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Optimization of Papain Enzyme Utilization in Non-Rennet Cream Cheese Production

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Abstract: In Indonesia, the method of cheese making by using papain enzyme which is obtained from papaya fruit has already been practiced since the Dutch colonial era, especially in Enrekang, regency at The South Sulawesi province. The product, *Dangke*, is a type of semi-soft cheese with notable bitter taste. This research aims to develop new cream cheese product based on papain-coagulated cheese with an acceptable appearance, aroma, texture and taste, made from fresh milk and commercially pasteurized milk. To reduce the bitterness, $CaCl_2$ were introduced to the formula to help the curd formation process with less papain enzyme addition. The unit of enzyme used was quantified using casein-tyrosine method with spectrophotometer analysis at 578 nm. The minimum enzyme capable of curd formation was $3.76x10^{-3}$ mol/s. Formula optimization was conducted with the variation of enzyme unit of $3.76x10^{-3}$, $7.52x10^{-3}$, and $11.28x10^{-3}$ mol/s and $CaCl_2$ addition of 0.25, 0.50, and 0.75 g for 1 L of milk. Full Factorial Design was used and curd quality and cream cheese viscosity were determined as responses. Optimization using Design Expert to cheese viscosity between 50,000-56,750 cP using less papain gives optimum formula of enzyme with unit of $3.76x10^{-3}$ mol/s and $CaCl_2$ addition of 0.5 g, for both type of milk. Cream cheese developed using the formula was tested for sensory evaluation. 54 untrained panelists gives score for hedonic test and cheese made from fresh milk was in general gives higher scores in term of appearance (6.80), aroma (6.39), taste and texture (6.63) and overall acceptance (6.65) compared to cream cheese made from commercially pasteurized milk, with the score for appearance of 6.06, score for aroma of 6.07, score for taste and texture of 5.52, and overall acceptance of 5.67. The cream cheese product developed in this research shows potential for further development as non-rennet based cheese, especially for utilization of papain.

Keywords: Papain enzyme, *Dangke*, cream cheese, viscosity

1. Introduction

The essential ingredients in cheese making are milk and protein coagulant. Milk coagulation is the primary step in the development of the texture and flavor of cheeses (Ozcan & Kurdal 2012). It depends on specific enzymatic proteolytic degradation of milk compounds especially protein in order to improve textural properties and the nutritional value of cheeses. Most cheese production uses rennet as the coagulant. There are others coagulating agents which can be used to make cheese. In Indonesia, the method of cheese making by using papain enzyme which is obtained from papaya fruit has already been practiced since the Dutch colonial era (Rahman 2014). Papain is found naturally in papaya (Carica papaya L.) manufactured from the latex of raw papaya fruits. The papain enzyme (EC 3.4.22.2)is able to break down organic molecules made of amino acids, known as polypeptides (Amri & Mamboya 2012). The cheese which is produced by using papain enzyme as coagulant is Dangke, it originates from Enrekang, a regency of The South Sulawesi province of Indonesia. Rahman (2014) stated that, traditionally the steps of Dangke production are fresh cow milk is heated until it reaches 70oC and added with papaya sap which contains papain enzyme. After the curds are formed, it was separated and molded into cheese forms. The texture of Dangke is similar to Indonesian tofu and the taste is slightly bitter. Figure 1 shows the form of Dangke.

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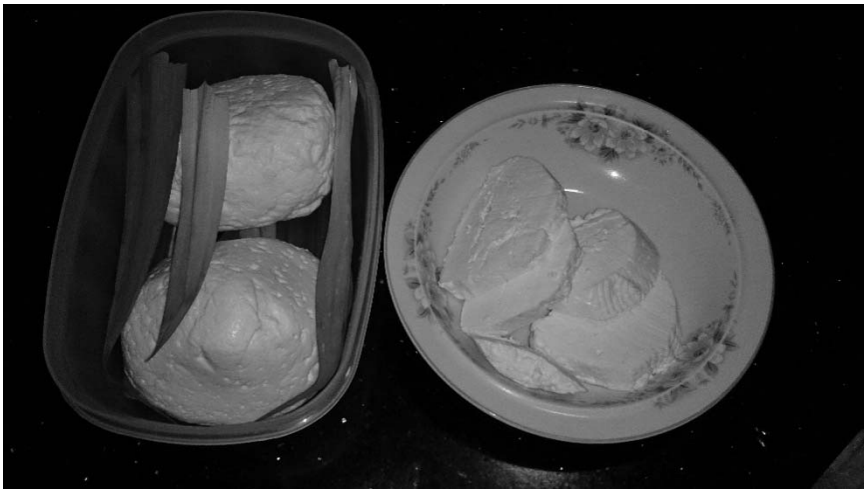

