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Research Article

Effects of Ginger Protease on Quality of Mozzarella Cheese

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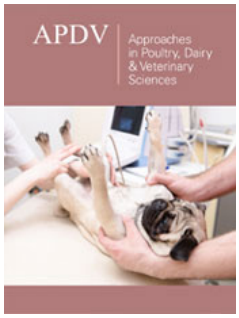

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Abstract

The study investigated the potential use of ginger protease as a coagulant in the preparation of mozzarella cheese. Control cheeses were prepared using calf rennet for comparison. Numerical optimization study revealed maximum milk clotting activity at pH5, temperature 35°C and enzyme concentration 15μL/mL of milk using ginger protease. Fat, ash, acidity, pH, calcium content and yield of the cheese were similar to the control. Ginger protease significantly enhanced the flavor of the cheese. While meltability and baking properties were comparable to the control, stretchability was relatively lower.

Keywords: Milk clotting; Plant rennet; Casein; Mozzarella; Coagulation; Purification

Abbreviations: BSE: Bovine Spongiform Encephalopathy; PA: Proteolytic Activity; MCA: Milk-Clotting Activity; RSM: Response Surface Methodology; ANOVA: Analysis of Variance; LSD: Least Square Difference; DAR: Direct Acidification Rennet; SCR: Starter Culture Rennet; SCGP: Starter Culture Ginger Protease

Introduction

Cheese is a valuable and nutritious dairy product containing a complex matrix of casein, fat, lactose, minerals and water [1]. The coagulation of milk protein (casein) is caused by the action of proteolytic enzymes from animal or plant origin [2]. There are 500-800 varieties of cheese available in the international market [3]. In Nepal, the important varieties produced are yak cheese, Kanchan cheese, mozzarella like cheese and processed cheese [4]. Mozzarella cheese is a soft, unripened cheese variety of the pasta-filata family, which had its origin in the Battipaglia region of Italy [5]. The demand for mozzarella cheese is increasing due to the expansion of pizza parlors and fast food chains and it is more suitable for pizza topping [6]. The most popular milk-coagulating enzyme is found in the stomach of infant animals and is known as rennet [7]. Rennet contains chymosin that causes coagulation of milk by cleavage of the bond between Phe105-Met106 linkage of k-casein [8]. Extraction and subsequent purification of calf rennet from the tissues of animal stomach require various steps, which makes this enzyme very expensive. Moreover, the reduced supply of calf rennet and calf diseases, like Bovine Spongiform Encephalopathy (BSE), have led to an increase in the demand for alternatives sources of milk coagulants [9,10]. The consumer constraints on the use of animal rennet for dietary (e.g. vegetarianism), religious as well as concerns over genetically engineered foods (e.g., Germany, Netherlands and France forbid the use of recombinant calf rennet). This led to a growing interest in vegetable-based coagulants [11]. Different plant proteases like papain, bromelin, ficin, oryzasin, cucumisin, sodom apple and

Jacaratia corumbensis have been identified in different parts of the world as an alternative to animal rennet [12]. Enzymes from plant extracts hydrolyse the κ- and β casein, leading to the curd formation [10]. The use of plant proteinases as milk coagulants is interesting because they are the natural enzymes and can produce cheeses suitable for lacto-vegetarian consumers and ecological markets [13]. Recent publications on new milk clotting proteolytic enzymes from the vegetable origin [14-17] revealed that vegetable coagulants are gaining growing interest in the dairy industry. These plant coagulants have been used for the preparation of different varieties of cheeses viz. French cheeses as Camembert and Gruy_ ere, Gu_õa cheese, Ovine cheeses, Warankashi and Tofu [18-22].

One of the potential plants that possesses proteolytic enzymes is ginger. Ginger protease is a cysteine protease characterized by a cysteine residue at the active center of the enzyme [23]. Majority of previous studies focused on the Proteolytic Activity (PA), purification, structural analysis, and meat tenderization properties of this enzyme [24,25]. The traditional use of ginger protease in producing milk curd has also been reported [26]. Ginger protease is, therefore, a rennet like enzyme that exhibits a strong coagulating activity and hydrolyzes α, β, and κ-casein, and have potential in preparing cheese and oriental dairy foods [27]. Unlike rennet, plant-derived protease possess broad substrate specificity and most of them are classified as cysteine proteases [28]. Chymosin from animal origin, on the other hand, hydrolyze the κ-casein at the Phe105-Met106 bond during the primary phase of milk curdling and destabilize the casein micelles, resulting in milk gelation [29,30]. This study aimed to investigate the feasibility of ginger protease on milk clotting potentiality during mozzarella cheese making. The physicochemical, functional and sensory parameters were analysed to determine technological suitability of ginger protease for mozzarella cheese production.

