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Effect Of Concentration Of Papaya Leaf Filtrate (*Carica Papaya L.*) And Citric Acid On Quality Of Goat's Milk Dangke

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ABSTRACT

Dangke is a dairy product that is shaped like cheese from Enrekang (South Sulawesi), which is made by coagulating milk with papain. The use of papaya leaf filtrate causes bitter taste in dangke, the addition of citric acid as a coagulant combination can control the proteolytic activity and cover an unfavorable after-taste. This study aimed to determine the effect of the concentration of papaya leaf filtrate and citric acid on the physicochemical and organoleptic characteristics of goat's milk dangke. This study used a factorial completely randomized design (CRD) with two factors: factor I was the concentration of papaya leaf filtrate (0.5%, 1%, 1.5%) and factor II was the concentration of citric acid (0.5%, 1 %, 1.5%). The data obtained were analyzed using ANOVA, and if there was a significant difference between treatments, it was continued with the DMRT follow-up test at a 5% level. This study showed a significant interaction between papaya leaf filtrate and citric acid on yield, water content, fat content, texture score, color score, and taste score. Dangke goat's milk with papaya leaf filtrate concentration of 1.5% and citric acid concentration of 0.5% is the best treatment with yield characteristics 10,6%; water content 54,47%; ash content 2,14%; protein content 21,25%; fat content 13,05% and pH 4,89 and hedonic score of texture 4,60 (slightly soft), aroma 3,30 (smells typical of goat's milk), color 2,25 (not very white), and taste 3,00 (bitter).

Keywords*: dangke, papaya leaf filtrate, citric acid, goat's milk*

INTRODUCTION

Dangke is a local cheese from Enrekang Sulawesi, Indonesia, which is made from buffalo or cow milk as raw material and processed with the help of milk clotting protease enzyme from papaya leaves and fruit (Zakariah et al, 2019). Dangke has a taste similar to cheese, but its appearance and texture are similar to tofu which is pure white to yellowish in color.

Goat's milk is milk obtained by milking goat's milk in the form of pure fresh milk without being mixed, reduced, or added (Zain, 2013). Goat's milk has an aroma that is not liked by consumers, processing goat's milk into dangke is expected to increase the added value of goat's milk and diversify dangke products. Rafiq et al. (2016) reported that goat milk is composed of total solid (12-13,5%), protein (3,5%), fat (3,8-4,5%), lactose (4,1%) and minerals (0,8%).

Dangke-making is an alternative to traditional cheese making because in general cheese is made by coagulating milk with the rennet enzyme while dangke is made by coagulating milk with the addition of papaya sap (papain enzyme). Making dangke using papain enzyme is an alternative natural coagulant substitute for rennet enzyme, which is available in limited quantities and has relatively expensive. The advantage of papain is its relatively high stability against temperature, pH, and alcohol solvents. The optimal temperature and pH range for papain are 60-70℃ and 6-7, respectively (Mahajan and Badgujar, 2010)

According to Amri and Mamboya (2012), The addition of the papain enzyme contained in papaya latex to milk with improper concentrations can cause the final product is not as expected as for example the product becomes bitter. Based on the research of Sulistyo et al., (2018), the

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use of acid in a combination of coagulants aims to control the proteolytic activity of the papain enzyme, because the papain enzyme has an optimum pH for proteolytic activity at pH 6-7.

Citric acid is an acidulant, an acidic chemical compound that is added to food processing for various purposes that can act as a flavor enhancer and color or cover an undesirable after-taste (Winarno, 2014). Based on the research results of Arifiansyah et al., (2014), the use of 1% synthetic citric acid coagulant produces the desired fresh cheese with an average moisture content of 58,81% and protein 12,26%, the value of preference for cheese texture shows a hedonic scale, the taste of the cheese is very like, the color is like and the total acceptance, that is, really like it.

Sulistyo et al., (2018), stated that the combination of papain enzymes at a fixed concentration (0,1%) with lemon juice concentration (5%) in making fresh cheese affects the water content, yield weight and shows the preferred preference value by comparison. organoleptic. Therefore, this study was conducted to determine the effect of a combination of papain enzymes derived from papaya leaf filtrate and citric acid as a coagulant in the formation of goat's milk dangke.

