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Study on Use of Lactoferrin for the Biopreservation of *Paneer*

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ABSTRACT. *Paneer is an Indian traditional milk product which occupies an important place as a base product for the production of various culinary preparations. The shelflife of paneer is a major constraint in its utilization. Lactoferrin, a member of transferrin family of proteins, is one of the most important milk proteins naturally found in the milk of mammals and is an iron-binding glycoprotein which has antimicrobial properties. Studies were conducted on incorporation of lactoferrin at different levels (10, 15 & 20 ppm) in paneer. It was observed that, as the level of lactoferrin in the product increased, there was a significant decrease in the bacterial growth when compared to the control; thereby increasing the shelflife of paneer. Lactoferrin-treated paneer, up to 20 ppm showed an increase in shelf life up to 7 days at room temperature (30 °C) and 15 days at refrigerated temperature (4 °C) compared with the control with a shelf life of 2 days at 30 °C and 7 days at 4 °C, respectively. Similarly, as the level of lactoferrin increases, paneer had significantly higher hardness, cohesiveness, springiness and chewiness. The sensory evaluation of the paneer samples treated with 20 ppm lactoferrin was acceptable up to 7 days at ambient temperature and up to 15 days at refrigerated temperature.*

Keywords: *Biopreservation, lactoferrin, paneer*

INTRODUCTION

India is the largest producer of milk with a production of over 108.5 million tones per annum in year 2008-2009 (Khanna, 2010). About 55 % of the total milk produced in the country is being utilized for preparation of various indigenous dairy products like *paneer*, *chhana* and *chhana*-based sweets, *khoa* and *khoa*-based sweets and fermented milk products etc. One of the major disadvantages of these traditional milk products, especially with *paneer*, is the low keeping quality and shelf life. The use of chemical preservatives to inhibit growth of spoilage bacteria in these products has been recognized as a deterrent to health because of their potential toxicity and the regulatory authorities have virtually banned the chemical preservatives to be used in these products. Hence, there is a considerable interest in the possible use of natural biopreservatives in these traditional milk products (Makhal, 2000). Various physiological functions like bacteriostatic, antimicrobial *etc*. have been reported for lactoferrin and these properties of lactoferrin have opened up a new approach to food safety (Weinberg, 2007). The lactoferrin isolated from whey and milk has a great potential to be used as biopreservative in extending the shelf life of traditional Indian dairy products. This study investigates the use of optimum level of lactoferrin for extending the shelf life of *paneer.*

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MATERIALS AND METHODS

Preparation of *paneer*

Paneer was prepared as per the procedure recommended by Sachdeva *et al.* (1991) with a slight modification.

Isolation of lactoferrin

Lactoferrin was isolated in the laboratory from cheese whey by gel filtration as per the method outlined by Oram and Reiter (1968).

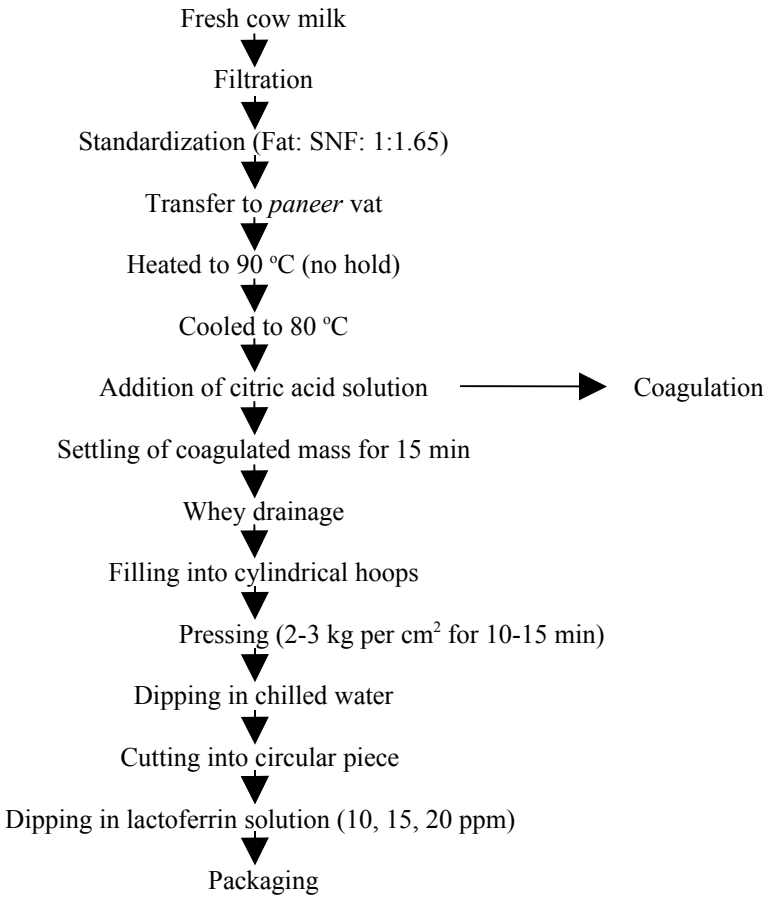

Fig. 1. Flow diagram for manufacturing *paneer*

Citical Dfference (CD)