Materials and Methods

The work was conducted in the Central Department of Food Technology, Central Campus of Technology, Sunsari, Nepal. Standardised milk was obtained from a local dairy, Nepal. The calf rennet and mixed culture consisting of streptococcus salavarious subsp. thermophillus and Lactobacillus delbrueckii subsp. bulgaricus were bought from Trisuli Traders, Kathmandu, Nepal. The ginger rhizome (Zingiber officinale var. Nashe) was collected from the local market of Dharan, Nepal.

Extraction of crude ginger protease extracts

The ginger protease was extracted from fresh ginger rhizomes using the method of Haliu [31] with some modifications. Briefly, the ginger rhizome was washed with de-ionized water, peeled and chopped to 2-3mm size. It was then homogenized using a blender (Model 38BL40, Blender 8010E, Christiano Scientific Equipment, USA) with 5 parts of cold acetone (w/v) (-23°C) and kept at 4°C for 20min. The homogenate was filtered through a cotton cloth and the residue was further washed with cold acetone. The filter cake was dried in a forced air oven at 40°C until no acetone odor was detectable. The air-dried material was powdered using a food grade-grinding mill (Model M20, KIKA®WERKW, Germany) and passed 100% through 500um sieve. The powder was homogenized in 200mL of 100mM citrate phosphate buffer (pH 7.0) for 2.4 min and the extract was filtered using a muslin cloth. The filtrate was centrifuged at 12,0000-×g for 20min and the supernatant was recovered as a crude extract.

Milk clotting activity

Milk-Clotting Activity (MCA) was measured using the standard assay procedure as described by Berridge [32] with slight modifications. Here, the pH of 4-6 was adjusted in 1.5L of standardized milk and 10-20μL of enzyme solution per mL of milk was added at 40°C. The time required to form curd fragments was measured. MCA, expressed in Otani units, was calculated as follows:

$$MCA\ units = \frac{2400}{T} \times \frac{S}{E}$$

Where:

T=Time (in sec) necessary for the curdling of milk

S=Volume (in mL) of milk

E=Volume (in mL) of enzyme.

Experimental design

The effect of three independent variables, namely enzyme concentration (10-20μL/mL of milk, X_1), and pH (4-6, X_2) and temperature (30-40°C, X_3) of milk on MCA of extracted protease from ginger rhizomes was investigated using Response Surface Methodology (RSM). The independent variables and their levels were selected based on literature and preliminary experiments. A three-factor, five-level central composite rotatable design was employed. MCA was the response variable in this design. The experimental design, data analysis and quadratic modeling were performed using the "Design Expert" software (Version 6.0.1, Stat-Ease Inc., USA). The responses MCA for different experimental combinations were related to the coded variables (xi, i=1, 2and 3) by a second-degree polynomial equation:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 . x_2 + \beta_{13} x_1 . x_3 + \beta_{14} x_2 . x_3 + \varepsilon$$

The coefficients of the polynomial were represented by β_0 (constant), β_1, β_2, β_3 (linear effects); β_{12}, β_{13}, β_{14} (interaction effects);

β_{11}, β_{22}, β_{33} (quadratic effects); and ε (random error). Data were modeled by multiple regression analysis. A complete second-order quadratic model employed to fit the data and adequacy of the model was tested considering R_2 (the coefficient of multiple determination, a measure of the amount of variation around the mean explained by the model), Adjusted R_2 (a measure of the amount of variation around the mean explained by the model, adjusted for the number of terms in the model), predicted R_2 (a measure of how good the model predicts a response value) and Fischer's F-test. The significances of all terms in the polynomial were judged statistically by computing the F-value at probability (p) of 0.05.

Preparation of mozzarella cheese

Four different experiments were undertaken to prepare mozzarella cheese using calf rennet and ginger protease employing two methods-starter culture and direct acidification. The experiments were performed in triplicates, and the mean was taken for analysis. Control cheese was prepared using calf rennet by starter culture [6] and direct acidification Panthi [33] methods. For ginger protease, the cheese-making method was modified as follows: One and a half liters of standardized milk was pasteurized at 72°C for 15s and cooled to 35°C. Milk pH was brought to 5 with 2.4 per cent (w/v) solution of citric acid or by addition of starter culture with agitation for two different methods. The crude enzyme was added at the rate of 15µL/mL of milk and mixed thoroughly. The coagulum was obtained at 45min. After cutting, the curd was cooked in whey to the temperature of 56°C and then separated from the whey. Sufficient quantity of hot water (85°C) was added to cover the curd for 5min. Hot water was drained and the curd was then plasticized manually with a wooden worker to gain elasticity. The curd was moulded in round blocks, salted at the rate of 1.75% and dipped in pasteurized chilled water (4°C) for 1 hr. Cheese blocks were then placed on stainless steel wire mesh at 10°C for 2hr to remove excess water. Hence prepared mozzarella cheese was packed in clean, sanitized polyethylene bags and stored at 5°C before analysis.

