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Physicochemical Properties of Dangke-cheese by Different Temperature Processing and Papaya-latex as Coagulant

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ABSTRACT

Background: Dangke-cheese is a traditional product of Enrekang, South Sulawesi, made by coagulating buffalo, cow, goat, or sheep milk, heating and adding papaya latex (*Carica papaya*).

Methods: Dangke-cheese was made from fresh-cow-milk heated at 75, 80, 85, 90, 95 and 100°C for 1 minute and added with papaya-latex 0.3, 0.4 and 0.5%. The curd was formed in a coconut-shell template, pressed until compact. Dangke-cheese was evaluated for physicochemical-properties.

Result: Dangke-cheese pH increased with increasing temperature, opposite to lactic-acid %. The best Dangke-cheese quality is heated at 75°C with a papaya-latex concentration of 0.5% and contains protein 17.94%, fat 24.295%, lactose 14.12%, pH 5.93 and lactic acid 0.296%.

Key words: Curd, Dangke-cheese, Incubation-temperature, Papaya-latex.

INTRODUCTION

Dangke-cheese is a traditional cheese of Enrekang in South Sulawesi, made by coagulating cow milk using papaya latex or another protein coagulant (Malaka *et al*., 2023). Dangke cheese is used as a side dish in Enrekang Regency. In general, farmers process the milk they produce into Dangke and if there is excess production, the Dangke cheese was sold in traditional markets or sold to other areas such as Makassar. Most household industries are dairy farmers (Contzen and Häberli, 2021), who use a pasteurization temperature of 65 - 80°C when coagulating with papaya latex (Rana *et al*., 2017). Pasteurization of dairy products is conducted to decrease the microbial burden in milk, specifically by restricting the abundance of spoiling microorganisms and preventing foodborne diseases (Tomar and Tiwari, 2024).

The process of heating milk can cause protein denaturation, which decreases the nutritional value of Dangke cheese. Likewise, using too much papaya sap will cause Dangke Cheese to taste bitter, while if too little papaya sap is used, then the yield will be low because there are only a few lumps of curd. The activity of papain enzyme contained in papaya sap has optimum activity at a temperature of 50-60°C, although this enzyme is resistant to higher heating. The coagulation of milk through lactic acidity and/or enzymatic activity is the initial step in cheese-making (Bounoua *et al*., 2024). Therefore, finding the optimal temperature and level of papaya latex is necessary to ensure the quality assessment of a good-quality Dangke-cheese. The process of milk-clotting to form curd by enzymes begins with the gelation process (Verdú *et al*., 2021). This involves extensive protein hydrolysis, thereby affecting product quality. Curd formation is also influenced by heating temperature and enzyme concentration (Nicosia *et al*., 2022).

The gel-formation process can be explained by observing changes in physicochemical and microstructural properties (Mende *et al*., 2020). If gelation continues, milk-protein coagulation can occur, leading to milk clotting (Beux *et al*., 2017) through the interactions of milk casein particles, whey protein, lactose and fat globules (Obeid *et al*., 2019). Casein can form milk curd and the gel or coagulum tissue determines the characteristics and textures of various high-

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protein (Jørgensen *et al*., 2019) and nutritious food products (Malaka *et al*., 2021). Formulating gels from protein depends on temperature and pH (Laursen *et al*., 2023). Dangke-cheese is expected to form gels and coagulums with smooth, shiny and compact surfaces (Asaduzzaman *et al*., 2021). Milk casein protein clumps occur at the isoelectric point, pH 4.8; dissociation occurs at pH 5.5. At that time, a separation occurs between polar and nonpolar molecules due to the enzyme's activity. The papain enzyme can create Dangke-cheese at specific concentrations and heating-milk temperatures (Marwah *et al*., 2023), causing the gelation and agglomeration processes to be hindered (Malaka *et al*., 2017).

The k-casein molecule contains 169 amino acids (Lajnaf *et al*., 2023). Proteolytic enzymes generally break at the amino acid bonds 105 (phenylalanine) and 106 (methionine) (Chen *et al*., 2021). The release of the 105-106 bond in the κ-casein-molecule is the initial phase of the gelation process, followed by the coagulum phase and syneresis (secondary phase). Furthermore, in the tertiary phase, the enzyme generally attacks casein, that is, α-casein and β-casein, which occur during ripening. A high pH value with prolonged milk heating can cause coagulation in Dangke-cheese by denaturating whey protein (Genene *et al*., 2019). The acid formation in pasteurized milk depends on the type of acid addition used and pasteurization time, which also affects the total levels of lactic acid (Edwards *et al*., 2021). Gaining a comprehensive understanding of the distinct attributes of milk, such as its composition and physical properties, before processing enables the selection of the appropriate technique of milk product technology for processing (Abduku and Eshetu, 2024).

