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The Effect of Fermentation Time on Product Quality of Cowpea (*Vigna unguiculata L.Walp*) Soft Cheese with Starter Culture of *Lactobacillus plantarum* B1765

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ABSTRACT: This research was conducted to determine the effect of fermentation time on lactic acid bacteria (LAB), pH, total titrated acid (TTA) and organoleptic (taste, flavor, color and texture) of cowpea soft cheese (*Vigna unguiculata L.Walp*) with starter culture *Lactobacillus plantarum* B1765. *L.plantarum* B1765 had been studied has a probiotic characteristics. The fermentation time was 0, 6, 18 and 24 hours. The methods were total plate count (TPC) to measure the number of LAB, pH meter to measure pH, acid-base titration to measure TTA, and hedonic test for organoleptic. The results showed that the fermentation time affected the number of LAB, pH, TTA and organoleptic. The number of LAB increased by 3 log cycles from 8.7 x 10^6 CFU/mL to 5,3 x 10^9 CFU/mL. The pH value decreased from 6.53 to 3.69. The TTA value increased from 0.45% to 1.79%. The highest level of preference for taste, texture, color and flavor was at 18 hours of fermentation with a value of 3.17; 2.77; 2.70; 2.97 (likes). Cowpea soft cheese with starter *L.plantarum* B1765 could be improve potentially as vegetable cheese and probiotic agent.

KEYWORDS: Cowpea, *L. plantarum* B1765, Product Quality, Probiotic, Soft Cheese.

INTRODUCTION

Cheese is one of the functional foods made from milk, it is highly nutritious so cheese can fulfill our body's needs. However, some people cannot consume cow's milk cheese. In addition to the relatively expensive price, cheese made from cow's milk cannot be consumed by people with cholesterol in large quantities because the high fat content is around 20-25% [1]. In addition, people who are allergic to cow's milk and vegetarians also cannot consume cow's milk cheese. Therefore, an alternative is needed to replace the cow's milk. Plant as the basis for making cheese, which is commonly known as vegetable cheese could be an alternatif to solve the problems. Some research showed vegetable cheeses used nuts, red bean, soybean, and green bean as the basic ingredients of vegetable cheese [2], [3],[4]. The principle of cheese making is the clumping of soluble protein in food by denaturation of acids. These acids precipitate proteins from the basic ingredients of cheese, both in vegetables and animals.

In this research, the acid was obtained from the metabolism results of lactic acid bacteria. The metabolism results also affect the taste and texture of the vegetables cheese products [5]. Based on previous research, soy cheese was fermented by a combination of starter *Lactobacillus bulgaricus, Lactobacillus lactis* and *Lactobacillus paracasei spp* results a number of total LAB of 4,1 x 10^7 CFU/g [3]. Another research also examined peanut cheese fermented with *Lactobacillus rhamnosus* NCDC18 with a total LAB of 2.34 x 10^{10} CFU/mL [6]. So, it can be concluded that vegetable cheese can be produced using Lactobacillus strains.

Cowpea (*Vigna unguiculata L.Walp*) is an alternative type of beans which could be develop potentially, because it is an local Indonesian commodity, cheap and there are not many diversified products from this plant. Cowpea belongs to the Leguminoceae family. The potential yield of cowpea seeds is quite high, reach 1.5–2 tons/ha, depends on the variety, location, growing season and cultivation applied. In addition, cowpeas can be grown with manure and low nitrogen [7].

Currently, the only parts of cowpea used are beans, young pods and leaves for vegetables and fresh vegetables. In 100 grams, cowpeas contain 331 kcal of energy, 1.90 g of fat, 56.60 g of carbohydrates, 24.40 g of protein, 481 mg of calcium, 399 mg of phosphorus, and 1.60 g of dietary fiber [8].