Physicochemical analyses

Samples of mozzarella cheese were analysed for pH and titratable acidity, as well as moisture, protein, calcium and ash contents according to the Official methods of AOAC [34]. Fat was determined using Gerber method [35]. The theoretical yield (Y) was calculated using Van Slyke yield equation [36]:

$$Y = \frac{(0.93\times\%M.fat)+(\%M.casein-0.1)\times 109}{100-moisture\,in\,cheese}$$

Where

%M. fat and casein refers to %fat and casein in milk

The 0.93x milk fat assumes that 93% of milk fat is retained in the cheese. The value for casein -0.1 approximates to a theoretical loss of 4% casein and casein retention of approximately 96%. The 109 is a 'constant' to allow for milk salts retention of whey protein and lactose. Actual yield was calculated by weighing the curd after pressing as described by Razzaq [3].

Determination of meltability of cheese

A suitable modification to 'Schrieber test' Muthukumarappan [37] was made for testing of the mozzarella cheese meltability. For simplicity and wider applicability, the bored sample having the fixed base area (1.75cm^2) and height (0.5mm) was heated on the aluminum dish in the boiling water for 5min. The whole assembly was covered with the plate during heating allowing the steam to escape. The dish was then taken out at the room temperature and cooled. The increase in melted cheese area was measured on the graph paper. The ratio of the melted cheese area to the original area was regarded as the indicator of cheese meltability.

Determination of stretchability of cheese

Stretchability test was based on the principle of 'stretch test' as described by Bhattarai and Acharya 2010 [6]. The stretchability was graded on 5 points arbitrary scale where 5 represents the best stretchable characteristics.

Sensory evaluation of the baking quality of cheese

About 100g of each shredded cheese was topped on unbaked pizza base separately, with tomato sauce, but without any vegetable fillings, and transferred to an oven maintained at 180-200°C. The pizza was baked for 20-25min to allow melting (till the shreds fused uniformly) of cheese. The baking characteristics of the cheese were assessed by the arbitrary numbers, where 5 points was awarded for the best pizza.

Sensory evaluation of cheese

The cheeses were organoleptically evaluated by semi-trained panelists, following the recommendations of IDF [38] The panelists evaluated cheese for appearance, flavor, texture, taste and overall sensory score) using a nine-point hedonic scale, with 1 being the worst, and 9 the best quality.

Statistical analysis

Collected data were analysed by GenStat Discovery Edition 3, GenStat Procedure Library Release PL15.2, Version 7.22 DE (Copyright 2008, VSN International Ltd) for Analysis of Variance (ANOVA). The significant for means was tested using LSD (Least Square Difference) method at 5% level of significance.

Results and Discussion

Numerical optimization for maximum Milk Clotting Activity (MCA)

The measured expansion of MCA varied from 17.23-65.36 Otani units (Supplementary information S1). Regression model fitted to experimental results of MCA (Supplementary information S2) showed that Model F-value of 55.51 was significant (P<0.05) whereas, lack-of-Fit F-value of 1.36 was not significant (P>0.05). The fit of the model was also expressed by the coefficient of determination R2=0.9804, indicating that 98.04% of the variability of the response could be explained by the model. The adjusted R2 of 0.9627 and adequate precision of 18.956 showed an adequate signal. Considering these criteria, the model (Equation 1) was selected for representing the variation of MCA and used for further analysis.

$$MCA = 62.45 + 4.29A + 3.53B - 2.06C - 5.89A^2 - 12.56B^2 - 12.45C^2 - 1.26AB + 3.24AC - 3.10BC \quad (1)$$

