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Comparative Study on Evaluation of Refrigerated (4±1ºC) Storage Stability of Paneer Incorporated with Crude Extract from Indian Curd, Nisin and Lactic Acid

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A B S T R A C T

The present study was conducted to evaluate the refrigerated storage (4±1˚C) stability of paneer incorporating crude extract (CE) prepared from Indian curd in comparison with conventional preservatives like lactic acid and nisin. Four different batches of paneer sample *viz.* control (C), paneer with CE (T1), 0.5% lactic acid (T2) and 10 ppm nisin (T3) were analyzed at every 3 days interval until day 12. The free fatty acids, thiobarbituric reacting species and peroxide value were significantly ($p<0.05$) higher in control than other treatments, but T1 showed significantly ($p<0.05$) lower values. Significant ($p<0.05$) decrease in pH and water activities were observed during the storage period. Microbial counts of the paneer samples were highly significantly ($p<0.01$) affected by storage days and treatments. There was a significant ($p<0.05$) decrease in sensory scores for all the products. However, the scores were significantly ($p<0.05$) higher in T1 towards later parts of storage period. The results showed a significant ($p<0.05$) effect of CE on most of the quality parameters as compared to other treated paneer samples. Hence CE from Indian curd was considered the most desirable biopreservative among all preservatives used in this study for extending the shelf life of paneer.

Introduction

Paneer is a heat and acid coagulated milk product obtained by coagulating the milk solids with the help of organic acids like citric and lactic acid, and then removing moisture by pressing. Paneer has its use in preparation of sweet dishes, salads, curries and various ready to serve foods. The normal shelf life of paneer is 7-9 days under refrigeration conditions. The problem with its limited shelf life can be overcome by the use of preservatives.

Nowadays, the health conscious consumers reject foods incorporated with synthetic compounds because of the potential harmful consequence of these compounds on health. Hence, there is need for application of natural food preservatives that will serve an alternative to the chemical compounds which

will reduce the consumer concerns about chemical preservatives. Biofunctional components of milk origin are regarded as potential ingredients of functional foods, nutraceuticals and pharmaceuticals. They can also be used as bio-preservatives to increase the shelf life of perishable food products.

Rizzello *et al.,* (2005) reported the presence of antibacterial peptides in water soluble extracts of Pecorino Romano (cheese variety made from ovine milk). Dionysius *et al.,* (2000) isolated a microbicidal peptide corresponding to αs1-casein f (1-9) with the sequence RPKHPIKHQ in a yoghurt product. Farvin *et al.,* (2010) used the crude peptide rich extract and different molecular weight peptide fractions from market yoghurt in the study conducted to evaluate antioxidant activity of yoghurt peptides in fish oil enriched milk emulsion.

Mendiratta *et al.,* (2007) studied the effect of citric acid and cultured whey treated samples of vacuum packaged skim milk paneer under both room temperature and refrigeration temperature. Ahmad *et al.,* (2014) found that the extent of spoilage of Kaladhi, an acid-coagulated milk product showed significantly ($p<0.05$) increasing trend throughout the period of storage in samples treated with different concentrations of sorbic acid as well as in control.

The rate of increase was indirectly related to the level of sorbic acid and the lowest rate of increase was observed for 0.3 % sorbic acid treated sample. Buch *et al.,* (2014) and Zanzad (1990) observed the shelf life of turmeric and carrageenan treated paneer respectively. Sachdeva and Singh (1990) observed microbial and sensory changes during the storage of paneer dipped in plain water, H_2O_2 solution (0.2%) and brine solution. A Study was conducted by Shashikumar and Puranik (2011) by incorporating lactoferrin in paneer and they observed that with increasing concentration of lactoferrin, there was a significant decrease in bacterial count as compared to control, thereby increasing shelf life of lactoferrin dipped paneer.

Considering the importance of research regarding the use of bio-preservatives to extend the shelf life of perishable foods like paneer and improve food safety, this study was undertaken to evaluate bio-preservative effect of crude extract (CE) from Indian curd on the refrigerated shelf life and quality of paneer in comparison to conventional preservatives like nisin and lactic acid.

Materials and Methods

Quality evaluation of the control paneer (untreated), test paneer sample dipped in crude extract (T1), paneer sample dipped in 0.5% lactic acid (T2) and paneer sample dipped in 10 ppm Nisin (T3) was done during refrigerated storage at 4+1˚C under aerobic packaging conditions packed with low density polyethylene (LDPE) (200 gauge) at every 3 days intervals until spoilage for the parameters *viz*. sensory evaluation, physicochemical tests like pH, thiobarbituric acid reacting substances (TBARS) value, peroxide value (PV), free fatty acid (FFA), water activity (a_w) and microbiological tests like total plate count (TPC), coliform count and psychrophilic count.

