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Kiwifruit enzyme: A new plant coagulant for the development of cottage cheese

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Abstract
In the present work, extraction of kiwifruit enzyme, partial purification, characterization, optimization of enzyme concentration, quality of cottage cheese prepared and their comparative study with animal rennet was studied. In the experiment, first enzyme was extracted at various stages of maturity followed by partial purification, characterization and optimization of enzyme concentration was done. Results showed that kiwifruit enzyme has maximum enzyme activity at immature stage and partial purification with ammonium sulphate precipitation method at 40-60 per cent shows the highest yield. Characterization of enzyme through Response Surface Methodology (RSM) shows that enzyme was stable at pH (8) and temperature (45^0C). The quality of cottage cheese prepared with kiwifruit enzyme was comparable to the rennet cottage cheese. Therefore, it was finally concluded that animal origin rennet can be completely or effectively replaced by employing plant kiwifruit partially purified enzyme at the rate of 0.5 per cent for cottage cheese production.

Keywords: kiwifruit enzyme, characterization, partial purification, cottage cheese, rennet

1. Introduction
Kiwifruit (*Actinidia deliciosa*) is native to Southern China [10]. 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. Kiwifruits are good sources of folate, potassium and contain large amounts of vitamin E in the seeds. Moreover, kiwifruit juice is known to contain highly active proteolytic enzymes [14]. Enzymes are widely used as technological aids in several food processes. Proteolytic enzymes are multifunctional class of enzymes [22]. Henceforth kiwifruit enzyme as a proteolytic enzyme can be used in food industry as milk clotting enzyme. Kiwifruit enzyme has the ability to form milk clots so that the enzyme is fully compatible with conditions used in cheese manufacture (optimum activity at 40-42ºC, mildly acidic pH values). Analysis of products produced by hydrolysis by using actinidin showed that the preferred substrate for this enzyme is *β*-casein, followed by k-casein, which is hydrolysed into a small number of larger peptides [18]. Milk coagulation is a basic step in cheese manufacturing and is most commonly achieved by addition of chymosin (rennet), milk clotting enzyme preparation obtained from the stomach contents of the unweaned calf [19]. 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 [6]. In addition, the use of animal rennet has consumer constraints due to religious reasons (e.g. Judaism and Islam), diet (vegetarianism), or bans on genetically engineered food [27]. For these reasons, enzymes extracted from plants have become a subject of growing interest in dairy technology. Henceforth, present study was planned to assess the effect of kiwifruit enzyme on quality attributes of cottage cheese as potential vegetable coagulant to replace the animal origin rennet.

2. Materials and methods
2.1 Procurement of raw material
The raw material i.e. kiwifruit (*Actinidia deliciosa)* was procured from Kiwifruit Orchard, Department of Fruit Science, milk from Dairy Farm, Department of Silviculture and Agroforestry, 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). However other materials used for producing cottage cheese viz. starter culture and salt were obtained from the local market of Nauni-Solan, Himachal Pradesh (India).

2.2 Estimation of soluble protein in kiwifruit
Protein concentration in kiwifruit was determined using procedure followed by Lowry [16].

2.3 Extraction of crude enzyme
The procedure followed by Thimmaiah [31] was employed for extraction of crude enzyme from kiwifruit. First the kiwifruit tissues were homogenized in pestle-mortar with phosphate buffer (pH 8.0) under cool condition (0-5^0C) and extract was centrifuged at 10,000 rpm for 10 min in refrigerated centrifuge. The supernatant was labeled as "Crude enzyme".

2.4 Partial purification of enzyme
The procedure followed by Sadasivam and Manickam [28] was employed for partial purification of enzyme extracted from kiwifruit. First partial purification of enzyme was done by using ammonium sulphate fractionation method as mentioned above. The crude extract of kiwifruit was precipitated by ammonium sulphate using different concentrations (0-90 per cent). Precipitation was carried out at 0-5^0C and the precipitate was recovered by centrifugation. The supernatant

was discarded and the sediment from each concentration was dissolved in phosphate buffer solution (pH 8.0) and dialyzed over night against the same buffer. The dialyzed enzyme was used for further studies.
Observations recorded dring partial purification are-

2.4.1 Specific activity [4]
Specific activity was calculated by dividing the total enzyme activity with total protein.

$$\text{Specific activity} = \frac{\text{Total enzyme activity}}{\text{Total protein}}$$