METHODS

Materials and Tools

The raw materials used in this research were goat's milk which is obtained at the Goat Milk Center in Surabaya from a farm in Lumajang, papaya leaves obtained from the Bangkok papaya garden in Surabaya, powdered citric acid, salt, and phosphate buffer pH 7. The tools used in the research included scales, measuring cups (Herma), thermometers, stoves, filters, and plastic containers. The tools used for analysis included spectrophotometry (Spectronic 21D by Milton Roy), Soxhlet apparatus, centrifuge (Hettich), analytical balance (Sartorius), oven (Memmert), vortex (Wizard), water bath, desiccator, pH meter, porcelain cup, Erlenmeyer (Duran), test tube, glass beaker (Duran), plastic containers, and labels.

The experimental design of the product

This method was compiled using a completely randomized design (CRD) with a two-factor factorial pattern, which involves the addition of papaya leaf filtrate at a concentration of 0,5%; 1%; 1,5%, and the addition of citric acid at a concentration of 0,5%; 1%, 1,5% to obtain 9 treatment combinations, each of which was repeated three times. The data were analyzed by analysis of variance (ANOVA), if there was a significant difference between the treatments, the Duncan test (DMRT) was continued at the 5% level. The organoleptic test was analyzed using a scoring test at a 5% level. The results obtained were then analyzed to determine the effectiveness index to obtain the best product. Determining the optimal formula is based on the chemical and sensory performance using the index effectiveness (DeGarmo et al., 2003).

Papain enzyme isolation from papaya leaves

The papain enzyme was isolated based on previous research by Zusfahair et al., (2014). Papaya leaves were pounded with a mortar, then the filtrate was taken by squeezing it with muslin fabric and adding 0.1M phosphate buffer (pH 7) at a ratio of 5:1 (papaya leaf: phosphate buffer). The filtrate was centrifuged at 3500 rpm for 15 min, to obtain a supernatant containing the crude papain enzyme extract.

The making of goat's milk dangke

Dangke was prepared based on previous research by Elwima (Elwima, 2017) with modification. Goat's milk used for each dangke is 200 ml. Milk was heated to a temperature of

50ºC, added with papaya leaf filtrate with a concentration of 0,5%; 1%; and 1,5% of the volume of milk while heated to a temperature of 60ºC. Then, 1% salt and citric acid (with concentrations of 0,5%; 1%; and 1,5% of milk volume) were added to the milk and stirred for ± 1 min.

The coagulating agents (papaya leaf filtrate and citric acid) were added and the mixture was heated (temperature 50-60ºC) and stirred until it coagulated. After that, the curd is separated from the whey by filtering and pressing it.

Analysis of goat's milk dangke

The goat's milk dangke was analyzed for moisture content (AOAC, 2005), ash content (AOAC, 2005), protein content using Lowry method (Sudarmadji, 2007), fat content (AOAC, 2005), pH using pH meter (Sudarmadji, 2007). The goat's milk dangke were analyzed for sensory properties using the scoring method (Susiwi, 2009).

Moisture content was analyzed using the AOAC method (AOAC, 2005) as follows: 1-2 g of sample is weighed and placed in a bowl that has been dried. Then, the sample and bowl were dried in a 105ºC temperature oven until a constant weight was obtained. Moisture content is the difference between the weight of the starting material and the weight of the final material after drying.

Ash content was analyzed using the AOAC method (AOAC, 2005) as follows: 1-2 g of sample is weighed and placed in a bowl that has been dried. Then, the sample and bowl were burned in a 400ºC temperature furnace until it becomes ashes. Ash content is the difference between the weight of the starting material and the weight of the final material after it becomes ashes.

Protein content was analyzed using the method of Lowry-Folin as follows: 0.1 g of sample was dissolved in 10 mL of distilled water. The sample was centrifuged for 5 minutes, 0,125 ml of the filtrate was taken, reacted with 2,5 ml Mix-Lowry reagent and left for 10 minutes. Then 0,25 ml of follin was added and left for 30 minutes until a blue color was formed. Aquades were added to a volume of 5 ml. The intensity of blue was measured by its absorbance using a spectrophotometer at a wavelength of 750 nm. The calculation of soluble protein levels was determined using the standard bovine serum albumin curve.

Fat content measurements were performed using a soxhlet extractor as follows: 2-5 g of finely ground sample wrapped in filter paper was inserted into a soxhlet extraction tube. Then, a fat dish and extraction tube are installed on the distillation apparatus. The soxhlet extractor that was filled with solvents was then drained with cool water and the appliance was turned on. Extraction was performed for 6 hrs. Then, the solvent was separated from the fat, while the fat-filled dish was dried in an oven at 100-105ºC for 1 hrs. The residual weight in the fat dish is expressed as fat weight.

