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Effect of coagulants on the chemical and microbial quality of fresh cheese

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Abstract

This experiment was conducted to explore the effect of various types of coagulants on the chemical and microbiological quality of cheese made from fresh milk. Fresh cheese was manufactured using four different coagulants; microbial rennet (0.7%), lactic acid (1.5%), papaya latex (5 drops in 100 mL water) and calf rennet (100 g calf abomasum soaked overnight in 1000 mL mixture of water and milk in 3:1 ratio and then filtrate) under laboratory condition and analyzed for cheese yield, proximate composition, acidity, pH and coagulation time. In addition, standard plate count and coliform count was also performed. Result revealed that using microbial rennet as coagulant gave the maximum yield of cheese (225 g/Kg milk) followed by calf rennet and lactic acid, and papaya latex had the minimum value (215g/Kg milk) in this regard (p=0.013). Microbial rennet took the lowest time (22 min) to coagulate the milk which was 14-34 min less than that of the other coagulants, and lactic acid had the slowest (54 min) coagulating action in cheese manufacturing process (p=0.000). In addition, cheese that are manufactured by microbial rennet and calf rennet were superior in terms of chemical composition compared to the cheese in which lactic acid and papaya latex was used as coagulant (p=0.000-0.047). But the calcium concentration was found maximum in papaya latex cheese. Acidity was found highest in lactic acid cheese and papaya latex cheese had the highest pH value. On the other hand, standard plate count and coliform count was found highest in cheese coagulated by lactic acid, where microbial rennet cheese had the lowest count, and the microbial count of other two cheese was intermediate of them (p=0.000-0.001). Overall, microbial rennet, lactic acid, papaya latex, and abomasum rennet could be used to make cheese successfully, however, with a preference to microbial rennet.

Keywords: Cheese, microbial rennet, lactic acid, papaya latex, calf rennet, cheese yield

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Introduction

Cheese making in simple sense is the separation of curd from whey and kneading into a mouldable consistency. It is a nutritious milk product made from the milk of cow, goat, sheep, buffalo, camel, and yak. It contains all the milk nutrients and even something more from the microbial fermentation. It is convenient and versatile, with a wide range of flavors and textures. In 2020, global cheese production amounted to about 21.69 million metric tons (Altafini *et al*., 2021). Worldwide the top cheese producer was the European Union with a production volume of around 10.4 million metric tons of cheese per year (Kossongo *et al*., 2020).

Coagulant has one of the most significant roles in cheese manufacturing, and the traditional and most commonly used milk coagulating enzyme is rennet, which comes from calf (Roseiro *et al*., 2003). In addition to milk quality, the cheese qualities are determined by milk coagulation, which is one of the most critical processes in the cheese making process. Coagulating agents that are used in the clotting process makes the differences in protein matrix degradation (by the hydrolysis of caseins and the production of hydrophobic peptides) that influences the cheese yield, texture profile (elasticity, fragility, adhesiveness, hardness, gumminess and chewiness) and flavour development (especially a bitter taste). Animal

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rennet, which contains two enzymes (chymosin and pepsin) that break the Met^{105}-Phe^{106} link of the casein found on the surface of the casein micelles (κ-casein), is the most widely employed rennet for the coagulation of milk during cheese making. However, rising demand for alternative milk-coagulating sources is being driven by increased cheese production and a diminishing availability of natural animal rennet. According to Jacob *et al*. (2011), only 20 - 30% of the total milk clotting enzyme requirement was fulfilled by the calf rennet. As a result of this scarcity along with a variety of other considerations (vegetarianism, religious convictions, concern for genetically modified foods and so on), is responsible for growing the interest in the use of microbial coagulants and plant-derived coagulants (Roseiro *et al*., 2003).

Proteases generated from *Rhizomucormiehei*, *Rhizomucorpusillus*, and *Cryphonectriaparasitica* are the most often used microbial coagulants, with the first of these, *R. Miehei*, having been used as a substitute for animal rennet for nearly 40 years (Jacob *et al*., 2011). The Ser^{104}-Phe^{105} bond in casein is cleaved by *C. parasitica* proteases, while the Phe^{105}-Met^{106} bond is cleaved by *R. miehei* proteases. On the other side, the enhanced heat stability of *R. miehei* derivatives may be due to excessive proteolysis, which is associated with shorter ripening times and bitter cheeses.