The milk-heating process at various temperatures greatly influences gel and curd-formation, essential in Dangke-cheese-making. Therefore, this article presents the results of evaluating the Dangke-cheese-making process by looking at its physicochemical properties.

MATERIALS AND METHODS

The experiment was conducted in August-December 2023 at the Laboratory of Biotechnology Milk Processing, Faculty of Animal Science in Hasanuddin University Makassar, Indonesia.

Papaya latex and milk sampling

Papaya-latex is collected, tapped from 10 fresh fruits on a tree, dried separately in an oven at 55°C and stored at 5°C before using in this study. Dangke-cheese was manufactured by pasteurizing fresh milk and adding dried papaya-latex (Malaka *et al*., 2021), diluted to 10% as a crude papain solution. Fresh cow milk 100 lt was collected from 12 smallholder farms in Enrekang Regency, South Sulawesi. Milk is transported to the laboratory in Makassar using cold milk containers.

Dangke-Cheese-Making

This study used a completely randomized design with two measures: Papain concentration and heating temperature. Each treatment contained fresh cow's milk in three 1000 ml Erlenmeyers, so all treatments used 15 Erlenmeyers. The entire treatment unit was heated at 50°C for 1 minute. Then add 0.3, 0.4 and 0.5% papain (w/v) to each Erlenmeyer, then increase the temperature to 80, 85, 90, 95 and 100°C for 1 minute, respectively. The study was repeated three times. The curd formed was molded in a coconut shell and pressed until compacted by whey draining.

Lactic-acid and pH

The percentage of lactic acid was measured by titration using NaOH 0.1 M with phenoptalien as an indicator. PH is measured using a pH meter (Schott Instrument, Germany). The 50 mg of dangke cheese was crushed in a mortar and put into a beaker containing 100 ml of distilled water, then stirred with a magnetic stirrer until the cheese solids dissolved. Before use, the pH meter is dipped in buffer pH 4, 7 and 10.

Fat

The Gerber method is used to determine fat content. The procedure follows: each butyrometer is filled with 10 ml of H_2SO_4, then 11 ml of samples are flowed slowly until two liquids remain separated. Amyl-alcohol 1 ml was added to the butyrometer, blocked with a rubber stopper, rotated and shaken vigorously, wrapped in a rag and shaken thoroughly until the fat globules were dissolved and the color became purplish. The butyrometer is placed in a water bath for 5 minutes at 65°C, then centrifuged for 3 minutes at 1200 rpm.

Protein

Protein analysis was using the Kjeldahl method. The cheese sample was weighed as much as one g and put into a Kjeldahl-flask, then 7 g of K_2SO_4 and 0.8 g of $CuSO_4$ were added. Next, 12 ml of H_2SO_4 solution was added to the fume cupboard. The digestion process was done in an acid chamber of heating milk in a Kjeldahl-flask using an electric stove until the color changed to turquoise green; then cooled by leaving it for 20 minutes. Then, 25 ml of distilled water, 50 ml of 40% NaOH and several boiling-stones were added to the Kjeldahl-flask. Then, 30 ml of H_3BO_3 was added to the Erlenmeyer flask by adding three drops of BCG-MR indicator to capture the distillate resulting from the distillation by adjusting the distillation equipment. The distillate obtained was titrated using a standard 0.1 ml NaCl solution until the color of the solution changed to light pink.

Lactose

Lactose levels were determined by weighing one gram of the sample and diluting it with 500 ml water; 2.5 ml of the dilution of the Dangke-cheese sample was taken and put into a closed test tube. Then 0.2 ml each of 5% $ZnSO_4$

and 4.5% $Ba(OH)_2$ were added and centrifuged at 1000 rpm for five minutes to form a precipitate. One ml supernatant was put into a large test-tube with a lid. Then 2.5 ml of Telles-Reagent was added and closed tightly. The tube was immersed in boiling-water for six minutes and then cooled under tap-water. Once cool, distilled water is added until the volume is 12.5 ml, then shaken repeatedly. An orange color will form and the absorbance can be read using a spectrophotometer at 520 nm. Standard-solutions are also treated the same as samples. Standard-solution: First, create a stock solution by dissolving 0.75 g of lactose in 100 ml and supernatant separate by centrifuge at 7500 rpm.

