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Microbiological Analysis and Nutritional Evaluation of Cottage Cheese Produced with Kiwifruit Enzyme

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A B S T R A C T

Cottage cheese (soft cheese) is an excellent source of protein, fats and minerals such as calcium, iron and phosphorus, vitamins and essential amino acids, thus making it an important food in the diet of both old and young. It is commercially made with the addition of chymosin (rennet). The worldwide increase in cheese production, alongside with the reduced supply of calf rennet and higher prices, have led to an increase in the demand for alternative sources of milk coagulants. Therefore, this study was designed to evaluate the effect of kiwifruit enzyme on the quality of cottage cheese. In the experiment, first enzyme was extracted from the kiwifruits followed by partial purification, characterization, optimization of enzyme concentration, microbiological analysis, nutritional evaluation and organoleptic analysis of cottage cheese was done. It is observed that cottage cheese prepared with kiwifruit enzyme showed comparable results to rennet in nutritional composition as well as in organoleptic properties during storage. Therefore, it was finally concluded that partially purified kiwifruit enzyme at the rate of 0.5 per cent can be used as potential vegetable source of coagulant for manufacturing of cottage cheese without any adverse effect on taste and nutritional characteristics.

Introduction

The Kiwifruit (*Actinidia deliciosa* also known as Chinese Gooseberry) is native to China and centre of origin is in the mountain ranges of South Western China where it occurs naturally as a deciduous fruiting vine. Kiwifruit is very popular in human diet due to its pleasant taste and high content of vitamin C, minerals (potassium, phosphorus, iron) and low calorific value. Moreover, kiwifruit juice is known to contain highly active protease enzyme (Kaur *et al.,* 2010). Protease enzymes are multifunctional class of enzymes (Marques *et al.,* 2010). Henceforth kiwifruit enzyme as a protease enzyme can be used in food industry as milk clotting enzyme. Calf rennet, which contains chymosin (E.C. 3.4.23.4) as a main enzymatic component, has been the most widely used milk-clotting enzyme preparation. Natural calf rennet is extracted from the inner mucosa of the fourth stomach chamber (the abomasum) of young, unweaned calves according to a complex and expensive procedure (Lopes *et al.,* 1988). The worldwide increase in cheese production, alongside with

the reduced supply of calf rennet, higher prices and due to consumer constraints such as religious reasons, diet (vegetarianism), or bans on genetically engineered food have led to an increase in the demand for alternative sources of milk coagulants (Cavalcanti *et al.,* 2010). For these reasons, enzymes extracted from plants have become a subject of growing interest in dairy technology. Henceforth, present study was planned to study the microbiological analysis and nutritional evaluation of cottage cheese produced with kiwifruit enzyme.

Materials and Methods

Procurement of raw material

The raw material i.e. kiwifruits (*Actinidia deliciosa)* and milk was procured from YSP University of Horticulture and Forestry, Nauni-Solan (India), while rennet was procured from Guru Angad Dev Veteniary and Animal Sciences University, Ludhiana, Punjab (India) and other material such as innoculator (yogurt) and salt was procured from local market of Nauni-Solan (India).

Extraction, partial purification and characterization of kiwifruit enzyme

Protein concentration and extraction of crude enzyme from kiwifruit was determined at different stages of maturity by using procedure followed by Lawrie (1998) and Thimmaiah (2006).The procedure followed by Sadasivam and Manickam (1998) was employed for partial purification of enzyme. The crude extract of kiwifruit was precipitated by ammonium sulphate using different concentrations (0-90 per cent). Response Surface Methodology (RSM) was used for the characterization of the enzyme by keeping the effects of pH, temperature, time of incubation as independent variables and enzyme activity as a dependent variable. As per the design treatments chosen were shown in Table 1. The different combinations were made as per the expert RSM design version 7.0 (Stat Ease, Inc, Minneapolis, USA). The procedure of Thimmaiah (2006) was employed for assessment of enzyme assay.

