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Proximate compositional, Texture Profile and Colour Profile Analysis of Mozzarella cheeses prepared using milk clotting enzymes extracted from Mustard and Sunflower oil seed cakes

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ABSTRACT
The present study was conducted to determine the proximate composition, textural profile and colour profile parameters of mozzarella cheeses prepared using enzyme extracts from mustard and sunflower oil seed cakes (experimental samples). Mozzarella cheese prepared using microbial rennet was taken as control. The results revealed that on the basis of all the studied parameters, mozzarella cheeses developed using enzyme extracts were found highly comparable to the control sample. Thus, these cheap plant sources of milk clotting enzymes (oil seed cakes) may prove a potential substitute of rennet in the dairy industry with promising results.
KEYWORDS: *Rennet, Rennet substitutes, Oil seed cakes, Mustard, Sunflower, Proximate composition, Texture profile, Colour profile*

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INTRODUCTION

Coagulating enzymes have been used in cheese making from thousands of years and they seem to be the oldest known application of enzymes (14). Calf rennet, the conventional milk clotting enzyme obtained from the fourth stomach of suckling calves, which consists of chymosin (EC 3.4.23.4) as the major component and another proteolytic enzyme, pepsin (EC 3.4.23.1). In general, milk coagulation using calf rennet is the most used procedure. However, the worldwide increase in cheese production, coupled with reduced supply and increasing prices of calf rennet and calf diseases like bovine spongiform encephalopathy (BSE) has led to search for alternative milk-clotting enzymes, as appropriate rennet substitutes [21; 3]. Apart from this, some religious factors (Islam and Judaism) and others related to vegetarianism of some consumers have greatly limited their use [21].

Several milk-clotting enzymes of microbial origin have been commercialized and are in use in cheese industry, such as aspartic proteases (APs) obtained from *Rhizomucor miehei, Rhizomucor pusillus and Cryphonectria parasitica* [25]. Microbial rennet produced by genetically engineered bacteria has proven suitable substitutes for animal rennet, but increasing attention has been directed toward natural rennet extracted from plants (1). The consumer concerns regarding genetically engineered foods (e.g., Germany, Netherlands and France forbid the use of recombinant calf rennet) have led to a growing interest in vegetable coagulants [7].

Due to this reason, much research interest has been aroused towards discovering new milk clotting enzymes from vegetable or plant sources which can satisfactorily replace rennet in cheese making process or production.

Plant coagulants includes enzymes such as from Cardoon (*Cynara cardunculus*) (18; 24), Gubbain (*Solanum dubium*) [1], fig (*Ficus carica*), pineapple (*Ananas sativa*) and castor oil seeds (*Ricinus communis*) [12] etc. The selection of an appropriate plant coagulant and the control of different gelation parameters are of great importance to obtain a better quality of final product [14].

Mozzarella cheese is a soft, white, unripened cheese which may be consumed shortly after manufacture. Its melting and stretching characteristics are highly appreciated in the manufacture of pizza where it is a key ingredient [13].

Oilseed cakes/oil meals are by-products obtained after oil extraction from the seeds. India holds a significant share in world oil seed production such as rapeseed, groundnut, castor seed, sesame, linseed, soyabean, and sunflower seeds [6]. The oil seeds have been identified as the plant sources for milk clotting enzymes [7; 9] and the enzymes extracted from oil seed cakes may prove a potential milk coagulant to fulfill the demand of cheese industry.

The crude extract of *Brassica napus* seeds showed a potent milk-clotting activity (164 U/g dry seeds) with firm clotting and minimum proteolytic activity at pH 4.5 (8). Similar to chymosin, exhibition of proteolytic activity by the seed extract of *Helianthus annuus* towards k-casein, α-casein and β-casein has been identified [7]. Oilseed cakes are rich in protein contents and can be utilized through development of new products and fortification of products [26]. Despite the widespread uses of mustard and sunflower oilseed cakes, its use as a source of milk clotting enzymes is not reported yet.

