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Use of Papaya Plant (*Carica papaya*) Latex for Making Cottage Cheese from Cow Milk

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Abstract

The study was carried out on milk clotting activity of the crude proteolytic enzyme (papain) from different parts of papaya plant (*Carica papaya*). Cottage cheese was prepared using whole cow milk collected from Bangladesh Agricultural University Dairy Farm. Diluted papaya latex (4 drops in 100 ml distilled water) was added and thoroughly mixed for coagulation of milk from different parts of the papaya plant. The treatment samples were named as A: mature papaya; B: immature papaya; C: papaya tree leaf; D: lower part stem and E: upper part stem. In case of organoleptic evaluation, it was observed that differences in scores for flavor, body and texture, finish, color, and total score were insignificant (p>0.05). In chemical analysis, significant (p< 0.05) differences were found in total solids, fat and ash contents among the different type of cheese samples. The highest total solids was found in samples B (56.24%) where the highest protein (30.97%), carbohydrate (9.30%), fat (22.67%) and ash (2.90%) content were observed in samples C, A, E and C, respectively. On the other hand, the lowest (p<0.05) acidity (0.73%) was recorded in sample B. Finally, it was evident that latex (papain) from different parts of papaya plant can be used in cottage cheese preparation.

Introduction

Cheese is a type of dairy product prepared from the coagulation of casein by proteolytic enzyme (Vairo-cavalli *et al*., 2005) that has given rise to a wide range of flavors, textures, and forms by coagulation of casein and played a significant role in human nutrition. Known for several types of cheese based on water content and hardness, one of them is soft cheese without ripening like cottage cheese. The various varieties of cheeses are basically for variation in production technologies (Mohamed Ahmed, *et al*., 2010). Cheese is categorized on the basis of appearance, manufacturing, ripening and chemical composition (Walstra *et al*., 2005; Farkye, 2004). More than 1000 Cheese varieties are available in the world (Sandine and Elliker, 1970). The coagulation process is a basic stage which determines the texture and flavour of the cheese (Jacob *et al*., 2011). From a long previous the animal rennet has been using extensively in making cheese (El-Shenawy and Marth, 1990; Sant'Ana *et al*., 2013; Khan and Masud, 2013; Murtaza *et al*., 2013). The enzyme rennet is obtained on a commercial scale from the fourth stomach of calves which are specifically slaughtered for this purpose. The milk clotting enzyme is important with regard to the quality and yield of cheese and firmer curd at cutting is positively correlated to yield of cheese (Raghunath *et al*., 2014). Cheese production has been increased worldwide. On the other hand, reduced supply and high price of calf rennet has become a great concern. Moreover, calf diseases like bovine spongiform encephalopathy (BSE) together have lead to search for alternative milk-clotting enzymes, as appropriate rennet substitutes (Anusha *et al*., 2014; Shah *et al*., 2014). Additionally, religious factors (Islam and Judaism) and others related to vegetarianism of some consumers have greatly limited their use (Shah *et al*., 2014). In recent time attention has been directed towards natural extract of plant origin (Mahajan and Badgujar, 2010) to replace the calf rennet (Anusha *et al*., 2014; Shah *et al*., 2014). A number of plant proteases have been proven to be effective in cheese making as papain, bromelin, ficin, oryzasin, cucumisin, sodom apple and Jacaratia corumbensis (Duarte *et al*., 2009; Luisa *et al*., 2003). Papain is found naturally in papaya (*Carica papaya L.*) manufactured from the latex of raw papaya fruits. Papain belongs to a family of related proteins with a wide

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variety of activities, including endopeptidases, aminopeptidases, dipeptidyl peptidases and enzymes with both exo and endopeptidase activity (Rawlings and Barrett, 1994). Papain breaks peptide bonds involve the use of a catalytic dyad with a deprotonated cysteine (Shokhen, 2009). Papain enzyme (EC 3.4.22.2) which is capable to break down polypeptides made of amino acids (Amri and Mamboya, 2012). With clotting and proteolytic properties papain enzyme activity causes structural changes to protein (Yit Hwei Low *et al*., 2006). A 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). Two studies were conducted by Rana *et al*. (2017a); Rana *et al*. (2017b) to standardize the desire level of papaya latex from papaya fruits on quality of cheese prepared from cow and buffalo milk. Crude papain enzyme obtain from different parts of the papaya plant may also have significant effect on cheese preparation. Our objective was to find out the efficacy of crude enzyme, papain from different parts of papaya plant, as alternative milk coagulant rather than employing the conventional rennet for the production of fresh unripened cheese upon optimizing the process parameters of cheese production.