Optimization of enzyme concentration

Enzyme concentration was standardized on the basis of milk clotting activity as mentioned by Arima *et al.,* (1970) with a slight modification. The substrate (skimmed milk) was prepared and the pH was adjusted to 6.5. The substrate (10 ml) was pre-incubated for 5 min at 37^0C and different concentration (0, 2, 4, 6, 8, and 10 %) of enzyme extract was added and the curd formation was observed at 37^0C while manually rotating the test tube from time to time. The end point was recorded when discrete particles were discernible. One milk clotting unit is defined as the amount of enzyme that clots 10 ml of the substrate within 40 min.

MCA (U/ml) = (2400/clotting time (sec)) x Dilution factor

Where MCA= milk clotting activity

Preparation of cottage cheese

Development of cottage cheese was done by using the method i.e. 2000 ml of pasteurized milk was heated in a pan and yogurt as starter culture (4-5 %) was added into pan when the temperature of 45^oC reached after which kiwifruit enzyme was immediately added to the milk using a sterile pipette and allowed for setting in incubator at 21^oC for 14 hrs. After the milk was fully coagulated and settled, cutting of cougulam into smaller pieces was done. For preservation, salt (at the rate of 1% of curd) was also added into cheese. Similar procedure of cheese making with rennet was followed in place of kiwifruit enzyme treated

as control. Developed cottage cheese was packaged in LDPE pouches and stored under refrigerated conditions (7±2^0 C) till it remains microbiologically and nutritionally acceptably.

Microbiological, physico-chemical and sensory characteristics of cottage cheese

The fresh as well as stored product was evaluated for microbiological and physico-chemical (AOAC, 1990) at an interval of 0, 2, 4 days till spoiled. Organoleptic characteristics were evaluated as per the procedure followed by Larmond (1977). In the study, sample of coded cheese was served in cleaned white plate to panelist of all age groups of both sexes at room temperature (25^{0}C) for sensory evaluation by using Hedonic scale where 1 = Dislike extremely and 9 = Like extremely.

Statistical analysis

The data pertaining to the sensory evaluation of cottage cheese were analyzed according to Randomized Block Design (RBD), while the data on chemical characteristics of product was analyzed statistically by following Completely Randomized Design (CRD) (Cochran and Cox, 1967).

Results and Discussion

The aim of the present study was to assess the effect of kiwifruit enzyme on microbiological stability, nutritional and organoleptic characteristics of cottage cheese compared to rennet cottage cheese. In this study, first kiwifruit enzyme was extracted at various stages of fruit maturity followed by their partial purification and characterization. Partially purified kiwifruit enzyme was employed for production of cottage cheese. Finally the quality attributes of cottage cheese produced with kiwifruit enzymes were compared with rennet cheese during storage period.

Extraction of kiwifruit enzyme

The highest protein content and enzyme activity (0.42mg/gm and 200.32µg/gm) was observed at immature stage of fruit (Table 2).

Partial purification of kiwifruit enzyme

Highest enzyme activity, yield and purification fold was found with 40-60 per cent concentration of ammonium sulphate 86 per cent protease enzyme yield of 1.65 purification fold and 0.86 /mg of protein specific activity whereas Hullikere *et al.,* (2014) reported protease enzyme with similar yield at 0.96 purification fold and 1.23 specific activities in latex of papaya (*Carica papaya)* (Table 3).

Characterization of kiwifruit enzyme

The figures (Fig. 1 a, b, c) depict the expected response of enzyme activity and correlation between the independent variables in three dimensional plots.

From figures it was clearly shown that with increase in pH, temperature and time of incubation up to a certain limit (pH 8.0, 20 min and 45^{0}C) significant increase in enzyme activity was observed but after this limit there was considerable decrease in the enzyme activity was noticed whereas Otani *et al.,* (1991) characterized the proteolytic enzyme of figure and found optimum temperature and pH 65^0 C and 7.5 respectively and further reported that ficin retained more than 90 per cent of its original activity after a period of 1 hr incubation at 55^{0}C.

Optimization of enzyme concentration

The enzyme concentrations i.e. 1 per cent rennet and 0.5 per cent partially purified kiwifruit enzyme were optimized for cottage cheese production.

