



Preparation of cottage cheese from buffalo milk with different levels of papaya latex

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Abstract

The study was conducted to standardize the desired level of papaya (*Carica papaya*) latex on quality of cottage cheese prepared from buffalo milk. Cheese sample was prepared using whole milk collected from Lal Teer Livestock Development (BD) Ltd. Dairy farm. This experiment was conducted on five treatments named as sample BC₂: buffalo control; A₂: 5 drops (0.16g); B₂: 6 drops (0.18g); C₂: 7 drops (0.21g); and D₂: 8 drop (0.24g). Overall score of cheese samples, B₂ was highest (91.67±0.58) and A₂ was lowest (77.67±9.29). Chemical analysis showed that, highest protein value 24.00±1.00 was found in B₂ and lowest value 21.00±1.00 was in C₂. On the other hand C₂ content the maximum fat percent 18.00±1.00 and A₂ content the minimum fat percent 12.00±1.00. Results indicate that carbohydrate, ash and acidity have significant differences ($p < 0.01$) but non-significant difference was found in total solids and moisture. Cheese from buffalo milk on coagulation time significantly different ($p < 0.01$) but non-significant differences were found in yield.

Key words: cheese, papaya, latex, coagulation, buffalo milk

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Introduction

Cheese is one of the most widely consumed fermented dairy products with a growing consumer demand. It is an excellent dietary source of high-quality protein, vitamins and minerals such as absorbable dietary calcium. Hundreds of types of cheese are produced in the world. There are numerous references to cheese making in the Bible while the writings of Homer and Aristotle indicate that cheese was made from milk of cows, goats, sheep, mares and asses (O'Connor, 1993). There is no definite list of cheese varieties while Sandine *et al.* (1970) cited that there are more than 1000 varieties. Milk coagulation is a basic step in cheese manufacturing. Calf rennet, the conventional milk clotting enzyme obtained from the fourth stomach of suckling calves was the most widely used coagulant in cheese making all over the world to manufacture most of the cheese varieties. The worldwide reduced supply of calf rennet and the increase in cheese production and consumption have stimulated research for milk clotting enzymes from alternative sources to be used as calf rennet substitutes. Further, if calf rennet is used as a coagulant for cheese making, the product has to carry a tag of non-vegetarian which may lead to non-consumption of cheese by

vegetarian population of the world. Thus, it was found necessary to discover milk clotting enzymes from alternative sources.

The essential ingredients in cheese making are milk and protein coagulant. Milk coagulation is the primary step in the development of the texture and flavor of cheeses (Ozcan & Kurdal 2012). It depends on specific enzymatic proteolytic degradation of milk compounds especially protein in order to improve textural properties and the nutritional value of cheeses. In Indonesia, the method of cheese making by using papain enzyme which is obtained from papaya fruit has already been practiced since the Dutch colonial era (Rahman 2014). Papain is found naturally in papaya (*Carica papaya L.*) manufactured from the latex of raw papaya fruit. The latex of the plant *Carica papaya* yields papain and several other proteases. It has powerful milk clotting activity but it is also highly proteolytic. Most enzymes used for preparation of cheese have been extracts from the stomachs of ruminants, but coagulants from microbes and plants were also used at very recently (Harboe *et al.*, 2010; Jacob *et al.*, 2011). Plant based milk coagulants include extracts from *Carica papaya* (papain), pineapple (bromelin), castor oil seeds (ricin) and the latex of the fig tree (Ficin) and the plant

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Calotropisprocera which grows abundantly in many parts of Africa. These extracts are suitable for softer curd cheeses which are consumed within a few days.

The aim of the present study was to utilize the country's rich plant resources, notably papaya tree for its enzyme, known as papain, which is found in the latex of the un ripened yet matured fruit for use as alternative milk coagulant rather than employing the conventional rennet for the production of fresh un-ripen cheese upon optimizing the process parameters of cheese production.

Materials and Methods

Duration and Site of the experiment:

The experiment was conducted during the period of April to October, 2016. Milk samples were collected from Lal Teer Livestock Development (BD) Ltd. Dairy farm and the experiment was carried out at Dairy Technology Laboratory, Department of Dairy Science, Bangladesh Agricultural University, Mymensingh.

Experimental procedure:

For each trial 2.5 litres of milk was used. Milk boiled at 81°C. After cooling at 40°C 0.03 % calcium chloride (CaCl₂) was added and allowed to stand for 10 minutes. Then bacterial culture was added to the milk sample.

