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Efficacy of whey based edible coating material for paneer preservation#

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Abstract

Paneer, a milk product coagulated by heat and acid, typically has a short shelf-life of one day at room temperature thus restricting its availability in the market. In this study, efforts were made to prolong its shelf-life at room temperature by applying an edible coating made from condensed whey mixed with antimicrobials (lactic acid and potassium sorbate). The condensed whey, concentrated to 45 per cent total solids, was combined with four percent glycerol as a plasticizer. A solution containing lactic acid and potassium sorbate (25per cent strength) was added at a 12per cent level to the condensed whey. Samples coated with this mixture and packaged in LDPE pouches showed a shelf-life of four days under ambient conditions. The standard plate count, coliform count and yeast and mould count expressed in Log_{10}cfu/g at the end of fourth day for this sample were 3.72 ± 0.026, 2.63 ± 0.047 and 2.42 ± 0.058 respectively and were within the FSSAI limits prescribed for paneer. This confirms the antimicrobial effect of the coating applied. The analysis of the samples' physicochemical properties indicated that the studied edible coating effectively prevented an undesirable rise in titratable acidity and helped maintain the product's moisture content. There was no significant difference observed in shelf-life between samples packaged using atmospheric and vacuum conditions; both methods provided a shelf-life of four days.

Keywords: *Paneer, edible coating, shelf-life extension*

Paneer is a popular indigenous milk product prepared by heat acid coagulation of cow or buffalo milk or a combination of thereof using citric acid or lactic acid or sour milk as the coagulating agent. It is characterised by a marble white colour, soft body, close knit texture and sweetish acidic nutty flavour. This traditional value-added dairy product contains around 26 percent of milk fat, 16 percent of protein, two percent of lactose and 1.5 to 2 percent of minerals which makes it highly

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nutritious. However, the availability of paneer in the Indian market is limited due to its poor shelf-life (Kanawjia and Singh, 2000). Paneer can be stored only for a day at room temperature and for about six days under refrigerated condition without deterioration in its chemical and microbiological quality. Lipolysis, proteolysis, increased acidity and lowering of pH are the chemical changes, while increase in yeast and mould counts are the major microbial changes that affect product quality during its course of storage.

Use of edible films and coatings is an emerging technique put into use for successful increase in the storage life of various products. "Edible coatings are a particular form of film designed for application directly onto the surface of target materials; they are normally regarded as part of the final product" (Han, 2002). The main advantage of edible films over traditional synthetics is that they can be consumed with the packaged products. Seydim and Sarikus (2006) reported that milk protein based edible coatings are excellent oxygen, lipid, and aroma barriers. Whey is obtained as a by-product during the preparation of chhana, paneer, cheese and casein. Much of this whey is not utilised, and it creates serious pollution and waste disposal problems. Ozdemir and Floros (2008) suggested that the development of whey based edible films and coatings can aid in effective utilisation of whey, improve the nutritional value of foods and prolong the shelf-life. Addition of antimicrobial agents like lactic acid and antifungal agents like potassium sorbate to this coating is expected to further ensure food safety and extension of shelf-life by reducing (or even preventing) growth of pathogenic and spoilage microorganisms. Hence treating paneer with whey based edible coating would certainly be effectual and practical (Archana *et al.*, 2023).

The present study focuses on investigating potential possibilities of employing whey based edible coatings containing glycerol (as plasticiser), lactic acid (as antimicrobial agent) and potassium (as antifungal agent) as an alternative shelf-life extension technique for paneer.

Materials and methods

Procurement of raw materials

Good quality buffalo milk used for paneer preparation was procured from Kerala Veterinary and Animal Sciences University Dairy Plant, Mannuthy. Freshly prepared whey obtained as a by-product of paneer manufacture was collected from University Dairy Plant, Mannuthy. Citirc acid, edible grade glycerol, lactic acid, potassium sorbate and analytical grade chemicals were purchased from Nice chemicals, Thrissur.

Microbiological media

Plate count agar, Violet Red Bile Agar and Potato Dextrose Agar procured from Himedia, Mumbai.

Packaging material

LDPE pouches of size 10 cm x 5 cm was purchased from Capricorn Polymer Packaging, Arimbur, Kerala. The pouches were rinsed with 30 per cent hydrogen peroxide solution followed by hot air blast at 80°C and then subjected to UV irradiation for sterilisation.

Preparation of paneer

Paneer was prepared as per procedure by Bhattacharya *et al*. (1971) using buffalo milk. The paneer block was then cooled and immersed in chilled water for one hour for texturisation. Later it was cut into uniform pieces of dimensions $5cm^3$.

Preparation of edible coating

Edible coating was prepared as per the procedure by Ramos *et al.* (2012) with slight modifications. Whey was vacuum concentrated using vacuum evapourator Anhydro, Lab E, 1688 to 45per cent total solids (TS). To this glycerol was added at level four per cent as plasticizer. The coating mixture was magnetically stirred for one hour. This was then heated in a water bath at 80°C for 20 min and subsequently cooled to room temperature. To this coating, antimicrobial solution of lactic acid and anti-fungal solution of potassium sorbate

each of 25 per cent concentration were added at 12 per cent level of coating mixture.

