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Preparation and Evaluation of Cottage Cheese using Enzyme Extracted from Mustard Oilseed Cake

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A B S T R A C T

The present study was aimed at preparing cottage cheese using an enzyme extract from mustard oilseed cake keeping in mind the plant alternative of rennet to be used as a source of milk clotting enzyme. Cottage cheese prepared using microbial rennet was taken as control. Under specific conditions of pH, temperature and concentration, the enzyme was used for the development of cottage cheese. Percent cheese yields, sensory evaluation and proximate composition of cottage cheese samples were determined. The results pertaining to these parameters revealed that the developed product using mustard enzyme extract was highly acceptable and comparable to the control and therefore the potential of mustard enzyme extract as a plant source of rennet substitute for the preparation of cottage cheese could be proved through this study.

Introduction

Cheese is one of the most widely consumed fermented dairy products with a growing consumer demand. It is an excellent dietary source of high-quality protein, vitamins and minerals such as absorbable dietary calcium (O'Connor, 1993). Milk coagulation is a basic step in cheese manufacturing. Calf rennet, the conventional milk clotting enzyme obtained from the fourth stomach of suckling calves was the most widely used coagulant in cheese making all over the world to manufacture most of the cheese varieties. 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). 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).

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). 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 (Ahmed *et al.,* 2009). 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). The use of plant proteases in cheese manufacturing promotes the greater acceptability by the vegetarians and may improve their nutritional intake (Duarte *et al.,* 2009).

Oilseed cakes/oil meals are by-products obtained after oil extraction from the seeds. Edible oil cakes have a high nutritional value; especially have protein contents ranging from 15% to 50% (Ramachandran *et al.,* 2007). The oil seeds have been identified as the plant sources for milk clotting enzymes (Egito *et al.,* 2007; El-Sayed *et al.,* 2013) and the enzymes extracted from oil seed cakes may prove a potential milk coagulant to fulfill the demand of cheese industry.

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). The typical shelf life of cottage cheese packaged in moisture-barrier containers is 3-4 weeks at 4°C (Chandan, 2003).

Keeping in view the above mentioned facts, the present study was planned to develop the cottage cheese using milk clotting enzymes from inexpensive and easily available mustard oilseed cake rather than employing the conventional rennet for the production of fresh un-ripen cheese upon optimizing the process parameters of cheese production.

Materials and Methods

Mustard (*Brassica spp*) oilseed cakes were procured from local market, Hisar from which the milk clotting enzyme (MCE) was extracted, called as Mustard Enzyme Extract (MEE). 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 (MR) was procured from amazon.in (Madmillie, Microbial vegetarian rennet tablets).

Bacterial cultures (Mesophilic) were procured from 'Esdee Marketing' Pune, Maharashtra. Table salt (Tata Chemicals Ltd., Mumbai) was procured from the local market. 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.

Cottage cheese preparation

Cottage cheese was prepared as per the methods mentioned by Sabikhi *et al.,* (2013) and Chandan (2003) with slight variation, using mesophilic starter culture (*Lactococcus lactis*

ssp. lactis) and milk clotting enzyme (enzyme extract from mustard oilseed cake) 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

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

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.

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.

Cheese yield

Cheese yield in percentage was calculated by the formula as given by Lucey and Kelly (1994).

$$\text{Per cent cheese yield} = \frac{\text{Amount of total cheese}}{\text{Amount of total milk}} \times 100$$

Sensory evaluation

A scale described by Tratnik *et al.,* (2000) was used to assess the sensory characteristics of the cheese samples.

The sensory qualities considered were flavour, body and texture, color and appearance, and total score of the products. A semi-trained panel of scientists and post graduate students evaluated the samples.

Proximate composition

Proximate composition was determined by following the standard methods of AOAC (2007). The moisture, protein, fat and ash contents of the products (control and experimental) were determined.

Statistical analysis

Data was analyzed statistically on 'SPSS-16.0' software package as per standard methods (Snedecor and Cochran, 1994).

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 skimmed milk (for cottage cheese)

Chemical composition of standardized skimmed milk (total solids- 9.5-9.95%) has been presented in table 1. The % protein, % fat, % ash and % total solids values of standardized skimmed milk were 3.74, 0.5, 0.82 and 9.94 respectively. The variation of skim milk composition had significant influence not only on the duration of milk fermentation and pH value of cheese curd at cutting, but also on composition of cheese, cheese yield and cheese yield efficiency (Tratnik *et al.,* 2000).

Cheese yield (%)

The % cheese yields for cottage cheese samples in both the treatments have been presented in table 2. No significant difference ($P \leq 0.05$) was noticed in the cottage cheese yields (%) of control (MR cheese) and treatment (MEE cheese). Steinsholt and Ystgaard (1966) concluded that there is a positive correlation between the yield of cheese and the casein fraction, fat content and total solids of milk. The yield values of cottage cheese samples were in the range of 19.17- 19.75% which were more than those reported by Qazalbash *et al.,* (2018) i.e. 14.3-18.7%.