The good potency of cowpeas and its relatively high protein content open the opportunities to develop these beans into vegetable cheeses. The carbohydrates, vitamins, and minerals can be a growth medium for lactic acid bacteria so that the pH of the medium can decrease. It is indispensable for coagulating cowpea protein into vegetable cheese. Cowpea antioxidants also add value to cowpea vegetable cheese.

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Lactobacillus plantarum B1765 had been studied has a probiotic bacterium characteristics including resistance to the gastrointestinal tract, namely pH 2-4, tolerance to bile salts, and tolerance to the generic amoxicillin clavulanate antibiotic tablet preparation at a concentration of 50 ppm because it is capable of producing lactamase enzymes [9]. So, cowpea cheese products inoculated with *L.plantarum* B1765 bacteria have the potential as probiotic vegetable cheeses.

Based on some research and the cowpea ingredients of this peanut potent to be processed into vegetable cheese and an alternative as a probiotic functional food. Therefore, this research examined the effect of the fermentation time of lactic acid bacteria *L.plantarum* B1765 as the starter culture on the quality of cowpea cheese. In this research, the variation of bacterial fermentation time of *L.plantarum* B1765 is expected to affect LAB, pH and Total Titrated Acid (TTA).

MATERIALS AND METHODS

Materials and Equipment

The equipment used in this research included blender (*Cosmos*), electric stove, pan, 200 mesh filter cloth, thermometer, autoclave (*Hirayama HVE-50*), micropipette (*D-LAB*), magnetic stirrer (*D-LAB*), centrifuge tube (*GP*), centrifuge (*Eppendorf 5810*), Incubator (*Memmert*), digital pH meter (*ATC*), stands and clamps, and glassware. The materials used in this research included cowpeas, skim milk (*Petit Eric*), salt, starter culture *Lactobacillus plantarum* B1765, MRS broth (*Merck*), NaCl (*PUDAK*), NaOH (*Merck*), $CaCO_3$ (*PUDAK Scientific*), phenolphthalein indicator (*Merck*), and aquademineral.

Methods

Preparation of Starter Culture L. plantarum B1765

1000µL *L.plantarum* B1765 isolate was inoculated in 9 mL MRS broth and incubated at 37°C for 24 hours. Furthermore, the growing culture was centrifuged for 5 minutes at 3500 rpm to be separated. The supernatant was decanted, and the pellet residue was suspended in 10 mL of a sterile 0.85% NaCl solution and then centrifuged. Next, the pellet was resuspended in 10 mL of sterile 0.85% NaCl solution for starter culture [10].

Preparation of Cowpea Cheese

The 100 g of cowpeas are washed and soaked for 24 hours. After that, the cowpeas are washed thoroughly and peeled. Then, cowpeas and aquademineral 1:4 (w/v) were mashed with a blender. After that, cowpea seeds are filtered to obtain cowpea extract. Cowpea extract and skim milk were pasteurized for 30 minutes and cooled to 37°C. Then, 5% inoculum was added to the cowpea extract. Cowpea extract was incubated for 0 hours, 6 hours, 18 hours, and 24 hours at 37°C. After fermentation, the cowpea extract was filtered to get the curd. 3% salt from the curd was added to the curd and stirred until homogeneous. The result was a cowpea analogue cheese paste. Cheese paste was packed in containers and stored in a refrigerator at 2°C (Modified Method [10]).

Enumeration of Total LAB

Enumeration of the Total LAB of cowpea cheese used the Total Plate Count (TPC) method. 1 mL of cowpea cheese sample was diluted in 0.85% NaCl solution to 10^{-1}-10^{-9}. Inucolation was done by adding 1 mL of the dilution results into a petri dish. Then, a mixture of MRS and 1% CaCO3 of ±15 mL was poured. The petri dish was moved in a circle so that the microbial cells evenly. Then, the media was allowed to stand to solidify. After that, the media was incubated upside down at 37°C for 48 hours. Growing colonies were counted as LAB [10]. Calculation of BAL is determined by the following formula:

$$\text{Total LAB (CFU/ml)} = \text{total colony x } \frac{1}{\text{dilution factor}}$$