Table.1 Range of values for the RSM

Variables	-1	0	+1
pH	4	8	12
Temperature (^{0}C)	30	45	60
Incubation Time (min)	**10**	**20**	**30**

Table.2 Protein content and enzyme activity of kiwifruit (Mean±SE)

Stage	Protein (mg/gm)	Enzyme activity (µg/gm)
Immature (TSS<6.5 ^{0}B)	0.42 ± 0.20	200.32 ± 0.20
Mature (TSS=6.5 ^{0}B)	0.28 ± 0.10	131.50 ± 0.20
Ripened (TSS<14 ^{0}B)	0.25 ± 0.20	130.25 ± 0.20

Table.3 Purification profile of kiwifruit enzyme

Observations					
Purification step	**Protein (mg/gm)**	**Enzyme activity (µg/gm)**	**Specific activity (/mg protein)**	**Purification fold**	**% Yield**
Crude enzyme	0.42	220.00	0.52	1	100
Ammonium sulphate precipitation (40-60%)	0.22	190.00	0.86	1.65	86

Table.4 Microbial load of cottage cheese samples

Samples	0 day	2 day	4 day
Control cheese	$<10^4$	$<10^5$	$>10^7$
Kiwifruit cottage cheese	$<10^3$	$<10^5$	$>10^7$

Table.5 Nutritional characteristics of cottage cheese samples

Treatments / Days	Moisture content (%)			Titratable acidity (%)			Crude protein(%) content			Calcium content			Total carbohydrates (%)			Energy (Kcal/100gm)		
	Rennet	Kiwifruit enzyme	Mean	Rennet	Kiwifruit enzyme	Mean	Rennet	Kiwifruit enzyme	Mean	Rennet	Kiwifruit enzyme	Mean	Rennet	Kiwifruit enzyme	Mean	Rennet	Kiwifruit enzyme	Mean
0	68.33	68.80	65.56	0.085	0.086	0.085	17.80	15.52	16.66	20.00	19.40	19.70	7.27	9.18	8.22	140.91	138.47	139.69
2	64.83	65.20	65.01	0.087	0.089	0.088	17.50	15.50	16.50	19.25	19.20	19.22	13.14	13.46	13.30	155.04	151.07	153.05
4	63.16	64.00	63.58	0.105	0.107	0.106	17.00	15.35	16.17	19.16	19.18	19.17	16.26	15.22	15.74	161.78	157.93	159.85
MEAN	65.66	66.00		0.092	0.094		17.43	15.45		19.47	19.26		12.22	12.62		152.57	149.15	
$CD_{0.05}$	T		0.812	T		0.002	T		NS	T		NS	T		0.100	T		0.213
	S		0.994	S		0.014	S		NS	S		NS	S		0.312	S		0.312
	TxS		1.406	TxS		0.106	TxS		NS	TxS		NS	TxS		0.140	TxS		0.122

