



## Characterization of Fermented Milk with the Addition of Gembili (*Dioscorea esculenta*) Flour

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### Authors' contributions

This work was carried out in collaboration among all authors. All authors have contributed to conduct this research activities and also read and approved this manuscript.

### Article Information

DOI: 10.9734/AFSJ/2021/v20i230267

#### Editor(s):

- (1) Dr. Amjad Iqbal, Abdul Wali Khan University Mardan, Pakistan.  
(2) Dr. Uttara Singh, Panjab University, India.

#### Reviewers:

- (1) Simona Maria Man, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania.  
(2) Fatma Coskun, Tekirdag Namik Kemal University, Turkey.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/65502>

Original Research Article

Received 10 December 2020

Accepted 13 February 2021

Published 02 March 2021

## ABSTRACT

**Aims:** Objective of this study was to determine the effect of addition gembili (*Dioscorea esculenta*) flour on the fermented milk was then evaluate the characteristics of fermented milk by analysis of pH, titratable acidity, viscosity, total phenolic content, crude fiber, sugar total, and total lactic acid bacteria.

**Study Design:** This study employs a completely randomized design with five treatments adding gembili flour and three replications.

**Place and Duration of Study:** Animal Product Technology Laboratory, Animal Product Technology Department, Faculty of Animal Science, Universitas Brawijaya, between April-October 2020.

**Methodology:** Fermented milk is prepared by pasteurizing of skim milk and gembili flour. The concentration of gembili flour added were (0 (P1); 0.5% (P2); 1% (P3); 1.5% (P4); 2% (P5). Furthermore, the characteristics of fermented milk is carried out including pH, titratable acidity, viscosity, total phenolic content, crude fiber, sugar total, and total lactic acid bacteria of fermented milk using combination of three bacteria namely, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Streptococcus thermophilus* as a starter culture.

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**Results:** The results in this study revealed that the pH value ranged from 3.5-4.3; titratable acidity 1.98-2.35%; viscosity 849-1856 cps; sugar total 2.8-4.11 g/100g; total lactic acid bacteria  $3.05 \times 10^7$  -  $3.21 \times 10^8$  cfu/ml respectively. Total phenolic content and crude fiber in P1 was not detected, but in P2-P5 had total phenolic and crude fiber ranging from 108,58 – 670,75 mg/kg and 0.57-2.16 g/100 g, respectively.

**Conclusion:** The addition of gembili flour to the fermented milk contributes to the quality of fermented milk product which include pH, titratable acidity, viscosity, total phenolic content, crude fiber, sugar total, and total lactic acid bacteria.

**Keywords:** Fermented milk; lactic acid bacteria; gembili flour; dioscorea esculenta.

## 1. INTRODUCTION

Fermented milk is one of the oldest methods that used to extend the shelf life of milk and widely produced in many countries. It is also famous dairy product which has advantageous with health promoting ingredients. Fermented milk is made from milk or combination or fully skimmed, concentrated milk or milk powder, buttermilk powder, whey protein (concentrated or powdered), soluble milk proteins, edible casein and caseinates, cream, butter, that have been pasteurized and then transferred with specific microorganisms, which decrease pH and coagulates [1].

The fermented milk contains lactic acid bacteria that are capable in the gut and can also act as probiotic [2]. Lactic acid bacteria that are commonly used as a starter culture in fermented milk such as yoghurt, are *Lactobacillus bulgaricus* and *Streptococcus thermophilus* [3]. Probiotics are living microorganisms which if sufficient in number are able to provide health effects for the body [4]. *L. plantarum* IIA-1A5 and *L. fermentum* B111K are lactic acid bacteria that have been investigated to have the ability to assimilate cholesterol through absorption and binding of cholesterol in the body [5,6]. In another study El-Gawad [7] explored that buffalo milk yoghurt containing *B. longum* Bb-46 found a significant reduction ( $p < 0.05$ ) in total cholesterol, LDL-cholesterol, and triglycerides of 50.3%; 56.3%; and 51.2%, respectively compared to the control.