Materials and Methods

Gross milk composition used for cheese preparation

Morning cow milk samples were collected from Bangladesh Agricultural University (BAU) Dairy Farm in Mymensingh. Gross milk compositions (Table 1) were analyzed using an automated milk analyzer (Lactoscan, SLP, MILKOTONIC Ltd., Nova Zagora-8900, Bulgaria) at Dairy Chemistry and Technology Laboratory, Department of Dairy Science of BAU.

Table 1. Proximate composition of cow raw milk

Parameters	Unit measurements
Water (%)	87.02
Fat (%)	3.5
SNF (%)	9.0
Protein (%)	3.3
Lactose (%)	4.22
Ash (%)	0.62
pH	6.8

Preparation and collection of crude enzyme

Fresh matured and immature papaya were punched with sterilized needle and 4 drops latex were collected drop by drop from each and immediately mixed with 100 ml distilled water in a beaker. Similarly, the upper, lower parts of the body of papaya plant and leaf also were punched with sterilized needle and latex from each part was collected drop by drop and immediately mixed with 100 ml distilled water in beaker (Rana *et al.*, 2017b).

Preparation of cottage cheese using papaya and papaya plant latex

Cottage cheese was prepared using the procedure followed by Rana *et al*. (2017b). Three liters of milk was used for each experimental trial. At initial milk sample was boiled at 81 $^{\circ}$C then cooled at 40 $^{\circ}$C. After prompt cooling calcium chloride ($CaCl_2$) was added @ 0.03% with milk sample and hold for 10 minutes. Thereafter bacterial culture through sour dahi (*S. thermophilus, L. bulgaricus and S.lactis*) was mixed thoroughly with boiled milk @ 1%. Then the milk sample was allowed for 1 hour at 40 $^{\circ}$C to reach desirable ripening. The starter culture was used to lower the pH which assists in easy precipitation of milk protein. After incubation diluted papaya latex (4 drops in 100 ml distilled water) collected from A: mature papaya; B: immature papaya; C: papaya tree leaf; D: lower part of the stem and E: upper part of the stem were added and thoroughly mixed for coagulation of milk. For complete coagulation milk sample was kept undisturbed for 2-2.5 hours. When curd formation was completed it was cut into small cubes (of 1/4 to 5/8 inch) with the help of wire knives followed to continuous stirring for removing whey then the temperature was raised to 46 $^{\circ}$C. The curd was cooked only for 20 minutes in this stage with continuous agitation to avoid uneven cooking or overcooking. Then whey was drained out and coagulated curd was collected when it almost free from whey. The curd was raked for complete drain out of whey. Salt (NaCl) was added at 1% by weight of the curd after complete milling. Following milling the curd was pressed into a stainless steel dices to form block shape. Finally, prepared cheese was kept under refrigeration (3-4 $^{\circ}$C).

Analysis of cheese

Organoleptic evaluation (flavor, body and texture, finish and, color) of five distinct cheese samples was carried out by using score card (Nelson and Trout, 1965) with help of an expert panel of judges explored from Department of Dairy Science, Bangladesh Agricultural University, Mymensingh. Total solids (TS) and ash content of different cheese samples were determined by oven drying and incineration method according to AOAC (2016). Babcock method, described by Aggarwala and Sharma (1961) was used to determine fat per cent and protein was determined by Kjeldahal procedure (AOAC, 2003).

Total carbohydrate was estimated by the anthrone method described in the book written by Sadasivam and Manickam (2008). Acidity was determined by titrating with N/10 sodium hydroxide solution (Aggarwala and Sharma, 1961).

Statistical analysis

The statistical model was Completely Randomized Design (CRD). Statistical differences among means of cheese samples were compared using one way analysis of variance (ANOVA). The statistical analysis was done using Statistical Package for the Social Science (SPSS) version-16. In case of significant differences DMRT was considered

Results and Discussion

Physical parameter

Flavor

The statistical presentation of flavor of five distinct cheese samples is showed in Table 2. The flavor score of different cheese samples prepared from papaya latex of different origin were found statistically similar (p>0.05). Out of score 45, cheese from immature papaya (B) had 43.67 which was found higher than cheese from mature papaya (A) and lower part stem (D) by 2.3 and 1 unit of score, respectively. Cheese from papaya tree leaf (C) and upper part stem (E) had flavor score nearly to cheese from immature papaya. Consumer acceptance is very much important in case of flavor of cheese (Young *et al*., 2004). Flavor score of cheese manufactured from cow milk was 40.1 reported by Mijan *et al*. (2010). The flavor score found in this study is in agreement with the findings reported by Rana *et al*. (2017b); Rana *et al.* (2017a).