Plant extracts can hydrolyze casein, resulting in curd formation, and they are also the major enzymes involved in casein hydrolysis (Roseiro *et al*., 2003). Different milk coagulating enzyme preparations have different site of action. The proteases from *Dregeasinensis Hemsl*. cleaved Ala^{90} - Gln^{91} (Zhang *et al*., 2015), rice wine chymosin acts on Thr^{94} - Met^{95} (Jiang *et al.,* 2007), *Albiziajulibrissin* protease breaks down Lys^{116}-Thr^{117} bonds and Sunflower seed proteases splits the bond between Phe^{105}-Met^{106} (Egito *et al*., 2007), and the ginger proteases breaks both the Ala^{90}-Gln^{91} and His^{102}-Leu^{103} (Huang *et al*., 2011) bonds of the κ-casein.

Papain, is normally found in the latex of papaya (*Carica papaya L.*), which has substantial milk clotting action and is particularly proteolytic. Its enzymatic activity is determined by the presence of a free sulfhydryl group. Papain is a proteolytic enzyme isolated from papaya latex that can degrade polypeptides, which are chemical molecules made up of amino acids (Amri and Mamboya, 2012).

The market growth and potential stimulates research into new microbial proteases for use as rennet substitutes, such as the studies conducted with *Nocardiopsis spp*. (Cavalcanti *et al*., 2005), *Mucor bacilliformis* (Machalinski *et al*., 2006), and *Bacillus subtilis* var. *natto* (Shieh *et al*., 2009). With the increased search for new coagulants, it is not unusual to hear about a new source. Keeping all these in consideration, the aim of this study was set to prepare fresh cheese by using calf rennet, lactic acid, microbial rennet and papaya latex with a view to compare the chemical, and microbial qualities of the prepared cheese.

Materials and Methods

Experimental site and materials

The experiment was conducted at the Dairy Chemistry and Technology Laboratory, Department of Dairy Science, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. Fresh Milk was collected from Bangladesh Agricultural University Dairy Farm. Abomasum of a calf of 11 months old was collected from Austogram (Kishorganj, Bangladesh). Papaya was collected from student resident area, BAU. Lactic acid (concentration 90%, w/v) was obtained from Merck (Darmastadt, Germany) and synthetic rennet from Chr. Hansen (Hoersholm, Denmark).Three trials were performed for each of the coagulants using six liters of milk for each. Chemical analysis of the cheese sample was conducted at Dairy Chemistry and Technology Laboratory, Department of Dairy science, BAU except protein and calcium. Protein and calcium determination were carried out in the Animal Nutrition Laboratory, BAU. In addition, standard plate count and coliform count was performed at Dairy Microbiology and Biotechnology Laboratory, Department of Dairy science, BAU.

Preparation of Coagulant

Around 8.4 g of microbial rennet powder was mixed with 1200 mL distilled water for the preparation of microbial rennet. Lactic acid coagulant was prepared by preparing a 1.25- 1.5% solution (v/v) of lactic acid with water (1.5 mL lactic acid in 120mL water). Six drops papaya latex was mixed with 120 mL of water to prepare papaya latex coagulant. In case of calf rennet, firstly 750 mL of water and 250 mL of milk was mixed to prepare a solution. Then, 100g of calf abomasum was soaked overnight in the solution at room temperature. Finally the filtrate was used as coagulant.

Preparation of fresh cheese

At first, freshly drawn whole milk was collected from Bangladesh Agricultural University Dairy Farm. The milk was heated to 90 °C and then cool down to 40 °C followed by the addition of 2% undefined lactic culture. After addition of culture, milk was mixed properly. Incubate the mixture for 1hr at 40 °C temperature. Incubated milk was divided into 4 equal parts (1.2 L each) and placed into 4 beakers. This was added with 20 mL $CaCl_2$ (10%) solution. After 5 min, the coagulants were added to the respective beaker's of milk. When coagulation was completed it was cut with the help of spatula. The curd was settled for 25-30 minutes. Whey was separated by muslin cloth. Curd was put into the pressing mould after addition of 2% common salt. It was kept in refrigerator at 5°C until further analyses.

