www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(9): 41-48

www.thepharmajournal.com
Received: 19-07-2020
Accepted: 21-08-2020

Yashvantha R
Dairy Technology Department, SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India

Suneeta Pinto
Dairy Technology Department, SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India

Dasharath Patel
Dairy Technology Department, SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India

Preeti Paul
Dairy Technology Department, SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India

Corresponding Author:
Suneeta Pinto
Dairy Technology Department, SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India

Evaluation of efficacy of lemon rind as a preservative in paneer

Yashvantha R, Suneeta Pinto, Dasharath Patel and Preeti Paul

Abstract
The present study was conducted to assess the shelf life of lemon flavoured paneer (LFP). Control paneer (CP) was prepared from standardized milk (4.5% fat/8.5% MSNF). LFP and control were vacuum packed in laminated pouches and stored at refrigerated temperature (7±1 °C). The sensory attributes, acidity (% LA), free fatty acid (% oleic acid), tyrosine content (mg/100g), colour value, textural properties and standard plate count, yeast and mold count and coliform count of stored samples were monitored at an interval of every 4th day. During storage there was a significant (P<0.05) increase in acidity, free fatty acid and tyrosine content. The hardness, springiness, chewiness, cohesiveness and gumminess values decreased significantly (P<0.05) during storage. After storage of 10 d, there was an increase in lightness, redness and yellowness of LFP. There was a significant (P<0.05) increase in SPC and yeast and mold count during storage. Throughout storage period coliform count was absent. The shelf-life of LFP and control was16 d and 12 d respectively when stored at 7±1 °C in 12 µ polyester + 50 µ LD/LLDPE laminated pouches under vacuum.

Keywords: Lemon, kagzi, paneer, shelf life, storage

Introduction
Paneer is an important indigenous, nutritious and protein rich dairy product. It is also good source of fat, minerals and vitamins. Paneer is used as a base for the preparation of a large number of culinary dishes. It is an acid coagulated dairy product, which is similar to western cottage cheese and tofu (Soy paneer). The use of flavorings in food products dates back as early as the 1660s with consumption of lemonade and orangeade (Hargitt, 2006) [8]. Consumer demand for foods flavoured with natural ingredients has increased, whereas the safety aspect of chemical additives has been questioned. Many plant extracts containing phenolic compounds have recently gained a great popularity and scientific interest (Melendez and Capriles, 2006) [20].

Lemon (Citrus limon) is the third most important species of citrus after orange and mandarin (Chanthaphon *et al*., 2008; Janati *et al*., 2012) [4, 14]. The rinds are an interesting source of phenolic compounds which include phenolic acids and flavonoids (flavanones, flavones, flavonols, anthocyanins and coumarins) and monoterpene hydrocarbons, exhibiting antimicrobial activity in several studies (Cushnie and Lamb, 2005) [5]. The lemon oil, which is present in lemon rind exhibits significant antimicrobial effect (Ortuno *et al*, 2006) [22]. It is also well known that the extracts from lemon rind are rich source of antioxidant and antimicrobial against various microorganisms.

Since paneer is a high moisture product, containing around 52-55% moisture, the shelf life is quite low and it loses freshness after two to three days when stored under refrigeration. It has been reported that the rind and pomace of lemon are a source of sugars, minerals and organic acids, dietary fibers and phenolics which have a wide range of actions which includes antioxidants, anti-mutagenic, cardio preventive, antibacterial and antiviral activities (Adams *et al*., 2006) [1]. The processed peel content in the lemon flavoured paneer was around 5.33%. Hence, it is expected to provide antibacterial, antiviral and antioxidant activities. Therefore, the study was carried out to assess the shelf life of LFP in comparison with control.

Materials and Method
Fresh, raw mixed (cow and buffalo) whole milk was procured from Anubhav Dairy, and standardized by mixing required quantity of skim milk and cream. The average composition of milk was 4.6+0.05% fat and 8.6+0.05% MSNF. Citric acid, β-cyclodextrin and zinc sulphate were procured from Loba-Chemical Pvt. Ltd.

D-limonen and orange oil were supplied by Hi-Media Laboratories Pvt. Ltd. Yellow coloured, Kagzi variety lemon were procured from local market, in Anand.

Debittering of lemon rind: Lemons of kagzi variety were soaked in 25 ppm chlorine solution for 10 min and peeled. The rinds were soaked in a 5% NaCl solution for 1 h at 7±1 ^{0}C. After draining, the rinds were soaked in pasteurized chilled water (about 4 times the weight of peel. Zinc sulphate (50 mg/ 100 ml of water) and β-cyclodextrin (200 mg/ 100 ml of water) were added in the water and kept overnight at 7±1 ^{0}C for debittering. The drained rinds were shredded to obtain debittered processed lemon rind shreds.