pH and TTA

The chemical quality test of this research included measuring the pH value and total titrated acid. The pH test used a pH meter. The total titrated acid test was conducted by diluting a 10 mL sample in a 100 mL volumetric flask. Then, 20 mL of liquid was put into Erlenmeyer and 3 drops of phenolphthalein indicator were added. Next, the sample was titrated with 1N NaOH until it turned pink [10]. The value of the titrated acid was calculated by the formula:

$$\frac{\text{FP x ml NaOH titration x N NaOH x BM x 100\%}}{\text{W sampel}}$$

FP = dilution factor
N = normality
BM = lactic acid molecular weight

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W = sample weight (g)

Data analysis

LAB, pH and TTA data were processed using the IBM Statistics SPSS 25 program. LAB data was processed using the *One-Way ANOVA* test and to be continued with the *Post Hoc LSD* (Least Significant Difference) test. The pH and TTA data were processed using the *Kruskal-Wallis* test and the results were calculated using the *Mann-Whitney Post Hoc* test. Organoleptic data were processed using the *Kruskal-Wallis* test and the *Post Hoc Mann-Whitney* test as significant difference.

RESULT AND DISCUSSION

Total LAB, pH and TTA

The microbiological (LAB) and chemical (pH and TTA) quality tests results could be seen in Table 1.

Table 1. Total LAB, pH and TTA of Cowpea Cheese at certain fermentation time

Fermentation Time	Total LAB (CFU/mL)	pH	TTA (%)
0 hour	8,7 x 10^{6a}	6,53[a]	0,45[a]
6 hours	3,4 x 10^{8b}	5,51[b]	0,92[b]
18 hours	5,3 x 10^{9c}	3,71[c]	1,78[c]
24 hours	5,3 x 10^{9c}	3,69[c]	1,79[c]

Note: Letters a, b, c in the same column indicates a significant difference at the 5% level.

Based on the statistical results, the LAB data was normally distributed and homogeneous, so the data fulfilled for the *One-Way ANOVA* test. The *One-Way ANOVA* test results showed that the fermentation time had a significant effect ($p<0.05$) on the total LAB. *The Post Hoc LSD* test results showed that the fermentation time had a significant effect on the total LAB, however, fermentation time of 18 hours and 24 hours did not significantly affect the total LAB. The length of fermentation time increased total LAB. Increase in the number of LAB occurred until the 18 hours fermentation time reached 10^9 CFU/mL, increase of 3 log cycles from the initial conditions. The addition of fermentation time up to 24 hours showed insignificant results on the total LAB. Meanwhile, the statistical tests results of pH and TTA data were not normally distributed and homogeneous, so the *One-Way ANOVA* test was not conducted because the data did not fulfill the requirements. The pH and TTA data were processed using the *Kruskal-Wallis* test because the data fulfilled the requirements. The *Kruskal-Wallis* test results showed that the fermentation time had a significant effect ($p<0.05$) on pH and TTA. Then, the *Post Hoc Mann Whitney* test was conducted. The *Post Hoc Mann Whitney* test results showed that there were significant differences at each fermentation time ($p<0.05$). However, the fermentation time of 18 hours and 24 hours was not different significantly. The length of fermentation time increased TTA and lowered the pH value. The TTA increase until 18 hours of fermentation time reached 1,78%. The pH value decreased until the 18 hour of fermentation time reached 3,71. The addition of fermentation time up to 24 hours showed insignificant results on pH and TTA.

Organoleptic Test

Organoleptic data could be seen in Table 2.

