Papain Enzyme and Lemon as Coagulants in Cottage Cheese

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Abstract. Cottage cheese is one type of cheese that is processed without ripening, which is made from milk coagulated using lactic acid bacteria or rennet enzyme. Another alternative as a substitute for rennet is the papain enzyme and lemon juice. This study aimed to access the influence of enzymes concentration and pasteurization temperature to the yield and quality of cottage cheese while lemon juice as fixed variable. Papain enzymes are isolated from immature papaya fruit sap, mixed with sulfite, the emulsion of gum is dried using vacuum oven to become crude, and lemon juice obtained from ripe lemon fruit. Cottage cheese made by pasteurization of fresh cow milk, addition papain enzyme and lemon juice, stirring, and the curd is separated. Analysis products are included the percentage of curd, lactic acid, protein content, water content and texture. The best results showed that from 200 ml of fresh cow milk obtained at 0.5% enzymes and 50°C with a weight of curd 30.16%, lactic acid 0.16%, dissolved protein concentrations 375.61 mg/l, and 5.33 g in texture.

1. Introduction

In general, cheese is made from cow milk, but can also be made from other types of milk. Known for several types of cheese based on water content and hardness, one of them is soft cheese without ripening like cottage cheese [1].

The majority of cheeses are made from heat-treated milk or pasteurized milk both full, low fat, and nonfat and non-pasteurized milk [2]. Pasteurization is a way to inhibit bacterial growth quickly without affecting the taste of food and drink [3].

Almost all the cheese marketed in Indonesia is hard cheese, that requires a longer maturation stage so that the production costs are higher. Cottage cheese with a short setting method is one alternative in reducing production costs [4].

This time Indonesia still import rennet enzymes from countries in the Continent of Europe, so it is necessary to find an alternative use of the enzyme rennet in making cheese to reduce the cost of cheese production. Alternative substitute rennet enzyme is the use of the enzyme papain which can make a big contribution to the development of processed cheese products. The papain enzyme can be used as a milk protein clotting material in the process of making cheese.

The papain is a proteolytic enzyme that can be obtained from papaya leaves and immature papaya fruit sap and is able to agglomerate protein, be resistant to temperature, and in low concentrations can function properly [5]. It is free of chemicals, easy to obtain, self-made, non-toxic, and no side reactions [6].

Lemon fruit contains acids which play a role in the formation of sour fruit, a source of vitamin C and antioxidants that are beneficial for human health. Acid at sufficient concentration can cause protein damage called denaturation. Because of microbial cells are formed from proteins, the...
administration of acid in a milk processing process will inhibit the growth of other types of microbes in milk [7].

This research aims to study the possibility of utilizing the papain enzyme and lemon juice as a milk coagulant while knowing the effect of the addition of papain enzyme concentrations and the temperature of pasteurization on cheese cottages product.

2. Materials and Methods

2.1 Crude Papain

Crude papain is obtained by scraping immature papaya skin. The tapping sap is mixed with 0.7% sulfite solution 4 times the amount of sap, stir it evenly with a mixer. This mixture will usually form a thick, milky white emulsion of gum. Furthermore, the emulsion of gum is dried using vacuum oven at 55 °C to become crude papain.

2.2 Cheese Cottage

Fresh cow milk is pasteurized at (50, 60, and 70) °C for 30 minutes. Added the papain enzyme concentration (0.35, 0.40, 0.45, and 0.50) % into pasteurized milk, 15% lemon juice, stirred until it is lumpy, added 1% salt then separated the whey and the curd using gauze. The curd is inserted into the mold, stored in the refrigerator at 10 °C before analyzed.

2.3 Product Analysis
The percentage of curd calculated from the weight of curd divided by the volume of milk. Lactic acid is determined with an acid-base titration method using 0.1 N NaOH and phenolphthalein as the indicator. Determination of dissolved protein levels in cottage cheese using the Lowry method by UV/Vis Spectrophotometer. The Bovine Serum Albumin standard solution obtained standard curve equation \( y = 0.00471x + 0.01909 \). The texture of product analysis by Texture analyzer Brookfield. The procedure flow diagram is shown in Figure 1.

