The effect of concentration of Ceremai (*Phyllanthus Acidus*) Fruit extract on FH (Friesh Holland) cattle milk clumping in cheese making

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**Abstract.** Most of the milk consumed by humans comes from cow's milk. The processed products are cheese as a result of the separation between whey and curd through the fermentation process. This process involves microbial activity that takes place in aerobes and anaerobes. Making cheese involves the process of clumping milk with helping of the rennet enzyme. There are difficult to obtain and the price is relatively expensive. So that a replacement for this enzyme is needed. Ceremai fruit extract can be used in the process of milk clotting. The research aims to find the concentration of it which optimally influences the clotting process of FH (Friesh Holland) cow milk in cheese making from the time of clumping. It is obtained by blending until smooth, then poured into a filter and squeezed to get extract. The extract was used for 5 treatments with a concentration variation of 50%, 60%, 70%, 80%, and 90% with 5 repetitions. The results showed at the concentration of 90% produces the optimal clumping time with an average clumping time of 5.4 minutes. So influences the time of clumping of cow's milk in making cheese and it can be used as an alternative ingredient for rennet enzymes.

1. Introduction

Milk is included in the additional nutrients needed by humans for their metabolic processes. Milk has become a daily drink for humans so milk needs are increasing. In Indonesia, milk comes from cow's milk and soy milk. But most of the most consumed milk comes from cow's milk. Cow's milk has high nutritional content. Among them, cow's milk contains: 3-6% fat in the form of triacil glycerol (98%), phenolic compounds, and sulfonamide antibacterials [1,2]. So that cow's milk is supplying fat to the body. However, the nature of milk is easily damaged because it is easily contaminated by bacteria, and when bacterial growth is uncontrolled in milk it will cause damage to milk, and pathogenic bacteria will cause food infections, thereby reducing the quality and nutrients contained in milk. So that it requires good handling and processing so that milk can be stored in a long and long lasting period. One way is the fermentation process. Fermentation is the process by which biomass will be converted into a product such as alcohol (ethanol, butanol), lactic acid, biogas, biohydrogen, volatile fatty acid (butyric acid, hexanoic acid) and amino acids [3-8]. Amino acids serve as precursors for the enzymes involved in making cheese. The fermentation process occurs aerobically and anaerobically by involving bacteria. The bacteria that are often involved are lactic acid bacteria and probiotic which play a role in the production of cheese, yogurt, and milk butter [9,10]. One of the fermentation processes is the separation of Whey and Curd which involves the enzyme so that the milk becomes lumpy and forms cheese. The
enzyme that is often used is the Rennet enzyme. The Rennet enzyme is a coagulant in cheese making because it contains protease enzyme [11,12]. This enzyme is relatively difficult to obtain and the price is expensive, so it is necessary to replace the enzyme in the process of clumping milk into cheese.

In this research, ceremai fruit extract was used as a coagulant in the process. Based on previous research showing that ceremai juice (Phyllanthus Acidus), citrus fruits and most of the fruits contains ascorbic acid [13-15]. This acid is a type of carboxylic acid which can cause coagulation of colloidal rubber [15]. In addition, from the results of the study, it is expected that the ceremai fruit can be used as an alternative coagulant substance, especially in making cheese from milk so that the community becomes more effective in its use.

2. Methods
To find out the effect of the concentration of ceremai fruit extract (Phyllanthus Acidus) on the time of clumping of FH (Friesh Holland) cow milk, several stages of the research procedure were carried out, namely:

1) Sample Preparation Stage.
   Before the pasteurization process, cow's milk is filtered first. Then, cow's milk is put into a 5-liter pan. Pasteurization was carried out for 5 - 10 minutes at 65 - 75°C while stirring. After the process is complete, cow milk is left until the temperature reaches 35°C. Then put the starter as much as 0.5% while stirring, after being evenly mixed just let stand for 3 hours.

2) Making Ceremai (Phyllanthus Acidus) Fruit Extract.