Preparations of crude extract (CE)

Pasteurized milk was procured from Dairy Technology Section of Indian Veterinary Research Institute (IVRI), Izatnagar and processed for the preparation of curd by inoculating it with 2% starter household culture (Aneja *et al.,* 2002). The crude extract (CE) from curd was prepared by the methods of Rizzello *et al.,* (2005) and Samlesh and Shilpa (2015) with slight modifications.

Briefly, 40ml of curd was taken in a sterilized beaker, homogenized for 3-5minutes followed by centrifugation at 8000 rpm for 20 minutes in a cooling centrifuge (Hermle, Z 32 HK, Germany). The supernatant was collected and filtered with Whatman no.1 filter paper and concentrated by lyophilization (Modulyo, Edwards) and drying (Ambay Biotech, India) in batches of 20ml volumes. Sterile distilled water (2 ml) (pH 7) was used for reconstitution of each batch of dried supernatant and pooled to obtain the crude extract (CE). These were aliquoted and stored at -20˚C until further use.

Preparation of paneer

Paneer was prepared from pasteurized mixed milk, after standardizing the milk to 4.5% fat and 8.5% SNF using method suggested by Bhattacharya *et al.,* (1971). The blocks were diced into approximately 1.5 cm^3 size to be subjected to different dipping treatments. The percentage yield of paneer was recorded.

Proximate composition

The moisture, protein, fat and ash content of the prepared paneer was estimated prior to the storage study as per methods described by AOAC (2003).

Optimization of incorporation level of CE

Different concentrations of crude extract were obtained by using sterile distilled water (pH 7) and subjected to antimicrobial activity against *Bacillus cereus* (ATCC 10876) and *Salmonella typhimurium* (clinical isolate 09/Epi) procured from Food Microbiology Laboratory, IVRI, Izatnagar using agar well diffusion assay on Mueller Hinton Agar (MHA) plates. The concentration of CE to be used for dipping of paneer was optimized based on sensory scores and Minimum Inhibitory Concentration (MIC) values (Table 2).

Physicochemical analysis

The pH of paneer samples (n=6) was determined as per Mendiratta *et al.,* (2007) with digital pH meter (pH tutor, Eutech instruments) equipped with a combined glass electrode. TBARS value of paneer was determined by the method described by Sanyal *et al.,* (2006) with some modifications. The PV was measured as per procedure described by Boghra *et al.,* (1997) with suitable modifications. The method as described by Buch *et al.,* (2014) was followed for the estimation of FFA. The a_w of paneer was measured using a hand-held, portable digital water activity meter (Aqua lab dew point water activity meter 4TE, USA) according to the method of Thippeswamy *et al.,* (2011) with little modification.

Microbiological analysis

Total plate count (TPC), pyschrophilic count and coliform count of the paneer samples were enumerated before and after treatment during the storage study as per the methods described by American Public Health Association (APHA, 2001).

Sensory evaluation

A six member experienced panel of judges consisting of scientists and students (postgraduate and doctoral) of Division of Livestock Products Technology, IVRI, Bareilly evaluated the paneer samples for the sensory attributes of colour and appearance, texture, flavour, aftertaste and overall acceptability using 8-point descriptive scale as given by Rajkumar *et al.,* (2010) with slight modification, where 8=excellent and 1=extremely poor.

Statistical analysis

The data obtained from the experiments were pooled and analyzed using SPSS 20 statistical

software (Version 20, IBM, USA). The samples were analyzed in duplicates for each parameter except sensory evaluation (6) and the experiments were repeated three times. Duncan's multiple range tests were used for comparing the means for significant differences (Duncan, 1955). The statistical significance was estimated at 95% confidence level ($p<0.05$).

Results and Discussion

Yield of paneer

The average paneer yield from pasteurized mixed milk in the present study was found out to be 17.035±0.241 percent.

Proximate composition of paneer

The average proximate compositions of the paneer used for this study is given in table 1. The results obtained in the present study agree with the observations of Bhattacharya *et al.,* (1971), Singh *et al.,* (1991), Dhole *et al.,* (2009) and Buch *et al.,* (2014) who estimated the moisture, fat, protein and ash content of paneer in the range of 47.68 to 59.70, 22.90 to 27.00, 16.81 to 33.27 and 1.30 to 2.18 % respectively.

Optimization of dipping level

The average weight of dried curd supernatant was found out to be 1.1 g. Sterile distilled water (2 ml) (pH 7.0) was added to the dried curd supernatant to obtain a 10-fold concentration (550 mg/ml) of crude extract (CE) which was further diluted to obtain 5-fold (275 mg/ml), 4-fold (220 mg/ml), 3-fold (165 mg/ml) and 2-fold (110 mg/ml) concentrated CE (Table 2). The levels of CE to be used for dipping of paneer was optimized based on MIC values (Table 2) and sensory scores (Table 3) of paneer samples treated with different concentrations of CE. The CE at different folds of concentrations showed significant differences ($p<0.05$) in terms of their antibacterial activities against standard bacteria *Bacillus cereus* and *Salmonella typhimurium*.