Figure 1. Raw Dangke from Enrekang, South Sulawesi

The specific bitter taste and traditional packaging and preservation techniques applied to Dangke made it difficult to extend the market. Developing the product into cream cheese that has more acceptable form and flavor is considered in this research. Cream cheese is a type of soft cheese which requires no pressing, ripening, or special humidity and temperature control (Kingsley 2008). The cheese will retain its original taste as the ripening stages that develop distinctive flavor of cheese is not included in the making of cream cheese (Smit et al. 2005). The signature bitter taste however, is a problem associated with the presence of papain from papaya sap. Reducing the amount of enzyme will reduce the bitterness; however it will also result in low quality curd for further processing.

Study by Everett and Auty (2008) stated that addition of calcium chloride or CaCl2 will increases the total calcium in milk and at the same time enhances casein-casein interactions, thereby reducing the melt ability and increasing the firmness of the cheese. Ionic calcium has a direct role in coagulation milk proteins, apart from a pH induced protein precipitation, when heated at 70oC. Addition of ionic calcium at levels >20 mM leads to the coagulation of milk even at the normal pH of milk. Ionic calcium combined with heat treatment coagulates milk to give a calcium-induced milk coagulum (Ramasubramanian et al. 2012). Addition of CaCl2 to help curd properties is potential in lowering papain enzyme usage, which later is expected to reduce bitterness of product. The effect and optimum amount of CaCl2 added to the milk however, needs to be evaluated.

This research aims to develop new cream cheese product based on papain-coagulated cheese with an acceptable appearance, aroma, texture and taste. To increase the community access for production of Dangke cream cheese, this research also evaluates the application of process for commercially available pasteurized milk in comparison to fresh milk. Making cheese using commercially pasteurized milk has some advantages namely the protein and calcium content in the milk is more likely to be stable, and it has less possibility of contamination. However, making cheese using the pasteurized will result in the lower quality of curd due to various reasons. Commercially pasteurized milk is formulated from fresh milk with addition of stabilizer and other additives. The additive quantity is very low, however it affecting the curding process. Addition of $CaCl_2$also shows potential to improve the curding properties of commercially pasteurized milk (Everett & Auty 2008).

2. Material and Method

2.1. Papain Enzyme

Papain enzyme which was obtained from papaya sap was taken from the private garden in Lippo Karawaci, Tangerang. Skin of papaya fruits 1 month old were scarred approximately 1 cm deep, 5-10 cm long, and the dripping latex was collected in glass bottle. 1 gram of papaya latex then mixed with 9 ml of aquadest to make the stock solution, stored in refrigeration. Stock solution was diluted to 1%, and the enzyme activity was measured. 250 µL of papain enzyme solution was added with 125 µL of 0.04

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mM $CaCl_2$ solution. Then, 250 µL of casein 1% and 250 µL of buffer pH 6 were added. The sample was incubated at 37 ˚C for 20 minutes. After incubation, the sample was added with 750 µL of 10 % Tri Chloro Acetic Acid solution and centrifuged at 8000 rpm for 10 minutes and at 4 ˚C. 300 µL of the supernatant of the sample was put into a test tube then 1000 µL of 0.5 M Na_2CO_3 and 200 µL of Folin – Ciocalteu (1:2) reagent were added. The absorbance of this solution was measured with spectrophotometer at 578 nm wavelength. Enzyme activity was measured by comparison with the Tyrosine standard curve.

2.2. Processing of Cheese

Fresh milk obtained from farm in Bogor, Indonesia, and commercially available pasteurized milk (Indomilk) obtained from local supermarket in Tangerang. Salt $CaCl_2$obtained from Eternal Buana Chemical Industries in Cikupa, Tangerang.