Table 2. Cowpea Cheese Hedonic Test Results

Parameter	Hedonic Test Mean Value		
	6 Hours	18 Hours	24 Hours
Taste	2.80 ± 0.714[a]	3.17 ± 0.648[b]	3.27 ± 0.740[b]
Texture	2.50 ± 0.682[a]	2.77 ± 0.774[a]	2.40 ± 0.814[a]
Color	2.33 ± 0.606[a]	2.70 ± 0.651[a]	2.40 ± 0.724[a]
Flavor	2.93 ± 0.583[a]	2.97 ± 0.414[a]	2.93 ± 0.583[a]

Description: 1= really dislike; 2= dislike; 3= like; 4= really like

Note: Letters a, b in the same column indicates a significant difference at the 5% level.

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The *Kruskal-Wallis* test results showed that the fermentation time had a significant effect ($p<0.05$) on the taste parameters. However, the fermentation time had no significant effect ($p>0.05$) on the texture, color and flavor. Then, the *Mann-Whitney* test was conducted on the taste parameters. The *Mann-Whitney* test results showed that there was a significant difference ($p<0.05$) in the fermentation time between 6 and 18 hours as well as 6 and 24 hours. However, there was no significant difference between 18 hours and 24 hours of fermentation time ($p>0.05$). The average level of preference for taste, texture, color and flavor, respectively, was 3.27; 2.77; 2.70; 2.97 in the like category.

Discussion

The corelation of total LAB, pH and TTA tests on cowpea cheese with fermentation times of 0, 6, 18 and 24 hours could be seen in Figure 1.

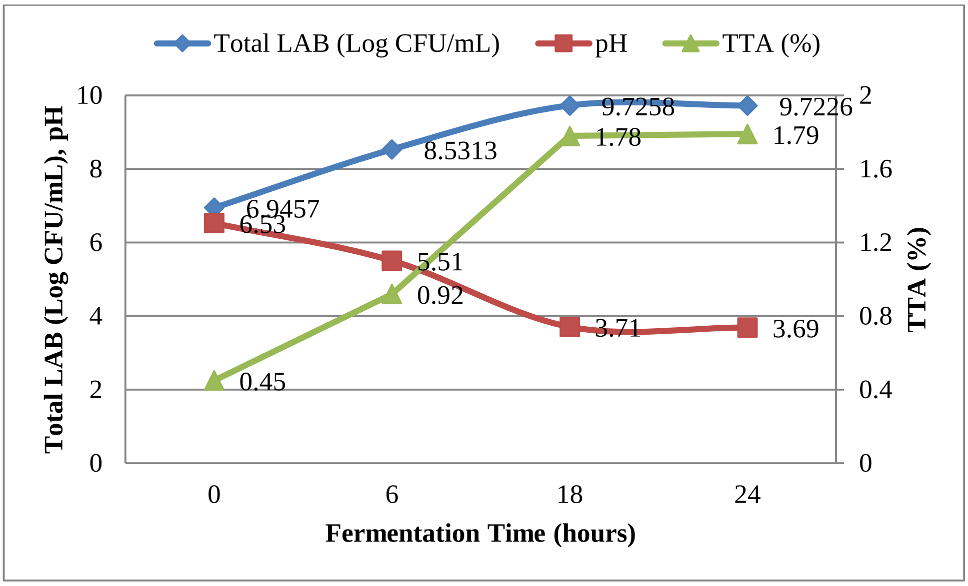

Figure 1. Graph of the correlation among total LAB, pH and TTA

Based on Figure 1., the length of fermentation time increased total LAB and TTA and lowered the pH value. Based on [11] research, increasing of fermentation time will increased the number of LAB. It was caused the fermentation time affected the proliferation of microorganisms so the microbes split glucose and produced primary metabolites (acid, lactic) and secondary metabolites (antibacterial activity and polyphenols) increased [11]. The increase of total LAB was due to the availability of nutrients needed by *L. plantarum* B1765. Carbohydrates of cowpea are metabolized by *L.plantarum* B1765 into lactic acid. LAB in cowpea cheese increased up to 3 log cycles from initial during 18 hours of fermentation. The minimum average amount of LAB was 8,7 x 10^6 CFU/ mL at 0 hours of fermentation, and the maximum average was 5,3 x 10^9 CFU/mL at 18 hours, and showed not different significantly with 24 hours of fermentation. Based ISO 19344:2015 standard, the minimum probiotic product is 10^6 CFU/g or CFU/mL [12], so the cowpea cheese product in this research could be a probiotic agent.

