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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(2): 277-281

www.thepharmajournal.com
Received: 16-12-2019
Accepted: 20-01-2020

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Proximate compositional, texture profile and colour profile analysis of cottage cheeses prepared using milk clotting enzymes extracted from mustard and sunflower oilseed cakes

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Abstract
Through this study, the proximate composition, textural profile and colour profile parameters of cottage cheeses prepared using enzyme extracts from mustard and sunflower oilseed cakes (experimental cheese samples) were determined. Cottage cheese prepared using microbial rennet was taken as control. The results revealed that cottage cheeses developed using enzyme extracts were found highly comparable to the control sample when all the above mentioned parameters were studied. Thus, this study could conclude that these cheap plant sources of milk clotting enzymes (oilseed cakes) may prove a potential substitute of rennet in the dairy industry with probable applications. Further, intense evaluation of the quality of cheese curd produced by their action will shed more light into their commercial suitability

Keywords: Microbial Rennet, Rennet substitutes, Oilseed cakes, Mustard, Sunflower, Proximate composition, Texture profile, Colour profile, Cottage cheese

1. Introduction

Enzymatic coagulation is very important in the manufacture of cheese. Calf rennet was the first and still is the most widely used in cheese making around the world. It is a milk-clotting enzyme preparation which is extracted from the calf's fourth stomach. This enzymatic preparation contains chymosin, which exhibits specific and limited proteolysis of the Phe105-Met106 bond in kappa-casein. High specific milk-clotting activity of calf rennet prevents excessive proteolysis during maturation. 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 (Anusha *et al*., 2014 ; Shah *et al*., 2014) [1, 17]. Apart from this, some religious factors (Islam and Judaism) and others related to vegetarianism of some consumers have greatly limited their use (Shah *et al*., 2014) [17].

Several milk-clotting enzymes of microbial origin have been commercialised and are in use in cheese industry, such as aspartic proteases (APs) obtained from *Rhizomucor miehei, Rhizomucor pusillus and Cryphonectria parasitica* (Sumantha *et al*., 2006) [21]. Most of the companies produce recombinant rennet of cattle calf origin in different microbial hosts (Seker *et al.,* 1999; Neelakantan *et al.,* 1999) [19, 13]. 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 such as *Carica papaya* (Cabezas *et al*., 1981) [5], *Cynara cardunculus* (Heimgartner *et al*., 1990) [12] etc. 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 (Egito *et al.,* 2007) [8]. Plant rennet have become a subject of growing interest in cheese industry, due to their easy availability and simple purification processes (Grozdanovic *et al*., 2013) [11]. Applications of plant coagulants allow target cheese production and hence contribute to improve the nutritional input of those populations on whom restrictions are improbable by the use of animal rennet (Silva and Malcata, 2005) [18]. Cottage cheese is a fresh, unripened cheese which evolved in rural American homes or cottages, hence, the popular name. The curd is formed by acid coagulation of skim milk, using either lactic acid fermentation or direct acidification (Raynes, 1992) [15]. Oilseed cakes/oil meals are by-products obtained after oil extraction from the seeds. The oilseeds have been identified as the plant sources for milk clotting enzymes (Egito *et al.,* 2007; El-Sayed *et al.,* 2013) [8, 10]. 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 (Elmazar *et al*., 2013) [10]. 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 (Egito *et al*., 2007) [8]. Oilseed cakes are easily available, of very low costs and are oil industry by products which are rich source of proteins (Ramachandran *et al.,* 2007) [14] and the enzymes extracted from oilseed cakes may prove a potential milk coagulant to fulfill the demand of cheese industry. Despite the widespread uses of mustard and sunflower oilseed cakes, their 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 cottage cheeses, in terms of their proximate composition, textural properties and colour profile analysis, which were prepared using enzymes extracted from oilseed cakes of mustard and sunflower.

2. Material and Methods

2.1 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'. Skim milk was 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. Table salt (Tata Chemicals Ltd., Mumbai) was procured from the local market.

2.2 Methods

2.2.1 Preparation and Standardization of the product (Cottage cheese)

Cottage cheese was prepared as per the methods mentioned in the manual on cheese technology, NDRI (Sabikhi *et al*, 2013) [16] and Chandan (2003) [7] with slight variation, using mesophilic starter culture (*Lactococcus lactis ssp. lactis)* and Milk clotting enzyme extracts (MCEE) at selected levels of pH, temperature and concentration. Control samples were prepared using microbial rennet as coagulant.

Standardized skim milk (9.5-10% TS) was pasteurised at 72.4 °C for 15 sec and was cooled to 32 °C.

Bulk mesophilic culture was added at the level of 6% and mixed thoroughly.