Raw *paneer* cut in to pieces of 1 cm cubes, was given surface treatment, that is, dipped in lactoferrin solution of different concentrations *viz*., 10, 15, and 20 ppm. The lactoferrin-treated *paneer* was subjected to physicochemical, microbial analysis and sensory evaluation for acceptability. Treated *paneer* was packed in Low Density Polyethylene (LDPE) and

stored at room temperature (30±1 °C) and refrigerated temperature (4±1 °C) in order to study the preservative effect of lactoferrin on *paneer*.

Chemical analysis of *paneer*

Fat content in *paneer* was estimated by Gerber method as per the procedure given in IS: SP18 (part XI) (1981). Moisture, protein and ash content were estimated according to IS: SP18 (part XI) (1981).

Microbiological quality of *paneer*

Paneer samples were analyzed for total bacterial count, coliform count, yeast and mold count using selective agar media (HI-MEDIA Laboratories Pvt. Ltd., Mumbai). The ingredients were dissolved in distilled water by steaming, cooled, pH adjusted to 7.0 and autoclaved at 121 °C for 15 min.

Texture analysis of *paneer*

Texture Profile Analysis (TPA) parameters were determined by using the Stable Micro-System TA-XT plus Texture Analyzer (UK) fitted with 25 kg load cell for two- stage linear compression of *paneer* samples.

Sensory evaluation

Sensory evaluation was carried out by serving control and experiment samples, to a panel of five experts. The samples were examined for colour and appearance, body and texture, flavor and overall acceptability on a 9-point Hedonic scale. The scores given by the panel of judges were then statistically analyzed (Dharampal & Gupta, 1985).

Storage studies

The samples were subjected to storage studies both at room temperature (30±1 °C) and refrigeration temperature (4±1 °C) along with a control. The analysis of the stored samples was carried out at regular intervals of storage.

Statistical analysis

The data were analyzed statistically for test of significance as per the procedure outlined by Siegal and Castlellan (1988). All the statistical tests were carried out at 5 % level of significance and all the data were analyzed using statistical packages for social sciences (SPSS version 10).

RESULTS AND DISCUSSION

Microbial quality

The results of the effect of lactoferrin on the microbial growth are shown in (Tables 1 and 2). The control sample showed total bacterial count, coliform count and yeast and mold count of 3.40 - 6.74 $\log_{10}$ cfu/g, 1.74 - 3.52 $\log_{10}$ cfu/g and 1.33 - 2.48 $\log_{10}$ cfu/g, respectively from 0 to 6th day. The total bacterial count, coliform count and yeast and mould count increased

proportionally. In case of lactoferrin incorporated *paneer*, there was no such increase and the total bacterial count, coliform count and yeast and mold count decreased significantly compared to the control *paneer*, the decrease being maximum in case of 20 ppm lactoferrin treated *paneer*. This clearly shows the bactericidal effect of lactoferrin in *paneer*, in combating the microbial growth. Batish *et al*. (1988), reported the bacteriostastic activity of natural lactoferrin against a wide range of microorganisms including Garm negative and also against some Gram-positive organisms. The results of the present study are in agreement with the result of above authors.

The microbial quality of control and lactoferrin treated sample packed in LDPE, stored at refrigeration temperature is presented in Table 3. In case of control the total bacterial count, coliform and yeast and mold counts increased from 3.46 to 7.68 $\log_{10}$ cfu/g, 1.74 to 3.68 and 1.33 to 3.27 $\log_{10}$ cfu/g from 0 to 12th day of storage, respectively. In case of samples treated with lactoferrin at 10, 15 and 20 ppm concentrations, Total Bacterial Count, Coliform count and Yeast and Mold count increased marginally, with the maximum bacteriocidal effect of lactoferrin in case of 20 ppm lactoferrin incorporated product. Lu *et al.* (2008) studied the antimicrobial activity of lactoferrin by Oxford cup method. The antimicrobial effect increased with the decrease of iron saturation. Richard (2003) reported that activity of lactoferrin was more at refrigeration temperature. Normal *paneer* without any preservatives can be kept for only 2 days at ambient temperature and 6 days at refrigerated temperature (Sachdeva, 1983).