Preparation of paneer Standardized milk (4.5% fat/ 8.5% MSNF) was taken in a clean stainless steel vessel and heated on a direct flame to 90 °C for 5 min and temperature was brought down to 80 °C. Debittered and processed lemon rind shreds (@ 0.8% w/w of milk) was added and stirred for one min. The milk was coagulated with citric acid (1.0% solution) at 75 °C. The coagulant was added to the milk with slow stirring until a curd and clear whey separated out. The pH of whey at this stage was 5.4 to 5.6. The coagulum was allowed to settle for 5 min and the whey was drained through a clean, sterile muslin cloth. Care was taken so that the temperature of whey was maintained above 70 °C. The curd was then collected and transferred to a rectangular shaped sterilized stainless steel hoop (15x10x9 cm^3) lined with a clean sterile muslin cloth. The coagulum was pressed for 15-20 min by applying a pressure of 2 to 3 kg/cm^2. The pressed block of paneer were removed from the hoop and immersed in pasteurized chilled water (3 to 5 °C) for 2 h. The paneer blocks were removed from chilled water placed in a clean stainless steel dish for allowing the water to drain off for 10 min. On completion of draining, paneer blocks were weighed and their representative samples drawn as per method given in IS: 5162 (1969) and subjected to compositional, sensory and rheological evaluation. After weighing and sampling, the chilled block of paneer was wrapped in 12 µ polyester + 50 µ LD/LLDPE laminated pouches and stored at refrigeration temperature (7±1 °C) until further use. Control paneer (CP) was prepared from milk standardized to 4.5% fat and 8.5% MSNF using the procedure outlined by Aneja *et al*., (2002) [2] using 1.0% citric acid as a coagulant.

Physico-chemical analysis
Moisture and titratable acidity content in paneer was determined by according to IS: 10484 (1983) [9]. Free fatty acid (FFA) of paneer samples was determined by the method described by Koniecko (1979) [17]. Tyrosine value of paneer samples were estimated by the method as described by Lowry *et al*., (1951) [19] with some modification.

Sensory Evaluation of Paneer
Each block of paneer was cut into approximately 25 g rectangular pieces and served in petri dishes which were labelled with three digit codes in randomized order. The paneer samples were tempered to 10±2 °C before judging. Sensory analysis of paneer samples was performed in a sensory evaluation laboratory. The sensory panel (n=10) was composed of staff members and post graduate students working in the department. Panelists were selected based on their consistent performance in triangle test. The samples were evaluated using 100 point score card as described in Indian Standards (IS: 15346, 2003) [10].

Textural analysis
Compression testing of paneer samples was done with Lloyd Instrument, Hampshire, UK (Model No. 01/2962) using 5 KN load-cells which moved at a speed of 20 mm/min. The paneer samples were taken for texture measurement after tempering the same at 10±1 °C for h. All the textural measurements were conducted in a room maintained at 23±1 °C temperature and 65±1% RH. Cubic samples of the experimental paneer, with edges of 2.00±0.06 cm, were placed in the compression support plate in such a manner that fibers were oriented perpendicular to the cylindrical compression anvil. The cubic samples were compressed up to 70% of their initial size. Five cubic samples were used for each experimental paneer under study and the average value of these readings was reported.

Microbiological analysis
For microbial analysis 11 grams of each of the paneer sample was aseptically weighed and transferred into 99 ml sterile citrate buffer flask. Further dilutions were prepared using 9 ml sterile citrate buffer blanks. Suitable dilutions (selected based on preliminary study conducted) of each sample was transferred (1.0 ml) aseptically into sterile Petri plates. For SPC, plates were prepared using standard plate count agar as per the procedure described in IS: 5402 (1969) [12]. For coliform count plates were prepared using VRBA as per the procedure described in IS: 5401 (1969) [11]. For yeast and mold count plates were prepared using PDA as per the procedure described in IS: 5403 (1969) [13].

Colour Analysis
Colour characteristics of the paneer samples were measured by using ColorFlex EZ Spectrophotometer. All the samples were tempered to 25-27 °C temperature before measurement. Samples were cut into 2 cm cubes and placed on the glass plate of the instrument. This plate was covered by the scanner top. Scanning was performed and the image was analyzed directly through the inbuilt software. The scanned image in JPEG format was opened in colour analysis software namely Adobe Photoshop Version CS4 Extended (11.0) running under the Microsoft Windows XP environment. This software was used to extract and analyse colour information from the scanned image in LAB mode: Lightness (L*), Redness (a*) and Yellowness (b*), from which data were converted in terms of L* [Lightness, ranges 0 (black) to 100(white)], a*[Redness ranges from +60(red) to -60(green)], and b* [Yellowness, ranges from +60 (yellow) to -60 (blue)].

Statistical analysis
Statistical analysis of data was carried out using Factorial Completely Randomized Design (FCRD).

Results and Discussion
Effect of Storage Period on Sensory Characteristics
As seen in Table 1, CP had a initial flavour score of 45.75 (out of 50) which was found to decrease significantly (P<0.05) to 35.72 after storage period of 12 d and it further decreased significantly (P<0.05) to 27.64 after 16 d of storage. The product was acceptable till 12 d of storage and at 16 d, the product was not acceptable due to development of bitter taste and stale off-flavour in the product. In case of developed LFP, the flavour score decreased from the initial value of 44.33 to 43.21 (on the 4th d) and further decreased to

34.45 (on the 16th d) and on 20th d the judging panel rejected the sample due to development of bitter taste and stale off-flavour in the product. The interaction between type of paneer (T) and storage period (P) was found to be statistically significant ($P<0.05$) for changes in flavour score of paneer samples over the storage period. Control flavour score showed a rapid decrease as compared to LFP. Reason behind higher flavour score for LFP could be the lemon rind present in LFP which would have been able to mask the development of off-flavours in the sample.