Body and texture

The statistical analysis explored non-significant (p>0.05) response among the cheese samples (Table 2). Out of 30 score, cheese sample B recorded with higher value (26.67) was almost similar to cheese C, D and E type. Lowest score (25.00) was found in cheese sample A. The result found for body and texture is in the line showed by Rana *et al*. (2017b) who reported body and texture score from 23 to 27.33 in cottage cheese. The result revealed by Rasheed *et al*. (2016) also supports our findings of body and texture of cottage cheese.

Finish

All the cheese samples were stated statistically similar (p>0.01) shown in Table 2. Among the finish score, cheese samples B, D, E were dominant with same score (14) which indicates papaya latex from immature papaya, lower part stem and upper part stem had the same significant effects on finish of cottage cheese. On the other hand, cheese sample A and C showed lowest score together than others. Mijan *et al*. (2010) delineated little bit lower score which may be due to difference in processing system. The reported score is in consistent with the score revealed by Rana *et al*. (2017b); Rana *et al.* (2017a).

Color

Color is a salient factor of visual perception because cheese color and appearance are ordinarily the first impression to select. Color score of different cheese samples showed non-significant difference (p>0.05) shown in Table 2. Cheese samples A and B recorded the highest score (9.00) than other three samples. Cheese sample E had 0.67 unit lower score than sample A and B. The results indicate that the cheese samples prepared from latex of mature and immature papaya had the perfect color which was desired. The color score found in this experiment is in consistent with other studies (Rana *et al*. 2017b; Rana *et al*. 2017a; Mijan *et al*., 2010).

Overall score

The overall score of cottage cheese prepared from papaya latex presented in Table 2. The average mean score of cheese samples ranged from 91.01 to 93.34. Statistical analysis specified that the overall score of various cheese samples showed non-significance (p> 0.05) difference. However, cheese prepared from latex of fresh immature papaya was preferred when compared to the other cheese samples.

Chemical parameters

Total solids (TS) content

The total solids content of cheese samples is given in Table 3. The highest total solids was obtained in cheese from latex of fresh immature papaya (56.24%) which was 14% more than D and E type cheese (p<0.05). However, D and E did not differ significantly between them with regards to their total solids contents (p>0.05). Though the total solid contents of A and C type cheese was 1 and 6%, respectively less than the B type cheese. Cheese samples A, B and C was found statistically similar in their total solids content. The difference of TS among cheese samples may be due to for variation in moisture loss during ripening (Buffa *et al*., 2003). The TS found in this study is in agreement with the finding of Raghunath *et al.* (2014); Rana *et al*. (2017b).

Protein content

The results expressing protein content of cheese samples prepared from milk with different source of papaya latex as coagulating agent are given in Table 3. It was observed that protein content of cheese samples was non-significant (p>0.05). Even the cheese sample C (papaya tree leaf) disclosed the highest protein value (30%) among the samples which was more than A, B, D and E type cheese by 4.7%, 1.2%, 3.6% and 2.4%, respectively. Lowest protein content was recorded in case of sample D. Papain is a proteolytic enzyme which produces nitrogenous products of intermediate size, such as proteoses, peptones, polypeptides, peptides and free amino acids. The protein content recorded in this study is higher (26.20-30.97%) than results reported by Rana *et al.* (2017b) (14.94-19.33%); Rana *et al*. (2017a) (21-24%). This variation might be for using different techniques in adding papaya latex. In a study Raghunath *et al*. (2014) found 15.7% protein in fresh cheese prepared from cow milk with papain as coagulant. Jain *et al*. (2019) reported 16.70% protein in cottage cheese which was coagulated with mustard enzyme extract.

Carbohydrate content

Carbohydrate content of prepared cottage cheese is shown in Table 3. The major part of carbohydrate in milk is lactose. In addition, milk also contains small quantity of glucose, galactose, and other saccharides. During coagulation of milk a major percentage of lactose goes into whey and the remaining is present in the curd. Lactose present in the curd is converted into lactic acid. Lactic acid inhibits the growth of undesirable micro-organisms. The average carbohydrate value of five distinct cottage cheese samples were non-significant (p>0.05). The highest carbohydrate content was found in the sample A (mature papaya): 9.30% followed by sample C (papaya tree leaf): 8.50% and E (upper part stem): 6.50%. Cheese samples B (immature papaya) and D (lower part stem) had the similar carbohydrate content. The reported value is in consistent with the value revealed by Rana *et al*. (2017b) who reported carbohydrate of cottage cheese samples range from 5.02 to 9.44%. Rana *et al*. (2017a) reported higher carbohydrate in cottage (14.04% to 20.46%) which might be for buffalo milk.