2.4.2 Fold purification [4]
Fold purification was calculated by dividing the specific activity of partially purified enzyme with specific activity of crude enzyme.

$$\text{Fold Purification} = \frac{\text{Specific activity of partially purified enzyme}}{\text{Specific activity of crude enzyme}}$$

2.4.3 Per cent yield / Recovery of enzyme [4]
Percent yield / Recovery was calculated by dividing the total enzyme activity of partially purified enzyme with total activity of crude enzyme.

$$\text{Percent yield} = \frac{\text{Total enzyme activity of partially purified enzyme}}{\text{Total activity of crude enzyme}} \text{X } 100$$

2.5 Characterization of enzyme
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 details of experimental design plan for characterization of enzyme are given in Table 2.

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

2.5.1 Experimental design and equation modelling
Second-order experimental design, *i.e.* Central Composite Design (CCD) with three factors at three levels was employed to investigate the first and higher-order main effects of each factor as well as interactions among them. The design involved eight center design points with 'α' value being ± 2. The three coded levels investigated in the current study were -1, 0, and 1 as shown in table. The enzyme assay conditions were optimized on the basis of highest enzyme activity.
The experimental results obtained by the characterization of enzyme were applied to obtain the regression models. Based on experimental design (Table 1), the cubic model could be fitted in case the lacks of fit of the quadratic or linear models were statistically significant ($p<0.05$), Where Y_i, evaluated response; b terms were coefficients estimated by the least square

$$\hat{y}_i = b_1^* x_1 + b_2^* x_2 + b_3^* x_3 + b_{12}^* x_1 x_2 + b_{13}^* x_1 x_3 + b_{23}^* x_2 x_3 + b_{123}^* x_1 x_2 x_3,$$

method; x terms were dependent variables [9]. Quality of the models fitness was evaluated by ANOVA, in which the repetition supplied the freedom degree to obtain the pure error. Calculations and graphics were performed by opstat. This was used at the beginning of this study.

Table 2: Experimental plan of characterization of enzyme as per the design

Treatments	Factor 1 A:pH	Factor 2 B:Temp (^{0}C)	Factor 3 C: Time of incubation (min)
T_1	8.00	45.00	20.00
T_2	4.00	30.00	10.00
T_3	1.27	45.00	20.00
T_4	8.00	45.00	20.00
T_5	8.00	45.00	20.00
T_6	12.00	60.00	30.00
T_7	8.00	45.00	20.00
T_8	8.00	45.00	36.82
T_9	12.00	30.00	10.00
T_{10}	8.00	70.23	20.00
T_{11}	4.00	30.00	30.00
T_{12}	12.00	60.00	10.00
T_{13}	8.00	45.00	20.00
T_{14}	4.00	60.00	30.00
T_{15}	4.00	60.00	10.00
T_{16}	14.73	45.00	20.00
T_{17}	8.00	45.00	20.00
T_{18}	12.00	30.00	30.00
T_{19}	8.00	19.77	20.00
T_{20}	8.00	45.00	3.18

2.6 Enzyme assay
The procedure followed by Thimmaiah [31] was employed for assessment of enzyme assay with slight modifications. For this, one ml of reaction mixture containing 1 per cent casein in 1 ml of 0.05 M phosphate buffer, pH 8.0 and 1 ml of enzyme was incubated at 45°C for 20 min. After 2hr, the reaction was stopped by adding 2ml of cold 10 per cent trichloroacetic acid (TCA) and for blank, immediately after incubation reaction was stopped with 10 per cent TCA. After 2 hour, the culture filtrate was centrifuged at 10,000 rpm for 10 min in refrigerated high speed research centrifuge (Model TC 4100 F/RC 4100 F) to remove the precipitate and absorbance of the supernatant was read spectrophotometrically at 660 nm. Enzyme activity was calculated by measuring mg of tyrosine equivalent released and compared with the standard. One unit (U) of enzyme activity represents the amount of enzyme required to liberate 1 µg of tyrosine under standard assay conditions.