Based on MIC values (Table 2) and sensory scores (Table 3), quality evaluation of the paneer samples including control paneer sample (untreated), paneer sample dipped in 5-fold (275 mg/ml) concentrated crude extract (T1), paneer sample dipped in 0.5% lactic acid (T2) and paneer sample dipped in 10 ppm nisin (T3) was carried out during refrigerated storage at 4+1˚C under aerobic packaging conditions packed in LDPE (200 gauge) at every 3 days intervals up to 12 days.

Changes in physicochemical properties during storage

The storage period and the dipping treatment had significant ($p<0.05$) effect on all physico-chemical parameters of the paneer. The interaction between treatments and storage period was also significant ($p<0.05$), except for pH (Table 4).

pH

There was a non-significant ($p>0.05$) decrease in pH of all the samples from 0 to 6th day of storage and the decrease was significant ($p<0.05$) thereafter. On day 12th, there was a non-significant ($p>0.05$) difference between T2 and T3 and control and T1.

But there existed significant ($p<0.05$) difference between T1 and other samples at the end of the study. The decrease in pH may be due to formation of lactic acid during storage period (Table 4).

Mendiratta *et al.,* (2007) and Bhattacharya *et al.,* (1971) observed similar decreasing trend in pH in citric acid and cultured whey treated

samples of vacuum packaged skim milk paneer and paneer prepared from standardized buffalo milk (pH from 6.60 to 5.80) respectively during storage under room refrigeration temperature. Similar results were also reported by Bamba (1989), during 28 days of storage of paneer at 7˚C.

TBARS

The mean TBARS values showed non-significant ($p>0.05$) increase from 0 to 3rd day and then a significantly ($p<0.05$) increasing trend was observed from 6th day of storage in all the treated samples as well as in control (Table 4). In treated samples, the rate of increase was slower as compared to control, the rate being lowest for T1 (dipped in crude extract).

The TBARS value increased from 0.254 to 0.890 and the rate of increase was indirectly related to the antioxidant properties. Similar findings were observed by Pal and Garg (1989), Kumar and Bector (1991), Pal *et al.,* (1993), Shukla and Vaid (2004) and Sanyal *et al.,* (2006) in paneer. Ahmad *et al.,* (2013) and Zanzad (1990) found that the increase in mean TBARS values of sorbic acid dipped Kaladhi (an acid-coagulated milk product) and paneer made with carrageenan throughout the storage period.

FFA

It was observed in this study that dipping in crude extract slightly reduced the FFA content of paneer samples (Table 4). Such effect may be attributed to antioxidant properties of crude extract. There was a significant increase ($p<0.05$) in FFA content of control and treatment samples with the advancement of storage days. There were no significant ($p>0.05$) differences between control and treatments on 0 day. There was significantly ($p<0.05$) lower FFA observed in T1 samples in comparison to others. Kumar and Bector (1991) reported a higher initial level of FFA in control paneer samples than the samples containing 0.05 % TBHQ and BHA. Venkateswarlu *et al.,* (2003) reported FFA content of 0.11 (% oleic acid) and Gokhale and Pandya (2009) reported a value of 0.04 %. Buch *et al.,* (2014) reported that the FFA content of control paneer ranged from 0-0.07% and addition of turmeric reduced the FFA content of paneer.

PV

There was a sharp increase in PV of T2, T3 and control samples as compared to T1 from day 9th to 12th. On day 12th, there was a significant ($p<0.05$) increase in PV of T2 and control than T3 and T1 (Table 4).

The results showed an antioxidant effect of the crude extract (CE). The increase in PV of paneer with the advancement of storage period, in this study can be correlated to the findings of Sindhu *et al.,* (2000) and Boghra *et al.,* (1997).

Water activity (a_w)

There were significant ($p<0.05$) decreasing trends in water activities of control and treatments during the storage period. However at the end of the storage, T2 had a significantly ($p<0.05$) lower water activity than other treatments, T3 and control had non-significant ($p>0.05$) differences and T1 sample had significantly ($p<0.05$) higher water activity than the other treatments (Table 4).

Thippeswamy *et al.,* (2011) observed that as the concentration of NaCl increased for dipping paneer from 1-3%, a_w decreased from 0.994 to 0.970. The reduction in a_w in paneer with several humectants was observed by Singh *et al.,* (1989), Jayaraj and Patil (1999), Singh and Rai (2004) during storage period.