The activity of lactic acid bacteria can increase when added with prebiotics [8]. One component that may be employed as prebiotic is fibers, which is usually found in tubers. Gembili (*Dioscorea esculenta*) is one local tuber from Indonesia especially in East Java, of carbohydrate source that has not been maximally utilized. Additionally, tubers are consumed by steaming or used in chips manufacturing. The

presence of fiber in gembili can also improve the quality of the product, so that its addition of fiber to food has become important. Since gembili contain carbohydrate, it is worthy to note that gembili could be used as stabilizer, which enhance the quality of fermented milk. Stabilizer can reduce the syneresis, increase viscosity and improving the emulsion stability of the processing product. Several researches reported that the addition of stabilizer could improve the quality of fermented milk [9,10,11]. Similarly, Utomo. et al. [12] found that the addition of porang tuber (*Amorphophallus oncophyllus*) could increase viscosity and decrease syneresis in yoghurt drink of 19.66 cp and 76.91, respectively.

Gembili also contain bioactive compounds that are beneficial to health including water-soluble polysaccharides, food fiber, diosgenin and inulin. Research indicated that gembili flour contains 5.05% water-soluble food fiber; 8.21% water-insoluble food fiber; 29.53% water-soluble polysaccharides; and 150.44 mg / 100 g diosgenin [13]. The level of inulin in gembili is 14.77%. Inulin is considered as a prebiotic due to its ability to stimulate the development of good bacteria in the intestine. A study shows that giving 18 g of inulin per day in men and women with hypercholesterolemia can significantly reduce LDL cholesterol levels by 14.4% [14]. The role of inulin as soluble fiber can help reduce blood cholesterol levels [15]. Another study reported that gembili contain oligosaccharides were lactulose, inulin and raffinose of 0.231%, 2.541%, and 1.485%, respectively [16]. Oligosaccharides can act as prebiotic and support the probiotics with prebiotic index optimal at 24 hours of incubation time [16].

Due to the health benefits obtained from fermented milk products, it is necessary to do research to improve the quality of fermented milk by providing the substrate that can increase the growth of lactic acid bacteria. Moreover, gembili flour was subjected into fermented milk to

stimulate the lactic acid bacteria, which will further examine the characteristics of fermented milk including pH, titratable acidity, viscosity, total phenolic content, crude fiber, sugar total and total lactic acid bacteria. The benefit of this research is to provide information on the characteristics of fermented milk added with gembili flour.

## 2. MATERIALS AND METHODS

### 2.1 Materials

The materials used in this study were gembili (Materia Medika Batu, Malang, East Java, Indonesia); skim milk; and lactic acid bacteria. The lactic acid bacteria, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Streptococcus thermophilus* were obtained from Food and Nutrition Culture Center Collection (FNCC), Center for Food and Nutrition Studies, Gadjah Mada University, Indonesia. The preparation of gembili flour is done using the method proposed by Fidyasari et al. (2017) [17]. The first step to make gembili flour were stripping the gembili, washing and thinly slicing it. Gembili is then soaked in a solution of 10% kitchen salt for 60 minutes, drained and dried using a cabinet dryer at a temperature of 60°C for approximately 5-6 hours. After drying, the gembili was ground and sieved using 80 mesh size, and packaging using aluminum foil before use.

### 2.2 Preparation of Starter Culture

The strains (*Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Streptococcus thermophilus*) were grown used by transferring a loopful of bacteria into 250 ml Erlenmeyer flask and each containing 50 ml of MRS broth (de Man Ragosa Sharpe) (Merck) medium and was then incubated for 24 hours at 37°C. Subsequently, they were subjected to centrifugation at 4500 rpm, for 5 minutes. The pellets were washed twice with 0.1% peptone saline solution (Merck), and then was inoculated into the 10% pasteurized skim milk. Subsequently incubated for 24 hours at 37°C. Furthermore, the starter culture begins by preparing *Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Streptococcus thermophilus* with ratio of 1:1:1 and were cultivated into the 10% pasteurized skim milk for 24 hours at 37°C. The starter culture was ready to use when the number of bacteria not less than 10<sup>6</sup> cells/ml.