2.7 Optimization of enzyme concentration
Enzyme concentration was standardized on the basis of milk clotting activity with a slight modification [3]. 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

2.8 Preparation of cottage cheese
Cottage cheese was manufactured with some modifications [12, 29]. About 2000 ml of pasteurized milk was heated in a pan while stirring on the induction heater. Yogurt as starter culture (4-5 %) was added into pan when the temperature of 45ºC reached after which kiwifruit enzyme was immediately added to the milk using a sterile pipette and allowed for setting in incubator at 21ºC for 14 hrs. After the milk was fully coagulated and settled, cutting of cougulam into smaller pieces was done. The process of cooking (1 hr) and then pressing out the remaining whey, tied and pressed with a known weight to dry to a constant weight (approximately after 2hrs) was performed. For preservation, salt (at the rate of 1% of curd) was also added into cheese. The cheese was then removed into a sterile container and stored in the fridge at 4ºC overnight or analyzed immediately. Similar procedure of cheese making with rennet was followed in place of kiwifruit enzyme treated as control.

2.9 Effect of kiwifruit enzyme on physico-chemical attributes of cottage cheese
2.9.1 Yield of cheese and whey
The fresh cottage cheese was studied for their quality characteristics i.e. Cheese yield and whey yield was calculated by the standard methods of Mahajan and Chaudhari [20].

2.9.2 Proximate analysis
The moisture content, crude protein, crude fat and ash content were determined in triplicates following methods of AOAC [2].

2.9.3 Calcium content
The AOAC method [2] was followed for estimation of calcium content. For that one gram of cheese sample digested in 20 ml concentrated sulphuric acid till 3-4 ml extract was remained after that it was diluted with distilled water to make 100 ml final volume. Then calcium content was estimated with flame photometer with standard procedure.

2.9.4 Total carbohydrates
The total carbohydrate content as per cent dry weight basis was determined mathematically.

Total carbohydrate (%) = 100 - (% crude protein + % crude fat + % moisture content + % ash content)

2.9.5 Energy value
The energy value of samples was calculated using the following relation.

Energy value in (Kcal) = (Crude protein x 4.1) + (Crude fat x 9.3) + (Carbohydrate x 4.1)

2.10 Texture analysis
The texture profile analysis of cheese samples were measured by using Texture Analyzer, TAXT2i (Stable 70 Microsystems, UK), equipped with a cell charge of 25 kg. The texture profile was obtained by a double compression test of the cheese samples, at room temperature, using a cylindrical compression probe with a 75 mm diameter (P/75). Test parameters included hardness, fracturability, adhesiveness, springiness, gumminess, chewiness, cohesiveness and resilience (Annexure IV). The instrument was operated at pre-test speed = 1.00 mm/s, test speed = 5.00 mm/s, post test speed = 5.00 mm/s, strain rate = 50%, trigger force = 0.04903 gm, force and data acquisition rate of 100 pps.

2.11 Sensory attributes of cottage cheese
The products were subjected to organoleptic analysis as per the procedure followed by Larmond, [15]. 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 (25^0C) for sensory evaluation by using Hedonic scale (annexure) where 1 = Dislike extremely and 9 = Like extremely.

2.12 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) [8].

3. Results and Discussion
The aim of the present study was to assess the effect of enzymes extracted from the kiwifruit on milk clotting and quality attributes of cottage cheese prepared. 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.

3.1 Extraction of kiwifruit enzyme at various stages of fruit maturity

This was done in order to estimate the right stage of enzyme extraction. Three different stages of fruit maturity i.e. immature stage (7 days before attaining the commercial harvest date (TSS<6.5^{0}B)), mature (commercial harvest date (TSS=6.5^{0}B)) and ripened stage (8-10 days after commercial harvest date (TSS<14^{0}B)) was taken. The data in Table 3 represents the protein content and enzyme activity of kiwifruit at various stages of fruit maturity as protein content indirectly represents the enzyme. The highest protein content and enzyme activity (0.42±0.20 mg/gm and 200.32±0.20 µg/gm) was observed at immature stage of fruit followed by mature (0.28±0.10 mg/gm and 131.50±0.20 µg/gm) and then ripened (0.25±0.20 mg/gm and 130.25±0.20 µg/gm) stage respectively. It is shown from the experiment that in kiwifruit maximum enzyme activity was shown at immature stage. Similar findings were reported by Whitaker, [32] that the enzyme activity of *Ficus carica* fruit was highest when they were unripened green.