Fig.1 Paneer samples on 0 day of refrigerated storage at 4±1ºC

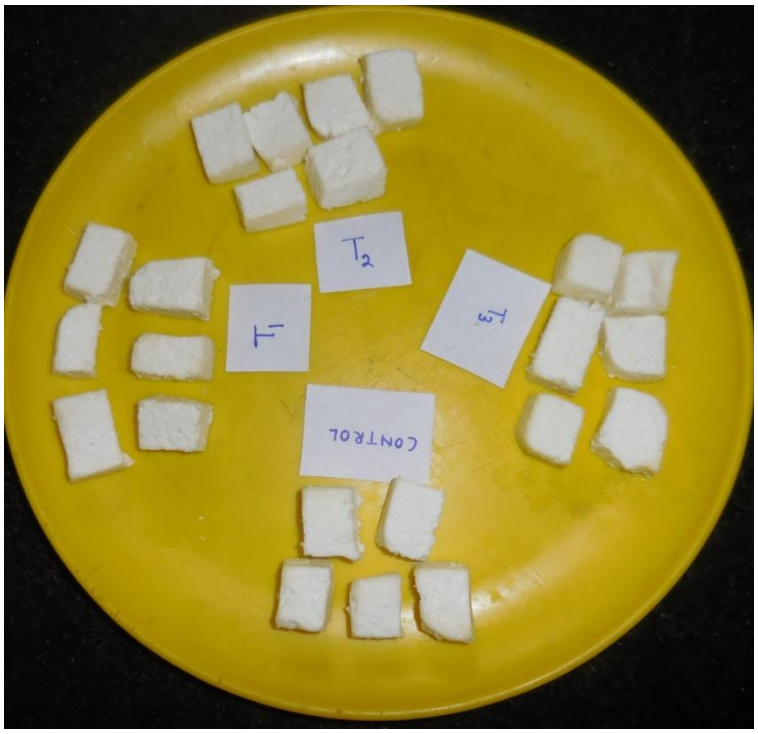

Fig.2 Paneer samples on 12th day of refrigerated storage at 4±1ºC

Table.1 Proximate composition of paneer (Mean±S.E.)

Moisture	Fat	Protein	Ash
53.56±0.74	24.33±0.75	17.37±0.39	1.43±0.03

Table.2 Minimum Inhibitory Concentrations (MIC) of different concentrations of CE (Mean±S.E.)

Zone of Inhibition (mm)		
Concentrations	***Bacillus cereus***	***Salmonella* Typhimurium**
2-fold (110 mg/ml)	11.83 ± 0.54^{E}	11.17 ± 0.31^{E}
3-fold (165 mg/ml)	16.67 ± 0.42^{D}	15.00 ± 0.37^{D}
4-fold (220 mg/ml)	21.17 ± 0.40^{C}	18.00 ± 0.45^{C}
5-fold (275 mg/ml)	25.17 ± 0.40^{B}	20.17 ± 0.54^{B}
10-fold (550 mg/ml)	27.67 ± 0.61^{A}	22.67 ± 0.42^{A}

Table.3 Sensory attributes of scores for optimization of dipping level, Mean±S.E.*

Parameters	Control	T1	T2
Appearance and colour	7.06 ± 0.08^{a}	6.86 ± 0.08^{a}	6.47 ± 0.11^{b}
Flavour	6.89 ± 0.06^{a}	6.75 ± 0.07^{a}	6.13 ± 0.06^{b}
Body and texture	6.93 ± 0.08^{a}	6.72 ± 0.09^{a}	6.13 ± 0.05^{b}
After taste	6.94 ± 0.04^{a}	6.74 ± 0.08^{b}	5.88 ± 0.08^{c}
Overall acceptability	7.00 ± 0.05^{a}	6.71 ± 0.07^{b}	5.85 ± 0.09^{c}

Mean±S.E.* with different superscripts (small letters) in a row differ significantly ($p<0.05$).
Mean values are scores on 8-point Hedonic scale, where 1= extremely undesirable and 8= extremely desirable
T1 = Paneer dipped in 5-fold concentrated crude extract, T2 = Paneer dipped in 10-fold concentrated crude extract, Control = without any dipping treatment, n= 18 for each treatment

Table.4 Changes in physico-chemical properties of paneer treated with different preservative solutions under refrigerated storage (4±1˚C), Mean±S.E.