The length of fermentation time decreased the pH value from 6.53 to 3.69 as shown in Figure 1. The pH of this research had a significant difference. However, 18 hours and 24 hours did not have significant difference. The pH value of this research was lower than the SNI standard for processed cheese food products, which is around 4.1-5.3 [13]. The research resulted pH 5.51 for with 6 hours of fermentation, which is close to the standard. However, the fermentation time reached 3.71. In research [2], the pH of red bean cheese with starter culture of *L. Acidiophillus* at 6 hours of fermentation was 4.853. In the same fermentation time, the pH of the research [2] was lower than this research because the difference of LAB and the concentration of LAB. In the peanut cheese inoculated with *L.rhanmosus* starter the pH decreased from 5.3 to 4.8 after 18 hours of fermentation and [6]. Cowpea cheese in this research has a lower pH than in peanut cheese [6]. It was caused the LAB concentration used in cowpea cheese was lower than in peanut cheese. Another research showed that the pH value of vegetable cheese from soybeans powder and fresh soybeans added with 5% inoculum and fermented with BAL isolated from curd for 6 hours generated pH of 6 and 5.5, respectively [1]. The pH

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value of the research [1] was higher than this research even though both of them had the same concentration and fermentation time. It was because the types of LAB were different.The decrease of pH in cowpea cheese was caused by the activity of LAB *L.plantarum* B1765 during the fermentation process, in which glucose was metabolized into organic acids so the pH of the substrate decreased.

As the total LAB increased and the pH decreased, the TTA increased. In Figure 1, the TTA value increased from 0.45% at 0 hour to 1.79% at 24 hours. In previous research [2], the TTA value of red bean cheese with 20% *L. Acidiophillus* and fermentation time of 6 hours was 1.52%. Another research showed that the TTA of vegetable cheese made from powdered soybeans and fresh soybeans added with 5% with BAL isolated from curd and fermented for 6 hours was 0.4 and 0.2 respectively [1]. The TTA value of the research [1] was lower than this research. It was because the types of LAB were different. The increase of TTA value at each fermentation time was because LAB metabolized to produce lactic acid as a primary metabolic product and continued to increase with LAB growth rate. The addition of lactic acid from carbohydrate metabolism increased acidity and lowered pH [3].

Hedonic test results could be seen in Figure 2.