The paneer had an initial body and texture scores of the two different type of paneer viz. CP and LFP were 32.57 and 32.45 respectively. The body & texture score of CP and LFP paneer during storage tend to decrease significantly ($P<0.05$) with the increase in storage period. The statistical analysis showed that storage period (P) had a significant ($P<0.05$) effect on the body and texture score of control and LFP. The treatment applied to samples and the interaction between treatment and storage period had a non-significant ($P>0.05$) effect on the body and texture score of both control and LFP. As the storage time elapsed, samples tended to get drier and harder body. They were observed to lose compactness of both samples as the storage period increased. Towards the end of the storage period, a slimy layer developed on the top surface of control. In the case of LFP, there was no slimy layer observed, instead the top surface became loose and harder, which could be due to the presence of lemon rind and zinc in LFP. Buch (2014) [3] also reported decrease in body and texture score of paneer (8.00 to 7.20 at the end of 12 d storage under refrigeration) incorporated with 0.6% turmeric.

The initial colour and appearance scores of the two different type of paneer viz. CP and LFP were 9.12 and 9.05 respectively. The colour and appearance score of CP and LFP during storage tend to decrease significantly ($P<0.05$) with the increase in storage period. The colour and appearance score of LFP was lower as compared to CP. This may be due to addition of lemon rind in developed paneer and developed paneer had a less white in colour as compared to control paneer. Statistical analysis showed that storage period had a significant ($P<0.05$) effect on the colour and appearance score of control and LFP. The interaction between treatment and storage period had a significant ($P<0.05$) effect on the colour and appearance scores of control and LFP.

At the end of storage period, CP had a lower score than control. This could be due to formation of dry and mouldy (slime formatiom) surface in control paneer. When lemon rind was used to prepare LFP, the final product has a much better colour than the control sample. As the storage period continued, the control sample started developing a deeper yellow colour. The LFP, on the other hand, remained slightly yellow colour (yellow colour of LFP paneer due to addition lemon rind) which could have influenced the decision of sensory panel while scoring for both the samples.

The initial total score of paneer i.e. control and developed were 92.44 and 90.83 respectively. The total score of CP and LFP was decreased with increases storage period. On the 0th day, total score for control was 92.44 which decreased to 63.48 on the 16th d. LFP also showed a decreasing trend of total score. On the 0th day total score for LFP was 90.83 that decreased to 72.27 on the 16th day of storage. Statistical analysis showed that storage period had a significant ($P<0.05$) effect on the total score of control and LFP. Treatment applied to each sample had a significant ($P<0.05$) effect on the total score of both the samples. The interaction between treatment and storage period had significant ($P<0.05$) effect on the total score of both control and LFP.

The total score of LFP was higher than control. This is due to a cumulative effect of higher colour and appearance, body and texture and flavor score of LFP when compared against control. This indicates that overall liking towards the developed product was slightly more than the control as expressed by the sensory panel. The observed reduction in total score of paneer could partly ascribed to the development of change in flavour owing to development of bitter after taste and dull colour and appearance to some extent. The overall sensory scores indicate a shelf life of control paneer was 12 d at refrigeration temperature and LFP was 16 d at refrigeration temperature.

Table 1: Changes in Sensory Characteristics of LFP and CP during storage at 7±1^0 C

Type of paneer (T)	Storage period in days (P)					Average for Treatment (T)
	0	4	8	12	16	
Flavour score (max. 50)						
CP	45.75 ± 0.66	43.57 ± 0.43	39.52 ± 0.80	35.72 ± 0.26	27.64 ± 0.09	38.90
LFP	44.33 ± 0.58	43.21 ± 1.00	41.66 ± 1.52	39.04 ± 1.45	34.45 ± 1.46	40.37
Average for Period(P)	45.04	43.28	40.59	37.06	32.20	
CD (0.05) T=0.73; P=1.15; TxP=1.63						
Body and Texture score (max. 35)						
CP	32.57 ±0.56	30.00 ± 0.81	28.41 ±0.56	26.93 ± 1.01	23.71 ± 0.62	28.42
LFP	32.45 ±0.49	29.92 ± 1.01	27.65 ±1.35	26.02 ± 0.79	24.38 ± 1.11	28.05
Average for Period(P)	32.51	30.22	28.03	26.39	24.02	
CD (0.05) T=NS; P=1.05; TxP=NS						
Colour and Appearance score (max. 10)						
CP	9.12 ± 0.11	9.01 ± 0.10	8.74 ± 0.10	8.37 ± 0.10	7.12 ± 0.05	8.54
LFP	9.05 ± 0.10	8.98 ± 0.15	8.80 ± 0.18	8.65 ± 0.10	8.44 ± 0.10	8.78
Average for Period(P)	9.09	8.99	8.77	8.51	7.95	
CD (0.05) T=0.08; P=0.13; TxP=0.19						
Total score* (max. 100) * 5 out of 5 marks allotted for packaging						
CP	92.44±0.70	87.58 ±1.97	81.67±1.18	76.02±1.39	63.48±0.95	80.24
LFP	90.83±1.66	87.11±1.79	83.11±0.98	78.71±0.63	72.27±1.10	82.40
Average for Period(P)	91.64	87.35	82.39	77.37	67.88	
CD (0.05) T=1.45; P=2.29; TxP=3.24						