Coagulation time and Cheese yield record

The coagulation time of milk for each of the coagulants were recorded in min. The complete coagulation was indicated by the cut of the curd and appearance of the whey. The obtained cheese was weighed and the yield was recorded as g/kg milk.

Chemical and microbial analysis

The pH was determined by a pH meter-215 (Ciba Coring Diagnostics Ltd. Sudhury, Suffolk, England). The acidity was assayed by titration method using 0.1 N NaOH and phenolphthalein. To determine the total solids content, the sample was heated in an oven (WTB oven, Germany) at 105 °C for 24 hours. This dried sample after necessary weighing and calculations for total solids content, were ignited in a muffle furnace (VULCAN A550 Furnace, USA)) at 600 °C for 5 hrs. The fat test was done by Babcock fat test method. For protein estimation Kjeldahl method was used where 6.38 was used as the conversion factor. The calcium content was measured by EDTA titrimetric method using calcon indicator.

For the standard plate count, SPC agar (Himedia, India) was used. Serial dilution of the samples were made in buffer solution (Buffer tablets pH 7.2, BDH, England) and poured into the agar plate. The plate was incubated at 34 °C in an oven (J. P. SELECTA, Barcelona, Spain) for 48 hrs. After that the independent assorted colonies were counted. The coliform count was carried out using VRB agar (Himedia, India). The diluted samples were plated and after solidification, the agar was poured again to ensure the anaerobic condition. The incubation was done at 32 °C for 24 hrs followed by counting of the independent, assorted colonies.

Statistical analysis

The statistical analysis was done according to Completely Randomized Design (CRD). The number of replication was three. Tukey's test was done for mean separation.

Results and Discussion

Milk coagulation time, cheese yield, and results on pH, acidity total solids, protein, fat, ash and calcium content, and standard plate and coliform count are presented in Table 1.

Coagulation time and cheese yield

The maximum time required for the milk coagulation was by lactic acid (54 min) and the lowest time required for microbial rennet (22 min). Time needed for papaya latex was close to lactic acid and the calf rennet required 13 min more than the microbial rennet. Alves *et al*. (2013) mentioned two different coagulation time while they were using protease from the fungus *Thermomucorindicae-seudaticae* N31 (35 min coagulation time) and commercial coagulant from *Rhizomucor spp*. (55 min coagulation time) during the production of prato cheese. Bittante (2011) reported average rennet coagulation time of 19 min in brown swiss cow milk with few samples with more time. On the other hand Abilleira *et al*. (2010) reported 11 - 15 min as rennet coagulation time of the sheep milk.

The maximum cheese yield (225g/kg) was obtained when microbial rennet was used as coagulant. The yield of papaya latex, lactic acid and calf rennet were 13, 6 and 5 g less, respectively than the microbial rennet cheese. Mahajan and Chaudhari (2014) obtained 234.9 g cheese from per kg of milk with papain enzyme. This is almost similar to the present findings. Alves *et al*. (2013) reported no significant differences in the adjusted yield of the cheese owe to different sources of coagulants. Barbano and Rasmussen (1992) found that the proteases from *Mucor miehei and Mucor pusillus* were less efficient in terms of cheese yield compared to fermentation produced chymosin. However, the efficiency was found identical to the calf rennet.

pH and acidity

The higher pH observed in papaya latex cheese, which was 5.90. pH of microbial rennet, lactic acid and calf rennet were 0.60, 0.41 and 0.21 unit less, respectively than the papaya latex cheese. Statistical analysis revealed that the pH of calf rennet and lactic acid cheese differ non-significantly ($p>0.05$) but papaya latex cheese differ significantly with microbial rennet cheese ($p=0.000$). The pH of cow quark cheese and cow milk ricotta cheese was 4.6 and 5.8, respectively (Boone, 2001). The pH of the Prato cheese was found 5.08-5.13 while using two different fungal protease as milk coagulating agents (Alves *et al*., 2013). Abdeen *et al*. (2021) used calf rennet, microbial rennet and milk coagulants from moringa during the manufacturing of soft cheese from goat milk and found a pH more than 6 in all types of cheese. However, with the advancement of the storage, it went low.