The general procedure of cheese making was referring to home style procedure (Barry & Tamime 2010; Kingsley 2008). Milk was heated until it reaches the temperature of 60^{o}C followed by addition of $CaCl_2$(0.25 gram, 0.50 gram, and 0.75 gram) and stirring for 1 minute.The addition of papain enzyme (2ml, 4ml, and 6ml) was following, and then the milk was stirred gently for about 15 minutes. The whey and the curd were separated and the curds were drained using filter cloth. The draining process was done for 15 minutes and the drained curds were put into the brine solution (5 % salt concentration). The papain cream cheese was brined about 1 minute, and then the curds were drained for the second time for 15 minutes. The drained curds were mixed gently to form cream texture. The cheese was stored in 50ml beaker glass and the viscosity of the cheese is measured by Viscometer (Brookfield type RVDVE using spindle 7, and 12 RPM) for 1 minute. Full Factorial Design was implemented and repeated for both type of milk. Experiment was conducted in duplicate.

Sensory analysis by hedonic test was conducted to determine papain cream cheese appearance, aroma, texture, and taste quality response. Number of panelist in hedonic test is 54. The panelists involved in this sensory test were untrained.

3. Results and Discussion

3.1. Enzyme Activity Determination

Papain enzyme used was standardized by its activity to ensure the similar properties in curding process. Enzyme activity was measured by comparison to Tyrosine standard and the enzyme unit of papaya latex utilized in this experiment was found of $1.88x10^{-3}$ mol/g.s. Addition of less than 2 ml of papaya latex ($3.76x10^{-3}$ mol/s enzyme activity) per liter of milk will result in separation of whey and curd, but no observable coagulation in curd. Upper level of enzyme addition, 6 ml ($1.13x10^{-3}$ mol/s enzyme activity) per liter of milk was determined by evaluating the bitterness of milk after addition. The standardization of enzyme activity is important to ensure that proper amount of enzyme was used in coagulation. The variation of enzyme activity in papaya latex is broad therefore coagulation properties of papaya latex obtained from various cultivar and ages might differs (Diouf *et al*. 2012).

3.2. Processing of Cheese

The papain cream cheese curd made from fresh milk has slight yellowish color and very soft and pudding-like creamy texture due to the high fat content inside the fresh milk. Curd which obtained from pasteurized milk has whiter color and the texture was slightly harder than and not as creamy as the papain cream cheese which is made of fresh milk. Figure 2 shows the differences in curd made from these two types of milk.Full Factorial Design was applied in this research to evaluate the effect of enzyme and $CaCl_2$addition to the cheese texture. Table 1 shows the viscosity evaluation of drained curd for each variation of process.

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A  B 

Figure 2. Papain based cheese curd made from fresh milk (A) and commercially pasteurized milk (B)

Table 1: Viscosity characteristics of cheese from various formulations

Addition		Fresh Milk		Pasteurized Milk	
Papain (ml)	$CaCl_2$ (g)	Average Viscosity (cp)	Std Deviation	Average Viscosity (cp)	Std Deviation
2	0.25	32700	253.41	36462.5	713.60
2	0.5	55000	207.29	57857.5	569.58
2	0.75	62750	694.06	68242.5	631.20
4	0.25	25170	70.13	25562.5	440.70
4	0.5	48000	790.87	52562.5	1005.84
4	0.75	55000	1336.04	58925	294.746
6	0.25	20000	884.59	24675	1042.53
6	0.5	33000	559.44	36287.5	427.75
6	0.75	43700	471.70	46812.5	948.93

Generally the viscosity of cream cheese produced in this research is within the range of standard identity of cream cheese (Cheng *et al*. 2011). From the table it could be seen that cheese texture is harder with the increasing addition of enzyme and $CaCl_2$salt. Figure 3 shows the texture of curd in various quantities of $CaCl_2$ addition.

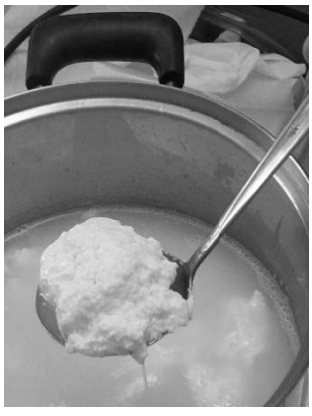
A  B  C 

Figure 3. Effect of addition of $CaCl_2$ to curd characteristics of fresh milk with 2 ml papain addition; $CaCl_2$ added per liter A) 0.25 gram, B) 0.5 gram, C) 0.75 gram

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From Figure 2 it can be seen that curd is more granular and crumbly when quantity of $CaCl_2$ was increased. This resulting in grainier and harder cheese which may not suitable for cream cheese. This finding confirmed the study by Everett and Auty (2008) and provides lowest possible papain addition to milk up to 2 ml/ liter of milk. Addition of 2 ml/ liter of milk is lower than traditional method which suggested that 3 – 6 ml of papain should be used for 1 liter of milk (Rahman 2014). By lowering the papain usage, the bitterness of product could be decreased. Further brining process implemented also reduce the bitterness of finished product.