Effectiveness Test (Degarmo, et al.,2003)

Determination of the best treatment is determined based on the index effectiveness method (DeGarmo et al., 1984). This method is based on the procedure as follows: variables are sorted by priority and contribute to results. Give value weight to each variable (BV) according to its contribution with relative numbers 0-1. This weight differs depending on the importance of each variable whose results are obtained as a result of treatment. Normal weight (BN) is determined from each variable by dividing the variable weight (BV) by the sum of all value weights. Divide the variables analyzed into two groups, namely: 1. Group A, consists of variables in which the greater the average the better the value (desired for the treated product). 2. Group B consists of variables which are the greater the worse (not desired). Determined the effectiveness value (NE) of each variable, using the formula: the treatment value - the worst value and the best value - the worst

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value, for the variable with the greater average the better, so the lowest value as the worst and the highest value as the best. Conversely for the variable with the smaller value the better, the highest value as the worst value and the lowest value as the best. Calculate the result value (NH) of each variable obtained from the normal weight multiplication (BN) with the value of effectiveness (NE).

Add the results value of all variables and the best combination is chosen from the treatment combination which has the highest result value (NH).

N Effectiveness = $\frac{treatment\ value - the\ worst\ value}{the\ best\ value - the\ worst\ value}$

Result value = NE x weight

RESULTS AND DISCUSSION

Enzyme activity of Papaya Leaf Filtrate

The enzyme activity of papaya leaf filtrate can be seen in **Table 1**.

Table 1. The results of the analysis of enzyme activity of papaya leaf filtrate.

Parameter	Analysis Results	Literature
Protease Activity (U/mL)	207,241	200-700[a]

Source: [a]Zusfahair et al., (2014)

According to Zusfahir et al., (2014), enzyme activity increases in proportion to the increase in temperature until it reaches its optimum temperature, this is because the kinetic energy of enzyme molecules increases before reaching the optimum. Saini (2010) stated that the papain enzyme works optimally at its optimum temperature, the optimum temperature of the papain enzyme is at 65-70°C. Wardhani et al., (2018) added, enzymes that work optimally able to produce more results a lot because there is an increase in reaction in breaking down casein into curds so that more curd is produced.

Chemical properties of Dangke

Table 2. The average moisture, ash,protein and fat content of goat's milk dangke

Coagulant Concentration		Moisture Content (%)	Ash Content (%)	Protein Content (%)	Fat Content (%)
PLF (%)	CA (%)				
0,5	0,5	56,46 [e] ± 0,185	2,12 [a] ±0,0055	16,23 [a] ±0,116	10,75 [a] ± 0,034
	1	54,00 [d] ± 0,250	2,13 [a] ±0,0036	16,35 [a] ±0,023	11,04 [b] ± 0,038
	1,5	53,06 [d] ± 0,739	2,13 [a] ±0,0046	16,19 [a] ±0,057	11,87 [c] ± 0,061
1	0,5	55,17 [d] ± 0,834	2,12 [a] ±0,0095	17,86 [b] ±0,563	12,02 [c] ± 0,079
	1	53,42 [d] ± 0,409	2,13 [a] ±0,0034	18,27 [b] ±0,029	12,14 [c] ± 0,146
	1,5	51,70 [c] ± 1,020	2,14 [a] ±0,0016	18,29 [b] ±0,021	12,51 [d] ± 0,086
1,5	0,5	54,47 [d] ± 0,443	2,14 [a] ±0,0156	19,15 [c] ±0,025	13,05 [e] ± 0,106
	1	49,27 [b] ± 0,383	2,14 [a] ±0,0093	19,26 [c] ±0,021	13,15 [e] ± 0,034
	1,5	47,98 [a] ± 0,552	2,14 [a] ±0,0158	19,27 [c] ±0,010	13,48 [f] ± 0,176

Note: [1)]PLF: Papaya Leaf Filtrate, [2)]CA: Citric Acid, [3)] different notations show a significant difference (p≤0.05).

Based on Table 2, the dangke moisture content ranged from 47,98% to 56,46%. Based on the analysis of variance, it can be seen that there is a significant interaction (p≥0,05). The

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higher the concentration of papaya leaf filtrate and citric acid will reduce the moisture content of dangke goat's milk.