pH					
Treatments	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**
Control	5.72 ± 0.02^{Aa}	5.69 ± 0.02^{Aa}	5.66 ± 0.02^{Aa}	5.58 ± 0.02^{Ab}	5.45 ± 0.02^{ABc}
T1	5.69 ± 0.02^{Aa}	5.67 ± 0.02^{Aa}	5.64 ± 0.02^{Aba}	5.57 ± 0.02^{Ab}	5.51 ± 0.01^{Ac}
T2	5.64 ± 0.03^{Aa}	5.62 ± 0.03^{Aa}	5.57 ± 0.02^{Ba}	5.45 ± 0.04^{Bb}	5.35 ± 0.03^{Cc}
T3	5.68 ± 0.03^{Aa}	5.65 ± 0.03^{Aa}	5.61 ± 0.03^{Aba}	5.52 ± 0.03^{ABb}	5.42 ± 0.03^{BCc}
TBARS value					
Treatments	**0 day**	**3 day**	**6 day**	**9 day**	**12 day**
Control	0.286 ± 0.009^{Ad}	0.341 ± 0.018^{Ad}	0.406 ± 0.016^{Ac}	0.775 ± 0.026^{Bb}	1.179 ± 0.026^{Aa}
T1	0.254 ± 0.003^{Bd}	0.278 ± 0.009^{Bcd}	0.318 ± 0.010^{Cc}	0.577 ± 0.018^{Db}	0.890 ± 0.027^{Ca}
T2	0.258 ± 0.011^{Bd}	0.296 ± 0.012^{Bcd}	0.353 ± 0.015^{BCc}	0.846 ± 0.023^{Ab}	1.237 ± 0.054^{Aa}
T3	0.274 ± 0.003^{ABd}	0.309 ± 0.008^{ABd}	0.368 ± 0.014^{ABc}	0.698 ± 0.023^{Cb}	1.059 ± 0.026^{Ba}
FFA value					
Treatments	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**
Control	0.033 ± 0.003^{Ae}	0.072 ± 0.004^{Ad}	0.107 ± 0.007^{Ac}	0.150 ± 0.009^{Ab}	0.230 ± 0.009^{Aa}
T1	0.030 ± 0.004^{Ae}	0.048 ± 0.004^{Bd}	0.077 ± 0.006^{Bc}	0.113 ± 0.007^{Bb}	0.137 ± 0.006^{Ca}
T2	0.030 ± 0.004^{Ae}	0.057 ± 0.006^{ABd}	0.090 ± 0.004^{ABc}	0.160 ± 0.007^{Ab}	0.237 ± 0.010^{Aa}
T3	0.032 ± 0.004^{Ae}	0.063 ± 0.006^{ABd}	0.093 ± 0.007^{ABc}	0.137 ± 0.010^{ABb}	0.203 ± 0.008^{Ba}
PV					
Treatments	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**
Control	0.32 ± 0.03^{Ae}	1.39 ± 0.04^{Ad}	1.78 ± 0.04^{Ac}	2.26 ± 0.05^{Ab}	2.64 ± 0.06^{Aa}
T1	0.24 ± 0.01^{Ae}	0.73 ± 0.05^{Cd}	1.17 ± 0.04^{Cc}	1.86 ± 0.03^{Bb}	2.25 ± 0.03^{Ca}
T2	0.28 ± 0.02^{Ae}	0.76 ± 0.02^{Cd}	1.25 ± 0.03^{Cc}	2.31 ± 0.03^{Ab}	2.73 ± 0.04^{Aa}
T3	0.32 ± 0.02^{Ae}	1.00 ± 0.05^{Bd}	1.39 ± 0.05^{Bc}	2.19 ± 0.04^{Ab}	2.48 ± 0.03^{Ba}
a_w					
Treatments	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**
Control	0.999 ± 0.000^{Aa}	0.980 ± 0.003^{Bb}	0.951 ± 0.003^{ABc}	0.923 ± 0.001^{Cd}	0.908 ± 0.001^{Be}
T1	0.998 ± 0.001^{Aa}	0.986 ± 0.001^{Ab}	0.955 ± 0.001^{Ac}	0.941 ± 0.001^{Ad}	0.921 ± 0.002^{Ae}
T2	0.994 ± 0.001^{Ba}	0.983 ± 0.001^{ABb}	0.948 ± 0.002^{Bc}	0.919 ± 0.001^{Cd}	0.899 ± 0.005^{Ce}
T3	0.994 ± 0.001^{Ba}	0.982 ± 0.001^{ABb}	0.950 ± 0.002^{ABc}	0.929 ± 0.002^{Bd}	0.908 ± 0.002^{Be}

Mean ± S.E. bearing different superscripts column wise (capital alphabets) and row wise (small alphabet) differ significantly ($p< 0.05$).