Further selection of formula was conducted by comparing the viscosity of product obtained from the addition of lowest papain quantity with commercially available cream cheese. Three market leaders in cream cheese were evaluated, namely Philadelphia, Arla, and Elle&Vire, which have the viscosity range of 45000-57000 cP. From the comparison, it could be seen that cream cheese made from addition of 2 ml papain with 0.5 g $CaCl_2$per liter of milk is considered the closest to commercial cream cheese character. This is applicable both for cheese made from fresh milk and commercially pasteurized milk.

Sensory evaluation was conducted to these cheese and the cheese made from fresh milk was in general gives higher scores in term of appearance (6.80), aroma (6.39), taste and texture (6.63) and overall acceptance (6.65) compared to cream cheese made from commercially pasteurized milk, with the score for appearance of 6.06, score for aroma of 6.07, score for taste and texture of 5.52, and overall acceptance of 5.67. The taste of commercially pasteurized milk is scored lower mostly due to bitterness that still observable as after taste. The bitterness might occur due to interaction between papain and $CaCl_2$with the additives and stabilizers used in the formulation of commercially pasteurized milk (Horne 2007). The bright white color of the cheese made from commercially pasteurized milk is apparently not preferred by panelist that is more familiar with slightly yellowish color of cheese.

Although the overall acceptance score is relatively even for cheese made from fresh milk, which is 6.65 in the scale of 9.0, the overall performance was increased significantly when sensory test was conducted with cream cheese served as condiment with other food. Cream cheese served with white bread and chicken ham gives higher overall performance score up to 7.41. The aroma and taste of the bread and chicken meat is capable to mask the bitter aftertaste of the cheese, while the creamy texture of cheese compliment the food in general.

4. Conclusions

Traditional cheese made by addition of papain enzyme could be optimized by the addition of $CaCl_2$salt to reduce the bitterness while maintaining the texture of the cheese. The quantity of enzyme used for coagulation of milk is $3.76x10^{-3}$ ml/s for every liter of milk. $CaCl_2$addition will improve the texture and the addition of 0.5 gram of $CaCl_2$per liter of milk will increase the viscosity close to commercially available cream cheese.

Cream cheese product sensory evaluation shows that the product is potential for further development. Improvement might be required to increase overall acceptance level. Addition of spices and flavoring to the product to mask the bitter after taste could be suggested for further study.

References

Amri E, Mamboya F. 2012. Papain, a Plant Enzyme of Biological Importance: A Review. *Am J Biochem Biotechnol*, 8(2): 99–104.

Barry AL, Tamime AY. 2010. *Technology of Cheesemaking* (2nd ed.). New Jersey: Wiley-Blackwell USA.

Cheng SG, Nellenback T, Fultz JL. 2011. Standard of Identity Cream Cheese That is Flowable at Refrigerated Temperature and Method of Making Same. USA.

Diouf L, Mallaye N, Mbengue M, Kane A, Diop A. 2012. Carica papaya leaves: a substitute for animal rennet in cheese-making tradition. *J Nat Prod Plant Resour*, 2(4): 517–523.

Everett DW, Auty MAE. 2008. Cheese structure and current methods of analysis. *Int Dairy J*, 18(7): 759–773.

ISSN: 2477-1538

Horne DS. 2007. Casein interactions: Casting light on the black boxes, the structure in dairy products. *Int Dairy J*, 1998(8): 171–177.

Kingsley KF. 2008. *The Home Creamery*. North Adams: Storey Pub.

Ozcan T, Kurdal E. 2012. The Effects of Using a Starter Culture, Lipase, and Protease Enzymes on Ripening of Mihalic Cheese. *Int J Dairy Technol*, 65(4): 585–593.

Rahman S. 2014. Studi pengembangan dangke sebagai pangan lokal unggulan dari susu di kabupaten Enrekang. *J Apl Teknol Pangan*, 3(1): 20–25.

Ramasubramanian L, Bruce DA, Deeth HC. 2012. Heat-Induced Coagulation of Whole Milk by High Levels of Calcium Chloride. *Int J Dairy Technol*, 65(2): 183–190.

Smit G, Bart A, Wim JM. 2005. Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. *FEMS Microbiol Rev*, 29(2): 591–610.