3. Results and Discussion

The purpose of pasteurizing milk is to kill pathogenic bacteria that can interfere with the production and quality of cheese. After pasteurization, the enzyme papain was added with various concentrations, namely 0.35%, 0.40%, 0.45% and 0.50%, and added 15% lemon juice after it was stirred to form a lump (curd). The curd is a part of milk that is coagulated after added coagulant so it will form cheese. The part that does not settle is called a slightly yellowish milk liquid (whey). Then the curd is separated by filtering and then added salt. The purpose of adding salt is as a flavor enhancer and preservative.

3.1 The Percentage of Curd

The percentage of curd is a parameter to find out the amount of curd formed after casein milk is coagulated. From the results of observations and calculations obtained data as seen in Figure 2. It can be seen that the highest percentage of curd is produced by adding the papain enzyme with a concentration of 0.5% at a temperature of 50°C pasteurization which is 30.16%. While the lowest percentage of curd was obtained by adding papain enzyme 0.35% at pasteurization temperature 60°C. This shows that the more concentration of the enzyme given, the more curd optimal clumping process occurs because the enzyme activity is quite good. Low enzyme concentration will result in enzyme activity not optimal for clotting reactions.

![Figure 2. Percentage of Curd in various concentration enzyme and temperature pasteurization](image)

The available substrate is inadequate with the needs of the activity of the enzymes. In addition, the addition of too many enzymes will also create a taste that consumers don't like (bitter).

3.2 Percentage of Lactic Acid

Lactic acid bacteria play a role in degrading lactose milk into organic acids (lactate and acetate) which causes a decrease in pH. Based on the results of the study, the amount of lactic acid in cottage cheese was made with four enzyme concentrations, which can be seen in Figure 3.

From Figure 3 it can be seen that the highest levels of lactic acid are found in cheese with 0.5% enzyme concentration and 50 °C pasteurization temperature which is 0.1638% and the lowest lactic
The acid content is in cheese with 0.35% enzyme concentration and pasteurization temperature 70 °C which is 0.0378%. This shows that the more concentration of the enzyme is added, the higher the lactic acid level. The higher the level of lactic acid, the better the cottage cheese produced because lactic acid is preservative.

![Graph](image_url)

Figure 3. Percentage of Lactic Acid in various concentration enzyme and temperature pasteurization

### 3.3 Protein levels by method Lowry

The concentration of enzymes is very influential on the levels of protein produced products. Protein content analysis is done by method Lowry. The results of the analysis show that the concentration of the papain enzyme affects the levels of the protein produced. This can be seen in Figure 4.

![Graph](image_url)

Figure 4. Protein content in various concentration enzyme and temperature pasteurization
The results of the analysis show that based on the addition of the enzyme papain concentration crude protein content of the largest cottage cheese obtained from the addition of enzymes 0.5% that is equal to 375,605 mg/l. Whereas the smallest protein content of cottage cheese was obtained from the addition of the papain enzyme concentration 0.4% ie 201.507 mg/l.

Addition of the enzyme papain as a biocatalyst affects the protein content because of papain is a protease. Protease degrades proteins in a way 18 hydrolyze peptide bonds.

The optimal clumping process occurs when the activity in the clumping process is good enough, this occurs when the amount of enzyme is sufficient for the reaction and the media for enzyme activity is sufficient. If the amount of enzyme is lacking, enzyme activity is lacking for the occurrence of clumping reactions, on the other hand, if too many enzymes are added, chances are the available media are inadequate with the needs of existing enzyme activity. Protein levels in the product decrease in the addition of excessive enzymes. In addition, the addition of an enzyme too much will cause a bitter taste.

3.4 Texture

Tests are carried out using the Texture analyzer test tool. The way it works from Texture analyzer is by pressing or pulling the sample, through an appropriate probe with the desired application. Data taken from each sample treatment was carried out three times repetition and taken the average value. The data observed included the value of Peak Load (compressive strength on the surface of cottage cheese). From observations as seen in figure 5 data obtained for texture.

![Figure 5. compressive strength on the surface of cottage cheese](image)

From Figure 5 it can be seen that the texture of the compressive strength on the surface of cottage cheese with the addition of enzymes at a concentration of 0.35% and a reaction temperature of 50 °C is greater than at concentrations of 0.40%, 0.45% and 0.50%. This is because the cottage cheese curd with an enzyme concentration of 0.35%, the texture tends to be rather hard and the water content is less than the other concentrations.

