PHYSICOCHEMICAL CHARACTERISTICS AND MICROSTRUCTURAL PROFILES OF COTTAGE CHEESE USING PINEAPPLE BROMELAIN ENZYME *[ananas comusus]* AS A NATURAL COAGULANT

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Abstract

Bromelin enzyme is a proteolytic that can help the hydrolisis of proteins into amino acids. In addition the bromelain enzym can also help the process of milk clumping, so that it can be aplied in cheese production. The research aims to analyse of the physicochemical characteristics and microstructural profile of cottage cheese using the enzyme bromelain from pineapple as a natural coagulant. The observed variables include protein, fat, moisture, ash, cheese yield . The research was conducted in a completely randomized design with 4 treatments [K0 =0%, K1 = 3%, K2 = 4%, K3 =5%], and 4 replications .All of the data were analysis of variance [Anova] and followed with significant difference[LSD] test. The results of the study concluded that a concentration of bromelin of 3% produced the best physicochemical properties of cottage cheese with the highest protein content and amendments as well as low water, fat and ash content, pH with a very noticeable difference of P≤0.01 from the treatment producing cheese containing 15.20±0.17% protein, fat content 2.12±0.01%, water content 44.31±0.12%, ash content 0.57±0.00%, yield of 10.18±0.09% and pH of 5.05. While the appearance of the microstructure indicates the presence of an interaction between the enzyme bromelin and the casein of milk protein. This suggests that the enzyme bromelin from pineapple extract can be used instead of cheap rennet in cheese making.

Keywords: *cheese, bromelin, pineapple, enzyme, proteolytic*

INTRODUCTION

Milk is one of the commodity products produced by livestock which is a source of animal protein which is quite in demand. Fresh milk has an excellent nutritional content, but because of this, fresh milk has perishable properties so that a processing process is needed that aims to extend shelf life and minimize damage to milk. One of the forms of milk processing is by cheese making. Cheese is a food made with a milk base that is produced by separating solid substances in milk through a thickening or coagulation process. This thickening process is carried out through the fermentation stage of lactic acid bacteria or by using rennet enzymes so that *curding* and separation of milk serum (Say et al, 2010).

Almost all cheeses marketed in our country are hard cheeses, which are cheeses that require a longer maturation stage, resulting in higher production costs. One of the factors that resulted in the use of the enzyme renin used in making cheese production is still expensive, so the price of rennin in Indonesia has soared and caused the cost of cheese production to increase (Mijan et al, 2014). On the other hand, the renin enzyme that is commonly used can come from animals or microbially produce, if it comes from animals, the source of the animal and the slaughter process must be the main focus of halal tracing. Meanwhile, if it is produced microbially, it must be clear which medium is used for its growth and production. (Kayagil, 2014)

In addition, the taking of rennet in calves can inhibit the growth of cattle farms as producers of meat and milk with higher economic value. In its development, it was found that pineapple (*Ananas comosus*) has

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the enzyme bromelin which is often used to process meat into a more tender texture by breaking down the protein from the meat. Despite this, the enzyme bromelin has not been utilized in the production of curd cheese.[Chandan et al, 2016]. Pineapple is a fruit that is generally known as a source of the enzyme bromelin which is relatively cheap and easy to obtain (Supartono, 2016). Some of the advantages that pineapple has as a source of enzymes to replace rennin are that they come from plants where their production does not depend on many slaughtered livestock to obtain enzymes so that they are safe from *animal welfare* regulations, the potential cost is relatively low due to the cheaper price of pineapple, and is natural and can reduce production costs compared to synthetic enzymes sold in the market (Jaya. F et al, 2009).

The purpose of this study was to determine the physico-chemical characteristics and microstructure profile of cottage cheese which uses the enzyme bromelin from pineapple fruit as a natural coagulant. This research method was designed with a Complete Randomized Design of 4 treatments and each treatment was repeated 4 times. The variables observed were: Amendment, pH, Protein, fat content, water content and ash content as well as microstructure profile.

The results of the study suggested that the addition of the enzyme bromelin 3% resulted in excellent physicochemical properties of cottage cheese in terms of the highest protein content as well as the yield, low content of water and fat and ash and pH with a very noticeable difference of $P \leq 0.01$. As for the microstructure profile, it shows the presence of an interaction between the enzyme bromelin and the casein of milk protein.