Table 3: 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

3.2 Partial purification of kiwifruit enzyme

In the Table 4, purification profile of kiwifruit enzyme was presented. With ammonium sulphate precipitation, the fractionation was carried out in different ranges i.e. 20-90 per cent. Each fraction was assayed for its protease activity. Highest enzyme activity, yield and purification fold was found with 40-60 per cent concentration of ammonium sulphate, i.e. 86 per cent protease enzyme yield of 1.65 purification fold and 0.86 /mg of protein specific activity were found in kiwifruit. However Chaiwut *et al.* [7] and Otani *et al.* [26] reported the highest activity of protease enzyme extracted from fig, precipitated with 50-70 per cent ammonium sulphate. Based on results, it is concluded that kiwifruit enzyme was precipitated in two steps, in which it was possible to recover more than 86 per cent of its initial activity with the purification factor of 1.65.

Table 4: 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

3.3 Characterization of kiwifruit enzyme

The results obtained from response surface design for enzyme activity are presented in figures 1(a-c). These figures depict the expected response of enzyme activity and correlation between the independent variables in three dimensional plots. Figure (a) shows pH at X- axis and time of incubation at Y- axis whereas figure (b) represents pH at X- axis and temperature at Y- axis and in figure (c) temperature at X- axis and time of incubation at Y- axis. From figure it was clearly shown that with increase in pH (8.0), temperature (45^{0}C) and time of incubation (20 min) up to a certain limit significant increase in enzyme activity was observed up to a certain level. After that there was decrease in enzyme activity with further increase in value of independent factors. Maximum enzyme activity of 342.00 µg/gm was observed at 8.0 pH and at 45^{0}C temperature. The regression equation coefficients were calculated and the data were fitted to a second-order polynomial equation. So that the response (enzyme activity) could be expressed in terms of the following regression equation:

Enzyme activity = 340.20 + 2.36A + 19.89B + 76.19C - 96.15A2 - 1.15 B2 + 13.88C2 - 24.25AB - 9.58AC + 6.08BC

Analysis of variance gave the enzyme activity as a function of the initial values of parameters. The coefficient of determination (R^2) was calculated as 0.5882 indicating that the statistical model can explain 58.82% of variability in the response. Adequate precision measures signal to noise ratio. For enzyme activity, an adequate precision of 4.815 was recorded (A ratio greater than 4 is desirable), which indicates an adequate signal.

The analysis of both pH and temperature dependence and stability of kiwifruit enzyme (Figures 1 a, b, c) suggests that the enzyme might be fully compatible with conditions used in cheese manufacture as well as with rennet action. Furthermore, the decrease in the enzyme activity observed after 20 min of incubation and that ensures a lowering of the proteolysis rate during the cheese making process, thus limiting the amount of short peptides that might be responsible for the enhancement of bitter taste.

However, Hullikere *et al.* [13]observed the protease enzyme of papaya with maximum activity at 40^0 C temperature and 7 pH, whereas Omar *et al.* [25] characterized the proteolytic enzyme of pineapple and found optimum temperature and pH 65^0 C and 7.5 respectively and further reported that bromelain retained more than 90 per cent of its original activity after a period of 1 hr incubation at 55^{0}C.

3.4 Preparation of cottage cheese

The preparation of cottage cheese required the optimization of enzyme concentration. The enzyme concentration was optimized on the basis of milk clotting activity.