The maximum acidity percentage (0.83%) of fresh cheese was found when lactic acid was used as milk coagulating agent which was 0.11% more than the lowest value of 0.72%, found in calf rennet cheese ($p=0.000$). The acidity of cheese prepared from cow and buffalo milk was found 0.73 and 0.67% respectively (Ghosh and Singh, 1996). These are in line with the findings of the present study. However, milk like acidity was reported by Abdeen *et al*. (2021) in the goat milk cheese.

Total solids

The total solids content of the experimental cheese made by using different coagulant were ranged between 47-49 g/100g of cheese. Statistical analysis indicated that microbial rennet and calf rennet cheese differ non-significantly ($p>0.05$). Cheese total solids was obtained maximum by using microbial rennet as coagulant whereas papaya latex cheese had the lowest total solids content. Similarly, Ghosh and Singh (1996) found total solids of cows and buffalo milk cheeses to be 497.0 g/kg and 501.9 g/kg, respectively. Alves *et al.* (2013) reported 56 - 59% total solids in cheese. All these studies support the result of present study. Cheese with 30 - 33% total solids were also obtained by using different kinds of milk coagulants (Abdeen *et al*., 2021). However, the total solids content of the cheese depends on the technological processes, the variety of the cheese etc.

Protein content

The protein content was found similar in cheeses (19.36-19.58%; $p>0.05$) except papaya latex coagulated cheese (18.90; $p<0.05$). Barbano and Rasmussen (1992) compared *calf rennet and adult bovine pepsin* with proteases from *Mucor miehei* and *Mucor pusillus* and fermentation produced chymosin in their experiment and found non-significant variations in the protein recovery in the manufactured cheese. This supports the findings of the present study. However, Alves *et al.* (2013) found a significant influence of types of the coagulant on the protein content of the cheese. Similarly Abdeen *et al.* (2021) reported significant variations in total nitrogen and water soluble nitrogen content in the goat milk soft cheese while using liquid calf rennet, microbial rennet powder, crude milk coagulating enzymes from moringa oleifera seed cake and partially purified milk coagulating enzymes from moringa oleifera seed cake as the coagulants. According to Jacob *et al*. (2011), the texture of cheese and transfer of casein to milk curd is related with the stiffness of the coagulated milk at cutting time.

Fat content

The fat content of the experimental cheese prepared by using different coagulant were ranged between 21.6-22%. The highest fat percent was found in microbial rennet cheese which differ non-significantly ($p>0.05$) with calf rennet. The lowest fat content was found in papaya latex (21.6%) which differ significantly ($p=0.000$) with rennet cheeses and lactic acid cheese (21.8%). Garcia *et al*. (2012) found no differences among the fat content of the cheese samples produced by using animal rennet, microbial rennet, and aqueous extracts of *Cynara cardunculus* subsp. *Cardunculus* and *C*. *cardunculus* subsp. *flavescens*as coagulant. Alves *et al*. (2013) also found non-significant differences in the fat content of the cheese samples while comparing the performance of coagulant from

Table 1: Coagulation time (min), cheese yield (g/kg milk), pH, acidity (%), chemical composition (%) and microbial count (log cfu) of fresh cheese using various types of coagulants.