MATERIAL AND METHOD

This research was carried out in June 2022 at the Livestock Product Technology laboratory of the Faculty of Animal Husbandry, Universitas Brawijaya Malang for sample preparation, and the process of making cottage cheese. Chemical Analysis of cottage cheese at the Labaratorium of Food Science, Faculty of Agricultural Technology, Universitas Brawijaya Malang.

Materials

The materials used in this study are: bromelin enzyme obtained from pineapple fruit extract, fresh milk, Tools used include: plastic containers, containers for whey, thermometers, heaters, pH meters, juicer tools.

Methods

Cottage Cheese Production

The procedure for cottage cheese is to extract the enzyme bromelin from the pineapple fruit by 1600 grams, then it is judged to take the juice. Cottage cheese is made by first pasteurizing 20 liters of fresh milk at a temperature of 70ºC for 15 minutes [HTST]. Then cooled until it reaches a temperature of 40ºC then add bromelin extract with each treatment, 3%, 4%, 5 % [w/w] then incubated for 35 minutes and filtered with a gauze sieve and added 1% salt [w /w]. The curd was then stored at a temperature of -17ºC for 24 hours after which it was analyzed.

Protein

Analysis of protein levels was carried out using the micro Kjedal AOAC method (2010) A sample of 3 grams was mashed and put into a 30 ml kjeldahl flask then added na_2SO_4 5 grams, 20 ml H_2SO_4 concentrated and 0.2 gr $CuSO_4$ samples simmered for 3-4 hours until the liquid was clear in color. The pumpkin and its contents are cooled and then the contents are transferred to the distillation tool and 100 ml of aqudes and 45% NAOH of 60ml are added. Heat the kjehdal flask, the Erlemeyer flask contains 50 ml of 0.1 N HCL which has been given a 1% phenolphalen indicator of 2-3 drops placed under the condenser. Distillation is terminated after the volume of distillate with a solution of NaOH 0.1 N until a change in color to pink occurs. The assignment of blanks is carried out in the same way.

Protein[%]=TotalNaOH[ml]-Total Sample[mL]x14x6,25/Sample weigh x 100

Fat Content

Analysis of fat content of fat flask was carried out using the AOAC method [2010]. The fat flask is first dried in the oven at 105°C and cooled in a desiccator and then calculated in weight. A sample of 5 grams in dry form wrapped in filter paper is put into the soxhlet extrasion device. A condenser is placed on top and a fat flask is placed under it. Hexane solvent is introduced into the fat flask to taste. Furthermore, refuks are carried out for 16 hours until the solvent that drops back into the clear fat flask. The fat flask containing the extrasy fat is dried in a 105°C oven to evaporate the remaining solvent until it reaches a constant weight, then cooled in a desiccator. Pumpkin fat is then weighed and the fat weight is known.

Calculation of fat content is calculated by the formula

Fat [%] = C-B/A x 100

A = sample weight
B = Initial boilling flask weigh
C = Final boilling flask weigh

Moisture

The moisture was analysis carried out using the AOAC oven method (2010). Samples that have been mashed as much as 2 grams in a saucer are weighed in weight. The sample is dried in an oven with a temperature of^{100} ^{0}C- 105 ^{0}C for 3 -5 hours. Then it is cooled in a desiccator for 30 minutes weighed. The treatment is repeated until a constant weight is reached.

Moisture content = Initial weight of the sample – final weight of the sample x 100 % / Initial weight of the sample

Ash Content

Prepared a clean saucer heated in a furnace at a temperature of^{400} ^{0}C for 1 hour, with cup clamp pliers taken from the furnace and cooled in a desiccator for 1 hour, then weighed (a), a cheese sample weighing 5 grams is put into the furnace and put into a desiccator to be cooled and then weighed (c).

Ash [%] = c – a x 100% / Sample weight [b]

pH

The pH testing procedure places each sample on the sample pot, prepares a pH4 buffer, pH buffer 7, prepares a pH meter tool and turns it on with the start button, before the pH meter is used the referring number must be calibrated using neutral pH after the pointer number on the pH meter stabilizes then the pH meter electrode is dipped into the blended sample and read the pH value obtained.

Cheese Yield

The yield test is measured based on the weight of the resulting cheese. The amendment is obtained by calculating / weighing the final weight of the material (b) produced from the process compared to the weight of the initial material (a) before undergoing the process.