Each observation is a mean ± SD of 3 replicate experiments; T=Type of paneer; P=Storage Period

Effect of Storage Period on Physico-Chemical Characteristics

Moisture

As seen in Table 2, the moisture of paneer decreased slightly with increase in storage period. Fresh control paneer had moisture of 53.72% which decreased non-significantly (P>0.05) to 51.32% after 16 d of refrigerated storage. Fresh LFP had moisture 52.01% which decreased non-significantly (P>0.05) to 50.74% after 12 d, 50.00% after 16 d of storage at refrigerated temperature. The treatment (T) showed significant (P<0.05) effect on moisture of paneer. The storage period (P) showed non-significant (P>0.05) effect on moisture of paneer samples. The interaction treatment (T) and storage period (P) showed non-significant (P>0.05) effect on moisture of paneer samples over storage period. No significance difference observed in moisture content. Because both LFP and regular paneer were packed in high barrier laminates under vacuum.

Table 2: Changes in Physico-Chemical Properties of LFP and CP during storage at 7±1^0 C

Type of paneer (T)	Storage period in days (P)					Average for Treatment (T)
	0	4	8	12	16	
Moisture (%)						
CP	53.72±1.82	53.04±1.26	52.49±1.44	52.09±0.0.91	51.32±0.69	52.53
LFP	52.01±1.00	51.88±0.83	51.12±1.06	50.74±1.55	50.00±1.55	51.16
Average for Period(P)	52.87	52.46	51.80	51.41	50.69	
CD (0.05) T=0.96; P=NS; TxP=NS						
Acidity (%LA)						
CP	0.457 ±0.045	0.522 ± 0.014	0.565 ± 0.047	0.670 ± 0.015	0.693 ± 0.026	0.586
LFP	0.550 ±0.027	0.586 ± 0.026	0.623 ± 0.051	0.679 ± 0.042	0.712 ± 0.055	0.630
Average for Period(P)	0.503	0.554	0.594	0.674	0.714	
CD (0.05) T=0.029; P=0.045; TxP=0.064						
Free Fatty Acids content (%oleic acid)						
CP	0.197 ±0.012	0.221 ± 0.009	0.263 ± 0.007	0.297 ± 0.008	0.315 ± 0.005	0.258
LFP	0.186 ±0.015	0.208 ± 0.019	0.252 ± 0.017	0.294 ± 0.007	0.311 ± 0.009	0.250
Average for Period(P)	0.191	0.214	0.257	0.295	0.313	
CD (0.05) T=NS; P=0.014; TxP=NS						
Tyrosine content (mg/100g)						
CP	14.67 ± 1.70	25.75 ± 2.24	41.77 ± 2.03	52.40 ± 2.65	60.39 ± 1.91	38.99
LFP	13.04 ± 1.38	24.05 ± 1.08	38.08 ± 1.65	43.90 ± 1.66	52.18 ± 1.77	34.25
Average for Period(P)	13.86	24.90	39.93	48.15	56.29	
CD (0.05) T=1.41; P=2.23; TxP=3.15						

Each observation is a mean ± SD of 3 replicate experiments; T=Type of paneer; P=Storage Period

Acidity

The changes in acidity of control and LFP, stored at refrigerated temperature are presented in Table 2. Acidity of the control sample increased from 0.457% LA on the 0th day to 0.693% LA on the 16th d. In the case of LFP, acidity increased from 0.550% LA on 0th day to 0.712% LA on 16th d. Treatments given to the samples showed a significant (P<0.05) effect on the acidity of the samples. Similarly, there was a significant (P<0.05) effect exerted by storage period on changes in acidity values of both the samples. The interaction effect of treatments with the storage period showed a non-significant (P>0.05) effect. During complete storage period, acidity of LFP was observed to be slightly higher than that of control. This behavior could be due to the addition of lemon rind to LFP. Lemon rind contains some organic acids like citric acid, which resulted in increase in acidity of LFP.

Free fatty acid content

The extent of lipolysis in paneer samples was measured using changes in free fatty acids (FFAs) value. The samples were analyzed for free fatty acids with view to know the rate of lipolytic changes during storage. Lipids undergo hydrolysis due to the production of microbial enzymes and thereby increasing the FFAs content leading to changes in flavour characteristics of the products.

Free fatty acid content in the control sample increased from 0.197% oleic acid on the 0th day to 0.315% oleic acid on the 16th day. In the case of LFP, FFA increased from 0.186% oleic acid on 0th day to 0.311% oleic acid on 16th d. Difference in applied treatments had a non-significant (P>0.05) effect on the free fatty acid content of the samples. Similarly, there was a non-significant (P>0.05) effect exerted by interaction between treatment and storage period on changes in FFA values of both the samples. The storage period had a significant (P<0.05) effect on FFA values of both LFP and control.