### 2.3 Preparation of Fermented Milk with the Addition of Gembili Flour

Gembili flour is added 0 (P1); 0.5% (P2); 1% (P3); 1.5% (P4); 2% (P5). Each flask contained 200 ml skim milk and gembili flour which had been pasteurized at a temperature of 80°C for 15 minutes, then, cool the milk to 40°C. An addition starter culture as much as 3% then incubated for 24 hours at 37°C [18]. All these experiments were performed in triplicate, and the pH, titratable acidity, viscosity, total phenolic content, crude fiber, sugar total, and total lactic acid bacteria were determined after the incubation time.

### 2.4 Determination of pH

pH was measured using pH meter (Mettler, Toledo). Calibration was carried out using a buffer solution at pH 4.01 and pH 7.01. Measurements were made by dipping the pH meter electrode into 10 ml of the sample and waiting until the pH meter showed a stable number [19].

### 2.5 Determination of Titratable Acidity

Titratable acidity was tested using the method proposed by Hadiwiyoto (1994) [20]. Total acid testing is done by taking a sample of 20 ml. Before titrating, the sample was dropped with 2% phenolphthalein (PP) solution as much as 2 drops. After that the sample was titrated with 0.1 N NaOH until it appeared pink. Calculation of the percentage value of total titrated acid (TAT) is determined from the multiplication of the normality of the titrant, the volume of the titrant, and the equivalent weight of the acid which is then divided by the sample volume multiplied by 10 [21].

### 2.6 Determination of Viscosity

The viscosity measurement was carried out using Brookfield Viscometer. The spindle is inserted into the yogurt until the spindle is immersed reaching the boundary mark. Viscosity data is taken every 10 seconds for 1 minute [22].

### 2.7 Determination of Total Phenolic Content

Total phenolic content (TPC) was performed according to the Folin-Ciocalteu method [23]. Samples that have been dissolved at a certain

concentration were taken as much as 0.5 ml, then added 2.5 ml of the 10% (v/v) Folin-Ciocalteu reagent. The mixture is homogeneous using vortex and incubated in a dark room for 5 minutes, then added 2 ml of Na<sub>2</sub>CO<sub>3</sub> 7.5% (b/v). The mixture is homogeneous using vortex and incubated in a dark room for 30 minutes. Absorbance was measured using a spectrophotometer (Shimadzu UV-1800) at a wavelength of 765 nm.

## 2.8 Determination of Crude Fiber

Crude fiber measurement was done by taking a sample of 1 ml of sample, put into 600 ml of Erlenmeyer and added 50 ml of 0.3 N H<sub>2</sub>SO<sub>4</sub> then heated for 30 minutes. Then it was continued by subsequently adding 25 ml of 1.5 N NaOH and cooked again for 30 minutes. Whatman paper number 41 was dried in a dryer at 105-110°C for one hour and put in a Buchner funnel. During filtering, the sludge was washed consecutively with hot distilled water, 50 ml of H<sub>2</sub>SO<sub>4</sub>, enough distilled water, and finally with 70% ethanol 70 ml. Filter paper and its contents were put into a porcelain cup and dried for 6 hours in an oven at a temperature of 105°C then cooled in an desiccator and weighed a porcelain cup and the contents were burned and baked in an electric furnace at a temperature of 400-600°C for 6 hours again, then cooled and placed in the desiccator and weighed [19].

## 2.9 Determination of Sugar Total

The determination of sugar total was carried out by the Antrone-Sulfate method [24]. Determination of sugar total was done by taking 1 ml of sample with 5 ml of anthrone reagent in a fume hood. Then the tube was closed and shaken so that the solution was evenly mixed. The solution was then heated over a water bath at 100°C for 12 minutes and cooled. Then the absorbance solution was observed in a visible spectrophotometer at a maximum wavelength of 580-680 nm.