Papain is a protease enzyme that can break down casein in milk. According to Fernández-Lucas et al. (2017), papain interferes with kappa-casein to form para-kappa-casein. Papain cuts the peptide bond between phenyl and methionine in the kappa-casein, damages its structure, and produces para-kappa-casein—which has a hydrophobic portion; therefore, as the concentration of papain increases, water content should be lower due to the peptide bonds being increasingly cut. According to Yuniwati et al., (2008) that the addition of coagulation ingredients that work optimally will result in a product yield that is not so large but the water content is small because the deposition is more perfect so that water is easily separated from the desired solids so that the texture is more sturdy or chewy.

The higher the concentration of citric acid, the lower the moisture content. According to Arifiansyah et al., (2014) that cheese with the highest pH will retain more water, whereas cheese with the lowest pH value or in more acidic conditions will lose the most water and have the lowest moisture content. The moisture content of dangke products can affect the shelf life of the product.

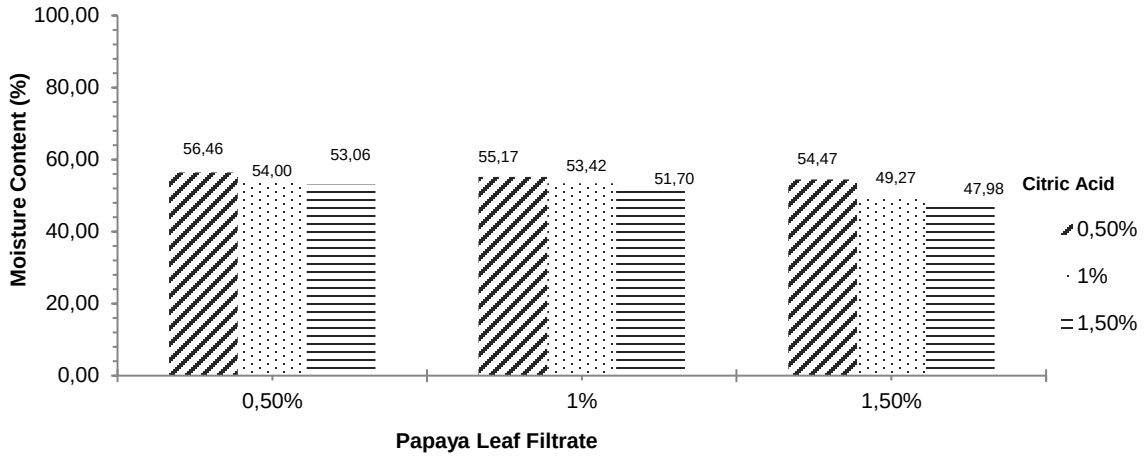

Figure 1. The moisture content of goat's milk dangke was influenced by the concentration of papaya leaf filtrate and lime extract.

The ash content of dangke goat's milk ranged from 2,129% to 2,139% according to the dangke ash content from Enrekang which was 1,9%-2,4%. Based on the analysis of variance, it can be seen that there is no significant interaction (p≥0.05). The ash content of dangke products is influenced by the minerals contained in goat's milk. According to Juniawati et al., (2015), milk minerals such as calcium, phosphorus, and magnesium are concentrated in the curd formed during the coagulation process.

The protein content of dangke goat's milk ranged from 16,19% to 19,17%. Based on the analysis of variance, it can be seen that there is no significant interaction (p≥0,05). the higher the concentration of papaya leaf filtrate added, the more papain enzyme content in papaya leaves which breaks down casein which causes milk to clot and increases dangke protein levels. This is in accordance with the statement of Yuniwati et al., (2008), that the greater the level of natural coagulation material from papaya latex, the greater the protein content produced because the

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reaction rate of an enzyme is directly proportional to the concentration of the enzyme. This citric acid treatment didn't give significantly different results at each concentration. This is because the addition of citric acid works by lowering the pH of the milk so that it clumps while according to Pellet and Young (1980) in Jamilatun (2009), soluble protein content or commonly called protein digestibility is the ability of a protein to be hydrolyzed into amino acids by protease enzymes.