The results obtained in this part of study are within the range reported by Khatkar *et al.* (2017) [16] and Patel (2014) [23]. Khatkar *et al.* (2017) [16] reported that the free fatty acid (% oleic acid) of control sample increased from an initial 0.175 to 0.517 in 10 d to 0.491 in 8 d and to 0.541 in 5 d when packed with MP, NP and LDPE, respectively. Patel (2014) [23] reported that FFA (% oleic acid) of control paneer was 0.050 on 0th d which increased to 0.167 on 15th d of storage and for sample with Na-sorbate had 0.037 on 0th d and increased to 0.095 on 15th d of storage. The FFA content in this study are similar to those reported by Kumar and Bector (1991) [18] reported that the FFA content of paneer increased from 0.28 to 0.5% on 22 days of storage at 5 °C.

Tyrosine content

Tyrosine content in the control sample increased from 14.67 mg /100 g on the 0th d to 60.39 mg/100 g on the 16th d. Similar trend was observed for LFP. Tyrosine value for LFP increased from 13.04 mg/100 g on 0th d to 52.18 mg/100 g on 16th d. Statistically there was noted that treatment, storage period and interaction effect between treatments and storage period had a significant (P<0.05) effect on changes in tyrosine value of both samples.

Khatkar *et al.* (2017) [16] also reported that tyrosine content (mg/100g) of control sample increased highly from an initial 12.192 to 28.642 in 10 d, to 32.531 in 8 d and to 39.336 in 5 d when packed with MP, NP and LDPE, respectively. Therefore, the results obtained in this study show similar increasing trend in tyrosine value during the refrigerated storage period. However, the values obtained for tyrosine content in LFP, in this study are higher than those reported by Khatkar *et al.* (2017) [16]. The differences could be attributed to variations in the spectrophotometric analysis of the samples, as well as differences in the initial quality of milk used to prepare the samples and processing parameters.

Effect of storage Period on Textural Properties

Textural properties play an important role in the quality of paneer. The texture of paneer depends upon the status of components and the temperature of storage. The changes in textural properties of CP and LFP were viz. hardness, cohesiveness, chewiness, springiness and gumminess is summarized in Table 3.

Table 3: Changes in Textural Properties of LFP and CP during storage at 7±1^{0} C

Type of paneer (T)	Storage period in days (P)					Average for Treatment (T)
	0	4	8	12	16	
Hardness (N)						
CP	14.09 ± 1.78	8.18 ± 1.15	7.65 ± 1.36	5.65 ± 0.57	4.63 ± 0.64	8.04
LFP	19.72 ± 1.82	16.06 ±1.38	14.92 ± 1.84	12.68 ± 2.29	10.54 ± 0.62	14.78
Average for Period(P)	16.90	12.12	11.29	9.17	7.58	
CD (0.05) T=0.97; P=1.54; TxP=NS						
Cohesiveness						
CP	0.481 ±0.024	0.456 ± 0.032	0.442 ± 0.027	0.426 ± 0.008	0.413 ± 0.008	0.443
LFP	0.453 ±0.018	0.414 ± 0.017	0.408 ± 0.017	0.399 ± 0.017	0.383 ± 0.030	0.411
Average for Period(P)	0.467	0.435	0.425	0.413	0.398	
CD (0.05) T=0.016; P=0.026; TxP=NS						
Chewiness (Nmm)						
CP	38.91 ± 1.70	36.63 ± 2.13	34.24 ± 1.78	15.18 ± 1.51	8.26 ± 1.36	26.64
LFP	26.13 ± 2.97	19.04 ± 3.06	16.48 ± 2.24	13.75 ± 1.25	4.74 ± 0.99	16.03
Average for Period(P)	32.52	27.83	25.36	14.46	6.50	
CD (0.05) T=1.53; P=2.42; TxP=3.42						
Springiness (mm)						
CP	6.20 ± 0.09	6.05 ± 0.05	5.91 ± 0.09	5.86 ± 0.21	5.58 ± 0.24	5.92
LFP	6.09 ± 0.35	6.03 ± 0.37	5.73 ± 0.16	5.62 ± 0.18	5.12 ± 0.12	5.71
Average for Period(P)	6.14	6.04	5.82	5.74	5.35	
CD (0.05) T=0.16; P=0.25; TxP=NS						
Gumminess (N)						
CP	8.00 ± 1.05	5.79 ± 0.80	4.05 ± 0.72	3.73 ± 0.25	3.23 ± 0.85	4.96
LFP	5.58 ± 0.35	4.00 ± 0.37	3.40 ± 0.16	3.22 ± 0.18	2.44 ± 0.12	3.73
Average for Period(P)	6.79	4.89	3.73	3.47	2.84	
CD (0.05) T=0.46; P=0.72; TxP=NS						

Each observation is a mean ± SD of 3 replicate experiments; T=Type of paneer; P=Storage Period

Hardness

Hardness of the control sample decreased from 14.09 N on the 0th d to 4.63 N on the 16th d. LFP showed a decreasing trend in hardness. Hardness decreased from 19.72 N on 0th d to 10.54 N on 20th d. The hardness of paneer samples decreased significantly (P<0.05) with increase in storage period. Treatments (T) given to the samples showed a significant (P<0.05) effect on the hardness of the samples. Similarly, storage period (P) of samples showed a significant (P<0.05) effect on the hardness of the samples. However, interaction effect of treatments (T) with the storage period (P) on changes in hardness values of both the samples showed a non-significant (P>0.05) effect.