Parameters	Microbial rennet	Lactic acid	Papaya latex	Calf rennet	P-value
Coagulation Time	22.33^{c}±2.08	54.33^{a}±4.04	50.33^{a}±3.51	35.67^{b}±4.04	0.000
Cheese Yield	224.67^{a}±5.62	217.67^{c}±4.43	215.33^{d}±4.58	218.67^{b}±5.51	0.013
pH	5.30^{c}±0.06	5.49^{b}±0.04	5.90^{a}±0.06	5.69^{b}±0.04	0.000
Acidity	0.81^{ab}±0.03	0.83^{a}±0.03	0.79^{b}±0.04	0.72^{c}±0.02	0.000
Total solids	49.24^{a}±0.36	47.43^{b}±0.81	47.13^{b}±0.36	48.36^{a}±0.64	0.047
Protein	19.58^{a}±0.95	19.39^{a}±0.76	18.90^{b}±1.17	19.36^{a}±0.82	0.045
Fat	22.02^{a}±0.07	21.80^{b}±0.05	21.61^{c}±0.07	22.02^{a}±0.05	0.000
Ash	1.41^{c}±0.10	1.80^{b}±0.20	2.37^{a}±0.15	2.29^{a}±0.10	0.041
Calcium	0.32^{b}±0.04	0.41^{b}±0.08	0.62^{a}±0.09	0.40^{b}±0.09	0.029
SPC	7.12^{c}±0.09	9.22^{a}±0.0 3	7.55^{b}±0.08	7.66^{b}±0.04	0.000
Coliform count	2.38^{b}±0.10	2.75^{a}±0.0 5	2.53^{b}±0.03	2.48^{b}±0.07	0.001

Means with different superscripts in a row are significantly different; SPC, standard plate count; cfu, colony forming unit.

Thermomucorindicae-seudaticae N31 and commercial coagulant in cheese making.The fermentation produced chymosin produce cheese with significantly higher fat content than the *Mucormieheiand Mucorpusillu* protease produced cheese, however, did not differ with the animal rennet and adult bovine pepsin coagulated cheese (Barbano and Rasmussen, 1992). According to Jacob *et al*. (2011), the transfer of fat from milk to curd depends on the stiffness of the coagulated milk at cutting. All these variable results correlate well with the results presented here.

Ash and calcium content

The highest ash 2.37 percent was found in papaya latex cheese. The ash percent of microbial rennet, lactic acid and calf rennet cheese were 0.96, 0.57 and 0.08% less, respectively than the papaya latex cheese. Calf rennet and papaya latex cheese differ non-significantly between them (p> 0.05) but they differ significantly with lactic acid and microbial rennet cheese (p<0.05). This findings are in line with the study of Rana *et al*. (2017) and they found significant difference (P<0.05) in the ash content of different cheese samples. Cheese made from different milk also showed variation in their ash content (Rasheed *et al*., 2016). The mean ash content of various cheese samples varied from 2.49% to 3.25%. These values covers the range of present work.

The highest calcium content was found in papaya latex cheese which was 0.62%. This was 0.30, 0.22 and 0.2% more (p<0.05) than microbial rennet, calf rennet and lactic acid cheese, respectively (p>0.05). The lowest value ranged from 0.32-0.41% found in microbial rennet and lactic acid, respectively. Farahani *et al*. (2013) found similar Ca content in the market cheese in Iran. The highest content of calcium was reported in White cheese (0.71%). Abdeen *et al*. (2021) reported the ash content of the cheese sample in the range of 3.27-3.80% while using four different coagulants in cheese making which varied among the coagulants. In another work, Alves *et al*. (2013) reported non-significant differences between the ash content of two cheese samples made by using two different coagulants. Different coagulation mechanism, processing conditions might have influence on the Ca content of the cheese samples.

Microbial count

The lactic acid coagulated cheese showed highest standard plate count (9.22 log cfu) and coliform count (2.75 log cfu) which is significantly higher than the other cheese samples ($p<0.05$). The microbial rennet cheese has the lowest SPC (7.12 log cfu) and coliform count (2.38 log cfu). The papya latex and calf rennet cheese were found statistically similar with regards to the SPC (7.55-7.66 log cfu; $p>0.05$), however, showed difference in coliform count (2.48-2.53 log cfu; $p<0.05$).

Conclusion

Four different coagulants *viz.* microbial rennet, lactic acid, papaya latex, and calf rennet were used for cheese manufacturing. Both the rennet showed good character in coagulation time. The enzyme preparation and purification and enzyme activity of the latex need to study further. All the coagulants were found promising in cheese yield. The chemical and microbial quality also indicates their suitability in cheese making. However, texture profiling, sensorial assessment and product stability is required to investigate before thinking of papaya latex as coagulant at industry level.

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