3) Ceremai (Phyllanthus Acidus) fruit is washed thoroughly and dry.
   The Ceremai fruit is put into the blander while the aquadest is added until the ceremai is submerged. After that, dilution is done until smooth. Then filtering 3 times to get extract from the ceremai in the form of a solution (fruit juice). The solution is recorded for volume. The volume of the ceremai (Phyllanthus Acidus) fruit extract solution is used as the basis for calculation. Assuming the content of the ceremai in a solution of 100% (v/v). The extract is diluted according to the concentration variation in each repetition using a dilution formula, namely: P1 x V1 = P2 x V2 [16].

2.1. Determination of optimal concentration on the time of clumping of FH (Friesh Holland) cow milk
The solution for the variation in concentration of ceremai (Phyllanthus Acidus) fruit extract was added to cow's milk (pasteurized results) which had been placed in a beaker glass with a volume of 100 mL for each treatment. Then stir until evenly distributed. Crushing is stopped when cow's milk has seen the cheese clots or the coagulation process has been reached. After that, recording the coagulation time for each treatment was carried out.

The flow of research starts from the preparation stage to the stage of determining the optimum concentration against the time of clumping of cow's milk type FH as follows:
3. Results and discussion
The extract used in this research is a ceremai (*Phyllanthus Acidus*) fruit extract in the form of a solution (fruit juice). This solution was obtained from the result of the dilution of the ceremai which was added by aquadest to produce fruit juice (extract) of the ceremai (*Phyllanthus acidus*) fruit. The results are filtered 3 times. This extract is used as a substance added to cow’s milk as a substitute for Rennet enzyme. Where it functions as a coagulant. The addition of this solution is based on the concentration variations that have been determined for each treatment, namely: 50%, 60%, 70%, 80%, and 90%. After adding the solution, it was then determined the timing of clumping of cow’s milk on each variation of concentration added. The data generated is in the form of the average clotting speed time (Table 1):
Table 1. Data on the clumping of FH (Friesh Holland) cow milk in cheese making.

| Repetition | Time Calculation Results Clumping of FH (Friesh Holland) Cow Milk in Cheese Making (minutes) |
|------------|--------------------------------------------------------------------------------------------------|
|            | C1 (50%) | C2 (60%) | C3 (70%) | C4 (80%) | C5 (90%) |
| 1          | 15       | 12       | 10       | 8        | 7        |
| 2          | 15       | 12       | 10       | 8        | 5        |
| 3          | 15       | 13       | 12       | 9        | 5        |
| 4          | 18       | 15       | 11       | 9        | 5        |
| 5          | 19       | 15       | 10       | 9        | 5        |
| Total      | 82       | 67       | 53       | 43       | 27       |
| Average    | 16.4     | 13.4     | 10.6     | 8.6      | 5.4      |

Information: C1 - C5 : Treatment  
1 – 5 : Repetition

From the table, the data shows that at the concentration of ceremai fruit extract 50%, the time to clot is longer (16.4 minutes). While the concentration of ceremai fruit extract was 90%, the clotting time was faster (5.4 minutes) compared to the time of clumping at the concentration of other ceremai fruit extracts. So that it can be concluded that for the optimum concentration of ceremai (Phyllanthus Acidus) fruit extract the most influential to the time of coagulation is the concentration of ceremai fruit extract 90%. Ceremai (Phyllanthus Acidus) fruit contains vitamin C or ascorbic acid. Ascorbic acid functions as a colloidal coagulant in latex [15]. This acid belongs to the carboxylate acid which has a carboxylic group which decomposes into H\(^+\) and COO\(^-\) ions. H\(^+\) ions will bind fat compounds in cow milk samples so that they will clump and separate into two phases, namely solid phase (fat) and liquid phase (water) [17,18]. In this study it was proven that when the concentration of ascorbic acid in the ceremai fruit extract was high, the process of coagulation of cow's milk was rapid.