Throughout storage period hardness of LFP was higher than that of control. This could be attributed to presence of fiber in lemon rind and cations such as Zn^{++} because of treated with zinc sulphate, which could have resulted in more closer linkages of protein resulting in increased hardness.

Moisture content of LFP was already lower than control which can be linked to the trend observed for hardness during storage.

The samples were criticized by panellists for their hard body, The results obtained in this study are similar to those reported by Kahraman and Ustunol (2012) [15], who reported that although zinc-fortified cheese was harder as determined by the texture analyzer.

Cohesiveness

The results indicated that the initial cohesiveness of lemon flavoured paneer was lower than control paneer. Cohesiveness of the control sample decreased from 0.481 on the 0th day to 0.413 on the 16th d. Cohesiveness of LFP decreased from 0.453 on 0th d to 0.383 on 16th day. The cohesiveness of paneer samples decreased significantly (P<0.05) with increase in storage period. Treatments (T) given to the samples showed a significant (P<0.05) effect on the cohesiveness of the samples. Similarly, storage period (P) of samples showed a significant (P<0.05) effect on the cohesiveness of the samples. However, interaction effect of treatments (T) with the storage period (P) on changes in cohesiveness values of both the samples showed a non-significant (P>0.05) effect.

The initial cohesiveness of lemon flavoured paneer was lower than control paneer. This could be due to differences in the matrix of both the products because of differences in the ingredients. Control paneer is a uniform coagulum consisting of only milk constituents, mainly caseins and milk fat. In the case of LFP, coagulum formed consists of casein as well as

lemon rind and also debittering agents viz. Zinc sulphate and β-cyclodextrin. The decrease in cohesiveness may be attributed to presence of fiber content in lemon rind.

Chewiness

Chewiness of the control sample decreased from 38.91 Nmm on the 0th d to 8.26 Nmm on the 16th d. Chewiness of LFP decreased from 26.13 Nmm on 0th d to 4.74 Nmm on 16th d. Treatments (T), Storage period (P) given to the samples and interaction between treatments (T) and storage period (P) showed a significant (P<0.05) effect on the chewiness of the samples.

Springiness

The springiness of paneer samples decreased significantly (P<0.05) with increase in storage period. Springiness of the control sample decreased from 6.20 mm on the 0th d to 5.58 mm on the 14th d. Similarly, springiness of LFP decreased from 6.09 mm on 0th d to 5.12 mm on 16th d storage under refrigeration. The treatment (T) showed significant (P<0.05) effect on springiness of paneer. The storage period (P) showed significant (P<0.05) effect on springiness of paneer samples. The interaction between treatment (T) and storage period (P) showed non-significant (P>0.05) effect on springiness of paneer samples.

Gumminess

The gumminess of paneer samples decreased with increase in storage period. Gumminess of the control sample decreased from 8.00 N on the 0th d to 3.23 N on the 16th d. Gumminess of LFP decreased from 5.58 N on 0th d to 2.44 N on 24th d. The treatment (T) and storage period (P) showed significant (P<0.05) effect on gumminess of paneer samples. The interaction between treatment (T) and storage period (P) showed non-significant (P>0.05) effect on gumminess of paneer samples.

Effect of Storage Period on Microbial Quality of Paneer

The microbiological quality of the paneer depends on the bacteriological quality of the milk, the practice of hygiene during paneer production and its subsequent handling, packaging and storage. Milk is exposed to heating temperatures of 90 ^{0}C, which leads to destruction of most of the pathogens in raw milk. However the growth of survivors and contaminants may be possible and hence limit the shelf life of such products, therefore the microbiological quality of fresh as well as product stored at refrigeration temperature, was assessed by enumeration of Standard plate count (SPC), Yeast and Mold count (Y & M) and Coliform count till the control and experimental samples were observed to spoil or were rejected by the judging panel. The SPC, Yeast and mould and Coliform count requirements as specified by FSSAI (2011) [7] for paneer is maximum 3, 50,000/g, 150/g and 100/g respectively. The microbiological status (viz., standard plate count, yeast and mold count, coliform count) paneer stored at refrigeration temperature is presented in Table 4.

Table 4: Changes in Microbial Quality of LFP and CP during storage at 7±1^0 C

Type of paneer (T)	Storage period in days (P)					Average for Treatment (T)
	0	4	8	12	16	
Standard plate count (log_{10}cfu/g)						
CP	3.80 ± 0.13	4.22 ± 0.26	4.84 ± 0.14	5.12 ± 0.11	5.35 ± 0.07	4.67
	(5.87 ± 0.99)	(13.67±1.25)	(75.49 ±2.07)	(141.33 ± 1.15)	(235 ± 2.00)	
LFP	3.71 ± 0.05	4.05 ± 0.05	4.79 ± 0.08	4.98 ± 0.06	5.13 ± 0.11	4.52
	(5.38 ± 1.32)	(11.17 ±1.19)	(69.33 ±0.58)	(130.67 ± 2.08)	(216.21±1.0)	
Average for Period(P)	3.76	4.13	4.81	5.05	5.24	
CD (0.05) T=0.09; P=0.14; TxP=NS						
Yeast and Mold count (cfu per g)						
CP	0.00 ± 0.00	2.10 ± 0.70	3.50 ± 0.44	8.92 ± 1.03	16.98 ± 1.13	6.30
LFP	0.00 ± 0.00	1.20 ± 0.82	2.68 ± 1.26	4.75± 0.70	10.70 ± 0.30	3.86
Average for Period(P)	0.00	1.65	3.09	6.83	13.84	
CD (0.05) T=0.73; P=1.16; TxP=1.64						