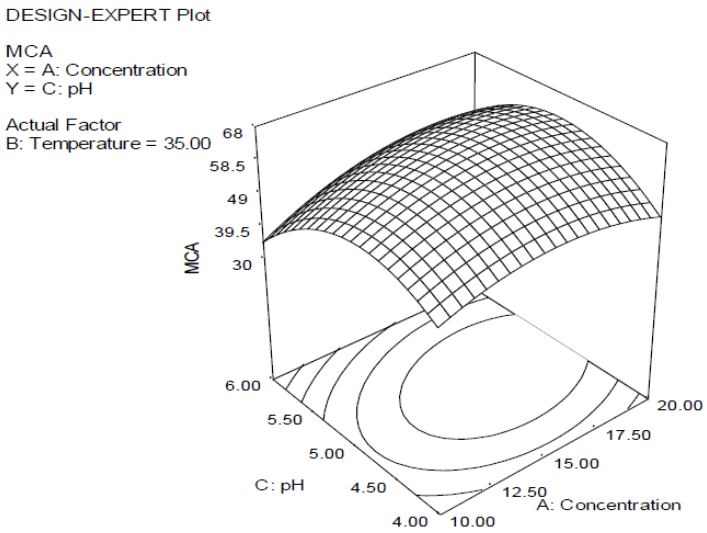

Figure 1: Response surface plot for MCA as a function of pH of milk and enzyme concentration at fixed temperature of 35°C.

Where A, B and C are the coded values of enzyme concentration, temperature and pH of the milk respectively. In this case, A, B, C, A2, B2, C2, AC and BC are significant model terms (P<0.05). The MCA in above quadratic equation 1 had significant (P<0.05) positive linear effect of enzyme concentration (A) and temperature of milk (B) at 95% confidence level. However, the linear term -pH of milk (C) had a significant negative effect on MCA (P<0.05). The quadratic terms of enzyme concentration (A) and temperature (B) and pH of milk (C) had highly significant (P<0.05) negative effect on MCA. The interaction term of enzyme concentration and pH of milk (AC) had significant (P<0.05) positive effect on MCA. Other interaction terms -temperature and pH of milk (BC) had a significant negative effect, while interaction terms of enzyme concentration and temperature of milk (AB) were found to be non-significant (P>0.05). An increase in temperature of milk resulted in a higher MCA (Figure 1). The activity reached a maximum when the temperature was at a certain level, with non-significant improvement thereafter. Campos, et al. [39] and Heimgartner, et al. [40] studied the effect of temperature on the proteolytic activity and reported that proteolytic activity increased with temperature to a maximum at 37°C. A possible explanation for this phenomenon could be the denaturation of proteins at higher temperature. The concentration of enzyme above the optimum level had a negative effect on the activity of ginger protease as shown in (Figure 1).

Amid, et al. [41] reported that increasing the buffer content above the optimum volume caused a decrease in the total activity of the extracted protease, which ultimately resulted in undue dilution of solution. The pH of the milk has an important linear effect on the final specific activity of the extracted enzyme. The maximum MCA was observed at a pH of 5.0. Successful milk clotting agents at optimum pH of substrate split the κ-casein at the bond between the phenylalanine (105) and the methionine (106) residues diffusing casino-peptide in the serum [42]. Below or above the optimum pH had a negative effect on the total activity of milk (Figure 2). It may be inferred that the large linear effect of pH on the final specific activity of the enzyme extracted arises primarily from the change in the intrinsic activity of the enzyme as a response to pH rather than from changes in the extraction yield [43]. Mohamed [44] observed the activity of Solanum dubium extract at pH 5.5. The discrepancy may be due to the source of milk clotting enzymes and the extraction method used.

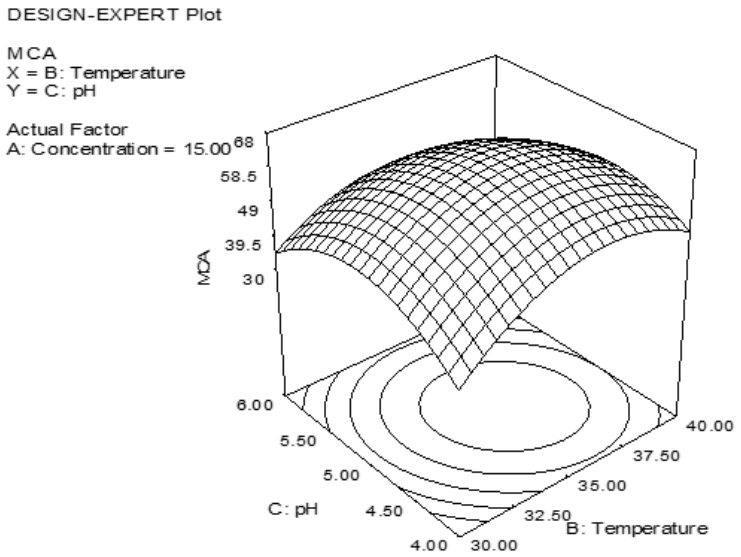

Figure 2: Response surface plot for MCA as a function of pH and temperature at fixed enzyme concentration of 15µL/mL of milk.

