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

Swati Sharma*, Devina Vaidya and Nilakshi Chauhan

Department of Food Science and Technology, Dr YSP University of Horticulture and Forestry, Nauni, Solan-173230, India

*Corresponding author

ABSTRACT

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.

Keywords
Kiwifruit enzyme, Cottage cheese, Partial purification, Characterization, Total bacterial count

Article Info
Accepted: 04 January 2018
Available Online: 10 February 2018

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 Veterinary 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°C and different concentration (0, 2, 4, 6, 8, and 10 %) of enzyme extract was added and the curd formation was observed at 37°C 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.

\[
\text{MCA (U/ml)} = \frac{2400}{\text{clotting time (sec)}} \times \text{Dilution factor}
\]

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°C reached after which kiwifruit enzyme was immediately added to the milk using a sterile pipette and allowed for setting in incubator at 21°C 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°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°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°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°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°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.
| Variables               | -1    | 0    | +1    |
|------------------------|-------|------|-------|
| pH                     | 4     | 8    | 12    |
| Temperature (°C)       | 30    | 45   | 60    |
| Incubation Time (min)  | 10    | 20   | 30    |

**Table.1** Range of values for the RSM

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

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

**Table.3** Purification profile of kiwifruit enzyme

| 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 | Moisture content (%) | Titratable acidity (%) | Crude protein (%) content | Calcium content | Total carbohydrates (%) | Energy (Kcal/100gm) |
|------------|----------------------|------------------------|---------------------------|----------------|-------------------------|---------------------|
| Days       | Rennet               | Kiwifruit enzyme       | Mean                      | Rennet         | Kiwifruit enzyme        | Rennet              | Kiwifruit enzyme   | Mean                     |
| 0          | 68.33                | 68.80                  | 65.56                     | 0.085          | 0.086                   | 17.80               | 15.52               | 16.66                    | 20.00                    | 19.40               | 19.70               | 20.00                    | 19.40                    | 19.70               | 20.00                    | 19.40                    | 19.70               | 20.00                    |
| 2          | 64.83                | 65.20                  | 65.01                     | 0.087          | 0.089                   | 17.50               | 15.50               | 16.50                    | 19.25                    | 19.20               | 19.22               | 19.25                    | 19.20                    | 19.22               | 19.25                    | 19.20                    | 19.22               | 19.25                    |
| 4          | 63.16                | 64.00                  | 63.58                     | 0.105          | 0.107                   | 17.00               | 15.35               | 16.17                    | 19.16                    | 19.18               | 19.17               | 19.16                    | 19.18                    | 19.17               | 19.16                    | 19.18                    | 19.17               | 19.16                    |
| MEAN       | 65.66                | 66.00                  | 65.02                     | 0.092          | 0.094                   | 17.43               | 15.45               | 19.47                    | 19.26                    | 12.22               | 12.62               | 152.57                   | 149.15                   | 157.93              | 159.85                   |

| CD_{hm}   | 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<br>NS              | 0.312          |
| T_{xs}    | 1.406               | T_{xs}                 | 0.106                     | T_{xs}         | NS                      | T_{xs}              | NS                  | T_{xs}                   | 0.140                   | T_{xs}              | 0.122          |
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.

References

AOAC, 1990. Official Methods of Analysis. 15th Edn. Association of Official Analytical Chemists, Washington, DC, pp.113-127.

Arima, K., Yu. J., and Iwasaki, S. 1970. Milk-clotting enzyme from Mucor pusillus var. Lindt. In: Methods in enzymology, G Perlmann and L Lorand (Ed.), Academic Press, New York., pp. 446–459.

Cavalcanti, M. T. H., M. F. S. Teixeira, J. L. Lima Filho and Porto A. L. F. 2010. Partial purification of new milk-clotting enzyme produced by Nocardiopsis sp. Bioresour. Technol. 93: 29-35.

Cochran, W. G., and Cox, C. N. 1967. Experimental Designs. John Wiley and Sons, Inc, New Delhi.

Galan, E., F. Prados, A. Pino, L. Tejada and Fernandez-Salgueiro, J. 2008. Influence of different amounts of vegetable coagulant from cardoon Cynara cardunculus and calf rennet on the proteolysis and sensory characteristics of cheeses made with sheep milk. International Dairy Journal. 18: 93-98.

Hullikere, M. M., C. G. Joshi, R. Vijay and Mahesh, M. 2014. Comparative analysis of papain from different varieties of papaya plant latex. International Journal of Agriculture and Food Science. 4: 123-127.

Kaur, L., S. M. Rutherford, P. Moughan, L. Drummond and Boland, M. J. 2010. Actinidin enhances gastric protein digestion as assessed using an in vitro gastric digestion model. Journal of Agricultural Food Chemistry. 58: 5068-5073.

Larmond, E. 1977. Laboratory Methods for Sensory Evaluation of Foods. Publication No. 1637 Department of Agriculture. Ottawa.
Lawrie, R. A. 1998. Meat Science. Technomic Publishing Company Incorporated, Lancaster. pp. 231.

Llorente, B., C. Brutti, C. Cimino, C. S. Vairo, C. Natalucci and Caffini, N. 1997. Presence of milk clotting proteinases in Cynara scolymus L. cv. Green Globe (Asteraceae). Medicinal and Aromatic Plants. 5: 249-258.

Lopes, A., G. Teixeira, M. C. Liberato, M. S. Pais and Clemente, A. 1988. New vegetal sources for milk-clotting enzymes. Journal of Molecular Catalysis Enzymatic. 5: 63-68.

Marques, A. C., M. R. Marostica and Pastore, G. M. 2010. Some nutritional, technological and environmental advances in the use of enzymes in meat products. Enzyme Research. 1: 1-6.

Otani, H., M. Matsumori and Hosono, A. 1991. Purification and some properties of a milk clotting protease from the young seeds of Albizia julibrissin. Animal Science and Technology. 62: 424-432.

Perveen, K., B. Alabdulkarim and Arzoo, S. 2011. Effect of temperature on shelf life, chemical and microbial properties of cream cheese. African Journal of Biotechnology. 10 (74): 16924-16928.

Sadasivam, S., and Manickam, A. 1998. Biochemical methods. New Age International (P) Limited, New Delhi.

Thimmaiah, S. K., 2006. Standard methods of biochemical analysis. Kalyani publishers. pp. 106-107.

---

**How to cite this article:**

Swati Sharma, Devina Vaidya and Nilakshi Chauhan. 2018. Microbiological Analysis and Nutritional Evaluation of Cottage Cheese Produced with Kiwifruit Enzyme. *Int.J.Curr.Microbiol.App.Sci.* 7(02): 1-7. doi: [https://doi.org/10.20546/ijcmas.2018.702.001](https://doi.org/10.20546/ijcmas.2018.702.001)