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The Effect of Using Crude Papain as Coagulant on Chemical Characteristics and Energy Value of *Susu Goreng* Made from Cow's Milk

Dea Ananda Hikmat[1], Bastari Sabtu[2], Yakob R. Noach[*3], Heri Armadianto[4]
[1,2,3,4] Faculty of Animal Husbandry Marine and Fisheries, Universitas Nusa Cendana

ABSTRACT: This study aimed to determine the effect of crude papain on the chemical characteristics and energy value of fried milk made from cow's milk. A completely randomized design (CRD) was used in this experiment with 4 treatments and 4 replicates. The levels of crude papain tested were P1 = 0.5%, P2 = 1%, P3 = 1.5% and P4 = 2.0%, of milk volume. The variables studied included protein content, total sugar, lactose, calcium and energy value. The collected data were processed according to the variance analysis procedure. The fried milk produced had protein content; 9.9 - 11.87%, fat 6.59 - 9.59%, lactose 4.84 - 8.72%; total sugar 28.25 - 36.75%; calcium 0.23 - 0.26% and energy value 2712.47 - 3388.25 calories/100g. The results of variance analysis showed that the treatment had a very significant effect ($P<0.01$) on fat content, total sugar, and a significant effect ($P<0.05$) on lactose content, but not significant ($P>0.05$) on protein, calcium, and energy value of fried milk. It was concluded that the use of crude papain produced fried milk with varying levels of fat, total sugar and lactose but protein, calcium and energy value tended to be the same. The best results were obtained with the use of crude papain 0.5%.

KEYWORDS: Crude papain, cow's milk, chemical characteristics, energy value, fried milk.

INTRODUCTION

Data from the Central Bureau of Statistics shows that the average milk consumption of Indonesians in 2020 is still low at 16.27 kg/capita/year, compared to neighboring countries such as Thailand 22.2 kg, Malaysia 36.20 kg and Myanmar 26.7 kg[1]. [2,3] Stated that milk consumption in the elderly population in Indonesia is among the lowest in the world, and two-thirds of the population is lactose intolerant. This may have an impact on energy and nutrient intake. However, there is a lack of information on the prevalence of inadequate nutrient intake in dairy users compared to non-dairy users, as well as population characteristics.

[4]reported that factors influencing low milk consumption are milk consumption habits, maternal knowledge about milk nutrition and low family income and milk allergy. [5] State that the low level of milk consumption in Indonesia is partly due to dislike due to the low culture of drinking milk, and cases of intolerance to lactose due to not consuming milk from an early age.

One possible strategy to promote milk consumption is to develop low-lactose processed products through certain processes or processing techniques to reduce lactose content so that it can be consumed without problems. In addition to fermentation, heating can also reduce lactose levels in milk due to the nature of lactose which will undergo browning (non enzymatic browning) if treated with heat. In Indonesia there are several dairy products traditionally made by certain communities based on buffalo and cow milk such as curd from West Sumatera[6,7], dangke made by the Enrekang community in South Sulawesi[8,9]. In East Nusa Tenggara (NTT) there is *susu goreng* made by people on the island of Rote [10,11], *Ei huhu pe ihi* by people on the island of Sabu[12], cologanti and suspesi in Timor[5,11]. The existence of traditional dairy products based on buffalo and cow milk is an indication that milk has been recognized by the community, although production and consumption are still limited.

Susu goreng made by people on Rote Island generally uses swamp buffalo milk mixed with palm sugar (locals call it *gula air* and *gula lempeng*) and processed by heating (cooking) until slightly dry brownish in color. This product is unique in the form of "amorphous" chunks resembling scrambled eggs with sweet, savory flavors and good nutritional value. A survey conducted by [10] in three production centers showed that the nutritional value of fried milk is quite good, namely moisture content of 36.23%, protein 29.01%, fat 32.15%, carbohydrates 30.66% and calcium 5.23%.

Currently, *susu goreng* has become a typical ole-ole from Rote in a 250 gram package with a price of 50 thousand rupiah (Figure 5), indicating that this product has prospects in the future even though it is still very dependent on limited buffalo milk production. [13] reported that in Indonesia, a buffalo herd can produce 1.5-3 liters of milk per day. According to [14] swamp buffalo milk production is very low, ranging from 1-1.5 l/head/day, compared to 6-8 l/head/day for dairy buffaloes.