T1 = Paneer dipped in 5-fold concentrated crude extract, T2 = Paneer dipped in 0.5% lactic acid, T3= Paneer dipped in 10ppm nisin, Control = without any dipping treatment

n= 6 for each treatment

Table.5 Changes in microbiological quality of paneer for control and different treatments during refrigerated storage (4±1˚C), Mean±S.E.*

TPC (log_{10} cfu/g)						
Treatments	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day15**
Control	2.47 ± 0.08^{Ae}	2.69 ± 0.03^{Ad}	3.06 ± 0.02^{Ac}	3.82 ± 0.01^{Bb}	5.43 ± 0.01^{Aa}	NP
T1	2.05 ± 0.05^{Be}	2.15 ± 0.07^{Ce}	2.33 ± 0.08^{Cd}	2.74 ± 0.02^{Dc}	3.53 ± 0.08^{Cb}	5.40 ± 0.05^{a}
T2	2.20 ± 0.06^{Bd}	2.33 ± 0.08^{Bd}	2.51 ± 0.05^{Bc}	3.91 ± 0.02^{Ab}	5.46 ± 0.01^{Aa}	NP
T3	2.23 ± 0.08^{Bd}	2.36 ± 0.04^{Bcd}	2.49 ± 0.05^{Bc}	2.95 ± 0.04^{Cb}	3.92 ± 0.04^{Ba}	NP
Coliform Count (log_{10} cfu/g)						
Treatments	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day15**
Control	ND	ND	ND	1.15 ± 0.07^{Bb}	1.97 ± 0.05^{Aa}	NP
T1	ND	ND	ND	ND	ND	1.60±0.05
T2	ND	ND	ND	1.41 ± 0.05^{Ab}	2.02 ± 0.06^{Aa}	NP
T3	ND	ND	ND	1.05 ± 0.05^{Bb}	1.56 ± 0.07^{Ba}	NP
Psychrophilic Count (log_{10} cfu/g)						
Treatments	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day15**
Control	ND	ND	2.19 ± 0.06^{Ac}	2.37 ± 0.04^{Ab}	2.66 ± 0.02^{Aa}	NP
T1	ND	ND	1.84 ± 0.07^{Cb}	1.99 ± 0.04^{Cb}	2.18 ± 0.06^{Ca}	NP
T2	ND	ND	1.95 ± 0.04^{BCc}	2.45 ± 0.02^{Ab}	2.75 ± 0.02^{Aa}	NP
T3	ND	ND	2.09 ± 0.04^{ABb}	2.17 ± 0.03^{Bb}	2.54 ± 0.02^{Ba}	NP

ND: Not Detected, NP: Not Performed, cfu/g: Colony forming units per gram, Mean±S.E.* bearing different superscripts column wise (capital alphabets) and row wise (small alphabet) differ significantly ($p< 0.05$). T1 = Paneer dipped in 5-fold concentrated crude extract, T2 = Paneer dipped in 0.5% lactic acid, T3= Paneer dipped in 10ppm nisin, Control = without any dipping treatment, n= 6 for each treatment

Table.6 Changes in sensory attributes of control and different treatment groups of paneer for different parameters during refrigerated storage (4±1˚C), Mean±S.E.*

Colour and appearance						
Treatments	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**
Control	7.15±0.06^{Aa}	6.90±0.07^{Ab}	6.28±0.05^{Ac}	5.08±0.06^{Cd}	3.01±0.06^{Ce}	Spoiled
T1	7.00±0.04^{Aba}	6.78±0.05^{Ab}	6.36±0.04^{Ac}	6.01±0.05^{Ad}	5.22±0.06^{Ae}	4.02±0.04
T2	6.94±0.06^{Ba}	6.58±0.05^{Bb}	6.04±0.04^{Bc}	4.99±0.05^{Cd}	2.54±0.07^{De}	Spoiled
T3	7.00±0.06^{Aba}	6.44±0.06^{Bb}	6.03±0.05^{Bc}	5.26±0.05^{Bd}	3.49±0.12^{Be}	Spoiled
Flavour						
Treatments	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**
Control	7.22±0.06^{Aa}	6.92±0.07^{Ab}	6.11±0.05^{Bc}	5.03±0.04^{Bd}	3.19±0.10^{Ce}	Spoiled
T1	7.03±0.05^{Ba}	6.81±0.05^{Bb}	6.28±0.05^{Ac}	5.88±0.05^{Ad}	5.17±0.06^{Ae}	2.93±0.06
T2	6.92±0.06^{Ba}	6.35±0.05^{Cb}	5.94±0.07^{Cc}	5.04±0.06^{Bd}	2.61±0.07^{De}	Spoiled
T3	6.94±0.07^{Ba}	6.68±0.06^{Bb}	6.25±0.06^{ABc}	5.21±0.09^{Bd}	3.44±0.10^{Be}	Spoiled
Texture						
Treatments	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**
Control	7.22±0.06^{Aa}	6.97±0.05^{Ab}	6.36±0.05^{Ac}	5.14±0.08^{Bd}	3.01±0.10^{Be}	Spoiled
T1	7.21±0.04^{Aa}	6.89±0.05^{Ab}	6.47±0.04^{Ac}	5.89±0.05^{Ad}	4.76±0.09^{Ae}	Spoiled
T2	6.97±0.05^{Ba}	6.51±0.05^{Bb}	6.03±0.05^{Bc}	4.83±0.09^{Cd}	2.56±0.09^{Ce}	Spoiled
T3	7.17±0.07^{Aa}	6.86±0.07^{Ab}	6.35±0.06^{Ac}	4.89±0.07^{Bd}	3.26±0.10^{Be}	Spoiled
After taste						
Treatments	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**
Control	7.17±0.05^{Aa}	6.83±0.07^{Ab}	6.00±0.06^{Bc}	5.03±0.07^{Bd}	Spoiled	Spoiled
T1	7.11±0.06^{Aa}	6.92±0.08^{Ab}	6.33±0.06^{Ac}	5.60±0.07^{Ad}	4.02±0.04	Spoiled
T2	6.90±0.06^{Ba}	6.47±0.05^{Bb}	5.43±0.08^{Cc}	4.24±0.06^{Cd}	Spoiled	Spoiled
T3	6.94±0.06^{Ba}	6.93±0.06^{Aa}	6.36±0.05^{Ab}	5.08±0.09^{Bc}	Spoiled	Spoiled
Overall acceptability						
Treatments	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**
Control	7.17±0.05^{Aa}	6.88±0.06^{Ab}	6.18±0.05^{ABc}	4.99±0.07^{Bd}	2.64±0.09^{Ce}	Spoiled
T1	7.11±0.06^{Aa}	6.72±0.04^{Bb}	6.26±0.04^{Ac}	5.82±0.04^{Ad}	5.13±0.05^{Ae}	2.88±0.06
T2	6.90±0.06^{Ba}	6.43±0.06^{Cb}	5.86±0.07^{Cc}	4.49±0.06^{Cd}	2.50±0.07^{Ce}	Spoiled
T3	6.94±0.06^{Ba}	6.42±0.05^{Cb}	6.04±0.05^{Bc}	5.04±0.06^{Bd}	3.32±0.05^{Be}	Spoiled