Generally 1% starter culture (sour dahi) was added. After addition of starter culture, the milk sample mixed thoroughly. Ripening was taken for one hour at 40°C. Lactic Acid Bacteria (LAB) were responsible for turning lactose into lactic acid, thereby reducing the pH of the solution. When the pH was lowered, the protein is more easily precipitated. This was vital for the production of cheese curds. At that time the milk was allowed to ripen for one hour upto reaches appropriate pH level. After the addition of starter culture 5, 6, 7, 8 drops papaya latex (papain) and 0.28g rennet was added for coagulation of milk. After collection of fresh papaya from the garden washed with alcohol and punched with syringe and latex was collected drop by drop. The papaya latex served to coagulate the milk protein and formed curds. Required quantity of papaya latex (papain) was added to the sample by thoroughly mixing and allowed to stand for 3-4 hours to complete coagulation. The coagulated

curd was cutted into small pieces like 1/4 to 5/8 inch cubes using stainless steel wire knife and allowed to continuous stirring and scalding for removing whey water from the curd and gaining appropriate pH. The curd was cooked at 46°C. To avoid uneven cooking or overcooking It was stirred constantly during this step and cooking only 20 minutes. When most of the whey was drain out from the coagulated curd it was collected. After that the curds were raked allowing whey to drainage.

When all of the curd was milled about 1% (by weight) salt was added. Then the curd was hooped and pressed into a stainless steel dices to form cheese block by forcing pressure. After collection of cheese it was kept in refrigerator for further use.

Analysis of the cheese samples

All experimental cheeses were judged by a panel of judges for organoleptic evaluation using a score card. The principal object of judging was to evaluate whether there prevailed any significant differences in cheeses of different groups in terms of color and appearance, flavor, finish, body and texture. The total solids and ash content of the different types of samples were determined by Oven Drying method according to A.O.A.C. (2003). Fat percent was determined by Babcock method using the procedure described by Aggarwala and Sharma (1961), protein content was determined by Kjeldahal procedure. Acidity was determined by titrating with N/10 sodium hydroxide solution using the procedure of Aggarwala and Sharma (1961).

Statistical analysis

The statistical model was Completely Randomized Design (CRD) and statistical analysis was done using Statistical Package software (SPSS).

Results and Discussion

Physical parameter

Flavor

There was non-significant differences within flavor score of different samples (Table 1). The highest flavor score was recorded in sample B₂ (6 drop papaya latex) whereas the lowest score was found in sample A₂ (5 drop papaya latex). This result indicates that judges appreciated the 6 drop papaya latex in cheese. The flavor of matured cheese is the result of the interaction of starter bacteria, enzymes from the milk, ripening

from the rennet and accompanying lipases, and secondary flora. Result of the experiment also agrees with the work of Urbach (1997).

Body and texture

There was significant difference ($p < 0.05$) within body and texture score of different samples (Table 1). Highest body and texture score was recorded in sample BC₂ (0.28g rennet) and B₂ (6 drops papaya latex). On the other hand, lowest score was found in sample A₂ (5 drop papaya latex). Body scores are accompanied by the conversion of part of the protein to water soluble compounds mainly proteases and peptones. The result is in agreement with the findings of Hossain (2006), where body and texture score was 25-27.

Finish

There was non-significant difference within finish score of different samples (Table 1). Highest finish score was recorded in sample B₂ (6 drops papaya latex) and lowest score was observed in sample A₂ (5 drops papaya latex). This result indicates that 6 drop papaya latex appreciable in cheese. The cheese sample had better appearance, smooth surface, free from cracks and practically free from molds.

Color

There was non-significant differences of color score among different samples (Table 1). Highest color score was recorded in case of sample B₂ (6

drops papaya latex) and lowest score was found in sample A₂ (5 drops papaya latex). It indicates that judges appreciated the 6 papaya latex drops in cheese. The result is also consistent with Dharam and Garg (1989) who reported appearance and color score of cheese to be 7.9 ± 0.2 .

Overall scores

There was significant difference ($p < 0.05$) within overall score of different samples (Table 1). Highest score was recorded in case of sample B₂ (6 drops papaya latex). On the other hand, lowest score was found in case of sample A₂ (5 drops papaya latex). The overall score of sample B₂ found (6 drops papaya latex) 91.67 ± 0.58 which indicates that judges appreciated the 6 drops papaya latex in buffalo milk for cheese manufacturing.

Chemical parameters

Fat content

The amount of fat content of cheese samples are demonstrated in Table 2. Differences were highly significant ($P < 0.01$) among those mean values. It was found that sample BC₂ contains highest score on the other hand sample A₂ contains lowest score. The results for cow's milk cheese coincide with those of Ghosh and Singh (1996a), Ahmed (2000) and Islam (2006) whose found (24.8%), (23.5%) and 23.5%, respectively.