Standardised skim milk was coagulated with

(i) Microbial rennet @1.5g/100 litres of milk at 32 °C at pH-6.3,

(ii) Mustard Enzyme Extract (MEE) @ 3 ml/1 litre of milk at 32 °C at pH- 6.0 and temperature was raised to 40 °C over a span of 30 minutes.

(iii) Sunflower Enzyme Extract (SFEE) @ 5 ml/1 litre of milk at 32 °C at pH- 6.0 and temperature was raised to 40 °C over a span of 30 minutes.

Waited for around 2 hours for fermentation until pH dropped to 4.8.

Cutting (horizontal and vertical) of curds was done into smaller cubes which were then allowed to mature for 10 minutes.

The temperature of curds was raised slowly to 52 °C over 1.5 h for cooking.

Draining was carried out after cooking for the removal of whey.

Washing of curds was carried out in 3 steps

With 25-27 °C wash water

With 15-17 °C wash water

With 5-8 °C wash water

Once again, curds were allowed to drain for 30 min.

Salting was done @ 1%.

Cottage cheeses thus obtained were packed in clean, polyethylene bags and aluminum foil and stored at refrigeration temperature (6±2 °C) for further analysis.

2.2.2 Parameters studied

2.2.2.1 Proximate composition

Proximate composition was determined by following the standard methods of AOAC (2007) [2].

2.2.2.2 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 Bourne (1978) [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. Textural attributes such as hardness, springiness, cohesiveness, gumminess and chewiness were analyzed.

Hardness (N) = maximum force required to compress the sample.

Springiness = ability of sample to recover its original form after a deforming force was removed.

Cohesiveness = extent to which samples could be deformed prior to rupture (A2/A1, A1 being the total energy required for first compression and A2 the total energy required for second compression);

Gumminess (N) = hardness × cohesiveness

Chewiness (N) = hardness × springiness × cohesiveness

2.2.2.3 Color Profile Analysis

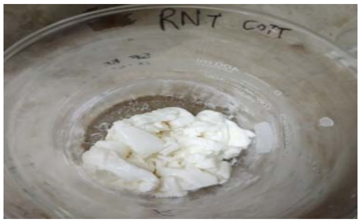
Konica Minolta Chromameter CR-400 (Konica Minolta Sensing, Inc., Japan) with 8 mm aperture was used for instrumental color evaluation. The instrument was calibrated with a white standard plate. Color scores were expressed as CIE Lab system as follows:

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*).

2.2.2.4 Statistical analysis

Data was analyzed statistically on 'SPSS-16.0' software package as per standard methods (Snedecor and Cochran, 1994) [20]. Data were subjected to ANOVA and Duncan's Multiple Range Test to find significant difference at 5% significance level in the mean values.

Drained SFEE cottage cheese



Drained Rennet cottage cheese

Drained MEE cottage cheese

All the three developed cottage cheeses before packaging

3 Results and Discussion
3.1 Chemical composition of standardized skim milk
Chemical composition of standardized skimmed milk (total solids- 9.5-9.95%) has been presented in table 4. The % protein, % fat, % ash and % total solids values of standardized skimmed milk were 3.74, 0.5, 0.82 and 9.94 respectively.

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

Components (%)	Standardized skim milk
Protein	3.74 ± 0.25
Fat	0.5 ± 0.20
Ash	0.82 ± 0.15
Total solids	9.94 ± 0.18

3.2 Parameters studied
3.2.1 Proximate composition of Cottage cheeses prepared using MEE, SFEE and MR
Data pertaining to the proximate composition of cottage cheeses prepared using MEE and SFEE has been shown in table 2. Pertaining to moisture content, values (%) ranged from 78.51– 79.80 amongst the samples. These findings are similar to the report of Tratnik *et al.* (2000) [22] who reported that total solids (%) in the cottage cheese curds manufactured from skimmed milks of different composition were in the range of 18.24 to 21.84. In turn, the moisture contents of the same could be judged and were found in the range of 78.16-81.76%.
With respect to protein % in the cottage cheeses, it was observed that values varied between 15.15-16.70%. Reports of Tratnik *et al.* (2000) [22] revealed that protein contents of different cottage cheese curds were in the range of 15.39-18.64%.
Values for fat % amongst the different cottage cheese samples varied from 0.66-0.78%. Work done by Tratnik *et al.* (2000) [22] on cottage cheeses manufacture revealed that fat content of cheese curd made from skim milk (with 0.05% fat) was 0.38%.
Ash content values (%) pertaining to different cottage cheese samples ranged between 0.61-0.70%. On the same note, the percent ash values pertaining to the cottage cheeses developed by Tratnik *et al.* (2000) [22] were in the range of 0.55-0.82%. Blanchette *et al.* (1995) [3] reports based on their work done on ‘production of cottage cheese using dressing fermented by Bifidobacteria’ reveal that the ash % of the control cottage cheese sample (creamed cottage cheese without *Bifidobacterium infantis* to obtain 4.5% fat in final cheese) was 0.97%.