## 2.10 Determination of Total Lactic Acid Bacteria

The lactic acid bacteria of fermented milk were determined using MRSA plate count. One milliliter of sample was diluted with 9 ml of 0,01% peptone saline solution. Samples were diluted to levels 1:10<sup>5</sup> and 1:10<sup>6</sup>. Moreover, samples were subjected to be plated in triplicate. Subsequently, they were incubated for 24 - 48 h at 37°C, and

then the viable cells were determined. The number of lactic acid bacteria is expressed in CFU / ml [25,26].

## 2.11 Statistical Analysis

Data processing was performed using the Completely Randomized Design (CRD) method. The test results were analyzed statistically with Analysis of Variance (ANOVA) by Microsoft Excel 2016 program. Subsequently, if there were any significant difference, then a further BNT test ( $p < 0.01$ ) were performed to know the significant different in each treatment.

# 3. RESULTS AND DISCUSSION

## 3.1 pH

Table 1 show the pH value of fermented milk with the addition of gembili flour. The results of the analysis of variance shows that there is a significant difference with the addition of various concentrations of gembili flour ( $p < 0.01$ ) to the pH of fermented milk. The pH value generated from this study ranged from 3.5-4.3. Jay (2005) reported that ranged pH of yoghurt of 3.65-4.40, respectively [27]. Based on the results, pH of fermented milk with the addition of 2% gembili flour is 4.3, while the control was 3.5.

The decrease in the pH value of fermented milk is due to an increase in the amount of H<sup>+</sup> ions due to an increase in total acid. This indicates that the use of probiotic bacteria has an influence on the pH of fermented milk. Lactic acid produced from carbohydrate metabolism will reduce the pH value of the growth environment and cause a sour taste. Gembili flour contain oligosaccharides and it used by bacteria as a substrate for growth of cells. During the fermentation process in fermented milk, lactic acid bacteria will utilize existing carbohydrates and sugars to accelerate the process of acid formation.

## 3.2 Titratable Acidity

Results on titratable acidity of fermented milk with gembili flour are provided in Table 1. The results of the analysis of variance indicated that there is a significant difference with the addition of various concentrations of gembili flour ( $p < 0.01$ ) to the acidity of fermented milk. The titratable acidity (TA) is used to determine the total acid concentration and forming of organic acids during the fermentation process.

**Table 1. The results of pH, titratable acidity, viscosity, and total phenolic content of fermented milk with the addition of gembili (*Dioscorea esculenta*) flour**

Treatments	pH	Titratable acidity (%)	Viscosity (Cps)	Total phenolic content (mg/kg)
P1	3.5 <sup>a</sup> ±0.05	2.02 <sup>a</sup> ±0.04	849 <sup>a</sup> ±2.08	0 <sup>a</sup> ±0
P2	3.6 <sup>ab</sup> ±0.12	1.98 <sup>a</sup> ±0.006	1093 <sup>b</sup> ±2.64	108.58 <sup>b</sup> ±4.92
P3	3.8 <sup>b</sup> ±0.05	2.01 <sup>a</sup> ±0.08	1238 <sup>c</sup> ±6.08	266.42 <sup>c</sup> ±3.96
P4	4.2 <sup>c</sup> ±0.05	2.30 <sup>b</sup> ±0.06	1552 <sup>d</sup> ±2.51	540.46 <sup>d</sup> ±0.71
P5	4.3 <sup>c</sup> ±0.05	2.35 <sup>c</sup> ±0.02	1856 <sup>e</sup> ±4.04	670.75 <sup>e</sup> ±3.02

Different superscripts in the same column indicate a highly significant effect ( $p < 0.01$ ). Data are shown as mean  $\pm$  SD