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Developing *susu goreng* in order to provide nutritious food for the community requires a comprehensive study related to the revitalization of buffalo livestock resources and the possibility of utilizing the milk of other types of livestock such as cows or goats as the basic ingredient for making *susu goreng* and modifying the process by adding coagulants. One practical coagulant is crude papain with its proteolytic activity that is able to coagulate milk. [15],[9] have utilized crude papain as a coagulant in the manufacture of *dangke* with the best results obtained at the level of use of 0.5%.

So far, there is no information available on efforts to modify the manufacture of *susu goreng* made from milk other than buffalo milk. This study was conducted to explore the possibility of making cow's milk-based *susu goreng* by modifying the process using crude papain coagulant and its effect on the nutritional value characteristics of the resulting product.

MATERIAL AND RESEARCH METHODS

Making *susu goreng* in the Laboratory of Animal Products Technology (THT) Faculty of Marine Animal Husbandry and Fisheries-Undana, while testing parameters at Chem-mix Pratama Laboratory Yogyakarta. The research took place from June to September 2022.

The research materials were 16 liters of fresh cow (FH) milk from a dairy farmer in Mandeu Village, Raimanuk District, Belu Regency; 1.6kg of solid palm sugar from Oeba Kupang market, 5g crude papain from papaya sap tapping which was dried and mashed and 500 ml distilled water. The tools used consisted of 1 liter pyrex measuring cup, 100g/0.001g digital analytical balance, 5kg x 1kg WJ-B0 analytical balance, water thermometer, waterbath, wok, stainless basin, 20ml spoit, 1900ml cup, sieve and stirrer.

The study used a completely randomized design (CRD) with 4 treatments repeated 4 times, so there were 16 experimental units. Each experimental unit used 1 liter of fresh cow's milk. The four levels of crude papain tested were P1 = 0.5%, P2 = 1.0%, P3 = 1.5% and P4 = 2.0% of milk volume.

Research Procedure

Crude papain is prepared by cutting the skin of young papaya fruit and the sap is collected, dried in the sun and then mashed into flour. Crude papain is activated following the instructions of [16], namely 5 g of crude papain dissolved in 100 ml of distilled water stirred for five minutes until homogeneous, let stand for fifteen minutes and ready to use.

Fresh cow's milk was pasteurized by HTST (high temperature short time) method at 70ºC for 15 seconds[17]. Pasteurization is done indirectly, namely 1000ml of milk is put into a measuring cup, placed in a pan of water that is being heated over low heat. During heating the milk is continuously stirred and the milk temperature is maintained at 70ºC for 15 seconds. Pour the milk into a special container and let stand until the temperature drops to 50-60ºC (optimum temperature for papain enzyme action). Add crude papain solution according to the treatment (0.5; 1.0; 1.5 and 2.0%) stir and let stand until coagulum (curd) is formed. Separate the curd and whey using a filter cloth, then weigh the weight of the curd obtained. Repeat this procedure for all experimental units.

The crushed solid palm sugar (*gula lempeng*) is weighed at 10% of the weight of the curd. Combine the sugar with the curd and put the mixture in a pan, then heated over low heat while stirring until the mixture slowly turns brownish or caramel color. After that, the heating is stopped and the fried milk obtained is transferred to a container, followed by a laboratory testing process to be ready for examination.

Variables Measured

1. Protein content

   Determination of protein content using the micro Kjeldhal method according to AOAC (2005)[18] procedures includes three stages, namely deconstruction, distillation and titration. The deconstruction stage begins with weighing 0.2 g of sample. The sample is then put into a 100 ml volumetric flask and then added 10 ml of concentrated H2SO4 with 2 g of catalyst and then the solution is deconstructed until it becomes clear and the deconstruction is continued for 10 minutes. The clear solution was cooled, diluted with distilled water as much as 3 ml, then added 5 ml of NaOH, 45% and a few drops of PP indicator and then distilled. The distillation results were collected in a 125 ml erlenmeyer containing 10 ml of 2% boric acid (H3BO3) containing 0.1% bromcherosol green and 0.1% methyl red indicators in a ratio of 2:1. Titration is done using 0.01 N HCl until the color of the solution in the erlenmeyer turns pink, then read and record the titration volume. Calculation of protein content using the formula:

   Protein content (%) =((V1-V2) x N x 0.0014 x fp x fk)/W x 100%

   Notes: W = Sample Weight; V1 = Volume of 0.01 N HCl for blank titration; V2 = Volume of 0.01 N HCl for sample titration; N = Normality of HCl; fp = Dilution factor; fk = Protein conversion factor 6.25.