Based on Table 2, dangke fat content ranged from 10,75% to 13,48%. Based on the analysis of variance, it can be seen that there is a significant interaction (p≥0,05). The higher the concentration of papaya leaf filtrate and citric acid, the higher the fat content. The use of two coagulants which is papaya leaf filtrate and citric acid will help optimize the clumping of dangke, the more protein clumps, the more fat will be trapped in the lumps and unite to form curd. This is in accordance with the statement of Fernández-Lucas et al., (2017) that the addition of papain shows proteolytic activity during the milk coagulation process, which causes the phospholipid-protein layer to be damaged, fat clumps will be trapped when the protein coagulates and eventually unites with the curd. In addition, this is also in accordance with the statement of Seth and Bajwa (2005) that the addition of acid during cheese production will increase the binding capacity of fat, because the addition of acid can cause protein coagulation which forms fat clots.

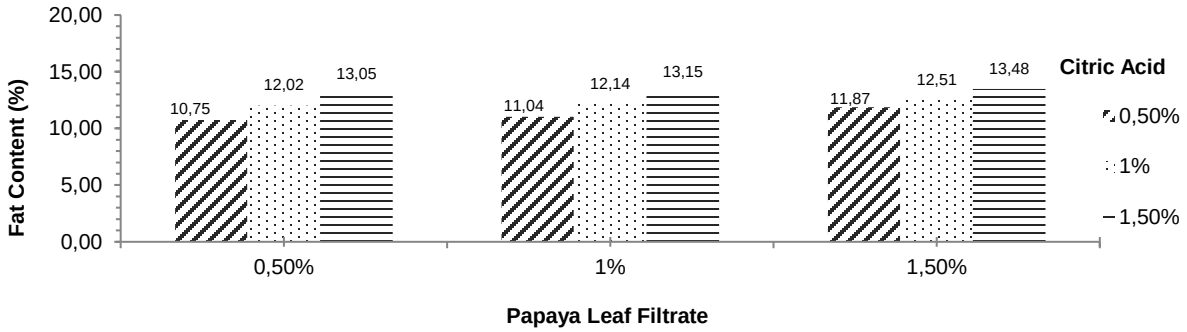

Figure 2. The fat content of goat's milk dangke was influenced by the concentration of papaya leaf filtrate and lime extract.

Sensoric characteristics of goat's milk dangke

The texture hedonic score of goat's milk dangke (Table 3) in the treatment of papaya leaf filtrate and citric acid concentrations ranged from 2,45-4,60. Goat's milk dangke treatment with 1.5% papaya leaf filtrate and 0,5% citric acid resulted in the highest texture score of 4,60 (slightly soft). Dangke texture is influenced by the water content factor contained in dangke. The smaller the water content, the stronger the protein gel network formed and the more compact the curd texture. Abu and Sri (2016) reported that, hardness level of the cheese texture is affected the presence of water content where the cheese matrix binds water higher so that the texture the cheese becomes softer, and less elastic. The addition of acid to the dangke causes the cheese to become softer. According to Harmayani et al, (2009), the very compact texture attribute is due to the higher concentration of agglutinating acid so that the higher concentration of coagulating acid results in the greater the attraction between proteins, which makes the protein gel network formed stronger and less water trapped so the texture will be softer.

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Table 3. The average hedonic score of texture, aroma, score and taste of goat's milk dangke.

Coagulant Concentration		Hedonic score of			
PLF (%)	CA (%)	aroma	texture	color	taste
0,5	0,5	2,90 [a] ± 0,447	3,65 [b] ± 0,745	4,40 [b] ± 1,046	4,45 [b] ± 0,510[b]
	1	3,10 [a] ± 0,447	3,35 [b] ± 0,745	3,85 [b] ± 0,587	4,40 [c] ± 0,503[c]
	1,5	3,00 [a] ± 0,459	2,45 [a] ± 0,686	4,00 [b] ± 0,562	5,75 [d] ± 0,444[d]
1	0,5	2,85 [a] ± 0,813	4,20 [c] ± 0,616	2,60 [a] ± 0,503	4,05 [b] ± 0,605[b]
	1	2,90 [a] ± 0,788	3,40 [b] ± 0,821	2,70 [a] ± 0,470	4,30 [b] ± 0,470[b]
	1,5	3,00 [a] ± 0,918	2,75 [a] ± 0,639	2,90 [a] ± 0,308	4,85 [b] ± 0,366[b]
1,5	0,5	3,30 [a] ± 0,733	4,60 [c] ± 0,503	2,25 [a] ± 0,550	3,00 [a] ± 0,324[a]
	1	3,00 [a] ± 0,918	3,55 [b] ± 0,510	2,20 [a] ± 0,410	3,20 [a] ± 0,410[a]
	1,5	3,15 [a] ± 0,745	2,90 [a] ± 0,641	2,15 [a] ± 0,671	3,35 [a] ± 0,489[a]

Note: [1)]PLF: Papaya Leaf Filtrate, [2)]CA: Citric Acid, [3)] different notations show a significant difference (p≤0.05).

