

THE EFFECT OF *Ananas comucis* EXTRACTED BROMELAIN ENZYME ADDITION UNDER DIFFERENT pH ON THE PHYSICOCHEMICAL PROPERTIES OF COTTAGE CHEESE

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ABSTRACT

The research aims to analyse the effect of bromelain enzyme addition from *Ananas comucis* under different pH condition to the physicochemical properties of cottage cheese. The observed variables include protein, fat, moisture, ash and cheese yield. The research was conducted in a completely randomized design with 4 treatments (K1 = pH 4.5; K2 = pH 4.6; K3 = pH 5.4; and K4 = pH 5.5) and 4 replications. All of the data were analysed with analysis of variance (ANOVA) and followed with least significant difference (LSD) test. The results showed that the bromelain addition under pH 4.6 showed the best physicochemical properties of cottage cheese that had lowest fat content (0.67 ± 0.53 %) and highest yield (18.15 ± 0.22 %) compared to other pH condition, while the protein and ash content did not show any significant difference on all pH condition.

Keywords: Enzyme; cheese; bromelain; yields

INTRODUCTION

Cheese is one of the processed milk products which contained high nutrients and become a preferred food products in Indonesian market. Cheese is commonly used both as the main dish or food flavouring agent. According to Mirza and Mulyani (2013), the cheese consumption in Indonesia has increased for 5.99% in 2001 to 2002 with the total cheese consumption around the year reached 5,127.58 tonnes as reported by PT. Kraft Indonesia. Moreover, the Indonesia has imported 7,869.67 kg of fresh cheese on 2010, and increased to 10,357.98 kg in 2011. The data showed that the demand for cheese product has increased as much as 2,488.31 kg a year or 207,359 kg monthly. The high consumption for cheese products thus demand a higher production of cheese as well. A sustainable cheese production by utilizing local resources starting from the milk as the main ingredients and enzyme for the cheese production offers a promising solution to meet the demand.

Enzyme is a group of protein that biologically catalyse chemical reactions such as hydrolysis, oxidation, reduction, isomerase, carbon chain cleavage and radical transfer (Sumardjo, 2006). The chemical reaction catalysed by an enzyme would be thousand times more optimal, while the catalysis could occur on wide range of pH condition and temperature with the reaction is commonly specific and selective to certain substrates (Chang, 2005). One of an important enzymes for cheese production is proteolytic enzyme. On cheese production, rennet enzyme is widely used as it contained rennin which has proteolytic abilities and chymosin as with

the coagulating ability. However, research has been done to find another potential proteolytic enzyme to be an alternative for rennet enzyme.

Ananas comosus or pineapple has been known as a cheap and widely available source for bromelain enzyme, an enzyme that is commonly used to tenderize a meat by breaking down the protein bonds. However, aside for tenderizing meat, the application of bromelain enzyme is still limited, especially for milk or cheese products even though it is a proteolytic enzyme. The utilization of bromelain enzyme from *Ananas comosus* would provide a safer alternative for rennet enzyme as it is originated from a plant, while also added with its relatively cheap price compared to the synthetic proteolytic enzyme.

The bromelain enzyme would catalyse proteolysis by hydrolysing protein into amino acids. The enzyme would also help milk coagulation, which is one of essential steps in cheese production. The cheese production would be affected by pH, temperature, time of incubation and enzyme addition (Kapoor and Metsger, 2008). Previous research by Jaya and Hadikusuma (2009) showed that the utilization of *Ananas comosus* bulb and skim could be used for cottage cheese production which had 10.34 to 12.77% protein, 70.5 to 85.78% moisture, pH at 4.36 to 4.75, and 11.85 to 15.77% cheese yield.

However, research on the utilization of bromelain enzyme from *Ananas comosus* extract for cheese production is still limited. In this research we aimed to observe the effect of *Ananas comosus* extracted bromelain enzyme under different pH condition to the physicochemical properties

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of cottage cheese. The *Ananas comucosus* used in this research is originated from Kotamobagu, North Sulawesi Province. The province has been known as the main producers of *Ananas comucosus*, the results of this research is then expected to also contribute on the knowledge of local resource utilization and product diversification for cheese production.

MATERIALS AND METHODS

The research was conducted in the Laboratory of Animal Products Technology, Faculty of Animal Science, Brawijaya University for the cheese production and cheese yield analysis and Laboratory of Food Technology, Faculty of Agricultural Technology, Brawijaaya University for the chemical analysis.