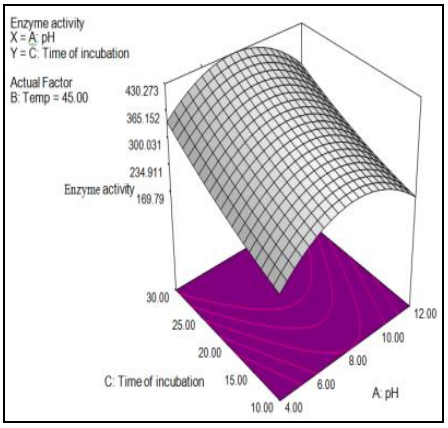

Fig 1(a): Effect of pH and time of incubation on enzyme activity

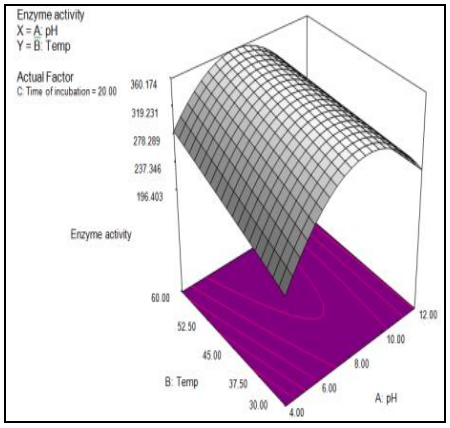

Fig 1(b): Effect of pH and temperature on enzyme activity

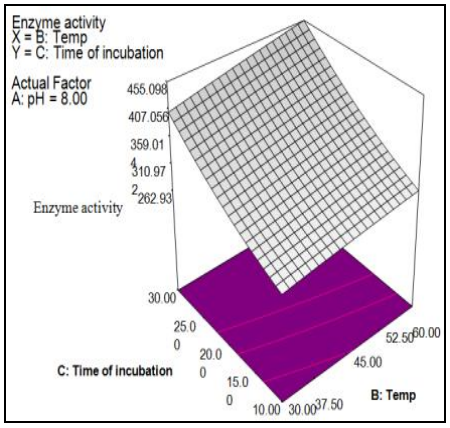

Fig 1(c): Effect of temperature and time of incubation on enzyme

3.4.1 Milk clotting activity

Perusal of data in Table 5 indicates the optimized concentration of kiwifruit enzyme and rennet on the basis of milk clotting activity for the preparation of cottage cheese. Kiwifruit enzyme shows milk-clotting activity, which is correlated with enzyme concentration. The enzyme concentrations i.e. 0.5 per cent partially purified kiwifruit enzyme (with milk clotting activity 1.45 U/ml and 30 min clotting time) were found best for cottage cheese production as indicated by first appearance of solid material in the milk. However milk-clotting activity estimated by Cavalcanti *et al.* [6] in different precipitation fractions of the *Nocardiopsis* sp were in the range of 1.14-20.00 U/ml whereas others reported the clotting time of proteinase isolated from mature flowers, immature flowers and leaves of artichoke were 8, 90, 180 min respectively [17,1].

Table 5: Optimized concentration of kiwifruit enzyme and rennet for the development of cottage cheese

Enzyme source	Concentration (%)	Milk clotting activity (U/ml)	Clotting time (min)
Rennet	1.00	3.81±0.1	10
Kiwifruit partially purified enzyme	0.50	1.45±0.1	30

3.5 Effect of kiwifruit enzyme on physico-chemical quality attributes of cottage cheese

3.5.1 Yield and protein content of cottage cheese

Results of the influence of kiwifruit enzyme on yield and protein content of cottage cheese are summarized in Table 6. The yield of kiwifruit cottage cheese was comparatively found lower as compared to rennet cheese i.e. 19.00 ± 0.30 and 23.16 ± 0.30 per cent whereas the whey yield was 73.91 ± 0.30 and 78.00 ± 0.10 per cent respectively. Despite of lower yield as compared to rennet, cottage cheese prepared from the kiwifruit enzyme may be considered a promising alternative of calf rennet for the coagulation of milk and/or for preparation of cheese.

In one of the related work Mahajan and Chaudhari [20] and Mahami *et al.* [21] reported 18.19-24.42 per cent yield of cottage cheese prepared with different concentrations (0.5-2.0 ml) of moringa seed extract. However whey yield 63.60 to 66.60 per cent was reported by Ojedapo *et al.* [23]. The protein content of 15.52 and 17.80 was recorded in both kiwifruit and rennet cottage cheese respectively whereas 12.14 to 15.71 percent protein content was recorded by Mahajan and Chaudhari, [20] in cheeses prepared with plant latex of Euphorbiaceae family.

Table 6: Effect of kiwifruit enzyme on yield and protein content of cottage cheese

Treatments	% yield of cottage cheese	Protein content in cheese (%)	Whey yield (%)
Rennet (1%)	23.16 ± 0.30	17.80 ± 0.30	73.91 ± 0.30
Partially purified kiwifruit enzyme (0.5%)	19.00 ± 0.30	15.52 ± 0.10	78.00 ± 0.10

3.5.2 Proximate analysis of cottage cheese

The effect of kiwifruit enzyme on proximate analysis of cottage cheese is presented in Table 7. The significant increase in moisture content was found in cottage cheese prepared with kiwifruit enzyme. However fat content was decreased in plant rennet cottage cheese as compared to animal rennet cottage cheese. The difference between two types of cheese might be due to change in proteolysis rate. Our findings were closely associated with the findings of Mahami *et al.* [21] whereas non-significant changes were observed in titratable acidity, ash and calcium content of kiwifruit cottage cheese. The results were parallel with the findings of Oladipo and Jadesimi [24].Total carbohydrates and energy value were found 7.27 to 13.72 per cent and 138.47 to 165.73 (Kcal) on calculation basis respectively, while Buriti *et al.* [5]; Mahajan and Chaudhari [20] found calcium, carbohydrate and energy value in the range of 1.38-12.29 g/kg, 6.14 to 6.84 per cent and 272.41 to 318.80 Kcal/g respectively in cheese prepared with plant extracts.