Mean±S.E.* bearing different superscripts column wise (capital alphabets) and row wise (small alphabet) differ significantly ($p< 0.05$).

Mean values are scores on 8-point Hedonic scale, where 1= extremely undesirable and 8= extremely desirable

T1 = Paneer dipped in 5-fold concentrated crude extract, T2 = Paneer dipped in 0.5% lactic acid, T3= Paneer dipped in 10ppm nisin, Control = without any dipping treatment

n= 18 for each treatment

Changes in microbiological quality during storage

The mean total plate count, psychrophilic count and coliform count (log_{10} cfu/g) for control and treated paneer samples during storage study are presented in table 5. This results revealed that TPC, coliform count and psychrophilic counts of the product were highly significantly ($p<0.01$) affected by storage days, treatments and the interaction of both.

TPC

The TPC of the paneer increased significantly ($p<0.05$) with increasing refrigerated storage period in all the groups. The rate of increase was significantly ($p<0.05$) lower for T1 as compared to control and other treatments. There was a non-significant ($p>0.05$) difference between total plate counts of T1, T2 and T3 on day 0. A significantly ($p<0.05$) lower microbial load was observed with T1 from 3rd day, which could be attributed partly to the antimicrobial activity of CE from Indian curd. On day 12th, there was a significantly ($p<0.05$) higher TPC for control and T2, whereas T1 and T3 had a significantly ($p<0.05$) lower microbial load.

As per the Bureau of Indian standards (IS: 1983), the TPC should not exceed $5x10^5$ cfu/g in paneer. Hence in this study, TPC of all the samples were within the BIS limit up to 9th day, but on 12th day the counts for control and T2 exceed the prescribed limit for TPC of paneer making them microbiologically unacceptable. Sachdeva and Singh (1990) also reported about the increasing trend of TPC of paneer dipped in different antimicrobial solutions like H_2O_2 solution (0.2%) and brine solution in comparison to the paneer samples dipped in plain water. The fresh paneer had a TPC of 10^1 to10^3 cfu/g which increased to 10^4 to10^6 cfu/g regardless of treatment. The results match with that of Kumar and Bector (1991), who reported the initial count of control, 3.0×10^3 cfu/g increased to 2.8×10^5 cfu/g on day four and 9.0×10^6 cfu/g on day seven during storage at 15°C.