Therefore, the aim of this research was to evaluate the physico-chemical properties of mozzarella cheeses, in terms of their textural properties and colour profile analysis, which were prepared using enzymes extracted from oilseed cakes of mustard and sunflower.

MATERIAL AND METHODS

Materials

Mustard (*Brassica spp*) oil seed cakes were procured from local market, Hisar and Sunflower (*Helianthus annuus*) oilseed cakes were procured from 'Pari Animal Nutrition' Khanna, Ludhiana'. Raw buffalo milk and Skim milk were obtained from the experimental dairy plant at the department of LPT. Dry Skim Milk Powder (SMP) (Sterling Agro Industries Ltd) was procured from local market, Hisar. Microbial Rennet was procured from Urban Platter (Madmillie, Microbial vegetarian rennet tablets, made from coagulant enzyme of *Mucor miehei*). Cultures of *Streptococcus thermophilus* (thermophilic) and *Lactococcus lactis lactis (*mesophilic) were procured from 'Esdee Marketing' Pune, Maharashtra. All the chemicals were procured from reputed firms like SRL, Qualigens, CDH, Hi-Media, Sigma-Aldrich etc. were procured through local dealers of reputed companies. Vivaspin® 20 centrifugal concentrators, Membrane 30,000 MWCO (Sartorius) (for desalting and concentration of proteins) were procured from Sigma- Aldrich. Table salt (Tata Chemicals Ltd., Mumbai) was procured from the local market.

Methods

Preparation and Standardization of the product (Mozzarella cheese)

Mozzarella cheese was prepared as per the methods mentioned by (15) with slight variation, using thermophilic starter culture *(Streptococcus thermophilus)* and MCEE at selected levels of pH, temperature and concentration. Control samples were prepared using microbial rennet as coagulant.

- Standardized buffalo milk (fat- 5.0%, Casein: fat- 0.68-0.70), having pH- 6.76 was pasteurized (72.4°C for 15 sec) and cooled to 38°C.
- Calcium chloride was added at the rate of 0.02% (w/v).
- Thermophilic starter culture was added at the rate of 2% (w/v).
- Standardized buffalo milk was coagulated with:
  - (i) Microbial rennet @1.5g/100 litres of milk at 38°C at pH-6.3,
  - (ii) Mustard Enzyme Extract (MEE) @ 3 ml/1 litre of milk at 38°C at pH- 6.0 and temperature was raised to 43°C over a span of 30 minutes.
  - (iii) Sunflower Enzyme Extract (SFEE) @ 5 ml/1 litre of milk at 38°C at pH- 6.0 and temperature was raised to 44°C over a span of 30 minutes.
- A firm coagulum was obtained within 30-35 minutes in all the treatments.
- Cutting of curd was done at
  - i. Rennet curd: at pH- 5.9
  - ii. MEE curd: at pH- 5.5
  - iii. SFEE curd: at pH- 5.5
- Cooking of rennet cheese curd was done by gradually raising the temperature from 38 to 42°C.
- Draining of whey was carried out at pH 5.2-5.4 range in all the treatments.
- All the curds were then salted at the rate of 1.5%.
- After about 5 minutes, the salted curds were plasticized by treating with hot water having temperature of 80-85°C.
- Curds were moulded into round balls and immersed first into clean water at room temperature for 15 minutes and then into clean chilled water (8-10°C) for 1 h.

Mozzarella cheeses thus obtained were packed in clean, polyethylene bags.

Parameters studied

Proximate composition

Proximate composition was determined by following the standard methods of [2].

Texture Profile Analysis

The textural properties of cheese samples (hardness, springiness, cohesiveness, gumminess and chewiness) were evaluated using Texture Analyzer (TA.HD plus), Stable Micro Systems Ltd., Surrey, England with the Texture Exponent Program. A compression platform of 75 mm diameter was used as a probe. The texture profile analysis was performed as per the procedure outlined by[4]. Samples of 20 mm diameter and height were compressed to 50% of their original height. A time of 5 sec was allowed to elapse between the two compression cycles. Force time deformation curves were obtained with a 50 Kg load cell applied at a cross-head speed of 2 mm/s.