Table 1. Overall score obtained by different types of cheese Samples from buffalo milk

Sample No.	Flavor (45)	Body and Texture (30)	Finish (15)	Color (10)	Total (100)
	Mean	Mean	Mean	Mean	Mean
BC ₂	41.33±0.58	28.00 ^a ±0.00	13.35±0.00	8.50±1.00	91.18 ^a ±1.15
A ₂	34.00±7.81	23.67 ^c ±2.08	11.67±1.15	8.33±1.15	77.67 ^e ±9.29
B ₂	41.33±1.15	28.00 ^a ±0.00	13.67±1.15	8.67±0.58	91.67 ^b ±0.58
C ₂	38.00±3.00	25.00 ^b ±2.65	12.67±2.08	8.33±0.58	84.00 ^c ±5.00
D ₂	35.67±5.13	24.33 ^b ±1.53	12.00±2.00	8.33±0.58	80.33 ^d ±5.86
LSD	4.650	1.736	1.558	0.858	5.702
LS	NS	*	NS	NS	*

**=Significant at 1% level, *=Significant at 5% level, LSD = Least Significant Difference, NS=Non significant, LS=Level of Significance, ^{abcd}=means with the different superscripts differed significantly within the same column. Where, BC₂= 0.28 g rennet, A₂= 5 drops papaya latex, B₂=6 drops papaya latex, C₂=7 drops papaya latex, D₂= 8 drops papaya latex.

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Protein content

Protein contents of cheese samples are demonstrated in Table 2. There was significant ($p < 0.05$) differences among the mean values of protein. It was found that sample B₂ contains highest score than the others. Proteolytic enzymes such as papain are responsible for the formation of nitrogenous products of intermediate size, such as proteoses, peptones, polypeptides, peptides and free amino acids. Enzymes of micro-organisms act on these and other substances to form products like amino acids, amines, fatty acids, esters, aldehydes, alcohols and ketones (Fox and McSweeney, 1996). Cheese serves as a store house of essential amino acids, having similar proportion of essential amino acids that is present in milk except the methionine and cysteine. Ahmed (2000) reported that the protein content of cheese prepared from cow milk was 22%, whereas the cheese from buffalo milk contained (19.4%). Islam (2006) found that the Mozzarella cheese of cow milk contained 21.9% protein, in agreement with the result of this study.

Carbohydrate (CHO) content

There was significant difference ($P < 0.01$) within the carbohydrate content of different samples (Table 2). Sample A₂ contained higher carbohydrate than the others group. Lactose is the major carbohydrate of milk. In addition to lactose, milk contains small amounts of glucose, galactose, and other saccharides. When milk is

coagulated, greater percentage of lactose is present in the whey and the remaining in the curd. For this reason, cheese that is prepared from the curd is low in carbohydrates (Penfield and Campbell, 1990). The result of this study is not similar to the results of Ali *et al.* (1977); Islam (2006), who found 6.3% and 5.5% carbohydrate respectively.

Total solids (TS) content

Total solids content of cheese samples are demonstrated in Table 2. Differences were not significant among those mean values. The weight loss in cheese during ripening has been attributed mainly to the loss of moisture (Buffa *et al.*, 2003). The result is partially similar with Ghosh and Singh (1996a), who reported total solids of cows and buffalo milk cheeses to be 49.7% and 50.2% respectively.

Ash content

Statistical analysis showed that there was significant difference ($P < 0.01$) within the ash content of different samples (Table 2). It was found that sample D₂ contains highest ash than the others. Ash is always higher than the milk from it made as because salt added in cheese, increase the amount of ash percentage in cheese. The result is similar to those of Ghosh and Singh (1996a); Ahmed (2000). They found ash content in cheese prepared from buffalo milk as 3.0% and 2.7%, respectively.

Table 2. Chemical composition of different types of cheese samples prepared from buffalo milk

Sample No.	Fat (%) Mean	Protein (%) Mean	CHO (%) Mean	TS (%) Mean	Ash (%) Mean	Acidity (%) Mean	Moisture (%) Mean
BC ₂	19.00 ^a ±1.00	21.35 ^b ±1.00	14.04 ^b ±1.00	57.00±1.00	2.75 ^b ±0.04	0.39 ^b ±0.00	42.00±1.00
A ₂	12.00 ^c ±1.00	21.87 ^b ±1.00	20.46 ^a ±1.00	57.43±1.00	2.99 ^b ±0.03	0.44 ^{ab} ±0.00	42.57±1.00
B ₂	15.00 ^b ±1.00	24.00 ^a ±1.00	16.09 ^b ±1.00	57.23±1.00	2.82 ^b ±0.03	0.41 ^b ±0.01	42.10±1.00
C ₂	18.00 ^{ab} ±1.00	21.00 ^b ±1.00	15.21 ^b ±1.00	57.23±1.00	3.19 ^a ±0.03	0.42 ^b ±0.01	42.77±1.00
D ₂	19.00 ^a ±1.00	21.35 ^b ±1.00	14.41 ^b ±1.00	57.84±1.00	3.79 ^a ±0.03	0.46 ^a ±0.01	42.16±1.00
LSD	1.050	1.050	1.050	1.050	0.033	0.011	1.050
LS	**	*	**	NS	**	**	NS