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2. Fat content
Determination of fat content using the Soxhlet method according to the AOAC (2005) procedure[18], namely as much as 1-2 g (W1) of sample weighed in filter paper and put into the Soxhlet tube, then the fat flask that has been weighed by its fixed weight (W2) is connected to the Soxhlet tube. The Soxhlet tube was inserted into the Soxhlet tube extractor chamber and flushed with 250 ml of n-hexane. The extraction tube was attached to the Soxhlet distillation device and then distilled for 6 hours. At the time of distillation the solvent will be collected in the extractor chamber, the solvent is removed so that it does not return to the fat flask, then the fat flask is dried in an oven at 105 ° C, after which the flask is cooled in a desiccator until its weight is constant (W3). Calculation of fat content using the formula:
Fat content (%) = (W3-W2)/W1 x 100%
Description: W1= Weight of sample (g) W2= Weight of fat flask without fat (g) W3= Weight of fat flask with fat (g)
3. Total sugar content
The total sugar content of fried milk is determined by the Luff Schoorl method, i.e. a mashed sample of 5 g is put into a 250 ml goblet, dissolved using 100 ml of distilled water, add Pb-acetate for clarification. Add Na_2CO_3 to remove excess Pb and then add distilled water to exactly 250 ml. Put 25 ml of the solution in Erlenmeyer then add 25 ml of Luff-Schoorl solution. Make a blank treatment by adding distilled water to 25 ml of Luff-Schoorl solution. Add a few boiling stones, connect the Erlenmeyer with a reverse cooler and boil for 10 minutes, after which it is immediately cooled and then add 15 ml of 20% KI and 2.5 ml of 26.5% H_2SO_4 carefully. The liberated iodine is titrated with 0.1N Na-Thiosulfate solution using 1% starch indicator as much as 2-3% (Titration is terminated after a milky beige color appears). Calculation of total sugar content using the formula:
Total sugar content (%): ((Blank Titration - Sample Titration) × Dilution Factor)/(mg sample) × 100
4. Lactose content
Determination of lactose content of fried milk is done as follows: 1 g sample is put in a 100 ml volumetric flask, dissolved with 100 ml of hot water. Precise with distilled water until the limit mark. Pipette 10.0 ml of the solution into a stop erlenmeyer and add 15.0 ml of distilled water and 25.0 ml of Luff Schoorl solution then cool with reverse cooling and heat for 10 minutes after that cool and then add 15.0 ml of H_2SO_4 6N and 15.0 ml of KI 20% and titrate with $Na_2S_2O_3$ 0.1 N until yellow. Add 1 ml of 1% amylum and titrate again until the TAT which is the exact blue color disappears. Calculation of lactose content of fried milk using the formula:
Lactose content (%) = (Sample x dilution factor)/(sample weight) x 100
5. Calcium (Ca) content
The calcium content of fried milk was determined by the titration method. A 4 mg sample was added to 100 ml of distilled water. The addition of 2N NaOH was done so that the pH was 12-13. The indicator used is murkesid 0.2% (w/b), then titrated using Na_2EDTA solution that has been standardized in the previous stage. The end point of the titration is marked by a change in color, which becomes purple with an initial color of pink. Calcium content was calculated with the formula:
Calcium content (mg/100 mg) = (M x Vb x 40 x 100)/(Vc)
Description:
M= Molarity of $Na_2EDTA.2\ H_2O$ solution (M)
V_b= Volume of Na_2EDTA used for titration
V_c= Volume of beverage pipetted (ml).
6. Energy value
The energy value of fried milk was determined using a bomb calorimeter expressed in kcalories/100g.

Data Analysis

The data obtained were tabulated and processed using analysis of variance to determine the effect of treatment on the variables studied and continued with Duncan Multiple Range Test (DMRT), to determine differences between treatment[19].

RESULTS AND DISCUSSION

The chemical characteristics and energy value of cow's milk-based fried milk produced from this study are presented in Table 1.

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Table 1. Chemical characteristics and energy value of *susu goreng*

Parameters	Treatment				P Value
	P_1 (0.5%)	P_2 (1%)	P_3 (1.5%)	P_4 (2%)	
Protein (%)	11.87	10.80	10.01	9.9	0.237
Fat (%)	6.59^a	7.19^a	9.59^b	7.51^a	0.001
Total Sugar (%)	28.25^a	35.39^b	33.20^b	36.75^b	0.004
Lactose (%)	6.95^b	8.72^c	7.54^{bc}	4.83^a	0.025
Calsium (mg/100mg)	0.24	0.23	0.26	0.23	0.692
Energy (kcal/100g)	3388.25	2858.29	2712.47	3107.97	0.089

Note: different superscripts on the same line indicate differences (P<0.05); P1= crude papain level 0.5%; P2= crude papain level 1%; P3= crude papain level 1.5%; P4= crude papain level 2%.