Color Profile Analysis

Konica Minolta Chromameter CR-400 (Konica Minolta Sensing, Inc., Japan) with 8 mm aperture was used for instrumental color evaluation. The instrument was caliberated with a white standard plate. Color scores were expressed as CIE Lab system as follows:

L* related to lightness, varying from black (zero) to white (100), and other two related to chromaticity, a* from green (-a*) to red (+a*) and b* from blue (-b*) to yellow (+b*).

Statistical analysis

Data was analyzed statistically on 'SPSS-16.0' software package as per standard methods [23]. Data were subjected to ANOVA and Duncan's Multiple Range Test to find significant difference at 5% significance level in the mean values.

RESULTS AND DISCUSSION

Chemical composition of standardized buffalo milk

Chemical composition of standardized buffalo milk has been presented in table 1. To standardize the casein: fat ratio (0.68-0.72) in milk (as prescribed in laboratory manual on cheese technology, NDRI Karnal, 20), the fat % of buffalo milk was standardized to 5.00 using skimmed milk. The pH value of standardized buffalo milk was 6.76.

Table 1: Chemical composition of standardized buffalo milk (Mean ± SD)

Components	Standardized buffalo milk
pH	6.76 ± 0.25
Titratable Acidity%	0.17 ± 0.15
Moisture%	84.75 ± 1.10
Protein%	4.38 ± 0.87
Fat%	5.00 ± 0.59
Ash%	0.78 ± 0.13
Total solids %	15.25 ± 0.80

Fig 1: Cutting the coagulum

Fig 2: Cooking in process

Fig 3: Curd structure starts contracting with hot water treatment

Fig 4: Curd structure fuses together at advanced stage of hot water treatment

Fig 5: The process of stretching

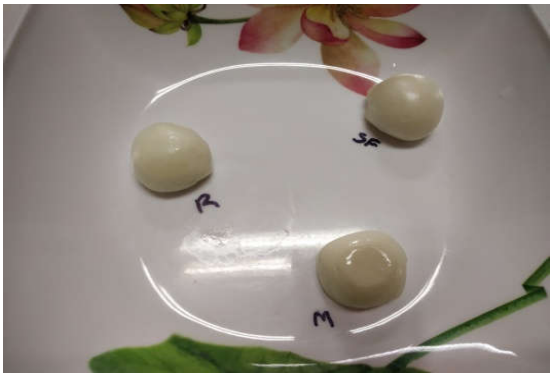

Fig 6: Mozzarella cheeses moulded into round balls after cold water treatment (pertaining to R-Rennet, M-Mustard, SF-Sunflower)

Parameters studied

Proximate composition of Mozzarella cheeses prepared using MEE, SFEE and MR

Proximate composition of Mozzarella cheeses prepared using MEE and SFEE has been presented in table 2. The reason for variations observed for moisture contents in different cheese samples has been explained by different authors differently. The reports of [17] revealed that a longer rennet coagulation time resulted in an increase in cheese moisture as well as an increase in cheese yield whereas [11] reported as a conclusion on their work on 'effect of vegetable coagulant, microbial coagulant and calf rennet on physicochemical, proteolysis, sensory and texture profiles of fresh goats cheese' that higher values of dry matter content were obtained in cheeses made with vegetable coagulants (extracted from *Cyanara cardunculus*) when comparison was done with microbial and calf rennets. They stated that the differences observed in dry matter content between cheeses made from microbial and vegetable coagulants can be explained by the three-dimensional structure of the curd, which would lead to variations in water retention.