In the concept of chemistry, when a chemical process takes place and produces a product, a chemical reaction has occurred. There are chemical reactions that take place quickly and slowly depending on the rate or speed of the reaction. The reaction speed is very influential on the chemical reaction process. The process of clumping cow's milk into cheese is an example of a chemical reaction. Factors that affect the rate or speed of a chemical reaction include concentration and temperature [19]. When the concentration of a substance added to a sample is high, the speed of its chemical reaction will be rapid and produce a product quickly too. From the results of the research it appears that when the concentration of 90% ceremai fruit extract is added to cow's milk (sample), the clotting process takes place faster than the addition of low concentrations. Other factors are temperature. When high temperatures of the molecules in the sample (cow's milk) move quickly to release each other's bonds. As a result, the reaction speed is fast. In this research, the fermentation temperature took place at high temperatures (65–75°C), so that the coagulation process took place quickly.

The influence diagram of the variation of the concentration of ceremai (Phyllanthus Acidus) fruit extract on the speed of clumping of cow milk type FH (Friesh Holland) in cheese making is shown in Figure 2.
Figure 2. The Effect of Concentration Variation of Ceremai (Phyllanthus Acidus) Fruit Extract on the Time of Clumping of FH Cow Milk (Friesh Holland).

To see how much influence the concentration of ceremai (Phyllanthus Acidus) fruit extract has on the speed of clumping of cow's milk type FH (Friesh Holland), a statistical test is performed. The statistical test used was the normality test and the Kruskal Wallis test [20]. From the results of the normality test for each concentration variation data (treatment) the following data are obtained (Table 2):

| Test of Normality | Shapiro-Wilk | Result |
|------------------|--------------|--------|
| Treatment        | Sig.         |        |
| 1                | 0.032        | Not normal |
| 2                | 0.086        | Normal |
| 3                | 0.046        | Not Normal |
| 4                | 0.006        | Not Normal |
| 5                | 0.000        | Not Normal |

From the table, it is found that for treatment 2 the data is normal. Statistically, the data is caused by the results of calculations, where the Sig value is obtained = 0.086 with α = 0.05. Because the value of Sig. (0.08) ≥ α (0.05), the distribution of data is normally distributed so that the speed of milk clotting in cheese making in the second treatment (concentration 60%) can be concluded to be normally distributed.

After the normality test, the hypothesis is tested to see whether or not there is an effect of the concentration of ceremai (Phyllanthus Acidus) fruit extract on the speed of clumping of FH (Friesh Holland) cow milk. The hypothesis test used is the Kruskal Wallis with the criteria, if the value of Sig. ≥ α then Ho is accepted and Ha is rejected, if the value is Sig. ≤ α then Ho is rejected and Ha is accepted. From the calculation results obtained the following data (Table 3):

| Test Statistics<sup>a,b</sup> |
|-------------------------------|
| Treatment                     |
| Chi-Square                     | 22,767 |
| df                             | 4      |
| Asymp. Sig.                    | 0,000  |
| a. Kruskal Wallis Test         |
| b. Grouping Variable: Group    |

Table 3. Data from the calculation of the Kruskal Wallis test.
Based on table 3 it is known that the significance (Asymp Sig) is 0.000. Because of significance (0.000) <0.05, Ho is rejected. So, it can be concluded that there is an effect of the concentration of the cermai (Phyllanthus Acidus) fruit extract on the speed of clumping of cow’s milk in the type of FH (Fries Holland) in making cheese.

Based on the results of the research obtained, the ceremai fruit extract (Phyllanthus Acidus) can be used as an alternative material to agglomerate milk in the cheese making process.

4. Conclusion
Based on the results of the research and discussion described above, it can be concluded as follows: 1). Each variation of the concentration given has an effect on the clumping of FH (Fries Holland) cow’s milk in making cheese 2). Concentration of the ceremai (Phyllanthus Acidus) fruit extract optimum on the clumping of FH (Fries Holland) cow milk in cheese making was at a concentration of 90% with the acquisition of an average clumping time of 5.4 minutes.

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