Fat content

There were significant differences (p<0.05) in fat content among cheese samples (Table 3). Cottage cheese sample E (upper part stem) was found with significantly (p<0.05) 4% higher value than cheese sample C, 8% higher than D and B and 9 % higher than sample A. Latex from upper part stem and papaya tree leaf may have positive effect on fat content of cheese. A legitimate ratio between fat and protein of milk must be keep up as it has significant effect on cheese (Abd El-Gawad *et al*., 2011). They also reported that fat is the principal element that expound body, texture and flavor of cheese. Our results coincide with Rasheed *et al*. (2016) who observed 23.6% fat in cottage cheese. Our results also supported by Rana *et al*. (2017b) who found 16.0 to 23.0% fat in cottage cheese prepared with papaya latex.

Ash content

According to the chemical compositions (Table 3) ash content of was found significant (p<0.05) among different cheese samples. The highest ash content (2.90%) was found in cheese samples C and D made from papaya tree leaf and lower part stem, respectively followed by cheese sample E.

Table 2. Organoleptic Scores (mean± SD) of cottage cheese prepared using papaya latex (papain)

Sample	Flavor (45)	Body and Texture (30)	Finish (15)	Color (10)	Total (100)
A	41.33±1.55	25.00±1.00	13.67±1.15	9.00±1.00	89.00±3.07
B	43.67±0.58	26.67±1.15	14.00±1.00	9.00±1.00	93.34±0.45
C	43.00±1.00	26.33±1.52	13.67±0.57	8.67±1.53	91.67±1.55
D	42.67±2.08	26.00±2.65	14.00±1.00	8.34±1.15	91.01±0.07
E	43.33±0.58	26.67±1.16	14.00±1.00	8.33±0.58	92.33±0.23
p- value	0.235	0.703	0.977	0.886	0.960

A= latex from mature papaya; B= latex immature papaya; C= latex from papaya tree leaf; D= latex from lower the part stem; E = latex from the upper part stem

Table 3. Chemical composition (mean± SD) of cottage cheese prepared using papaya latex (papain)

Sample	TS (%)	Protein (%)	CHO (%)	Fat (%)	Ash (%)	Acidity (%)
A	55.47^a±3.51	26.20±4.12	9.30±2.56	13.53^b±0.50	2.00^b±0.22	0.87±0.17
B	56.24^a±1.90	29.77±2.07	5.83±1.76	14.53^b±1.29	2.14^b±0.33	0.73±.0.15
C	50.48^a±1.07	30.97±2.07	8.50±2.18	18.33^{ab}±2.08	2.90^a±0.10	0.95±0.28
D	42.45^b±1.03	27.39±2.06	5.97±1.76	14.33^b±2.52	2.90^a±0.10	0.90±0.00
E	42.50^b±0.84	28.58±3.58	6.50±1.80	22.67^a±3.06	2.70^{ab}±0.10	0.97±0.20
p- value	0.042	0.351	0.205	0.002	0.006	0.580

^{ab}=means with the different superscripts differed significantly within the same column. A= latex from mature papaya; B= latex from immature papaya; C= latex from papaya tree leaf; D= latex from the lower part stem; E = latex from the upper part stem

The lowest value was in cheese sample A (2.0%) made from mature papaya. The reported value concurs with Rana *et al*. (2017b) who reported 2.19-2.70% ash content in cottage cheese prepared from cow milk with different level of papaya latex. In another study Rana *et al*. (2017a) reported ash content (2.75-3.79%) in cottage chasse which is inconsistent with the finding of this study. Rasheed *et al*. (2016) also found 2.79% ash in cottage cheese which is in line with our findings.

Acidity

Comparative analysis of acidity percent of cottage cheese samples are presented in Table 3. Acidity percent was non-significant (p>0.05) among cheese samples. In spite of non-significance higher acidity percent (0.97%) was found in cheese sample (E) followed by sample C and D with (0.90% and 0.95%), respectively. The lowest acidity (0.73%) was recorded in cheese sample B (immature papaya) which implies the freshness. In a study Rana *et al*. (2017b) found a range of acidity from 0.50 - 0.61% in cottage cheese coagulated with latex from papaya. In another study Rana *et al*. (2017a) also reported comparatively lower acidity per cent than the reported acidity of this study. Here, it is observable that latex from papaya tree stem and leaf may have positive effect on acidity per cent.

Conclusion

It was found that all the cheese samples were satisfactory in terms of organoleptic score, though cheese sample B (latex from immature papaya) obtained higher score. From the compositional analysis, it was found that total solids, fat and ash contents were significantly (p<0.05) differed among the cheese samples where sample B (latex from immature papaya) obtained higher total solids content. Upon the evaluation of all the physical and compositional parameters it may be concluded that latex (papain) from different parts of papaya plant can be successfully used in the preparation of cottage cheese

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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