Yield [%]= Final product weight/total ingredients weight x 100%

Data Analysis

This study was conducted with laboratory experiments with a Complete Randomized Design with 4 treatments [K0 = 0%, K1= 3%, K2= 4%, K3 = 5%.] and 4 repeats. All data were analyzed for variance [ANOVA] and continued with the smallest real difference test [LSD] using SPSS 16.0 (Subali, 2010).

RESULTS AND DISCUSSIONS

Chemical and Physical Properties of Cottage Cheese

Data and results of the analysis of various values of protein content, fat content, water content, fat content, ash content, amendment and pH with differences in the application of bromelin enzyme concentrations [0%, 3%, 4%, 5%] and the average values of chemical properties and physical properties are shown in table 1.

Table 1. Physicochemical Characteristics of Cottage Cheese

Characteristics	Bromelien Enzyme Concentration			
	K0	K1	K2	K3
Protein%	11.07 ± 0.00 [a]	15.20 ± 0.17 [b]	14.11 ± 0.08 [c]	12.30 ± 0.06 [d]
Fat %	7.70 ± 0.00 [a]	2.12 ± 0.0 [b]	3.16 ± 0.02 [c]	5.25 ± 0.01[d]
Moisture%	80.20 ± 0.00 [a]	44.31 ± 0 12 [b]	47.50 ± 0.50 [c]	56.25 ± 0.21[d]
Ash%	0.78 ± 0.00 [a]	0.57 ± 0.00 [b]	2.47 ± 0.09 [c]	2.48 ± 0.93[d]
Yield%	6.04 ± 0.02 [a]	10.18 ± 0.09 [b]	8.06 ± 0.02 [c]	6.81 ± 0.48[d]
pH	6.8 ± 0.00 [a]	5.05 ± 0.58 [b]	6.55 ± 0.12 [c]	6.32 ± 0.55[d]

Description:Different notations show very noticeable differences between treatments [P≤0.01]

Protein

The results of the variety analysis showed that the addition of the bromelin enzyme concentration gave a very noticeable difference [p≤0.01] to the cottage cheese protein content with the highest protein content at a concentration of 3% whose protein was 15. 20 ± 0. 17%. The difference in protein content is due to the proteolytic properties of the enzyme bromelin. [Wuryanti et al, 2014], said that the enzyme bromelin has a specific activity of 4.5 units/mg of protein for a total of 128 units x $10^{2.}$ In the pineapple fruit, there is a protease enzyme, namely the enzyme bromelin. Bromelin enzyme is a protease enzyme that is able to break down proteins through a hydrolysis, therefore it can increase protein levels and at a concentration of 3% it also has a more efficient protease activity that affects protein content (Komansilan et al, 2021), found that the acids added at the beginning of cheese processing can affect the proteins contained in the cheese. The activity of different vegetable enzymes will show different proteolytic activity. (Shakeel et al, 2013) Purwadi's discovery 2010 that improperly added acid causes the resulting curd to be mushy due to whey loss. In this study, cottage cheese containing protein of 12.30-15.20% has met the standards.

Fat Content

The results of the variety analysis showed that the addition of bromelin enzyme concentrations gave a very noticeable difference [P≤0.01] to cottage cheese fat levels. The fat content of cottage cheese ranges from 2.12-5.25 %. The highest cheese fat content is found in K0[7.70 ±0.00] and the lowest in [K1 2.21±0.01]. Scoumacker et al., say that the fat content of cottage cheese commercially is 0-7%. In this study, the overall fat content of cottage cheese produced was worthy of being marketed. [Kayagil, 2006] states that the decrease in cheese fat content is due to the process of fat degradation with the help of the enzyme lipase during the cheese making process.

Moisture

The results of the analysis showed that the addition of the bromelin enzyme concentration gave a very noticeable difference of P≤0.01 to the moisture content of cottage cheese. This difference is due to the addition of the concentration of the enzyme bromelin which causes the occurrence of different coagulation processes. Water is an important component in processed food because it can affect the texture and taste of the entire product stored (Komansilan et al, 2020). Water content in processed food products is the accumulation of water content in food which is added by the addition or processing of other external materials. In the process of cheese processing, the significantly lost moisture content can affect the weight loss during the ripening period. Added salt can also affect the loss of moisture in cottage cheese.