The aroma hedonic score of goat's milk dangke (Table 3) was not significantly different, ranging from 2,85 to 3,30. Goat's milk dangke treatment with 1,5% papaya leaf filtrate and 0,5% citric acid resulted in the highest aroma score of 3,30 (smells typical of goat's milk). The aroma of dangke is influenced by the raw materials used and the ingredients added. The addition of papaya leaf filtrate and citric acid has no effect on goat's milk dangke results. According to Setyawati et al., in Sari (2014) stated that citrate fermentation in dairy products such as cheese causes changes due to volatile flavor and aroma-forming compounds.

The color hedonic score of dangke goat's milk (Table 3) in the treatment of papaya leaf filtrate and citric acid concentrations ranged from 2,15 to 4,40. Goat's milk dangke treatment with 0,5% papaya leaf filtrate and 0,5% citric acid resulted in the highest color score of 4,35 (slightly white). The color value in each treatment of papaya leaf filtrate concentration and citric acid showed a significant difference. The more papaya leaf filtrate added to the milk, the greener the cheese will be. This is due to the presence of green pigment (chlorophyll) in papaya leaves so that the final color of the dangke product is slightly greenish.

The taste hedonic score of goat's milk dangke (Table 3) in the treatment of papaya leaf filtrate and citric acid concentration ranged from 3,00 to 5,75. Goat's milk dangke treatment with 0,5% papaya leaf filtrate and 1,5% citric acid resulted in the highest taste score of 5,75 (not bitter). The taste value in each treatment of papaya leaf filtrate concentration and citric acid showed a significant difference. The addition of papaya leaf filtrate affects the bitter taste formed in dangke. According to Miskiyah and Mulyorini (2011), factors that need to be considered in using enzymes as a substitute for rennet are the presence of excessive proteolytic activity, excessive concentrations of proteolytic enzymes can cause a bitter taste because peptide bonds are formed which do have a bitter taste. The addition of citric acid aims to reduce the bitter taste of the resulting dangke. According to Winarno (2010), the addition of citric acid lowers the pH of milk so that the proteolytic activity of the papain enzyme can be controlled. The addition of citric acid with a high concentration also causes the resulting dangke have sour taste.

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Effectiveness Test

The effectiveness test is carried out to determine the best improver treatment used in goat's milk dangke. Effectiveness testing is carried out on all parameters. The treatment with the highest NH value of the product was the best treatment.

Based on Table 4, the effectiveness test of goat's milk dangke with treatment (PLF 1,5% and CA 0,5%) has the highest effectiveness value of 0,75 with characteristics: water content of 54.47%, ash content of 2,14%, protein content of 21,25%, fat content of 13,05%. Goat's milk dangke sensory test has the highest effectiveness value of 0,55 with characteristics: the color of the dangke with a value of 2,25; aroma with a value of 3,30; texture with a value of 4,60; taste with a value of 3,00

Table 4. Determination of the best formula using the effectiveness index method.

Coagulant Concentration		NH Value	
PLF (%)	**CA (%)**	**Chemical**	**Sensoric**
0,5	0,5	0,33	0,51
	1	0,36	0,54
	1,5	0,30	0,52
1	0,5	0,48	0,36
	1	0,50	0,32
	1,5	0,58	0,37
1,5	0,5	**0,75**	**0,55**
	1	0,69	0,25
	1,5	0,64	0,2

CONCLUSION

The use of Papaya leaf filtrate concentration and citric acid affect the physicochemical and organoleptic characteristics of goat's milk dangke. Based on effectiveness test (DeGarmo et al., 2003), The optimal formula for goat's milk dangke used 1.5% papaya leaf filtrate and 0.5% citric acid concentration which produced goat's milk dangke with water content 54.47%, ash content 2.14%, protein content of 21,25%, fat content of 13,05% and hedonic score of texture 4.60 (slightly soft), aroma 3.30 (smells typical of goat's milk), color 2.25 (very not white), and taste of 3.00 (bitter).

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