Optimization

A numerical response optimization technique was applied to determine the optimum combination of enzyme concentration, temperature and pH of milk for the maximum MCA. Under the assumptions by a design expert, the optimum operating conditions for maximum MCA of milk were enzyme concentrations of 15µL/mL of milk, pH 5.0 and milk temperature 35°C. The responses predicted by the software for these optimum process conditions reported MCA of 62.45 Otani units. (Table 1) shows the different conditions of the constraints for optimization.

Table 1: Different constraints for optimisation.

Name	Goal	Lower Limit	Upper Limit
Enzyme concentration	is in range	10	20
Temperature of milk	is in range	30	40
pH of milk	is in range	4	6
Milk clotting activity	maximize	17.23	65.36

Verification of the model

Within the scope of the variables investigated in central composite rotatable design, additional experiments with different processing conditions were conducted to confirm the adequacy of the model equations. The conditions and the results of the confirmatory experiments are presented in (Table 2).

Table 2: Predicted and actual values of the responses at the optimized condition.

	Conditions					
Trial No.	Enzyme Concentration (µL/mL of Milk)	Temperature of Milk C	pH of Milk	Predicted Value	Observed Value	% Deviation
1	15	35	5	62.45	60.39	3.29
2	15	35	5	62.45	64.26	2.89

Physicochemical properties

Chemical composition: The chemical composition of the mozzarella cheeses made from Direct Acidification Rennet (DAR), Starter Culture Rennet (SCR), direct acidification ginger protease (DAGP) and Starter Culture Ginger Protease (SCGP) have been shown in (Table 3). It was observed from (Table 3) that there was a significant difference (P<0.05) in the moisture content of cheeses prepared from two coagulants. The moisture percentage in all cheeses are in line with the findings of Johnson, et al. [45] but lower than the findings of Nawaz, et al. [46]. The variation in results might be due to the difference in milk composition, the activity of the coagulant and processing techniques. The moisture contents in mozzarella cheese prepared using ginger protease were significantly (P<0.05) higher compared to rennet ones. Utsumi, et al. [47] explained that the molecular forces involved in the coagulation of casein by crude enzyme results in a greater water-binding capacity of the protein matrix. Another justification for more moisture retention might be due to the longer coagulation time for ginger protease as outlined by Johnson, et al. [45]. Regarding fat, (Table 3) revealed non-significant (P>0.05) difference among cheese samples. While the fat levels correlated well with the findings of Fasale, et al. [48], these were higher than reported by Sameen, et al. [49]. Cheese made using ginger protease showed comparatively lower fat content. Khan, et al. [50] revealed that plant protease take a longer time for coagulation compared to rennet and thus retain less fat in the final product.

Table 3: Chemical composition of mozzarella cheeses prepared from ginger protease and rennet.

Parameters	DAR	SCR	DAGP	SCGP	LSD
Moisture (%)	48.99^{a} (0.47)	48.07^{a} (0.75)	51.79^{b} (0.65)	51.2^{b} (0.21)	1.06
Fat (%)	24.53^{a} (1.17)	23.43^{a} (1.30)	22.5^{a} (0.82)	23.03^{a} (0.89)	2.005
Protein (%)	20.03^{a} (0.96)	19.27^{a} (0.68)	21.90^{b} (0.86)	22.94^{b} (0.65)	1.506
Ash (%)	2.52^{a} (0.15)	2.32^{a} (0.040)	2.433^{a} (0.12)	2.57^{a} (0.13)	0.2182
pH	5.27^{a} (0.15)	4.933^{a} (0.058)	5.333^{a} (0.058)	4.97^{a} (0.12)	0.196
Acidity	0.22^{a} (0.02)	0.25^{a} (0.01)	0.21^{a} (0.01)	0.24^{a} (0.01)	0.0304
Ca (mg/100gm)	628^{a} (7.94)	610.3^{a} (7.64)	624.7^{a} (6.50)	618.3^{a} (5.50)	13.11

