidants, including various vitamins and other phytochemical compounds that can inactivate free radicals thereby contributing to prevention of damaged cell membranes (Zhang & Liu, 2015).

#### 4. CONCLUSIONS

FCE can be used as a starter in improving FMF characteristics through the fermentation process. Longer fermentation time makes decreases the levels of phytic acid, tannin and total phenolic, followed by an increase in antioxidant activity of FMF. In addition, there was a decrease in pH value and an increase in the total LAB of FCE liquid as the fermentation time used increased. Optimal fermentation time to produce FMF with the best characteristics (low anti-nutrients and high antioxidant activity) is 48 hours.

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