Each observation is a mean ± SD of 3 replicate experiments; T=Type of paneer; P=Storage Period (Bracket data indicate cfu×10^3 /g value)

Standard plate count

The control and developed paneer stored at refrigeration temperature (7±1 °C) were analyzed for its standard plate count (SPC) during storage as presented in Table 4. It can be seen from the Table 4 that SPC count of CP and LFP increased significantly (P<0.05) with increase the storage period. Fresh control paneer sample had a SPC count of 3.80 log_{10} cfu/g which increased significantly (P<0.05) to 5.12 log_{10} cfu/g after 12 d, 5.35 log_{10} cfu/g after 16 d under refrigeration storage. Fresh LFP had SPC count 3.71 log_{10} cfu/g which increased significantly (P<0.05) to 5.13 log_{10} cfu/g after 16 d under refrigeration storage. The treatment (T) showed significant (P<0.05) effect on SPC of paneer. The storage period (S) showed significant (P<0.05) effect on SPC of paneer. The interaction between treatment (T) and storage period (S) showed non-significant (P>0.05) effect on SPC of paneer over storage period.

As shown in Table 4 SPC count of LFP was lower than control paneer throughout the storage period. This could be attributed because of lemon peels had an anti-bacterial and anti-viral properties. Lemon peels are a source of sugars, minerals and organic acids, dietary fibers and phenolics which have a wide range of actions which includes antioxidants, anti-mutagenic, antibacterial and antiviral activities (Adams *et al.*, 2006) [1].

Eresam *et al.* (2013) [6] also reported a rise in SPC of paneer samples prepared by the addition of 0.6% (w/w) cardamom from 3.49 log cfu/g on 0th day to 6.18 log cfu/g on 28th day of storage under refrigeration.

According to FSSAI requirements the microbiological criteria which separates marginally acceptable quality from unsatisfactory quality for SPC count is should not be more than 3,50,000 cfu/g. The product met the requirements for SPC on the 16th d of storage.

Yeast and Mold Count

The yeast and mold count (cfu/g) of control and developed paneer during storage were found to increase with the increase in storage period. It can be seen from the Table 4 that yeast and mold count of CP and LFP increased significantly ($P<0.05$) with increase the storage period. Fresh CP had a yeast and mold count nil which increased significantly ($P<0.05$) to 8.92 /g after 12 d, 16.98 /g after 16 d of refrigerated storage. Fresh LFP had yeast and mold count nil which increased significantly ($P<0.05$) to 10.70 /g after 16 d under refrigeration temperature. The treatment (T), storage period (P) and interaction between treatment (T) and storage period (P) showed significant ($P<0.05$) effect on yeast and mold of paneer.

Yeast and mold count LFP had a lower than that of control paneer. This could be attributed to the addition of lemon peel for preparation of LFP. Lemon peels are a source of sugars, minerals and organic acids, dietary fibers and phenolics which have a wide range of actions which includes antioxidants, anti-mutagenic, antibacterial and antiviral activities (Adams *et al.*, 2006) [1].

According to FSSAI requirements the microbiological criterion which separates marginally acceptable quality from unsatisfactory quality for yeast and mold count should not be more than 150/g. Paneer samples met this requirement up to 16th d of storage.

Coliform count

The fresh as well as stored samples of control paneer and lemon flavoured paneer were analyzed for the presence of coliforms. Throughout storage period coliform were found to be absent in all samples of paneer.

Effect of Storage Period on Colour Characteristics of Paneer

The colour of food is one of the major attributes which affect the consumer perception and its quality, and it is also a powerful tool for quality control and marketing. The change in colour characteristics of paneer during storage, was measured for both control as well as optimized sample and the observations are given in Table 5.

As seen from the values quoted in tables, it could be noted that the L* values of CP as well as LFP increased after 10 d of storage. While a* and b* values were seen to be increasing with period of storage. The L*, a* and b* value represent lightness, redness and yellowness. The figures in the Table 5 reveal that for CP, initial value 84.55 of L* (lightness) increased to 86.61 after 10 d of storage, while for the LFP, it increased from 84.12 to 86.60. However, the lightness value of LFP was lower than for CP, due to presence of lemon rind in LFP. The initial yellowness value of CP and LFP were 14.78 and 16.00 respectively, which increased after 10 d to 16.57 and 18.62 respectively. However, the yellowness value of LFP was higher than for CP, due to presence of lemon rind in LFP. There was a increase in redness a* of LFP after 10 d, while increase in redness of CP after 10 d. Shrivastava *et al.* (2013) [25] also found that the lightness value of paneer samples packaged under 1 atm, 2 atm, 3 atm, 4 atm and 5 atm decreased during storage at 3±1^{0}C for 10 d.