Sensory scores of cottage cheeses prepared using MEE and MR

Sensory scores of cottage cheeses prepared using MEE and MR have been presented in table 3. No significant difference was observed in the sensory scores of MEE CC and MR CC for any of the sensory evaluation parameter i.e. flavour (1-10), body and texture (1-5), colour and appearance (1-5) and total score (1-20). The scores for all the parameters were highly comparable between the two samples (MEE CC and MR CC). Qazalbash *et al.,* (2018) while working on effect of different storage conditions on coagulating properties and cheese quality prepared by using *Withania coagulans* extract, reported that the optimum proteolytic activity resulted in higher retention of fat contents in cheese.

Abd El-Gawad *et al.,* (2007) mentioned that fat contents are linked to the characteristic body, texture and flavour of cheese.

Proximate composition of Cottage cheeses prepared using MEE and MR

Data pertaining to the proximate composition of cottage cheeses prepared using MEE and MR has been shown in table 4. It was noticed that control (MR CC) (79.21) had significantly higher moisture content (%) as compared to MEE CC (78.51). Tratnik *et al.,* (2000) 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%.

For percent protein, MEE CC (16.70) was observed to have significantly higher values as compared to control (15.15). Reports of Tratnik *et al.,* (2000) revealed that protein contents of different cottage cheese curds were in the range of 15.39-18.64%.

Values for fat % amongst the cottage cheese samples varied from 0.66-0.78%. Work done by Tratnik *et al.,* (2000) on cottage cheese manufacture revealed that fat content of cheese curd made from skim milk (with 0.05% fat) was 0.38% (Fig. 1–4).

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

Table.2 Cheese yield (%) (Mean ± SD, n=6)

Treatments	% cheese yield (Cottage)
MEE Cheese	19.66 ± 0.85
MR cheese	19.17 ± 0.79

MEE cheese- Cheese from Mustard enzyme extract
MR cheese- Control; Cheese from Microbial Rennet

Table.3 Sensory scores of cottage cheeses prepared using MEE and MR (Mean ± SD, n=18)

Treatments	Parameters			
	Flavour (1-10)	**Body & Texture(1-5)**	**Colour & Appearance(1-5)**	**Total Score (1-20)**
MEE CC	8.61±0.47	3.64±0.16	3.67±0.25	15.92±0.47
MR CC	8.61±0.50	3.69±0.25	3.69±0.27	16.00±0.48

MEE CC- Cottage cheese from Mustard enzyme extract
MR CC- Control; Cottage cheese from Microbial Rennet

Table.4 Proximate composition of Cottage cheeses prepared using MEE and MR (Mean ± SD, n=6)

Treatments	Parameters			
	Moisture (%)	**Protein (%)**	**Fat (%)**	**Ash (%)**
MEE CC	78.51^{a} ± 0.35	16.70^{b}± 0.10	0.72^{a}±0.05	0.69^{b} ± 0.04
MR CC	79.21^{b}± 0.32	15.15^{a} ± 0.12	0.78^{a} ±0.07	0.61^{a} ± 0.03

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

Fig.1 Drained MEE cottage cheese

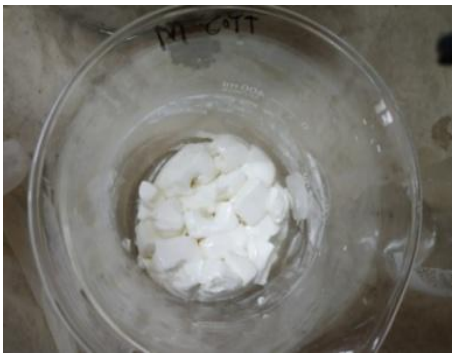

Fig.2 Developed MEE cottage cheese before packaging

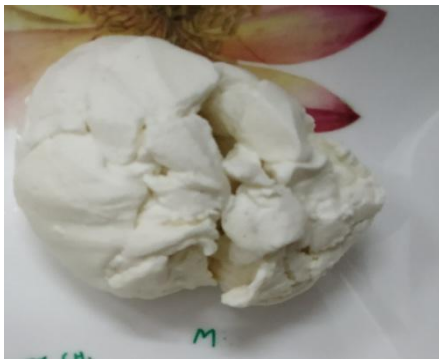

Fig.3 Drained Rennet cottage cheese

Fig.4 Developed Rennet cottage cheese before packaging

Pertaining to ash %, MEE CC (0.69) had significantly higher values as compared to control (0.61). The percent ash values pertaining to the cottage cheeses developed by Tratnik *et al.,* (2000) were in the range of 0.55-0.82%.

The study was conducted to prepare cottage cheese using enzyme extract from mustard oilseed cake which was used as a source of milk clotting enzyme to replace rennet. It was found that enzyme extract from mustard oilseed cake (MEE) at specific conditions of pH, temperature and concentration could result into a successful coagulation and preparation of cottage cheese. The results pertaining to the percent cheese yield and scores of sensory evaluation indicated that the product prepared using MEE was highly acceptable and found very close to the control prepared using microbial rennet. To the best of our knowledge, this is the first report of the development of cottage cheese using enzyme extract from mustard oilseed cakes and its evaluation based upon its % yield, sensory scores and proximate composition. However, further studies on the complete purification and characterization of this promising enzyme together with the intense evaluation of the quality of cheese curd produced by its action will shed more light into its commercial suitability.

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