Ash

The ash content of the material is determined by the dry ashing method. The porcelain-cup was dried in an oven at 105°C for 15 minutes, cooled in a desiccator and weighed. A total of 1-2 g of DC sample was weighed. The sample was placed in a porcelain-cup and put into an electric-furnace for ashing. After the ashing process, the crucible was cooled in a desiccator and weighed until the weight was constant.

Water content

The empty-cup was placed in the oven for 2 hours, then put in a desiccator for 15 minutes. Once the empty cup has cooled, weigh it to determine its weight and weigh 5 grams of cheese. The cup containing the oven-ready sample is placed in the oven for 24 hours at a temperature of 105°C. The cup containing the sample was placed in a desiccator for 15 minutes and then weighed. Calculation of water-content uses the formula of Jurcovic *et al*. (2019).

Data analysis

The collected data were analyzed using variance-analysis with ANOVA using Excel and SPSS. The ordinal data were analyzed using non-parametric statistics. Results are best interpreted based on statistical inferences related to Dangke-cheese quality.

RESULTS AND DISCUSSION

Powdered papaya-latex

The drying rate of papaya latex using an oven at 55°C for 5 hours in Fig 1 showed that varied between 17.39% and 33.33%, with an average of 24.89%. Papaya-latex contains the papain enzyme (Ningrum *et al*., 2018). The advantage of using papain enzyme for milk coagulation is its optimal activity at 75°C, with activity maintained across a broad temperature range of 70-90°C. The papain enzyme breaks peptide bonds at asparagine-glutamine, glutamate-alanine, leucine-valine and phenylalanine-tyrosine residues.

pH and Lactic-acid

The average pH of Dangke-Cheese at different heating milk and papain concentrations shows in Table 1. When the pH reaches 5.6, curd formation begins. A gel forms at the bottom with a soft texture and white color (Tarapata *et al*., 2021). The curd formation process occurs at pH 4.6 (isoelectric point) and casein becomes hollow, slightly stiff and white, with a sour taste and aroma, while the whey is greenish-yellow (Tserovska *et al*., 2002). Papain coagulates casein micelles by cleaving the peptide bond between phenylalanine and methionine in κ-casein, leading to the formation of para-κ-casein and exposing hydrophobic residues. When the pH approaches the isoelectric-point, the casein-micelles attract each other due to the different ionic properties of each molecule, resulting in clumping (Asaduzzaman *et al*., 2021).

Variance analysis showed that the heating milk temperature treatment had a very significant effect ($P<0.01$) on the Dangke-Cheese pH. The Dangke-Cheese pH at 75°C heating milk ranges from 5.91-6.12; at 80°C is 6.08-6.27. The lactic-acid produced will lower the pH value (Hetényi *et al*., 2011). The results of the least significant difference test (LSD) showed a high difference between 75°C and other heating milk temperatures, except for 80!. Papain concentrations of 0.3% and 0.5% were significant differences in the pH of Dangke-Cheese, indicating the

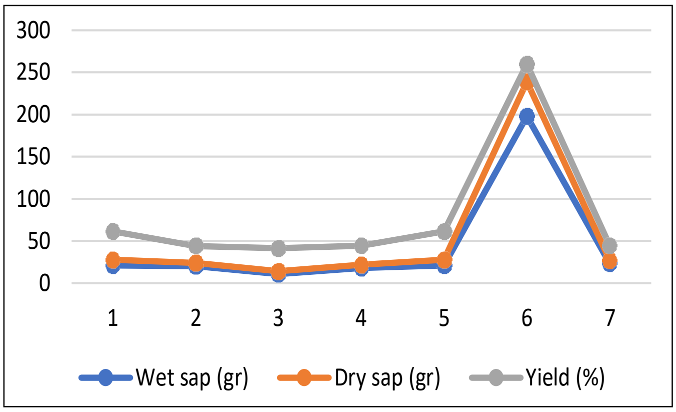

Fig 1: The average yield of papaya-latex extracted from papaya fruit.