The acidity of fermented milk is due to lactose breakdown by lactic acid bacteria which can be measured by titration using NaOH 0.1 N. Based on the results of the study, the titratable acidity ranged from 1.98-2.35% with the highest titratable acidity in the P5 treatment with 2% of gembili flour added. This shows that lactic acid bacteria have an effect on increasing the total value of the titrated acid of fermented milk through the large amount of lactic acid produced during the fermentation process. During the fermentation process, lactic acid bacteria produce primary metabolism in the form of lactic acid and secondary metabolites in the form of organic acids [28]. The acid in fermented milk is the result of the metabolism of lactic acid bacteria that convert nutrients such as lactose to lactic acid. Basically, the milk fermentation process breaks down lactose into lactic acid and its components of aroma and flavor. Not only utilizing lactose in milk, bacteria can also use other sugars as energy for metabolism [29]. It is interesting to note that the addition of gembili flour provide nutrient for the growth of lactic acid bacteria. The highest gembili flour added the titratable acidity will increases, which indicates the formation of lactic acid during fermentation. This is a line with the results from Mustika et al. [30] that purple sweet potato rich oligosaccharides and fiber that can increase the growth of lactic acid bacteria and produce metabolite of lactic acid on yoghurt.

### 3.3 Viscosity

Determination of viscosity in fermented milk with the addition of gembili flour are presented in Table 1. Analysis of variance showed that there is a significant difference due to the addition of various concentrations of gembili flour ( $p < 0.01$ ) to the viscosity in fermented milk. This study revealed that the viscosity ranged from 849-1856 cps. The highest average viscosity value of 1856 cps was obtained in treatment with the addition

of 2% gembili flour (P5), while the lowest viscosity was fermented milk with 0% gembili flour concentration (P1). The difference in effect is due to interactions between casein particles and gembili flour which plays a role in the separation of whey causing it to increase in its viscosity. Polysaccharides plays a role in shaping the structure of yogurt [31]. The higher the addition of the flour concentration, the viscosity value will increase.

The level of viscosity of fermented milk can be influenced by several factors, such as the main constituent ingredients of yogurt and its storage time [8]. In addition, fiber can also affect the texture and consistency of the product produced [32]. The addition of inulin can increase the thickness of the fermentation product. The consistency of yogurt with the addition of fructooligosaccharides (FOS) and inulin increases during storage of 21 days in the refrigerator [8]. In this study also reported that after 21 days storage at refrigeration, the volume of separated whey decreased from 5.3% to 1.3%, respectively [8]. It is interesting to note that the addition of prebiotic could reduce yoghurt syneresis.

In addition, an increase in the activity of lactic acid bacteria also affects the viscosity of fermented milk because lactic acid bacteria will break down carbohydrates into simple sugars to form lactic acid. Lactic acid bacteria that produce the enzyme lactase can also affect the thickness of milk. Lactase enzymes are used to decompose lactose and produce lactic acid which causes instability of milk protein, causing an increase in viscosity [33]. This is in accordance with the increasing concentration of adding gembili flour gave the highest viscosity of fermented milk.

Moreover, through their metabolism, some lactic acid bacteria (e.g. *S. thermophilus*) can produce

polysaccharides, namely exopolysaccharides (EPS). EPS are produced by various kinds of lactic acid bacteria e.g mesophilic and thermophilic. The EPS can increase the consistency index and yoghurt viscosity [34]. It is worthy to note that EPS derived from lactic acid bacteria may play a role in improving of the rheology, texture, mouth feel of fermented food formulations in food industry [35].

### 3.4 Total Phenolic Content

Results on total phenolic content (TPC) analysis in fermented milk with the addition of gembili flour are presented in Table 1. Additionally, in this experiment all treatments were considered significant statistically ( $p < 0.01$ ) to the total phenolic in fermented milk.

The polyphenols content in gembili flour before its addition to milk was not detected in this study. Moreover, fermented milk with the addition of 2% gembili flour has the highest total phenolic content, which is 670.75 mg / kg, respectively, while in P1 treatment phenolic content was unidentified. The difference is due to gembili tubers containing phenolic compounds that affect the total phenolic content of the yogurt produced. The water and chloroform extract of the *Dioscorea esculenta* (Lour.) Burkill were shown to antioxidant content which is analogues to the total phenolic concentration of 116 and 168, 5 ppm, respectively [36]. Gembili are one type of plant that contains phenolic compounds and flavonoids as natural antioxidants. Antioxidants play a role in stopping oxidative reactions by removing free radicals by oxidizing them [37].