Materials

The materials used in this research include cottage cheese, crude bromelain

enzyme extracted from *Ananas comucosus*, fresh dairy milk. The equipment for cheese production include plastic container, whey container, heater, thermometer, gauze filter, mixer, and pH meter.

Methods

Cottage cheese production

The crude bromelain enzyme extraction from *Ananas comucosus* and procedure for cottage cheese production is presented in Figure 1 and 2, respectively. The cottage cheese was made by firstly pasteurize 28 L of dairy milk at 70°C for 15 min (High Temperature Short Time/HTST pasteurization). The milk was then cooled down until reached 40°C and conditioned for the pH according to the treatments then added with bromelain enzyme as much as 3% (w/w). The milk was then incubated for 35 min and filtered through gauze filter and added with 1% (w/w) salt. The curd was then incubated at -17°C for 24 h before analysed.

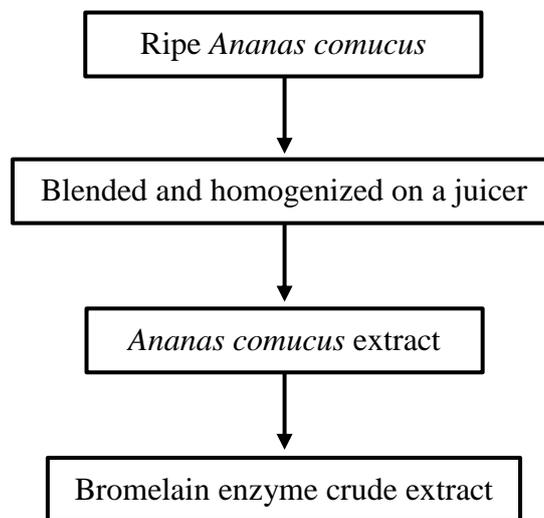


Figure 1. Crude extraction of bromelain enzyme from *Ananas comucosus* (Manoi, 2007)

Protein

The protein content was analysed by using Kjeldahl method (AOAC, 2005). As much as 3 g of cottage cheese was ground and placed in 30 mL Kjeldahl flask and added with 5 g Na₂SO₄, 20 mL H₂SO₄ and 0.2 gr CuSO₄. The solution was then boiled

for 3 to 4 h until became transparent. The flask was then cooled down and placed into distiller, added with 100 mL aquadest and 60 mL of 45% NaOH. The flask was heated again, added with 50 mL HCL 0.1 N until turned to pink colour. The blank standard was done with the same procedure.