Ash

The results of the variety analysis showed that the addition of the bromelin enzyme concentration gave a very noticeable difference [P≤0.01] to the ash content of cottage cheese. Ash content indicates the mineral content contained in a food. In the process of processing the ash content of cheese represents the miniral in the last product and depends on which salting

process is used. In this study, K3 addition of bromelin 5% resulted in the highest ash content of 2.48% and the lowest was K1 0.57%.

Yield

The results of the variety analysis showed that the addition of bromelin concentrations gave a very noticeable difference [p≤0.01] to the cottage cheese yield. The most important parameter as an alternative to cheese making is the yield or weight of the resulting cheese. The result obtained is that cottage cheese is important for optimal cheese production because it can determine the cost or benefit. (Likah et al, 2012). The results obtained in the process of processing cheese during processing are influenced by several factors such as: temperature, pH, enzyme concentration during the heating process. In this study, the total cheese yield differed very markedly between treatments, namely 6.04%±0.02, [K0], 10.18%±0.09, [KI] 8.06%±0.02, [K2] 6.81%±0.48 [K3] it turned out that the use of bromelim enzymes was 5%, resulting in a low cheese yield, while a bromelin concentration of 3% had a more optimune cheese yield compared to other concentrations. States that the optimum pH and enzyme concentration can initiate efficient coagulation of milk can result in a compact curd and higher resulting cheese (Liepa et al., 2017).

pH

The results of the variety analysis showed that the addition of bromelin enzyme concentrations gave a very noticeable difference [P≤0.01] to the pH of cottage cheese, in this study K0 [6.8], K1 [5.05], K2 [6.55] K3 [6.32] the highest in K0 without the addition of the enzyme bromelin while the lowest in K1 [5.05]. A low pH will cause the environment to become acidic so that the whey protein comes out of the curd so that the breakdown of the bond between the protein and hydrogen will be filtered. Similarly, a high pH will contain higher humidity (Lobato et al., 2007).

Profile Microstructure

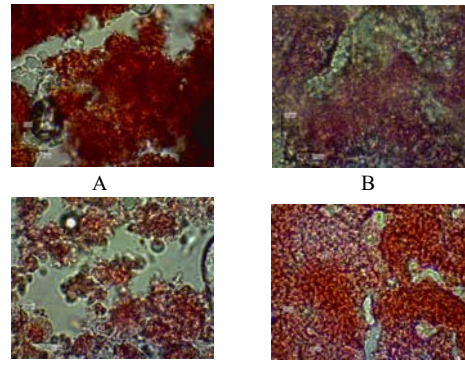

Fig.1 Cottage cheese microstructure CLSM method enlargement 400 x . Milk protein casein[A], without the addition of bromelin. B [bromelin 3%], C [bromelin 4%], D [bromelin 5%]

Cheese microstructure is the spatial arrangement of casein particles that combine into a bond and chain to form an overall matrix of proteins dispersed by water, fat globules and minerals. [Raikos, 2018] Microstructure is one of the main control factors of the texture and completeness function of the cheese. Observations from cottage cheese are observations made using a mincroscope aid to look at the images and structures of cottage cheese microscopically (Mounsey et al., 2011). Suggests that microstructures are the smallest arrangements or components contained in a fat or lipid material contained in milk in the form of millions of small balls with a centerline between 1-2µ with an average midline of 3µ. There are usually approximately 1000 x 106 fat grains in each ml of milk. Suggest that the typical microstructure picture of fat cheese is slight, its structure is filled mostly of a protein matrix with a small amount of fat globula scattered in the matrix.

CONCLUSIONS

The results of the study concluded that a concentration of bromelin of 3% produced the best physicochemical properties of cottage cheese with the highest protein content and amendments as well as low water, fat and ash content, pH with a very noticeable difference of P≤0.01 from the treatment producing cheese containing

15.20±0.17% protein, fat content 2.12±0.01%, water content 44.31±0.12%, ash content 0.57±0.00%, yield of 10.18±0.09% and pH of 5.05. While the appearance of the microstructure indicates the presence of an interaction between the enzyme bromelin and the casein of milk protein. This suggests that the enzyme bromelin from pineapple extract can be used instead of cheap rennet in cheese making.

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