Values are the means of triplicates. Figures in the parentheses represent the standard deviation. Values in the row bearing similar superscript are not significantly different at 5% level of significance. There was a significant difference (P<0.05) observed in protein contents of cheeses prepared from two coagulants. The protein in mozzarella cheeses made using ginger protease was higher than that of rennet. The protein contents of DAR and SCR are in line with the findings of Seth, et al. [51] but higher than the findings of Sameen, et al. [49]. These disparities could be due to the use of crude enzyme extract as it contains proteinous material in it. Omueti and Jaiyeola [22] reported that an increase in protein content might be due to retention of whey in the final cheese. It was observed from (Table 3) that there was a non-significant (P>0.05) difference in the ash among cheese samples. The ash contents of mozzarella cheese are similar to the findings of Mijan, et al. [52]; Masud, et al. (1993) and Patel and Gupta (1986), who reported ash contents in the range of 2.50 to 3.20%. (Table 3) also revealed non-significant difference (P>0.05) in the pH of all cheese samples. The results correlated with the findings of Mijan, et al. [52] and Waheed, et al. [53] but lower than the reported values of Khan, et al. [50]. The type of coagulating agent, processing technique and source of milk might have caused such differences in results. (Table 3) also revealed a non-significant (P>0.05) effect on the acidity of cheeses prepared by both coagulants. However, a slight increase in acidity was observed in mozzarella cheese prepared with calf rennet. The present findings follow a similar trend to Nunez, et al. [54] who concluded higher acidity for animal rennet cheese in their study was due to higher whey retention and subsequent lactose fermentation.

The difference found in the calcium contents of the cheeses produced by two coagulants was non-significant (P>0.05). The values ranged from 610-628mg/100gm of cheese, which correlated with the findings of Panthi [33]. Among four treatments, calcium content was more in DAR followed by DAGP, SCGP and SCR. According to Keller, et al. [55] calcium content inversely correlated to the moisture content of the cheese. Similarly, Joshi, et al. [56] reported that caseins are more hydrated as the level of bound calcium decreases in milk. The higher calcium in cheese prepared with direct acidification method (DAR and DAGP) may be due to the effect of citric acid. Shakeel-Ur, et al. [57] reported citric acid chelates more calcium than by any other acid.

Theoretical and actual yield: The theoretical and actual yield of mozzarella cheeses prepared by using animal and plant

coagulant by direct acidification and starter culture method have been presented in (Figure 3). A non-significant difference was observed in the theoretical yields of cheese samples prepared from rennet and ginger protease. The findings are in accordance with the results of Nawaz, et al. [46] but lower than the results of Seth, et al. [51]. The slight variation in theoretical yields might be due to the moisture contents in the final cheese [58]. Similarly, a non-significant difference was observed in actual yields among all treatments. Actual yields are similar to the findings of Zedan, et al. [59] and, Bhattarai and Acharya [6].

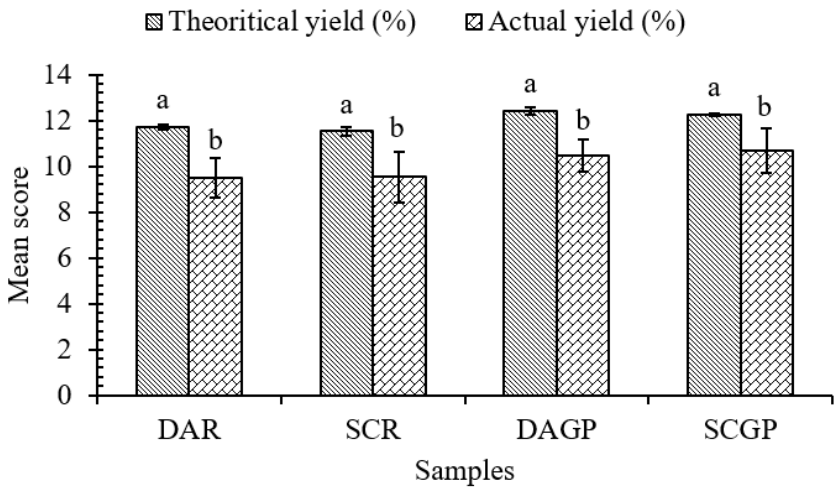

Figure 3: Theoretical and actual yield of mozzarella cheeses.