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

Treatments	Parameters			
	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
MEE CC	78.51^{a} ± 0.35	16.70^{c}± 0.10	0.72^{ab}±0.05	0.69^{b} ± 0.04
SFEE CC	79.80^{c} ± 0.46	15.67^{b}± 0.10	0.66^{a} ±0.06	0.70^{b}± 0.05
MR CC	79.21^{b}± 0.32	15.15^{a} ± 0.12	0.78^{b} ±0.07	0.61^{a} ± 0.03

Means with different superscripts within a column differ significantly ($P \leq 0.01$)
MEE CC- Cottage cheese from Mustard enzyme extract
SFEE CC- Cottage cheese from Sunflower enzyme extract
MR CC- Control; Cottage cheese from Microbial Rennet

3.2.2 Texture Profile Analysis of Cottage cheeses prepared using MEE, SFEE and MR
Data pertaining to the Texture Profile Analysis of Cottage cheeses prepared using MEE and SFEE has been shown in table 3. Pertaining to each parameter, MEE CC samples had significantly higher scores as compared to control and SFEE CC. According to Calvo *et al.* (2007) [6], in Majorero cheese, hardness and firmness significantly increased as a consequence of the low moisture content, which might be related with our results regarding values related to different parameters of texture profile analysis. 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.

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

Treatments	Texture Profile Analysis				
	Hardness (N)	Springiness	Cohesiveness	Gumminess (N)	Chewiness (N)
MEE CC	$3.44^{c} \pm 0.13$	$0.87^{b} \pm 0.04$	$0.73^{b} \pm 0.04$	$2.49^{c} \pm 0.16$	$2.18^{c} \pm 0.18$
SFEE CC	$2.48^{a} \pm 0.08$	$0.79^{a} \pm 0.03$	$0.68^{a} \pm 0.02$	$1.68^{a} \pm 0.09$	$1.32^{a} \pm 0.07$
MR CC	$2.70^{b} \pm 0.11$	$0.80^{a} \pm 0.02$	$0.69^{a} \pm 0.02$	$1.86^{b} \pm 0.11$	$1.50^{b} \pm 0.10$

Means with different superscripts within a column differ significantly ($P \leq 0.05$)
MEE CC- Cottage cheese from Mustard enzyme extract
SFEE CC- Cottage cheese from Sunflower enzyme extract
MR CC- Control; Cottage cheese from Microbial Rennet

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

Table 4 indicates Colour Profile Analysis of cottage cheeses prepared using MEE and SFEE. Values with respect to lightness (L*) were in the range of 82.03- 85.41, for redness (a*) in the range of -2.88 to -1.76 and for yellowness (b*), were in the range of 10.31- 11.82.

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

Treatments	Colour Profile Analysis		
	Lightness (L*)	Redness (a*)	Yellowness (b*)
MEE CC	$85.41^{c} \pm 0.86$	$-2.38^{b} \pm 0.14$	$10.31^{a} \pm 0.47$
SFEE CC	$83.93^{b} \pm 0.96$	$-1.76^{c} \pm 0.14$	$10.50^{a} \pm 0.42$
MR CC	$82.03^{a} \pm 0.90$	$-2.88^{a} \pm 0.18$	$11.82^{b} \pm 0.56$

Means with different superscripts within a column differ significantly ($P \leq 0.05$)
MEE CC- Cottage cheese from Mustard enzyme extract
SFEE CC- Cottage cheese from Sunflower enzyme extract
MR CC- Control; Cottage cheese from Microbial Rennet

4. Conclusions

The proximate composition, textural characteristics and colour profile of cottage cheese samples prepared using enzyme extracts from mustard and sunflower oil seed cakes (experimental samples) and microbial rennet (control) were analyzed in this study. Pertaining to proximate composition of cottage cheese samples, it was found that moisture content (%) was the lowest and protein content was the highest in MEE CC samples. For fat content (%), no significant difference was noticed in control and MEE CC samples. % 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 CC whereas the lowest in SFEE CC. Regarding springiness and cohesiveness values, MEE CC samples had significantly higher values as compared to control and SFEE CC. For colour profile, lightness and redness values were found higher and yellowness values were lower in experimental samples as compared to those with respect to control. Therefore, it could be concluded that cottage 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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