Table 1. Effect of lactoferrin on microbial count of *paneer* packed in LDPE stored at 30±1 °C

Storage	$\log_{10}$ (cfu/g)												
	Control			10 (ppm)			15 (ppm)			20 (ppm)			CD
	TBC	C	Y&M	TBC	C	Y&M	TBC	C	Y&M	TBC	C	Y&M	
0	3.4	1.74	1.33	3.4	1.72	1.3	3.42	1.72	1.32	3.40	1.74	1.32	0.02
2	4.8	2.34	1.94	4.64	2.28	1.90	4.58	2.24	1.74	4.25	2.10	1.68	0.23
4	5.88	2.98	2.44	5.78	2.88	2.22	5.74	2.86	2.20	5.56	2.48	1.90	0.16
6	6.74	3.52	2.84	6.58	3.44	2.54	6.42	3.42	2.36	6.24	3.34	2.20	0.14

*All values are average of three trials
TBC- total bacterial count, Y&M- yeast and mold, C- Coliform count

Table 2. Effect of lactoferrin on microbial count of *paneer* packaged in LDPE stored at 4±1°C

Storage (day)	$\log_{10}$ (cfu/g)												
	Control			10 (ppm)			15 (ppm)			20 (ppm)			CD
	TBC	C	Y&M	TBC	C	Y&M	TBC	C	Y&M	TBC	C	Y&M	
0	3.46	1.74	1.33	3.42	1.70	1.30	3.40	1.68	1.32	3.44	1.74	1.32	0.02
2	4.24	1.90	1.64	4.00	1.82	1.58	3.78	1.80	1.48	3.68	1.78	1.40	0.12
4	5.18	2.12	1.78	4.64	1.96	1.70	4.52	1.90	1.69	4.26	1.83	1.52	0.16
6	5.92	2.34	1.94	5.56	2.12	1.84	4.98	2.02	1.78	4.68	2.00	1.68	0.24
8	6.28	2.58	2.24	5.84	2.44	2.08	5.54	2.24	1.89	5.1	2.18	1.79	0.30
10	6.92	3.08	2.60	6.46	2.86	2.38	6.17	2.52	1.96	5.54	2.38	1.90	0.22
12	7.40	3.48	3.18	7.16	3.42	2.80	6.92	2.94	2.24	6.08	2.74	2.05	0.28

14	7.68	3.68	3.27	7.40	3.46	3.00	7.16	3.40	2.84	6.92	3.34	2.55	0.40

*All values are average of three trials
TBC- total bacterial count, Y&M- yeast and mold, C- Coliform count

Chemical composition

The fresh and stored lactoferrin treated *paneer* at different levels were subjected to chemical analysis and the results obtained during the process are shown in Table 3. In case of fresh control *paneer* the moisture, fat, protein and ash contents were 53.90, 24.80, 17.60 and 1.40 per cent, respectively. Whereas *paneer* treated with 20 ppm lactoferrin had the moisture, fat, protein and ash content of 54.50 %, 24.50 %, 17.38 % and 1.32 %, respectively. The moisture content of lactoferrin treated *paneer* samples increased with the increase in the level of lactoferrin. Similar observations were made in case of stored lactoferrin treated *paneer*.

Table 3. Effect of different levels of lactoferrin on chemical composition of fresh *paneer*

Concentration Levels	Chemical composition (%)							
	Moisture		Fat		Protein		Ash	
	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored
Control	53.90	53.80	24.80	24.85	17.61	17.75	1.40	1.45
10 ppm	54.20	54.00	24.72	24.80	17.50	17.58	1.38	1.40
15 ppm	54.35	54.20	24.60	24.72	17.42	17.52	1.37	1.39
20 ppm	54.50	54.35	24.50	24.55	17.38	17.43	1.32	1.35
CD	0.25	0.25	0.08	0.08	0.15	0.16	0.08	0.08

*All values are average of three trials

Rheological characteristics

The rheological characteristics of fresh and stored *paneer* treated with lactoferrin are presented in Table 4. The lactcoferrin treated *paneer* had significantly lower hardness (14.384), cohesiveness (0.601), springiness (0.736), chewiness (6.368) than the control sample. The decrease in the hardness of lactoferrin treated products could be due to higher moisture content. The finding of this study related to untreated *paneer* is in accordance with Desai *et al.* (1991) who studied the rheological properties of market *paneer*.