The fermentation process can increase biological activity since microbial enzymes cause the content of polyphenols to become more active components. Polyphenols have the ability to regulate the activity of various enzymes and play a role in signaling mechanisms of various processes in cells so that they are considered to influence the metabolic oxidation-reduction reactions of cells. Polyphenols can reduce reactive oxygen species (ROS) and malondialdehyde (MDA), metabolites that are formed when oxidized ROS and LDL attack fatty acids in cell membranes [38].

### 3.5 Crude Fiber

Results on crude fiber measurement in fermented milk with the addition of gembili flour are provided in Table 2. Analysis of variance

shows that there is a significant difference with the addition of various concentrations of gembili flour ( $p < 0.01$ ) on the total crude fiber in fermented milk.

As shown in Table 2, the highest crude fiber was ranged from 0.57-2.16, while the crude fiber in P1 (without gembili flour) treatment was undetected. The difference in effect is due to gembili tubers containing fiber so that it affects the levels of crude fiber in fermented milk. The level of crude fiber will increase with the addition of gembili flour. The highest crude fiber content is 2.2 g / 100 g in the treatment of adding 2% gembili flour. This result is similar to that reported by Pramono et al. (2020) who found that crude fiber of yoghurt will increase with the higher concentration of the addition lesser yam tuber flour [39]. Additionally, fiber is a polysaccharide that cannot be digested by enzymes in the digestion of the human body and reaches the human intestine intact. Fiber is divided into two, namely water-soluble fiber and water insoluble fiber. Water-soluble fiber is better known as dietary fiber (dietary fiber) while water-insoluble fiber is known as crude fiber (crude fiber). Dietary fiber (inulin) can be reduced which will be used by lactic acid bacteria as nutrients in their growth.

Crude fiber content in gembili flour employed in this study was 5.7 g/100 g, but lower than reported by Retnowati et al. (2019) of 9.04%, respectively [40]. Food fiber is useful to inhibit the growth of harmful bacteria while crude fiber is useful to prevent constipation [41]. Fiber can also be a source of prebiotics that can affect the number of bacterial colonies. This is in accordance with the statement of Biggs et al. (2007) that oligosaccharides can be used as prebiotics because they are able to change the condition of microflora colonies [42]. This was confirmed by Retnati et al. (2009) which states that the natural fibers of oligosaccharides stored in sweet potatoes can be useful as prebiotics which will be used by lactic acid bacteria as nutrients in their growth [25].

### 3.6 Sugar Total

The results of the sugar total in fermented milk with the addition of gembili flour can be seen in Table 2. Results of analysis of variance shows that there is a significant difference with the addition of various concentrations of gembili flour ( $p < 0.01$ ) to the sugar total in fermented milk. The difference is due to the addition of gembili flour which increases lactic acid bacteria growth

**Table 2. The results of crude fiber, sugar total, and total lactic acid bacteria of fermented milk with the addition of gembili flour (*Dioscorea esculenta*)**

Treatments	Crude fiber (g/100 g)	Sugar total (g/100 g)	Total Lactic acid bacteria (CFU/ml)
P1	0 <sup>a</sup> ±0	4.11 <sup>d</sup> ±0.09	3.05 x 10 <sup>7a</sup> ±0.06
P2	0.57 <sup>b</sup> ±0.11	3.8 <sup>c</sup> ±0.01	2.9 x 10 <sup>8b</sup> ±0.25
P3	1.19 <sup>c</sup> ±0.04	3.2 <sup>b</sup> ±0.05	3.1 x 10 <sup>8b</sup> ±0.20
P4	1.94 <sup>d</sup> ±0.03	2.8 <sup>a</sup> ±0.05	3.13 x 10 <sup>8b</sup> ±0.08
P5	2.16 <sup>e</sup> ±0.08	2.8 <sup>a</sup> ±0.02	3.21 x 10 <sup>8b</sup> ±0.01

Different superscripts in the same column indicate a highly significant effect ( $p < 0.01$ ). Data are shown as mean  $\pm$  SD

allowing it to increase total acid and reduce sugar levels. This is proven by the results of the analysis (Table 2) which shows that the higher concentration of gembili flour results in decreased sugar levels.