breakdown of the three-dimensional network in the curd structure, which could release lactose in the curd. Lactic acid bacteria have the ability to convert lactose into lactic acid. Additionally, lactic acid plays a role in the creation of tiny peptides and amino acids through the action of their proteolytic enzymes during the process of cheese manufacture (Nurye and Wolkero, 2022). The pH of curd ranges from 4.24 to 4.63 (Usmiati *et al*., 2011). Variance analysis indicated that heating milk significantly affected Dangke-Cheese lactic acid (P<0.01). The average percentage of Dangke-Cheese lactic-acid at 75°C is in the range of 0.60-0.77%; at 80°C is 0.34-0.62%; at 85°C is 0.32-0.73% and it continues to decrease as the Heating-milk-temperature increases.

The LSD test resulted in a high difference between 75°C and other Heating-milk-temperatures except for 0.4% papain concentration, but there was no difference between 80 and 85°C, 85 and 90°C. The concentration of papaya-latex in Dangke-Cheese-making does not affect lactic-acid at 75°C. The percentage of lactic-acid is inversely proportional to pH. On average, fresh milk contains lactic acid between 0.14 and 0.19%. When milk is stored at room temperature, lactic-acid increases to 0.25%. If acidity continues to increase, casein precipitation occurs when the acidity reaches 0.5-0.65%, or pH reaches 4.64-4.78.

Protein

The protein content determines a food product's quality. Table 1 shows the results of testing Dangke-Cheese-Protein levels at different heating-milk temperatures and concentrations of wet-papaya-sap. Variance analysis results that different heating-milk-temperatures and papain concentrations significantly affected Dangke-Cheese-protein (P<0.01). The average Dangke-cheese-protein at 75°C is between 16.13-17.67; at 80°C is 15.88-17.49%; at 85°C is 15.82-17.32; at 90°C 14.25-15.89; at 95°C is13.98-15.38 and 100°C is 11.94-14.12%. The insoluble protein in milk, casein-protein, will quickly degrade when heated above 75°C (Qian *et al*., 2017).

Proteins consist of polypeptide chains of amino acid residues through peptide bonds and forming disulfide bonds. The acidic carboxyl group and the weakly basic amino group both combine *via* a hydrocarbon chain that is unique to each amino acid. Casein is round and consists of smaller units known as submicelles. Casein is a globular protein with primary, secondary, tertiary and quarter bonds. This bond can break at certain heating levels and will be damaged and broken due to heating above 75°C.

Protein is the primary nutrient in milk and contains various essential-amino-acids. The protein in milk consists of a water-insoluble-protein, namely casein. Other protein is water-soluble protein, namely α-lactalbumin and

Table 1: Fat, protein, lactic-acid and pH of Dangke-cheese by different papain concentrations.

Papain-concentration (%)		Heating-milk-temperature (°C)					
		75	80	85	90	95	100
0.3	Fat (%)	26.23^{a}	23.88^{b}	22.89^{c}	20.18^{d}	17.21^{e}	13.82^{f}
0.4		23.84^{b}	23.55^{c}	22.86^{c}	20.67^{g}	17.12^{e}	16.89^{e}
0.5		24.31^{h}	24.20^{h}	23.97^{b}	22.42^{c}	18.78^{i}	17.24^{e}
0.3	Protein (%)	16.13^{a}	15.88^{b}	15.82^{b}	14.25^{c}	13.98^{d}	11.94^{e}
0.4		17.45^{f}	17.28^{g}	16.72^{h}	15.69^{b}	14.05^{d}	12.67^{h}
0.5		17.67^{i}	17.49^{g}	17.32^{g}	15.89^{b}	15.38^{i}	14.12^{b}
0.3	Lactic-acid (%)	0.77^{a}	2.41^{b}	0.43^{b}	0.52^{c}	0.34^{d}	0.19^{e}
0.4		0.65^{a}	0.62^{a}	1.73^{a}	0.36^{b}	0.41^{b}	0.37^{d}
0.5		0.31^{d}	0.34^{d}	0.32^{d}	0.28^{d}	0.23^{e}	0.28^{d}
0.3	pH	6.12^{a}	6.27^{b}	6.62^{c}	6.53^{c}	6.73^{c}	6.64^{c}
0.4		6.12^{a}	6.19^{b}	6.40^{c}	6.37^{c}	6.50^{c}	6.68^{d}
0.5		5.91^{a}	6.08^{a}	6.06^{a}	6.12^{a}	6.38^{c}	6.72^{d}

Note: Different signs indicate a very significant difference (P<0.01).