Protein content of *susu goreng*

The protein content of *susu goreng* obtained in this study ranged from 9.92 - 11.87% with an average of 10.65%. Table 1 shows a decrease in protein content as the level of crude papain use increases where the highest protein content is at the 0.5% level and the lowest at the 2% level. According to [20] the addition of too much papain concentration can result in less optimal enzyme activity due to insufficient available substrate. However, the results of variance analysis showed that the treatment had no significant effect (P>0.05) on the protein content of *susu goreng* produced. This means that the use of crude papain with a level of 0.5 -2.0% produces fried milk with protein levels that tend to be the same.

The average protein content of *susu goreng* obtained from this study is lower than the study of [11] on *susu goreng* made from buffalo milk which is 13.98%, even much lower than the results reported from three *susu goreng* production centers on the island of Rote which is 28.84%[10]. This difference is due to the milk base material used is cow's milk while the previous research on fried milk was based on buffalo milk. Buffalo milk does have advantages in terms of nutritional composition over cow's milk. Buffalo milk has a protein content of 5.14%[14] while Frissian Holstein cow's milk contains 3.16% protein[21]. [22] states that buffalo milk contains 4 - 6% protein and is higher than cow's milk with a protein content of 4%.

Based on the value of protein contained in the fried milk produced from this study, this product can be relied upon as one of the sources to fulfill the *Angka Kecukupan Gizi* (AKG) of Indonesians. The recommended protein adequacy at various ages and statuses include children 7-9 years as much as 40g, adolescent boys 16-18 years = 75g, adult men 30-49 years = 65g, elderly men 65-80 years = 64g, adolescent girls 16-18 years = 65g, adult women 30-49 years = 60g, elderly women 65-80 years = 58g, pregnant women in the third trimester = 90g and nursing mothers in the first 6 months 80g[23]. Based on this AKG, by consuming 100g of *susu goreng* made from cow's milk with a protein value of 10.65%, it can fulfill around 11.83 - 26.63% of the protein adequacy rate for the age group and status as mentioned above.

Fat content of *susu goreng*

The fat content of fried milk obtained in this study ranged from 6.59 - 9.59% with an average of 7.72%. Table 1 shows an increase in fat content up to the level of 1.5% crude papain usage and decreased again at the level of 2% usage. The results of variance analysis showed that the treatment had a very significant effect (P<0.01) on the fat content of fried milk produced. This means that the use of crude papain with a level of 0.5 -2.0% produces fried milk with varying fat content. Duncan test results proved that the fat content of fried milk in the treatment pair P1-P3; P2-P3; P3-P4 was different while the pair P1-P2; P1-P4 and P2- P4 were not different. The decrease in fat content at the 2% crude papain usage level is thought to be related to the coagulation process where not all fat is bound in the curd formed due to less than optimal enzyme activity. This is in line with the statement of [20] that adding too much papain concentration can result in less optimal enzyme activity.

The average fat content of fried milk obtained from this study is much lower than fried milk made from buffalo milk reported by [11] which was 23.65%, and [10] from three fried milk production centers on Rote island, which was 32.14%. The lower fat content of fried milk in this study is due to the milk base material used is cow's milk, where there is a significant difference between the fat of cow's milk and buffalo milk. [22] stated that the components of buffalo milk are generally the same as cow's milk, except that the fat content of buffalo milk is higher at 7-10% compared to 3% in cow's milk.

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Total sugar content of *susu goreng*

The total sugar content of *susu goreng* obtained in this study ranged from 28.25 - 36.75% with an average of 33.4%. Table 1 shows a fluctuating increase in total sugar content as the level of crude papain usage increased up to 2%. The results of variance analysis showed that the treatment had a very significant effect (P<0.01) on the total sugar content of *susu goreng* produced. This means that the use of crude papain with a level of 0.5 -2.0% produces fried milk with varying total sugar content. Duncan test results proved that the total sugar content of *susu goreng* in the treatment pairs P1-P2; P1-P3; P1-P4 was different while the P2-P3; P2-P4 and P3- P4 pairs were not different. This difference is thought to be due to differences in the quantity of curd obtained in the preliminary process, where the amount of sugar added is based on the amount of curd produced.

The average total sugar content of *susu goreng* from this study if assumed to be equivalent to total carbohydrates, the results obtained are not far from those reported by [10] from three *susu goreng* production centers on the island of Rote which is 30.66%.