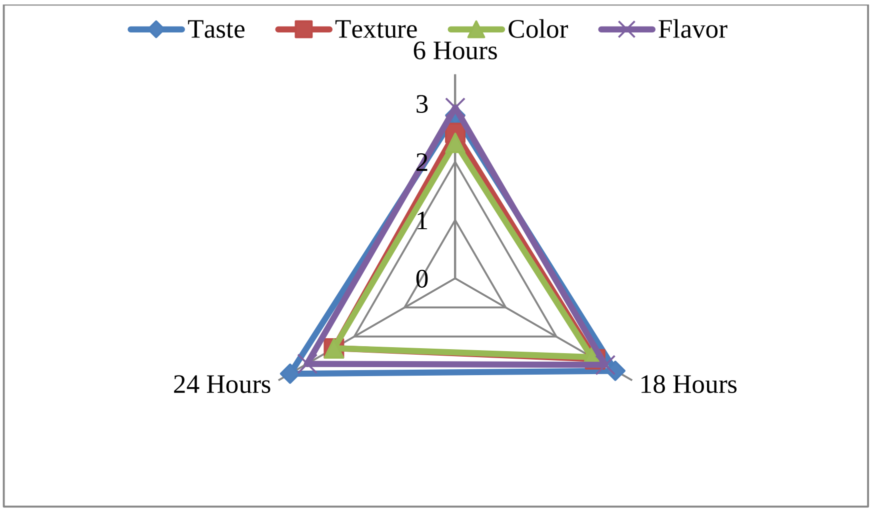

Figure 2. Cowpea Cheese Hedonic Test Results

Figure 2 showed the overall test result of hedonic test of the cowpea cheese used nontrained panelists for, cowpea cheese with a fermentation time of 18 hours, the best fermentation time for chemical and microbiological aspect. The long fermentation time generated the sour taste of cowpea cheese. It was related to the decreasing pH value. In addition, there was an increase of total BAL and TTA. The sour taste of cheese comes from the carbohydrates metabolized by *L. plantarum* B1765. The most preferred taste by panelists was the 24 hours of fermentation time in which the pH of 3.27 with the like category. This pH value lower than pH standard for cheese product, because fermented cheese like in this research has a different taste and flavor characteristics.

Fermentation time did not show a significant difference in texture with a preference level of 2.40–2.77 and at dislike-likes category. It was because of the panelists perception of the dense form of cheese. In fact, the fermentation time affected the texture as shown in Figure 3.

Figure 3. Cowpea Cheese

Figure 3 showed that the texture of cowpea cheese at a long fermentation time was thick (solid). At 6 hours of fermentation, the texture of cowpea cheese was runny. Then, the texture of cowpea cheese at 18 and 24 hours of fermentation was thick. It was because the long fermentation time lowered the pH value. The decrease of pH caused the cowpea extract protein to coagulate when it reached the isoelectric point. When the pH of cowpea cheese reached the isoelectric point, the protein denatured, clumped and separated into curd and whey [2]. The isoelectric point is the condition of the protein molecules in cowpea extract with a zero charge. When the bacteria *L.plantarum* B1765 produced lactic acid, the pH of cowpea cheese decreased and approached the isoelectric pH. When it reached the isoelectric point, the protein solubility of cowpea cheese decreased, agglomerated and formed a [14]. Cowpeas contain high protein which is around 24.4% [8]. Cowpea proteins include albumin and globulin dang lutein. The research results [15] showed that the minimum solubility pH of cowpea protein was albumin protein and globulin dang lutein, respectively, was pH 4.0; 4.2; 5.0 and maximum pH was 10, 12 and 10. According to the pH value and texture of this research, the texture was thick (solid) when approaching the isoelectric point it is thick (solid). It was because the protein solubility of cowpea cheese decreased [14].

The long fermentation time caused the cheese to be more pungent. The flavor of cowpea cheese was due to other organic acids and volatile ingredients from bacterial fermentation. So, the flavor of cheese stinged [16]. Flavor at 24 hours had the most pungent flavor.

Cowpea cheese in this research was yellowish white. This was happened because there was a heating process during the pasteurization of cowpea extract. It was due to non-enzymatic reactions (Maillard) during the pasteurization. The Maillard reaction was a browning reaction because reducing sugars and compounds containing an amine group (amino acids, proteins, peptides and ammonium) react [17]. During the fermentation process, the color of the cowpea cheese remains stable.

Apart from organoleptic data, the best fermentation time was also seen from the microbiological and chemical quality. The microbiological quality of cowpea cheese at 18 hours of fermentation generated a total LAB of 5,3 x 10^9 CFU/mL. These results fulfilled the standards of probiotic products [12]. Meanwhile, the chemical quality of cowpea cheese at 18 hours of fermentation generated a pH of 3.71 and a TTA of 1.78%. The pH value was close to the SNI pH standard for processed cheese products [13].

CONCLUSION

Based on the data, it can be concluded that the long fermentation time affects the microbiological, chemical and organoleptic qualities. The best time for cowpea cheese fermentation is 18 hours with a total LAB of 5,3 x 10^9 CFU/mL, pH value of 3.71 and TTA of 1.78%. The level of preference for taste, texture, color and flavor, respectively, is 3.17; 2.77; 2.70; 2.97.

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