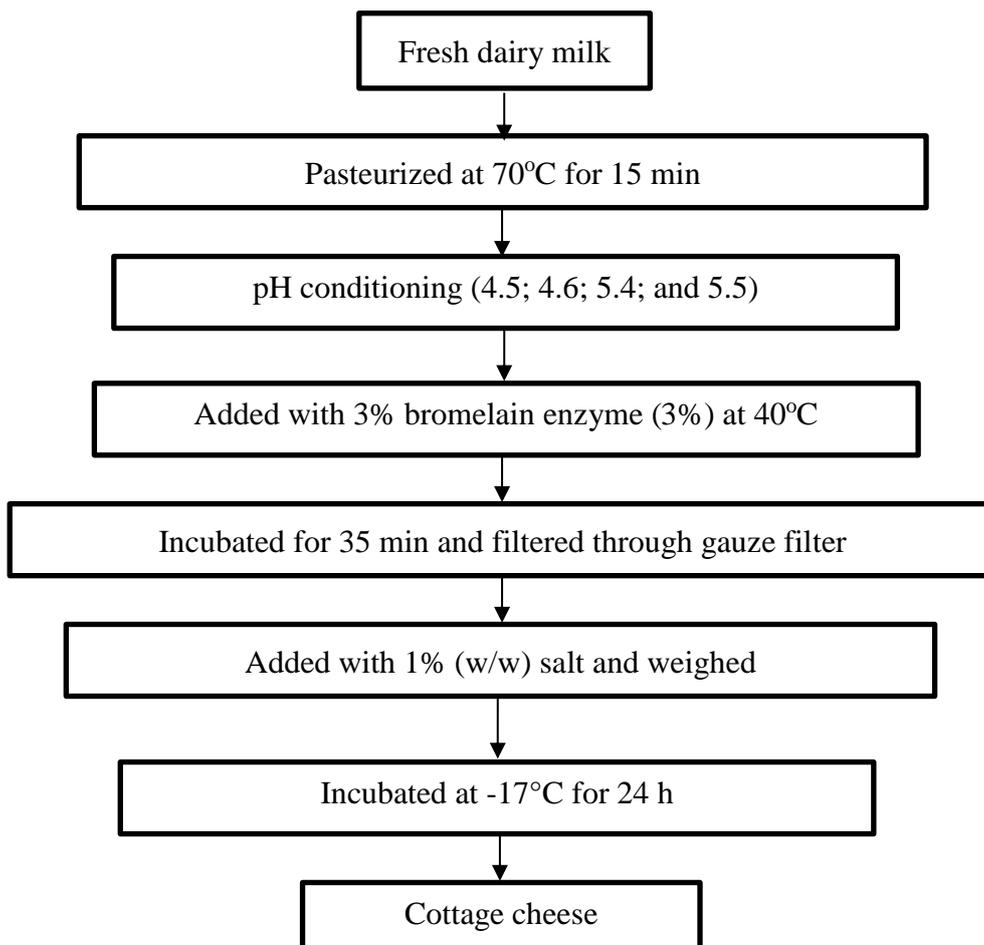


Figure 2. Cottage cheese production

Fat

The fat content was analysed by following AOAC (2005). The boiling flask was firstly heated on the oven at 105 °C and then weighed after cooled down in the desiccator. As much as 5 g of dry sample was placed into the filter paper and then into the Soxhlet extractor. A condenser was then placed above the boiling flask. Hexane solvent was added to the boiling flask accordingly.

Sixteen hours reflux was then performed until the sample solution turned clear color. The extracted sample was then

dried in the oven for 105 °C to evaporate any solvent residue and reached a constant weight. The sample was then cooled in the desiccator and weighed. The fat content is measured by the following formula:

Moisture

The moisture was analysed by following AOAC (2005). The sample was dried in the oven at 100 – 105 °C for 3 – 5 h. The sample was then cooled down on the desiccator for 30 min. The initial weight and final weight of the sample was then recorded to be calculated by the following formula:

$$\text{Protein (\%)} = \frac{\text{mL NaOH} - \text{mL sample} \times 14 \times 6.25}{\text{Sample weight}} \times 100\%$$

$$\text{Fat (\%)} = \frac{C - B}{A} \times 100\%$$

A = Sample weight

B = Initial boiling flask weight

C = Final boiling flask weight

$$\text{Moisture (\%)} = \frac{\text{Initial sample weight} - \text{final sample weight}}{\text{Final sample weight}} \times 100\%$$

Ash

The petri dish was heated at 400°C for 1 h, and then weighed after cooled down on the desiccator for 1 h (A). The cheese sample for 5 g was heated for 400°C along with the petri dish for 1 h, the sample along with the petri dish was then weighed after cooled down on the desiccator (B). The ash

content is calculated as follow (AOAC, 2005):

Cheese yield

The cottage cheese texture was measured by using Penetrometer (Shakeel-Ur-Rehman *et al.*, 2003). The texture was then calculated by the following formula:

$$\text{Ash (\%)} = \frac{B - A}{\text{Sample weight}} \times 100\%$$

$$\text{Yield (\%)} = \frac{\text{Final product weight}}{\text{total Ingredients weight}} \times 100\%$$

Data analysis

The research was conducted as laboratory experiment in a completely randomized design with 4 treatments (K1 = pH 4.5; K2 = pH 4.6; K3 = pH 5.4; and K4 = pH 5.5) and 4 replications. All of the data were analysed with analysis of variance (ANOVA) and followed with least significant difference (LSD) test by using SPSS 16.0 software (Subali, 2010).

RESULTS AND DISCUSSIONS

The physicochemical properties of the cottage cheese in this research, which include protein, fat, moisture, and ash content as well as cheese yield is presented in Table 1.

Different pH to protein content of cottage cheese

The analysis of variance showed that different pH condition did not give significant effect ($P > 0.05$) to the protein content of cottage cheese. This can be seen from the relatively similar protein content of the cottage cheese (Table 1). In this research, the highest protein content was achieved in K2 (16.88 %), followed with K4 (16.72 %) and K3 (16.04 %), while the

lowest was shown on K1 (16.02 %). The protein content in cheese is categorized as easily digested protein due to the protein breakdown during the cheese production. The increased protein content was caused by the addition of *Ananas comosus* which contained protein coagulating agent. Moreover, *Ananas comosus* contained bromelain enzyme, a proteolytic enzyme which hydrolyse protein into amino acids. According to Purwadi (2010) incorrect acid addition during cheese production could cause mushy curd and loss of whey. In this research, the protein content has fulfilled the standard for cottage cheese (12.70 to 21.00%).