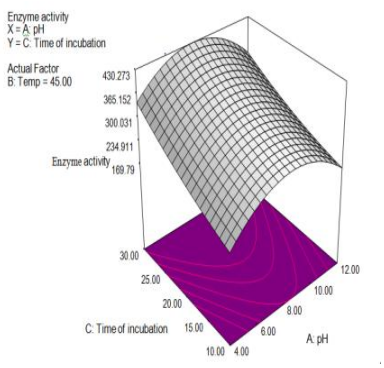


Fig.1 (a) Effect of pH and time of incubation on enzyme activity

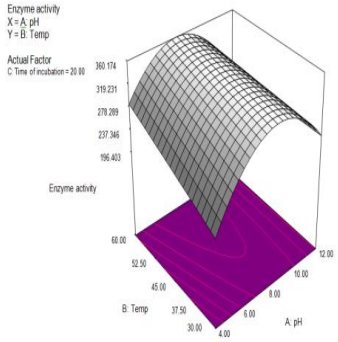


Fig.1 (b) Effect of pH and temperature on enzyme activity

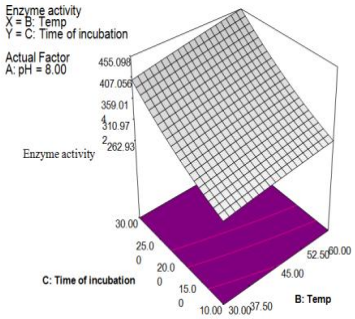
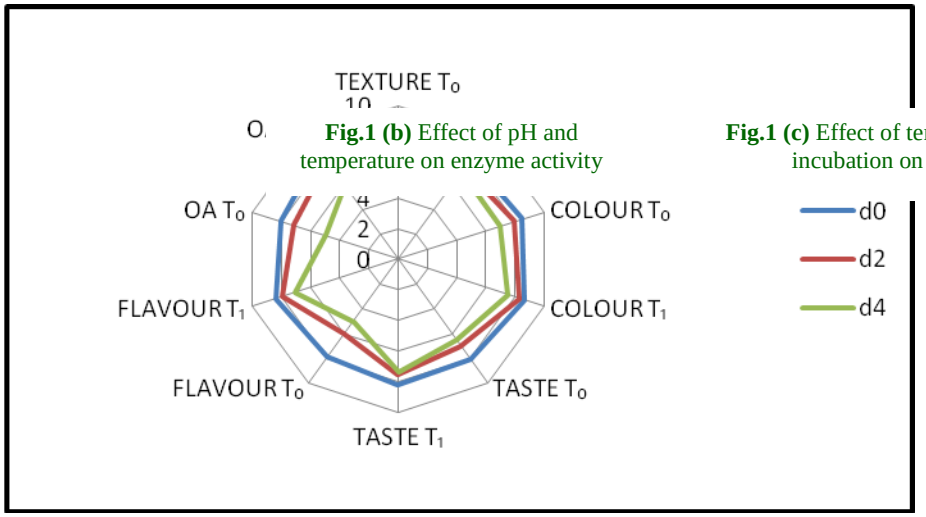


Fig.1 (c) Effect of temperature and time of incubation on enzyme activity

Fig.2 Effect of storage on sensory characteristics of cottage cheese



T_0- control cheese and T_1- cottage cheese prepared with kiwifruit enzyme
OA- overall acceptability, d0- zero day, d2- 2nd day, d4- 4th day

However milk-clotting activity estimated by Cavalcanti *et al.,* (2010) in different precipitation fractions of the *Nocardiopsis* sp were in the range of 1.14-20.00 U/ml whereas Llorente *et al.,* (1997) reported the clotting time of proteinase isolated from mature flowers, immature flowers and leaves of artichoke were 8, 90, 180 min respectively.

After the optimization of enzyme concentration, cottage cheese was prepared and then studied fresh as well as stored for their microbiological, nutritional and organoleptic characteristics.

Microbiological, nutritional and organoleptic characteristics of fresh and stored cottage cheese

Table 4 shows the effect of storage on microbial load of cottage cheese samples. With the storage there was increase in microbial load in both the samples. Both the samples were found unacceptable after the 4th day of storage.

Data presented in Table 5 shows the effect of storage on nutritional characteristics of cottage cheese samples. With the storage (4

days) there was decrease in moisture content whereas titratable acidity (%), total carbohydrates (%) and energy value (Kcal/100 g) increased respectively. The reduction in moisture content of cheeses with increase in storage period was due to increase in acidity which caused the protein matrix in the curd to contract and squeeze out moisture (syneresis) whereas increase in acidity during storage of cheese was mainly due to the lactic acid formed by a predominating lactic acid bacteria. However for other parameters, the overall effect of storage was found insignificant except carbohydrate and energy value which were on calculation basis. Similar trend of results were reported by Perveen *et al.,* (2011).

Organoleptic characteristics of cottage cheese

The data pertaining to the effect of storage on organoleptic characteristics of cottage cheese (Fig. 2) showed that evaluators had given higher scores for the overall acceptability of cottage cheese prepared with kiwifruit enzyme compared to reference cheese. There was decrease in acceptance of product by the evaluators with the advancement of storage period.

The results were parallel with Galan *et al.*, (2008), reported that cardoon (vegetable) rennet cheese exhibited softer texture and higher creaminess scores as compared with the calf rennet cheeses.

From this study it was concluded that kiwifruit enzyme is potential vegetable source of coagulant for manufacturing of cottage cheese without any adverse effect on taste and nutritional characteristics. Henceforth, kiwifruit enzyme can be considered a promising alternative of natural calf rennet for the coagulation of milk leading to new dairy products.

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