Coliform count

The coliform counts were not detected up to day 6 in all the samples and up to day 12 in T1. Coliform counts (log_{10} cfu/g) of control, T2 and T3 increased significantly ($p<0.05$) from day 9 to day 12. The coliforms for T1 were detected on day 15 only (Table 5). The detection of coliforms in later part of storage study may be due to contamination during storage or while performing the experiment. Sanyal (1997) also observed coliform count of 2.65 log_{10} cfu/g on 4th day of storage in low fat buffalo milk paneer. Sachdeva and Singh (1990) determined coliform count of paneer treated with different dipping materials like H2O2 solution (0.2%), brine solution and plain water, and reported that the initial coliform count was not more than 3 to 4 colonies in the first dilution of all the paneer samples and this increased to a maximum of 30-50 colonies over the storage period. Kumar and Bector (1991) reported the initial level of coliform count of control increased from 90 per g to 3.5×10^3 per g after 4 days and 8.0×10^6 per g after 7 days of storage. These results are in contrast to the findings of Buch *et al.*, (2014), who observed that coliforms were absent in control as well as turmeric treated paneer during refrigerated storage of 7 days.

Psychrophilic count

Psychrophiles were not detected in control and treated paneer samples up to 3rd day of storage. The psychrophilic count of control was significantly ($p<0.05$) higher than other treatments. On day 6 and afterwards, the

counts of control and T2 were significantly ($p<0.05$) higher than T1 and T3 (Table 5). Agnihotri and Pal (1996) also observed significantly higher psychotrophic counts in goat milk paneer after three days of storage under refrigeration.

Changes in sensory quality during storage

There was a significant ($p<0.05$) decrease in colour and appearance scores for all the products and the scores decreased for control, T1, T2 and T3 from 0 to 12 days of storage period. The score for control and the product treated with CE (T1) were comparable up to the 6th day of storage.

However, the scores were significantly ($p<0.05$) higher in T1 than all the other products afterwards (Figure 1 and 2).

There was a significantly ($p<0.05$) decreasing trend observed in flavor scores for all the products and the scores decreased for control, T1, T2 and T3 from 0 to 12th day of storage. Up to 3 days, the score of T1 was significantly ($p<0.05$) lower than the control and comparable to other treatment products on 0 day and to nisin treated product on 3rd day. After 3rd day, the T1 was observed to receive significantly higher ($p<0.05$) score than all other products, throughout the storage period. Initial lower scores in T1 than the control may be because of a different taste of CE on the surface of the product however afterwards it was absorbed into the product and the flavour was improved.

There was a significant ($p<0.05$) decrease in mean texture scores for all the products from 0 to 12th day of storage. Up to 9th day of storage, the score for control, T1 and T3 were comparable, however, afterwards, the score for T1 was significantly ($p<0.05$) higher than all the other products, although the score was very low.

There was a significantly ($p<0.05$) decreasing trend observed after taste scores for all the products from 0 to 9th day of storage. Up to 3 days, the score of T1 was comparable with that of control, on 6th day it was comparable to that of nisin treated product and significantly ($p<0.05$) higher than control and lactic acid treated product and on 9th day it was significantly higher than all the other products. The results showed good sensory acceptability of CE treated product.

The mean overall acceptability for control, T1, T2 and T3 showed a significantly decreasing trend from 0 to 12th day of storage. Up to 6th day, the overall acceptability score of T1 was comparable to that of the control product and significantly higher than T2 and T3, however, afterwards the score was significantly ($p<0.05$) higher than control, T2 and T3. The results indicate the sensory acceptability of CE treated product.

Visible sliminess and discoloration were observed in all the samples on 12th day of storage except T1 where it was observed on 15th day. There was a reddish-brown discolouration in T2 and yellowish-brown discolouration in T3 and control (Figure 2).

Similar results were reported by Sachdeva and Singh (1990), who observed gradual deterioration in the flavor score during the storage of paneer dipped in plain water for 2 hours packed in polyethylene pouches. Mendiratta *et al.,* (2007) also observed similar trend in sensory scores of control and treated samples of vacuum packaged skim milk paneer during advancement of storage period.

On the basis of storage study, the overall quality of CE treated paneer was found to be acceptable for 12 days under refrigeration storage temperature (4±1˚C), although the paneer was microbiologically safe up to 15 days, which may be attributed to the

antioxidant and antimicrobial activities of CE. Lower shelf life and inferior quality was observed with control, lactic acid and nisin treated paneer samples as compared to CE treated paneer. Although the nisin treated paneer samples were microbiologically safe up to 12 days (might be due to the antimicrobial activity of nisin), the scores for different physico-chemical quality parameters were low on 12th day, hence remained acceptable up to 9 days. The lactic acid treated paneer and control samples were found to be acceptable up to 6th day and were spoiled on 9th day. Hence a better physico-chemical, sensory and microbial quality of CE treated paneer was observed at refrigerated storage temperature (4±1˚C) as compared to other treatments (10 ppm nisin and 0.5% lactic acid). The CE treated paneer was successfully stored under aerobic packaging conditions for 12 days at 4±1˚C temperature with an acceptable sensory, oxidative and microbial quality attributes. Further studies may be carried out to identify the antioxidant and antimicrobial factors in CE.

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