Different pH to fat content of cottage cheese

The analysis of variance showed that different pH give a highly significant effect ($P \leq 0.01$) to the fat content of cottage cheese. Fat content was decreased along with bromelain enzyme addition. The lowest fat content was found in K2, this indicates that at pH 4.6, the bromelain enzyme has met its isoelectric condition. This would initiate higher fat dissolve and thrown away along with whey during pressing of the cheese. Cottage cheese with the highest fat content

in this research was found in K4 (3.22 %), followed with K3 (2.47 %) and K1 (1.92 %), while the lowest was found in K2 (0.67 %). The maximum fat content of cottage cheese according to USDA (2001) is 0.5%. This showed that the only cottage cheese that fulfilled the standard was K2. Sukotjo (2003) added that cottage cheese is categorized as low fat soft cheese, thus the fat content of cottage cheese is one of essential parameters to determine its quality.

Different pH to moisture of cottage cheese

The analysis of variance showed that different pH gave a highly significant effect ($P \leq 0,01$) to the moisture of cottage cheese. Cottage cheese with the lowest moisture in

this study was found in K1 (56.33 %), followed with K2 (57.23 %) and K3 (57.80 %), while the highest was found at K4 (58.48 %). The low pH would cause acidic environment which pull out the whey protein from the curds, and the breakdown of protein bond with hydrogen will be filtered. On the other hand, the high pH would contain higher moisture. The different moisture of cottage cheese in this study was due to several factors, which were: (1) bound water in the curd structure; (2) bound water on hygroscopic particles in curd; and (3) free water. Lobato *et al.* (2007) added that higher protein content in cheese would increase the casein matrix capabilities to bind water (Lobato *et al.*, 2007).

Tabel 1. Physicochemical properties of cottage cheese

Physicochemical properties	Treatments			
	K1	K2	K3	K4
Protein (%)	16.02 ± 0.548	16.88 ± 0.87	16.04 ± 0.80	16.72 ± 0.26
Fat (%)	1.92 ± 0.40 ^b	0.67 ± 0.53 ^a	2.47 ± 0.52 ^{ab}	3.22 ± 0.76 ^b
Moisture (%)	56.33 ± 0.60 ^a	57.23 ± 0.74 ^{ab}	57.80 ± 0.73 ^{bc}	58.48 ± 0.84 ^c
Ash (%)	1.61 ± 0.55	0.72 ± 0.39	1.10 ± 0.51	1.34 ± 0.50
Yield (%)	16.68 ± 0.29 ^c	18.15 ± 0.22 ^d	15.36 ± 0.01 ^b	14.00 ± 0.004 ^a

Description: Different superscripts indicate significant difference on different pH conditions ($P \leq 0.01$). K1 = pH 4.5; K2 = pH 4.6; K3 = pH 5.4; and K4 = pH 5.5.

Different pH to ash content of cottage cheese

The analysis of variance showed that different pH did not give significant effect ($P > 0.05$) to the ash content of cottage cheese. As presented in Table 1, the ash content of cottage cheese produced under different pH condition are relatively similar. The lowest ash content of cottage cheese in this study was found in K2 (0.72 %), followed with K3 (1.10) and K4 (1.34 %), while the highest was found in K1 (1.61 %). Moreover, the ash content of cottage cheese in this study has fulfilled the standard at around 0.21 to 1.10 %.

Different pH to cottage cheese yield

The analysis of variance showed that different pH gave a highly significant effect ($P \leq 0,01$) to the yield of cottage cheese. The

lowest yield was found in K4 (14.00 %), followed with K3 (15.36%) and K1 (16.68 %), while the highest was found in K2 (18.15%). Cheese yield indicates the amount of produced cheese from the total input during cheese production, which include milk, bacterial started and salt (McSweeney, 2007). The different cheese yield in this study was due to the different casein coagulation, which mainly determined from the pH condition. Moreover, the bromelain enzyme addition also contributes to the casein coagulation process. Geantaresa and Supriyanti (2010) showed that protease enzyme will breakdown k-casein (kappa casein) in the surface area of casein micelle into para-kappa-casein. Moreover, when the pH is closer to the casein isoelectric (pH 4.6 to 4.7), the casein micelles will be coagulated into gel through hydrophobic

interaction. Pardede *et al.* (2013) added that calcium also contributes to the casein coagulation by aggregating the casein micelles.

CONCLUSION

The research concludes that 3% bromelain enzyme addition at pH 4.6 produced the best physicochemical properties of cottage cheese.

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