Table 7: Effect of kiwifruit enzyme on physicochemical characteristics of cottage cheese

Parameters Treatments	Moisture (%)	Titratable acidity (%)	Crude fat (%)	Ash (%)	Calcium (%)	Total Carbohydrates (%)	Energy value (kcal/100g)
Rennet (1%)	68.33	0.085	4.10	2.50	20.50	7.27	140.91
Kiwifruit partially purified enzyme (0.5%)	68.80	0.086	4.00	2.50	20.47	9.18	138.47
$CD_{0.05}$	0.03	NS*	0.07	NS*	NS*	0.17	0.67

*NS- non significant

3.6 Texture profile analysis (TPA) of cottage cheese

Figure 2(a-b) shows the effect of rennet and kiwifruit enzyme on the TPA characteristics of cottage cheese. Regarding TPA parameters both kiwifruit enzyme cottage cheese and rennet cheese presented significant differences between each other. With the addition of kiwifruit enzyme, the cottage cheese had the lowest hardness (6668.65 N), fracturability (7349.70 N), guminess (4908.08 N), chewiness (4250.39 N.mm) and highest cohesiveness (0.73) and springiness (0.86 mm) and the latter had the highest hardness (9600.85 N), fracturability (11910.19 N), Guminess (N), chewiness (N.mm) and lowest springiness (mm) and cohesiveness. Differences can be related to differences in the degree of proteolysis during preparation. Similar behaviors in findings were reported by Sheibani *et al.* [30] i.e. hardness and gumminess decreased from 13.17 to 11.06 N and 10.00 to 7.06 N respectively in butter cheese.