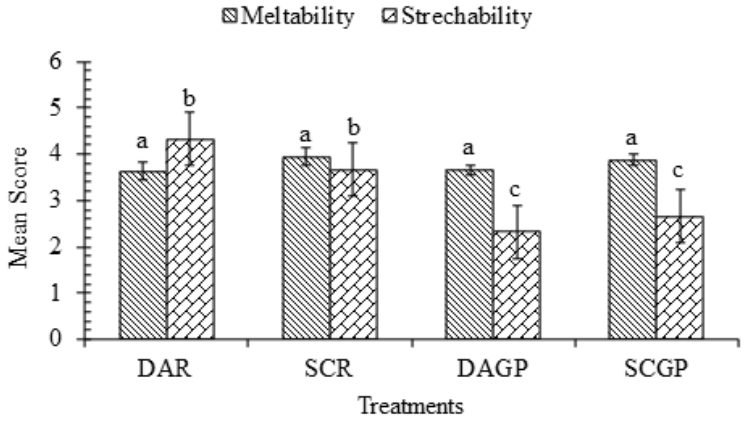

Figure 4: Meltability and stretchability of mozzarella cheeses.

Meltability and stretchability: The meltability and stretchability of cheeses prepared using animal and plant coagulants by direct acidification and starter culture method are presented in (Figure 4). Analysis of variance of meltability revealed a non-significant ($p>0.05$) difference among all treatments. On 2nd day of storage, the cheese sample SCR (3.94±0.035) had highest meltability ratio followed by SCGP (3.89±0.015), DAGP (3.67±0.03) and DAR (3.64±0.04). The increased meltability in SCR and SCGP may be due to the decreased calcium content and higher amount of moisture present [60,61] compared to sample DAR and DAGP. Fresh cheese was typically firm and had poor melting properties although it was stretchable. However, as the cheese matures, the texture softens and there is an increase in the melt [62]. The improvement in meltability is due to dislodgement of para-casein matrix [63]. As time increased, the meltability of all cheese samples increased significantly, but the trend of increase remained the same Sameen, et al. [49]. The statistical analysis of stretchability showed samples were significantly different ($p<0.05$). The stretchability of sample DAR (4±0) was found to be highest followed by SCR (3.67±0.58), SCGP (3.89±0.015) and DAGP (2.33±0.58) as shown in (Figure 4). According to Joshi, et al. [56], a decrease in the calcium content would lead to the decreased structural rigidity of the cheese matrix consequently increasing the stretchability. Keller, et al. [55] and Oberg, et al. [64] reported an inverse relationship between stretchability and meltability of mozzarella cheese.

Sensory evaluation of cheese

The graphical representation of sensory scores of the cheese samples is shown in (Figure 5). The data regarding appearance showed a non-significant (p>0.05) difference among the samples. The average appearance value was comparatively higher for SCR (6±0.87) followed by SCGP (6.11±0.78), DAR (6±0.87) and DAGP (6±0.71). According to Delahunty, et al. [65], the appearance of cheese is a function of the interaction between cheese color and texture, and coagulant type used. Cheese made using ginger protease had significantly (p<0.05) higher score for flavor as compared to cheese made using rennet. The average flavor score was highest for DAGP (6.78±0.8) followed by SCGP (6.67±0.71), SCR (5.78±0.83) and DAR (5.67±0.71). This variation in cheese flavor might be attributed to the inherent property of the crude extract of ginger rhizome (an aromatic plant used as a spice) that provides a pleasant aroma and enhances the flavor of the cheese [66]. Chen, et al. [67] and Roseiro, et al. [10] reported that intense proteolytic action displayed by plant-coagulant enzyme enhances the flavor of the cheese. The data regarding taste, texture and overall acceptability showed a non-significant (P>0.05) difference among the samples. The average taste score was slightly higher for SCR (6.44±0.89) followed by DAR (6.22±0.97), SCGP (5.78±0.67) and DAGP (5.67±0.71). A mild bitter taste was observed in cheese made from plant coagulant, which may be the reason for low taste score in SCGP and DAGP. In a study by Dinakar, et al. [68], an aqueous extract of berries of Withania coagulans also showed a bitter taste in the final product This bitterness is associated with accumulation of the bitter peptides that contain more hydrophobic amino acid residues when coagulants from plant sources are used [69].

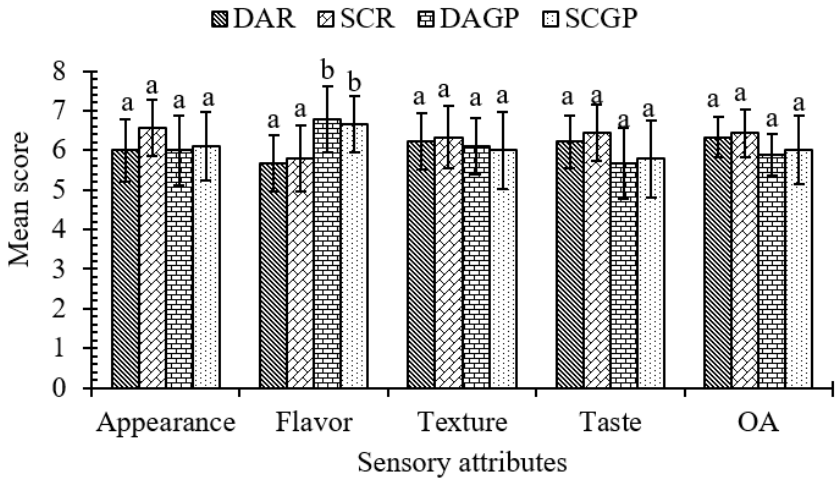

Figure 5: Graphical view of mean sensory scores of mozzarella cheeses.