Lactose content of *susu goreng*

The lactose content of *susu goreng* obtained in this study ranged from 4.83 - 8.72% with an average of 7.01%. Table 1 shows an increase in lactose levels at the 1% crude papain usage level with the highest number (8.72%) then decreased to the lowest number of 4.83% at the 2% crude papain usage level. The results of variance analysis showed that the treatment had a very significant effect (P<0.01) on the lactose content of *susu goreng* produced. This means that the use of crude papain with a level of 0.5 -2.0% produces *susu goreng* with varying lactose levels. The Duncan test results proved that the lactose levels of P1-P2; P2-P4; P1-P4 and P3-P4 treatments were different while the P2-P3; P1-P3 pairs were not different.

The presence of lactose in milk or processed products is often associated with the health problem of lactose intolerance as one of the causes of low milk consumption in Indonesia. Milk or dairy products that contain high lactose content are usually avoided by certain groups of people because they often cause problems, such as abdominal pain and diarrhea. People with lactose intolerance are advised to choose milk or dairy products that contain low lactose.

The *susu goreng* made from cow's milk produced in this study has a high average lactose content of 7.1%. This indicates that this product should not be recommended for people with lactose intolerance. [24]Stated that people with lactose intolerance should be encouraged to limit rather than avoid lactose in terms of how to consume milk, through good education about the comparison of dairy and non-dairy products in meeting daily nutritional needs. [25] featured several dairy products with low lactose content such as butter, probiotic yogurt, hard cheese, kefir and heavy cream.

Calcium content of *susu goreng*

The calcium content of *susu goreng* obtained in this study ranged from 0.23 - 0.26% with an average of 0.24%. Table 1 shows that calcium levels fluctuate in response to the use of crude papain with different levels. The results of variance analysis showed that the treatment had no significant effect (P>0.05) on the calcium content of *susu goreng* produced. This means that the use of crude papain with a level of 0.5 -2.0% produces *susu goreng* with protein levels that tend to be the same. This tendency is related to the protein content of fried milk which is also not different. The average calcium content of *susu goreng* from this study is much lower than the results reported from three fried milk production centers on the island of Rote which is 0.82%[10].

Energy value of *susu goreng*

The energy value of *susu goreng* obtained from this study ranged from 2712.47 - 3388.25 kcal/100g with an average of 3016.75 kcal/100g. Table 1 shows that there was a tendency to decrease the energy value up to the level of using 1.5% crude papain but increased again at the level of 2%. The results of variance analysis showed that the treatment had no significant effect (P>0.05) on the energy value of *susu goreng* produced. This means that the use of crude papain with a level of 0.5 -2.0% produces fried milk with energy values that tend to be the same. The energy value of this *susu goreng* is lower than *susu goreng* made from buffalo milk which is 3400 kcal/100g[11]. This difference is due to the milk base used is cow's milk while the previous study on fried milk made from buffalo milk.

Based on the energy value contained in *susu goreng*, this product can be relied upon as one of the sources to fulfill the *Angka Kecukupan Gizi* (AKG) for Indonesians. The recommended energy adequacy at various ages and statuses include children 7-9 years: 1650 kcal, adolescent boys 16-18 years: 2650 kcal, adult male 30-49 years old: 2550 kcal; elderly men 65-80 years: 1800 kcal, adolescent girls 16-18 years: 2100 kcal, female adults 30-49 years: 2150 kcal, female seniors 65-80 years: 1550 kcal, third trimester pregnant women: 2480 kcal and breastfeeding mothers in the first 6 months: 2450 kcal[23]. Based on this AKG, consuming

100g of fried milk made from cow's milk with an energy value of 3016.75kcal can cover the energy adequacy for the age group and status as mentioned above.

CONCLUSION

Based on the results of the study, it can be concluded that the use of crude papain as a coagulant with a level of 0.5 to 2% produces fried milk with varying chemical characteristics. The use of crude papain level 0.5% is the best where the fried milk produced has the characteristics of the highest protein content and energy value, the lowest fat content and total sugar. Consuming 100g of cow's milk-based fried milk can contribute 11.83-26.63% to the protein sufficiency rate and exceed the recommended energy sufficiency rate for Indonesians.

RECOMMENDATION

Making fried milk by adding coagulants in the form of crude papain should be used at a level of 0.5% and the fried milk produced needs to be tested publicly to determine the level of consumer acceptance of the product.

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Profile of *susu goreng* made from Cow's milk

Fig. 1. Using crude papain 0,5%

Fig. 2. Using crude papain 1,0%

Fig. 3. Using crude papain 1,5%

Fig. 4. Using Crude papain 2,0%

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Fig. 5. The original *susu goreng* made from buffalow milk

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