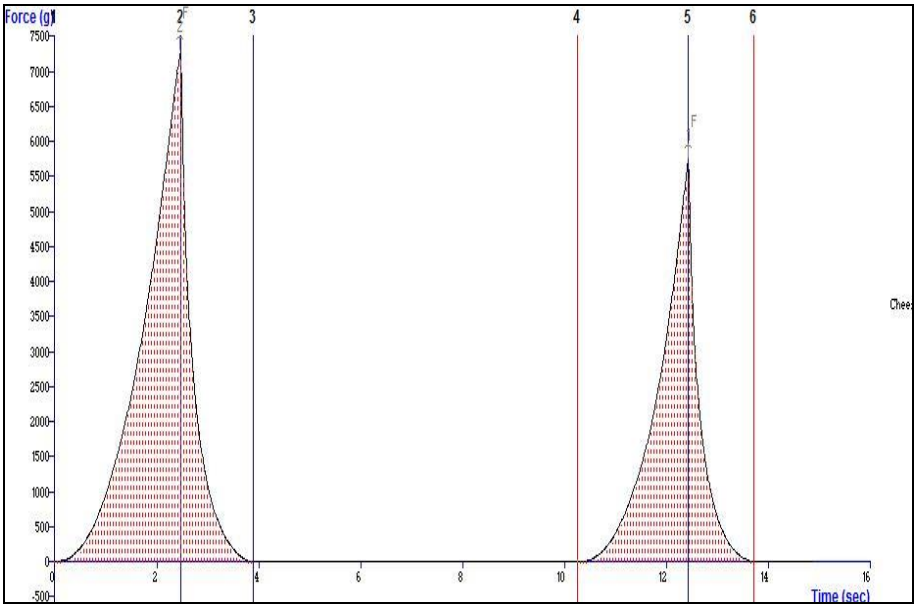

Fig 2(a): Texture profile analysis (TPA) of cottage cheese with rennet

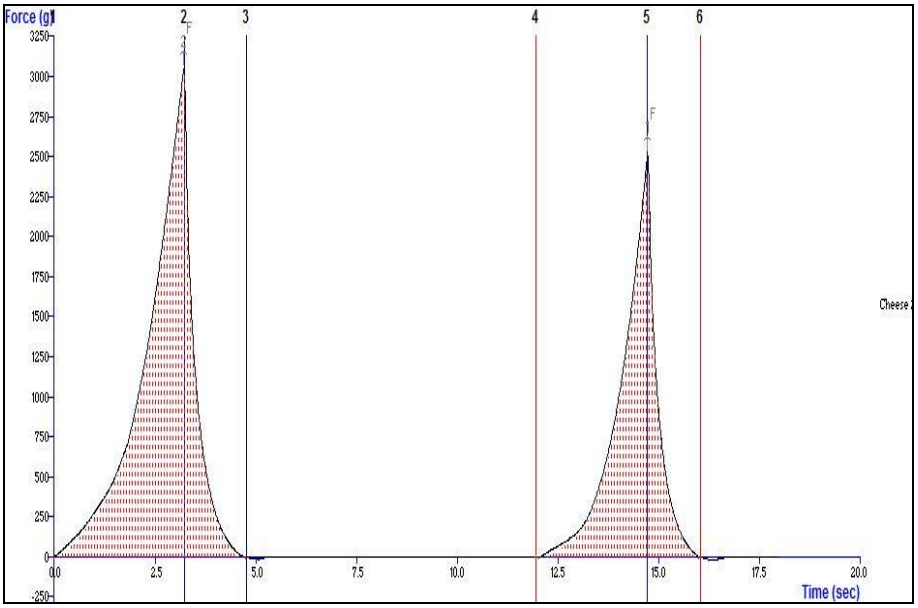

Fig 2(b): Texture profile analysis (TPA) of cottage cheese with kiwifruit enzyme (protease)

3.7 Sensory characteristics of cottage cheese

Appraisal of data presented in fig 3 indicates the sensory/organoleptic characteristics i.e. texture, colour, taste, flavor and overall acceptability of cottage cheese. Cottage cheese was prepared as per the standardized recipe with kiwifruit enzyme and compared with cheese prepared with commercial rennet (microbial source) for sensory characteristics. The cottage cheese prepared with partially purified kiwifruit enzyme scored highest for overall acceptability compared to cottage cheese prepared with rennet.

The results were parallel with Galan *et al.* [11] reported that cardoon (vegetable) rennet cheese exhibited softer texture and higher creaminess scores as compared with the calf rennet cheeses.

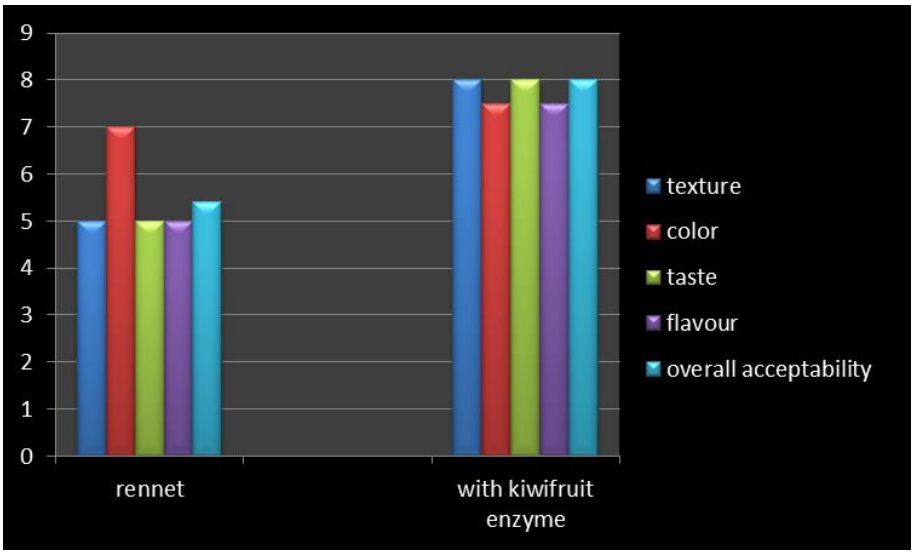

Fig 3: Effect of kiwifruit enzyme on sensory characteristics* of cottage cheese *on 9-point hedonic scale

4. Conclusion

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 texture. The immature stage of fruits is superior for extraction of enzyme with maximum proteolytic activities for milk-clotting and preparation of cottage cheese. Further, it is also show that the enzyme is fully compatible with the physical–chemical conditions utilized during cottage cheese manufacture (40–45 °C, sub-acid pH values). Henceforth, kiwifruit enzyme can be considered a promising alternative of natural calf rennet for the coagulation of milk leading to new dairy products.

5. Acknowledgements

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