The reason for the higher protein contents in the experimental cheeses (MEE MC and SFEE MC) can be explained with the help of reports of [19]. They mentioned that the protein contents of cheese prepared from *Withania coagulans* (DAW) (20.360%) were little higher than that of cheese prepared using

microbial rennet (DAR) (19.220%) which could be because of crude enzyme extract having higher concentration of proteins.

The fat contents of control cheese were found slightly and significantly higher than those of experimental samples. This observation is in close agreement with the findings of (19) where they observed that the fat contents of DAR (25.00%) were higher than that of DAW (23.30%) which may be because of the crude enzyme extract used coupled with more coagulation time as compared to calf rennet.

Ash contents (%) of cheese samples were in the range 2.20-2.45. The results are consistent with the study of (19), who reported that % ash levels of DAW and DAR were 2.33 and 2.40, respectively.

Table 2:Proximate composition of Mozzarella cheeses prepared using MEE and SFEE (Mean ± SD, n=6)

Treatments	Parameters			
	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
MEE MC	$50.80^{a} \pm 0.52$	$22.09^{c} \pm 0.16$	$22.15^{b} \pm 0.36$	$2.45^{b} \pm 0.11$
SFEE MC	$53.53^{c} \pm 0.65$	$21.21^{b} \pm 0.23$	$20.40^{a} \pm 0.13$	$2.40^{b} \pm 0.10$
MR MC	$52.85^{b} \pm 0.52$	$20.26^{a} \pm 0.18$	$22.30^{b} \pm 0.42$	$2.20^{a} \pm 0.09$

Means with different superscripts within a column differ significantly (P≤0.01)

MEE MC- Mozzarella cheese from Mustard enzyme extract,

SFEE MC- Mozzarella cheese from Sunflower enzyme extract,

MR MC- Mozzarella cheese from microbial rennet

Texture Profile Analysis of Mozzarella cheeses prepared using MEE, SFEE and MR

Data pertaining to the Texture Profile Analysis of Mozzarella cheeses prepared using MEE, SFEE and MR has been shown in table 3. Hardness values (N) ranged from 4.16 to 7.51 in fresh samples. These findings relate to the reports of (10) who worked upon the process optimization for mozzarella cheese from cow and buffalo milk. The reports for the hardness values (N) of their cheeses (using combined milks of cow and buffalo milks in different proportions) confirm that these values varied with the values such as 0.432 kg (4.23 N), 0.694 kg (6.80 N) and 0.722 kg (7.07 N) etc for different mozzarella cheese samples, based upon the proportion of cow and buffalo milk used for cheesemaking. According to [5], in Majorero cheese, hardness and firmness significantly increased as a consequence of the low moisture content, which may be related with our results regarding hardness values.

Springiness values ranged from 0.89 to 0.93 whereas cohesiveness values ranged from 0.79 to 0.83 amongst different mozzarella cheese samples. According to the reports of [10], the cohesiveness values (N) of their cheese samples (using combined milks of cow and buffalo milks in different proportions) varied from 0.492 - 0.636 for different mozzarella cheese samples, based upon the proportion of cow and buffalo milk used for cheese making. (16) reported that springiness values of various mozzarella cheese samples (control and experimental) varied in the range of 0.85- 1.00 which are in close agreement with the present springiness values. The different agents used for cheese making did not influence cheese springiness or cohesiveness.

Results pertaining to gumminess and chewiness values follow the same pattern as followed for hardness values as the magnitude of both of gumminess and chewiness values depends directly on the magnitude of hardness values as depicted previously in the formulas. Moreover, the values are consistent with the findings of (10), according to whom, the gumminess values of the various cheese samples varied in the range of 0.213-1.147 (kg) i.e. 2.08- 11.24 (N).