Note: Values are the means of 9 panelists. Values on top of bars bearing similar superscript are not significantly different at 5% level of significance; where DAR, Direct Acidified Rennet; SCR, Starter Culture Rennet; DAGP, Direct acidified ginger protease and SCGP, Starter culture ginger protease.

The average texture score was comparatively higher for SCR (6.33±0.71) followed by DAR (6.22±0.97), DAGP (6.11±0.78) and SCGP (6±0.71). The lower primary proteolysis by chymosin might be the reason for slightly hard and rubbery texture in samples-DAR and SCR. Bansal, et al. [70] also expressed similar views. Proteolytic activity of ginger contributed to texture softening during ripening of Iranian ultra-filtrate white cheese [71]. El-Aziz, et al. [66] indicated that fortification of buffalo milk with ginger extract caused an increase in cohesiveness and a decrease in firmness of the curd, which resulted in more softness and smoothness of the cheese. Chen, et al. [67] and Roseiro, et al. [10] also reported the textural defects in cheeses made with plant-protease. Despite the slightly lower overall sensory score, mozzarella cheese made with ginger protease is comparable to the cheese made with calf rennet. (Figure 5) shows the statistical analysis of the baking characteristics (melting, flavor, browning) of the mozzarella cheese which are not significantly (p>0.05) different. However, an arbitrary number of DAGP (4.33±0.71) showed highest score followed by DAR (4.11±0.78), SCR (3.78±0.67) and SCGP (3.44±0.88).The non-significant (P>0.05) difference in the baking characteristics might be due to higher temperature (180-220°C) of the oven during baking. Browning was not detected in sample DAGP and DAR. According to the Innovate with dairy (1998), direct-acidified cheeses are less prone to browning because little secondary proteolysis occurs in the absence of starter culture [72,73] (Figure 6).

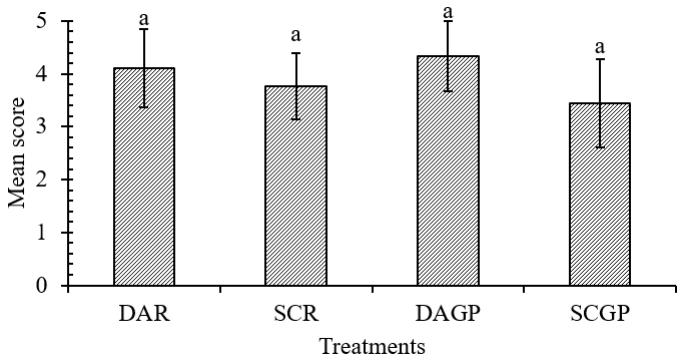

Figure 6: Graphical view of mean scores for baking quality of mozzarella cheeses.

Note: Values are the means of 9 panelists. Values on top of bars bearing similar superscript are not significantly different at 5% level of significance.

Conclusion

Protease enzyme was extracted from ginger (var. Nashe) rhizome by simple extraction method. The extracted enzyme displayed optimum milk clotting activity at pH5, temperature 35°C and enzyme concentration 15µL/mL of milk. The physico-chemical analysis, showed that fat, ash, acidity, pH, calcium and yield were similar (P>0.05) between cheeses prepared using ginger protease and calf rennet. Likewise, appearance, texture, taste and overall acceptance of the cheeses prepared using ginger protease were also similar (P>0.05) to that of control cheese prepared using calf rennet. Meltabilility and baking properties of the cheeses prepared using ginger protease were comparable to that of control cheeses. However lower stretchability was observed in cheese prepared using ginger protease. The results of this study revealed that physicochemical, functional and sensory attributes of mozzarella cheese prepared from ginger protease is comparable with the cheese made from calf rennet. This shows that the ginger enzyme is a potential substitute for rennet in mozzarella cheese preparation. Further studies using a pure enzyme from ginger are required to improve the potential use at commercial scales.

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