The highest sugar total in fermented milk was obtained in the P0 treatment without the addition of gembili flour which was 4.11 g / 100 g, while the lowest sugar total was 2.8 g / 100 g in the P4 and P5 treatments. Basically, microbes need energy for their survival. Energy is needed to maintain cell life and for cell proliferation. The easiest substrate is sugar. This study uses the addition of a combination starter, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Streptococcus thermophilus* in making fermented milk. With faster growth, more sugar will be used both for growth and to form lactic acid, so sugar levels decline. As already known, bacterial activity will depend on conditions during fermentation. Basically, the milk fermentation process breaks down lactose into lactic acid and its components of aroma and flavor. Not only utilizing lactose in milk, bacteria can also use sugars as energy for metabolism [29]. The substrate used must contain carbon components, such as carbohydrates. Lactic acid bacteria will convert lactose of milk to lactic acid. The decrease in sugar levels is caused by the use of sugar as a fermentation media for *Lactobacillus plantarum* [43]. The higher the lactic acid bacteria activity, the lower the level of sugar produced.

### 3.7 Total Lactic Acid Bacteria

Total lactic acid bacteria in fermented milk with the addition of gembili flour was provided in Table 2. Results of various analysis, it shows that there was a significant difference with the addition of various concentrations of gembili flour ( $p < 0.01$ ) to the total lactic acid bacteria in fermented milk. The difference is due to

gembili flour which functions to increase lactic acid bacteria growth, in other words, as a prebiotic. Lactic acid bacteria have the ability to break down prebiotic compounds and is used as an energy source for breeding and metabolism.

The total lactic acid bacteria found in this study were ranged from 3.05 x 10<sup>7</sup> to 3.21 x 10<sup>8</sup> CFU/ml. This shows that the fermented milk with the addition of gembili has fulfilled the standard amount of lactic acid bacteria contained. This is in accordance with SNI (2009) that the number of bacteria in yogurt must meet the minimum standards of 10<sup>7</sup> CFU / ml or 7 log CFU / ml [44]. The amount of lactic acid bacteria contained in fermented milk can be influenced by several factors, such as growth media components, temperature during inoculation, fermentation time, storage time, storage temperature, substrate nutrient content and pH. Sources of nitrogen used for the growth of lactic acid bacteria are obtained from amino acids or peptides produced from the proteolysis process [45]. Gembili flour contains 5.9 g / 100 g protein, therefore the treatment group with the addition of gembili flour has a higher value than the control (P1). On the other hand, gembili flour also contains inulin and fructooligosaccharides, which can increase the number of lactic acid bacteria [8]. Boeni et al. (2012) reported that the addition of 2% inulin to yogurt can increase the amount of lactic acid bacteria [46]. Lactic acid bacteria are gram-positive bacteria that are anaerobic, do not form spores, and can ferment carbohydrates to produce lactic acid [47]. Fermentation of lactic acid bacteria produces organic acids such as propionic acid, glucuronic acid, folic acid, and lactic acid [48].

## 4. CONCLUSION

The addition of gembili flour to the fermented milk contributes to the quality of fermented milk

product which include pH, titratable acidity, viscosity, total phenolic content, crude fiber, sugar total, and total lactic acid bacteria. In conclusion, pH value ranged from 3.5-4.3; titratable acidity 1.98-2.35%; viscosity 849-1856 cps; sugar total 2.8-4.11 g/100g; total lactic acid bacteria  $3.05 \times 10^7$  -  $3.21 \times 10^8$  cfu/ml. Total phenolic and crude fiber in P1 was not detected, but in P2-P5 had total phenolic and crude fiber ranging from 108,58 – 670,75 mg/kg and 0.57-2.16 g/100 g, respectively.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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