Table 3: Texture Profile Analysis of Mozzarella cheeses prepared using MEE and SFEE (Mean ± SD, n=6)

Treatments	Texture Profile Analysis				
	Hardness (N)	Springiness	Cohesiveness	Gumminess (N)	Chewiness (N)
MEE MC	$7.51^{c} \pm 0.16$	$0.93^{a} \pm 0.02$	$0.83^{a} \pm 0.03$	$6.22^{c} \pm 0.24$	$5.81^{c} \pm 0.26$
SFEE MC	$4.16^{a} \pm 0.24$	$0.89^{a} \pm 0.03$	$0.80^{a} \pm 0.03$	$3.31^{a} \pm 0.34$	$2.95^{a} \pm 0.35$
MR MC	$6.27^{b} \pm 0.15$	$0.92^{a} \pm 0.02$	$0.79^{a} \pm 0.03$	$4.93^{b} \pm 0.28$	$4.55^{b} \pm 0.35$

Means with different superscripts within a column differ significantly (P≤0.05)

MEE MC- Mozzarella cheese from Mustard enzyme extract

SFEE MC- Mozzarella cheese from Sunflower enzyme extract

MR MC- Control; Mozzarella cheese from microbial rennet

Color Profile Analysis of Mozzarella cheeses prepared using MEE, SFEE and MR

Data pertaining to the Colour Profile Analysis of Mozzarella cheeses prepared using MEE, SFEE and MR has been depicted in table 4. Results showed that lightness values varied in the range of 80.33- 82.27. As reported by (11), the lightness values varied in the range of 87.82- 89.47 for various types of mozzarella cheese samples whereas the work done by (22) on manufacture of shredded mozzarella cheese revealed that L* values varied in the range of 77.3- 82.03 among various fresh mozzarella cheese samples. Results pertaining to a* (redness) values were in close agreement with (22), which indicated the variation of a* values in the range of -2.67 to -2.47 for various fresh (shredded) mozzarella cheese samples. Results with respect to b* (yellowness) values were found similar to the findings of (22), who reported that the b* values of various fresh mozzarella cheese samples were in the range of 17.22- 18.49.

Table 4: Color Profile Analysis of Mozzarella cheeses prepared using MEE and SFEE (Mean ± SD, n=6)

Treatments	Color Profile Analysis		
	Lightness (L*)	Redness (a*)	Yellowness (b*)
MEE MC	82.27^{b} ± 0.85	-2.60^{b} ± 0.25	10.50^{a} ± 0.34
SFEE MC	81.86^{b} ± 0.76	-2.06^{c} ± 0.29	10.71^{a} ± 0.22
MR MC	80.33^{a} ± 0.74	-3.20^{a} ± 0.30	11.25^{b} ± 0.30

Means with different superscripts within a column differ significantly (P≤0.05)
MEE MC- Mozzarella cheese from Mustard enzyme extract
SFEE MC- Mozzarella cheese from Sunflower enzyme extract
MR MC- Control; Mozzarella cheese from Microbial Rennet

CONCLUSIONS

The proximate composition, textural characteristics and colour profile of mozzarella cheese samples prepared using enzyme extracts from mustard and sunflower oil seed cakes (experimental samples) and microbial rennet (control) were analyzed in this study. It was found that pertaining to proximate composition of the cheese samples, moisture content (%) was the lowest and protein content was the highest in MEEMC samples. For fat content (%), no significant difference was noticed in control and MEE MC samples but both were found to have significantly higher fat level as compared to SFEE MC. % ash was higher in experimental samples as compared to that found in control. For textural characteristics, hardness, gumminess and chewiness values were found the highest in MEE MC whereas the lowest in SFEEMC. Springiness and cohesiveness values were found comparable amongst all the samples. For colour profile, lightness and redness values were found higher whereas yellowness values were lower in experimental samples as compared to those with respect to control. Therefore, it could be concluded that mozzarella cheeses prepared using enzyme extracts from oil seed cakes of mustard and sunflower were found highly close to the cheese prepared using microbial rennet, in terms of all the characteristics, we studied and thus, these oil seed cakes may prove a good option as plant sources of milk coagulating enzymes for substitution of rennet.

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