While the lowest Peak Load value is produced from cheese with the addition of the papain enzyme at a concentration of 0.50% and a reaction temperature of 50 °C which is 8.2 grams. This shows that cottage cheese with an enzyme concentration of 0.50% has a smooth texture and more water content compared to the concentration and temperature of other reactions.

3.5 Water Content Percentage

A total of 2 grams of sample was put into a porcelain dish whose weight was known and put into the oven at 105°C for 3 hours until the weight was constant. The sample is cooled in a desiccator, then weighed until a constant weight is obtained. From the results of observations and calculations obtained data as seen in Figure 6.
From Figure 6 it can be seen that the water content in cottage cheese with the addition of enzymes at a concentration of 0.35% and a reaction temperature of 50 °C is less than at concentrations of 0.40%, 0.45% and 0.50%. This shows that cottage cheese is hard and not soft.

While the highest water content is found in cottage cheese with the addition of the papain enzyme at a concentration of 0.5% and a reaction temperature of 70 °C which is 76%. This shows that cottage cheese is smooth and not hard compared to the concentration and temperature of other reactions. Characteristics of cottage cheese according to the United States Department of Agriculture (2001). Mention that good cottage cheese has a fine texture range, not like flour, not sticky and not runny.

4. Conclusion

The mixture of the enzyme papain and lemon juice used has the ability to agglomerate milk casein in the process of making cottage cheese. The most curd is produced in cottage cheese with the addition of papain enzyme concentration of 0.5% and at a temperature of 50 °C pasteurization which is 30.16%. The highest levels of lactic acid were found in cheese with an enzyme concentration of 0.5% and a reaction temperature of 50 °C which was 0.1638%. The lowest Peak Load value is produced from cheese with the addition of the papain enzyme at a concentration of 0.50% and a reaction temperature of 50 °C which is 8.2 grams showing a smooth texture. The highest water content is found in cottage cheese with the addition of the papain enzyme at a concentration of 0.5% and a reaction temperature of 70 °C at 76%.

References

[1] Betti. et al., 2013. Utilization of Papain Enzymes from Papaya Fruit Sap (Carica Papaya L) in Making Cottage Cheese Using Bacteria Lactobacillus Bulgricus. Chemistry Study Program, Faculty MIPA, Tanjungpura University.

[2] Egrina G. And FM titin S., 2010, Utilization of Papain Rough Extracts as Coagulants in Making Cottage Cheese Using Bacteria, Journal of Chemical Science and Technology, Vol. 1, No. 1, things: 38-43, ISSN 2087-7412.

[3] Fachraniah, 2002. Making Pepton From Soybean Meal And Beer Waste With Enzymes Papain for Media Bacterial growth. Postgraduate Program in Arjana, Bogor Agricultural University.

[4] Murni Yuniwati. et al 2008, Utilization of the Papain Enzyme as a Clot in Making Cheese. Chemical Engineering Department, FTI, AKPRIND IST Yogyakarta.

[5] Bogor Agricultural Institute (1994) in Ullyl Mi’raj Nizhar (2012), LevelOptimum SariBuahLemon (citruslimon) As Clumping Material in Formation of Cheese Cage.

[6] Malacca. R, 2010, Introduction to Milk Technology, Masagena Press, Makassar.

[7] Nurhidayati, T, 2003, Effect of Concentration of Papain Enzymes and Temperature of Fermentation on Quality of Cheese Cage.
[8] Aline P.K and Rita P. D, 2005, *Determination of Optimum Condition of Papain enzyme From Papaya Var Java (Carica Papaya)*, Indo. J. Chem., Vol. 5, No. 2, hal: 147-151.

[9] Daulay, D. 1991. Fermentasi Keju. Pusat Antar Universitas Pangan & Gizi Institut Pertanian Bogor. Bogor.

[10] Sulistyorini. 2010. *Fermentasi enzim rennet*. http://ebookpangan.com/Artikel/pembuatan%20ekstrak%20dan%20enzim%20rennet.pdf. [23 Januari 2014].

[11] Sukmasari, D.D. 2009. *Pengaruh penggunaan getah pepaya dan sari buah markisa terhadap warna dan konsistensi curd sebagai bakal keju*. http://princessrainblog.blogspot.com/. Diakses [23 Januari 2014].