Table 5: Comparison of Colour Constants of Fresh and Stored Samples (10 d) of LFP with CP

Storage period (days)	Lightness (L*)		Redness (a*)		Yellowness (b*)	
	CP	LFP	CP	LFP	CP	LFP
0	84.55±1.31	84.12±1.31	0.06±0.14	0.23±0.30	14.78±0.44	16.00±0.72
10	86.61±2.93	85.60±1.84	0.43±0.03	0.46±0.08	16.57±0.89	18.62±1.07

Conclusion

Based on the results obtained in this part of study it can be concluded that the product has shelf-life of 16 d when stored at 7±1°C in 12 µ polyester + 50 µ LD/LLDPE laminated pouches under vacuum compared to 12 days for control paneer (CP) prepared from standardized milk. It also met the microbial standard as per FSSAI.

References

1. Adams LS, Seeram NP, Aggarwal BB, Takada Y, Sand D, Heber D *et al*. Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells. J Agric Food Chem. 2006; 54(3):980-985.
2. Aneja RP, Mathur BN, Chandan RC, Baneerjee AK. Heat acid coagulated products. In: Technology of Indian milk products. A Dairy India Publication, Delhi, India, 2002, 133-158.
3. Buch S, Pinto S, Aparnathi KD. Evaluation of efficacy of turmeric as a preservative in paneer. J Food Sci Technol. 2014; 51(11):3226-3234.
4. Chanthaphon S, Chanthachum S, Hongpattarakere T. Antimicrobial activities of essential oils and crude extracts from tropical Citrus spp. against food-related microorganisms. Songklanakarin. J Sci Technol, 2008, 30.
5. Cushnie TT, Lamb AJ. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents. 2005; 26(5):343-356.
6. Eresam EKK, Pinto S, Aparnathi KD. Concise and informative title: evaluation of selected spices in extending shelf life of paneer. J Food Sci Technol. 2015; 52(4):2043-2052.
7. FSSAI. Food Safety and Standards Authority of India. Ministry of health and family welfare. Government of India, 2011.
8. Hargitt B. Introduction in Carbonated Soft Drinks, Formulation and Manufacture. In Ashurst, P. R., Eds. Blackwell Publishing Ltd: Oxford, 2006, 1-14.
9. IS: 10484. Specification for Paneer. Bureau of Indian Standards, Manak Bhavan, New Delhi, 1983, 1-11.
10. IS: 15346. Method for Sensory Evaluation of Paneer/Chhana [FAD 19: Dairy Products and Equipment] Indian Standard Institution, New Delhi, 2003.
11. IS: 5401. Methods for detection and estimation of coliform bacteria in food stuffs. Bureau of Indian Standards, Manak Bhavan, New Delhi, 1969.
12. IS: 5402. Method for plate count of bacteria in food stuffs. Bureau of Indian Standards, Manak Bhavan, New Delhi, 1969.
13. IS: 5403. Method for yeast and mold count in food stuffs. Bureau of Indian Standards, Manak Bhavan, New Delhi, 1969.
14. Janati SSF, Beheshti HR, Feizy J, Fahim NK. Chemical

composition of lemon (Citrus limon) and peels its considerations as animal food. GIDA- J Food. 2012; 37:267-271.
15. Kahraman O, Ustunol Z. Effect of zinc fortification on Cheddar cheese quality. J Dairy Sci. 2012; 95:2840-2847.
16. Khatkar AB, Ray A, Kaur A. Studies on shelf life extension of Paneer with the addition of plant essential oil and different packaging materials. Int J Curr Microbio App Sci. 2017; 6(9):376-389.
17. Koniecko ES. Handbook for Meat Chemists. Wayne, New Jersey, USA: Avery Publishing Group Inc, 1979, 68-69.
18. Kumar P, Bector BS. Enhancement of shelf life of paneer with food additives. Indian J Dairy Sci. 1991; 44:577-584.
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biolo Chem. 1951; 193(1):265-275.
20. Melendez PA, Capriles VA. Antibacterial properties of tropical plants from Puerto Rico. Phytomedicine. 2006; 13(4):272-276.
21. Mishra D, Rao KJ, Bhardwaj R, Sutariya H, Kavitkar RS, Subhash WS *et al*. Effect of pH on sensory, textural, microbial quality and shelf-life of paneer. Int J Food Fermen Technol. 2016; 6(2):405-414.
22. Ortuno A, Baidez A, Gomez P, Arcas MC, Porras I, Garcia-Lidon A *et al*. Citrus paradisi and Citrus sinensis flavonoids: Their influence in the defence mechanism against Penicillium digitatum. Food Chem. 2006; 98(2):351-358.
23. Patel RB. Evaluation of sorbic acid and its salts as a preservative for paneer (M.Tech. thesis, Anand Agricultural University, Anand), 2014. Retrieved from http:// krishikosh. egranth.ac.in /handle/1/5810001257.
24. Ranmode AK. Evaluation of efficacy of Nisin as a preservative for paneer (M.Tech. thesis, Anand Agricultural University, Anand), 2010. Retrieved from http:// krishikosh. egranth.ac.in /handle/1/5810024245.
25. Shrivastava S, Goyal S, Goyal GK. Effect of Modified Atmosphere Packaging on colour profile of paneer stored at 3±1ºC. J Agric Sus. 2013; 3(2):195-204.
26. Suthar JS. Evaluating the effect of homogenization on the quality of paneer (M.Tech thesis, Anand Agricultural University, Anand, India), 2015. Retrieved from http:// krishikosh. egranth.ac.in/handle/1/5810047475