**=Significant at 1% level, *=Significant at 5% level, LSD = Least Significant Difference, NS=Non significant, LS=Level of Significance, ^{abcd}=means with the different superscripts differed significantly within the same column. Where, BC₂= 0.28 g rennet, A₂= 5 drops papaya latex, B₂=6 drops papaya latex, C₂=7 drops papaya latex, D₂= 8 drops papaya latex.

Acidity content

The acidity percentage of cheese samples are demonstrated in Table 2. Statistical analysis showed that there was significant difference ($P<0.01$) within the acidity content of different samples. This result is nearly in agreement with the result of Ghosh and Singh (1996b). They found acidity of cheese made from buffalo milk as 0.67%.

Moisture content

There was non-significant difference within the moisture content of different cheese samples (Table 2). Moisture content of different cheese sample BC₂, A₂, B₂, C₂ and D₂ were and 42.00±1.00, 42.57±1.00, 42.10±1.00, 42.77±1.00, and 42.16±1.00. The result followed by Hine (1994) and Islam (2006), who reported moisture content of cows' milk cheese as (40%-60%) and 46.8%, respectively.

Table 3. Effect of rennet (control group) and papaya latex on curd coagulation of different type of cheese prepared from buffalo milk

Sample No.	Time of Curd Coagulation(H)	Yield (g/kg)
	Mean± SD	Mean± SD
BC ₂	0.50 ^b ±0.00	220.00±0.00
A ₂	4.35 ^{ab} ±0.05	216.67±2.89
B ₂	4.30 ^a ±0.05	220.00±0.00
C ₂	4.25 ^{ab} ±0.05	220.00±0.00
D ₂	4.20 ^{ab} ±0.05	216.67±2.89
LSD	0.047	1.438
LS.	**	NS

**=Significant at 1% level, *=Significant at 5% level, LSD = Least Significant Difference, NS=Non significant, LS=Level of Significance, ^{abcd}=means with the different superscripts differed significantly within the same column. Where, BC₂= 0.28 g rennet, A₂= 5 drops papaya latex, B₂=6 drops papaya latex, C₂=7 drops papaya latex, D₂= 8 drops papaya latex.

Effect of rennet (control group) and papaya latex on curd coagulation of different type of cheese prepared from buffalo milk

Time of Curd Coagulation (H)

There was significant difference ($P<0.01$) within different cheese samples (Table 3). It was found that sample BC₂ required the lowest time in curd

coagulation than the others. Whereas, sample A₂, B₂, C₂ and D₂ were required much time on curd coagulation is not agreement with others study.

Yield g/kg

There was no significance different among the different cheese samples (Table 3). It is a very important parameter, the higher the recovered percentage of solids, the greater is the amount of cheese obtained and therefore gains in economic terms (Mona *et al.* 2011). Mahajan and Chaudhari (2014) reported 234.9g/kg cheese made from milk coagulating with papain enzyme is similar with the result of this study.

Conclusion

Milk coagulation is the primary step in the development of texture and flavor of cheeses depend on specific enzymatic proteolytic degradation of milk compounds specially proteins. In order to improve textural properties as well as nutritional value of cheeses and in addition to reduce the decreasing number of young animals has lead the producers to investigate alternative milk clotting enzymes of different origins. These include especially microbial and plant enzymes are commonly accepted for lacto vegetarians. The results showed that milk clotting enzymes of plant papaya have cheese making property. The papain enzyme had high milk clotting activity and better solubility. It could be useful in the dairy industry as a rennet substitute. The present research work was undertaken to evaluate the quality of cheese prepared from buffalo milk by using different concentration of papaya latex. Time of Curd Coagulation and yield observed, physical and chemical tests were performed to evaluate the effect of different level of papaya latex on quality of cheese. After the analysis of all parameters (physical, chemical, time required on curd coagulation and yield), it may be concluded that cheese from buffalo milk with 6 drops papaya latex (papain) had highest acceptability for preparation of desired quality cheese.

Conflict of interest

The authors declare that they have no conflict of interests.

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