Table 4. Effect of lactoferrin incorporation on rheological characteristics of fresh and stored *paneer*

Sample	Rheological characteristics							
	Fresh *paneer*				Stored *paneer*			
	Hardness	Cohesiveness	Springiness	Chewiness	Hardness	Cohesiveness	Springiness	Chewiness
Control	17.416	0.645	0.767	8.625	17.560	0.657	0.767	8.864
10 ppm	17.235	0.598	0.753	7.775	17.340	0.627	0.760	8.276
15 ppm	16.359	0.589	0.743	7.170	16.574	0.613	0.756	7.956
20 ppm	14.384	0.601	0.736	6.368	14.608	0.616	0.735	6.623
CD	0.0064	0.0064	0.0049	0.0094	0.007	0.003	0.006	0.004

*All values are average of three trials

Sensory characteristics

The sensory scores with regard to the effect of incorporation of lactoferrin for colour and appearance, body and texture, flavor and overall acceptability are presented in Table 5. The overall acceptability scores were 7.70 in the control, compared to 7.51, 7.46 and 7.33 for 10, 15 and 20 ppm in lactoferrin-treated *paneer*, respectively. The overall acceptability of *paneer* samples treated with lactoferrin at different levels did not vary significantly and were similar to the control. Hence it could be inferred that lactoferin could be incorporated up to 20 ppm concentration in *paneer* samples without significant changes in the sensory attributes of *paneer*.

Table 5. Effect of incorporation of different levels of lactoferrin on sensory characteristics of *paneer*

Concentration levels	Color & appearance	Body & texture	Flavor	Overall acceptance
Control	8.11	7.72	7.27	7.70
10 ppm	7.78	7.52	7.24	7.51
15 ppm	7.60	7.45	7.23	7.46
20 ppm	7.45	7.32	7.22	7.33
CD	0.13	0.17	0.08	0.20

*All values are average of three trials

CONCLUSIONS

Lactoferrin was incorporated in *paneer* at three different levels *viz*, 10,15 and 20 ppm. The microbiological counts of *paneer* stored at room temperature (30±1 ºC) and as well as at refrigerated temperature (4±1 ºC) were estimated at every three day-interval. The growth of total bacterial count, coliform and yeast and mold counts in control sample were observed to be higher when compared to experimental *paneer* samples. The *paneer* sample treated with 20 ppm had significantly lower microbial growth, thus indicating the higher keeping quality upto 6 days at room temperature (30±1 ºC). Similar observations were made in case of *paneer* treated with 20 ppm lactoferrin and stored at refrigeration temperature (4±1 ºC), where the keeping quality was observed to be 14 days. Hence, lactoferrin could be easily used as a biopreservative for extending the shelflife of *paneer*.

REFERENCES

Batish, V.K., Chander, H., Zumdegeni, K.C., Bhatia, K.L. and Singh, R.S. (1988). Antibacterial activity of lactoferrin against some common food-borne pathogenic organisms. Aust. J. of Dairy Tech. *5*, 16-18.

Desai, H.K., Gupta, S.K., Patel, A.A. and Patel, G.R. (1991). Studies on texture of *paneer*. Jap. J. Dairy. Food Sci. *40(1),* A14-A21.

Dharam Pal, P.V. and Gupta, S.K. (1985). Sensory evaluation of dairy products. Indian Dairyman *34(3)*, 473-477.
IS: SP 18 part XI, (1981). ISI hand book of food analysis, Dairy products, Bureau of Indian standard, Manak Bhavan, New Delhi, India.

Khanna. R.S. and Sharad Gupta, (2010). Market efficiency: Key to Indian Dairy Sector. Proceedings of 38th Dairy Industry Conference, pp18-31.

Lu,R.R., Xu,S., Yang,R. and Sun, Z. (2008). Antimicrobial activity of lactoferrin and its mechanism involved. Food Sci. China. *29(2)*, 238-243.

Makhal, S. (2000). Preservation of *paneer* with 'GRAS' additives- M.Tech. Dissertation. West Bengal Univ. Animal and Fishery Sciences, Mohanpur Campus, Nadia (WB). pp: 126-129.

Oram, J.D, and Reiter, B., (1968). Inhibition of bacteria by lactoferrin and other chelating agents. Biochem. Biophys. Acta*, 170*: 351.

Richard, A.H. (2003). Use of lactoferrin and lactoferricin to inhibit growth of food patens and spoilage microorganisms in meat. Agri. Food Research and Development Initiative. ARDI-Project # 01-521.

Sachdeva, S. (1983). Production, packaging and preservation of *Paneer*. Ph.D. Thesis submitted to Kurukshetra University, Kurukshetra, Haryana, India.

Sachdeva, S., Prokopek, D. and Reuter, H. (1991). Technology of *paneer* form cow milk. Japaneese J. Dairy and Food Sci. *40(2)*, A85-A90.

Siegel, S. and Castlellan, J.N. (1988). Non-parametric Statistics, Second Edition, McGraw-Hill International Editions, U.S.

Weinberg, E.D. (2007). Antibiotic properties and applications of lactoferrin. Curr. Pharm. Design, *13*, 801-811.