Table 2: Physicochemical properties of dangke cheese at different heating-milk-temperatures and papaya-latex-concentrations.

	Heating-milk-temperature								
	70°C			75°C			80°C		
Papaya latex concentration (%)	0.3	0.4	0.5	0.3	0.4	0.5	0.3	0.4	0.5
Carbohydrate (%)	2.81^{a}	7.30^{b}	6.92^{b}	3.41^{c}	7.32^{b}	5.92^{d}	3.49^{c}	5.18^{e}	5.00^{e}
Ash (%)	1.99^{a}	1.97^{a}	1.98^{a}	2.31^{b}	2.23^{b}	2.45^{c}	2.47^{c}	2.48^{c}	2.49^{c}
Water (%)	70.22^{a}	70.34^{a}	60.28^{b}	68.23^{c}	70.12^{a}	70.10^{a}	63.81^{d}	67.21^{c}	68.13^{c}

Note: Different signs indicate a very significant difference (P<0.01).

β-lactoglobulin. The k-casein molecule contains 169 amino acids. The isoelectric point for the binding of amino-acids 105 (phenylalanine) and 106 (methionine) is easily broken by proteolytic enzymes (renin, papain, bromelain). Starting from amino-acids 106-169, dissolved amino-acids are hydrophilic polar, which are also dissolved in the whey when making cheese. Curd formation increases if the enzyme that separates polar and non-polar amino-acids works optimally.

Fat and carbohydrate

The highest Dangke-Cheese fat content is at 75°C heating-milk, with a papaya-latex level of 0.3%, around 17.31%. Table 1 shows that fat decreases with increasing temperature. Table 2 explains the effect of 3 heating milk temperatures on carbohydrate, ash and water content using papain with different concentrations. The carbohydrate content, which represents lactose, means there is a fundamental difference between each heating milk temperature, with a range of 2.81-7.32%.

In cheese-making, lactose is generally included in the whey when the curd and whey are separated. The lactose content in cow's milk is 4.6%, so when using 0.3% papain, the lactose content is only 2.8 at 70°C, 3.41% at 75°C and 3.49% at 80°C heating-milk-temperature. However, what is surprising is that lactose-levels increased sharply when 0.4% and 0.5% papain were added to all heated milk. This may be due to lactose joining the curd during curd formation with increased enzyme activity at papain concentrations above 0.3%.

Minerals

Table 2 shows that changes in mineral content significantly differ between heating milk 70, 75°C and 80°C but not between heating milk 75 and 80°C. This indicates that increasing milk heating will increasingly precipitate and release minerals from protein. Calcium-caseinate-phosphate -micelle is a protein molecule bonded with calcium and phosphate and determines the process of forming curd using acid or enzyme.

In the coagulation process using papain, the casein binds with other casein, releasing calcium from the casein-micelles, increasing the mineral reading and heating-milk-temperature. At least 21 types of minerals are found in milk. The most important mineral is calcium, whose composition is balanced with phosphorus. Calcium in milk is mainly found as calcium-phosphate, calcium-carbonate and calcium-chloride, so it is easily absorbed in the intestine.

Water content

The water content in Dangke-cheese in Table 2 ranges from 60.28 to 70.34%. This water content is still relatively high, so it is still classified as soft cheese, although it is still lower than cottage cheese's, around 78.3%. The water content is higher at a papaya latex concentration of 0.3% because the 0.3% concentration has not caused all the casein to coagulate and much water is still stored in the curd. This condition causes curd to store water in a three-dimensional-network, fat-protein-water. Data is essential for understanding how heating temperatures and papaya sap concentration impact dangke cheese's physicochemical properties, crucial for consistent high-quality cheese production.

CONCLUSION

The higher the heating-milk-temperature, the lower the Dangke-cheese protein, fat and lactose. Meanwhile, as the heating-milk-temperature increases, pH and lactic-acid percentages approach lactic-acid percentages in fresh milk. The best Dangke-cheese-structure is heated at 75°C with a papaya-latex concentration of 0.5%, protein 17.94%, fat 24.295%, lactose 14.12%, pH 5.93 and lactic-acid 0.296%.

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Conflict of interest

The authors have declared no conflict interest.

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