Application of edible coatings

The uniformly cut paneer pieces, were dipped completely into the edible coating solutions using sterilized tongs. The samples were then dried at room temperature for an hour. The coated paneer samples were aseptically packed into the previously sterilized pouches and sealed under normal and vacuum conditions.

Proximate composition

Moisture and total solids in paneer samples were determined as per Sachdeva (1983). pH of the sample was estimated as per the procedure followed by O' Keeffee *et al*. (1976). The titratable acidity was determined by the method recommended by AOAC (1990) for cheese.

Sensory analysis

The paneer samples were evaluated organoleptically for different quality attributes like flavour, body and texture, colour and appearance and overall acceptability by a selected panel of judges comprising of five members. A nine-point hedonic scale score card was used for evaluation Mishra *et al*. (2016).

Microbiological quality

Standard plate count (SPC) of each paneer sample was estimated by pour plate technique, as described by Mortan (2001). Coliform count and yeast and mould counts of each paneer sample were enumerated using the method given in IS: 1224, 1981.

Storage studies

Sensory evaluation was performed for normal packed and vacuum-packed samples stored at room temperature. The free fatty acid (FFA) content of paneer samples was determined by extraction titration method suggested by Deeth *et al.* (1975). Thiobarbituric acid (TBA) value of paneer sample was determined according to the method recommended by Sidwell *et al.* (1955). Tyrosine value of paneer was estimated as per the modified method of Juffs (1973). Microbiological parameters like standard plate count, coliform count, yeast and mould count were also enumerated during storage. Paneer samples were analysed daily for a period of five days when stored at room temperature.

Statistical analysis

The statistically analysis of data for physico-chemical changes and microbiological changes were performed using repeated measures ANOVA and pair wise comparison was done using t-test. The data obtained from sensory analysis was statistically analysed using Friedman's test and Mann Whitney U test. All the statistical analysis was performed employing suitable computer packages under the guidance of a statistician.

Results and discussion

Storage studies

In the present study storage stability of paneer coated with whey based edible coating was studied at room temperature storage under atmospheric and vacuum packaged conditions. The results of physico-chemical changes, microbiological changes and sensory changes are represented in Tables 1, 2 and 3 respectively.

Changes in pH and acidity

The pH of the atmospheric packaged and vacuum packaged samples changed from initial mean value of 5.95 to 5.78 and 5.95 to 5.79 respectively over a period of four days storage at room temperature. The F values for pH in both the samples were significant ($p \leq 0.01$) during the storage period. The acidity of the atmospheric and vacuum packaged samples changed from initial mean value of 0.21 to 0.28 and 0.20 to 0.27 percentage lactic acid over four days at room temperature. A significant difference ($p \leq 0.05$) for atmospheric packaged samples and significant difference vacuum packaged samples ($p \leq 0.01$) was also observed. Khatkar *et al.* (2017) reported that paneer gave a titratable acidity of 0.25 percentage lactic

Table 1: Changes in physico-chemical attributes of coated paneer stored at room temperature

Sample	DAYS OF STORAGE						
	0	1	2	3	4	5	F value
A	CHANGE IN pH DURING STORAGE						
Atmospheric packaging	5.95± 0.008^{a}	5.94 ± 0.006^{a}	5.94 ± 0.006^{a}	5.87 ± 0.008^{b}	5.78 ± 0.006^{c}	spoiled	105.05**
Vacuum packaging	5.95 ± 0.003^{a}	5.95 ± 0.003^{a}	5.94 ± 0.003^{a}	5.88 ± 0.01^{b}	5.79 ± 0.005^{b}	spoiled	179.62**
t- value	0.00^{ns}	-1.34^{ns}	-1.00^{ns}	-0.66^{ns}	-0.38^{ns}	-	
B	CHANGE IN ACIDITY DURING STORAGE (per cent LACTIC ACID)						
Atmospheric packaging	0.21 ± 0.008^{a}	0.22 ± 0.006^{a}	0.23 ± 0.008^{a}	0.24 ± 0.006^{ab}	0.26 ± 0.006^{b}	spoiled	10.21*
Vacuum packaging	0.20 ± 0.003^{a}	0.20 ± 0.003^{ab}	0.21 ± 0.003^{a}	0.24 ± 0.018^{a}	0.27 ± 0.009^{b}	spoiled	88.86**
t- value	1.02^{ns}	2.68^{ns}	1.41^{ns}	1.20^{ns}	1.77^{ns}		
C	CHANGE IN TOTAL SOLIDS DURING STORAGE (per cent)						
Atmospheric packaging	41.21 ± 0.12	41.22 ± 0.11	41.35± 0.10	41.61 ± 0.20	41.79 ± 0.23	spoiled	2.30^{ns}
Vacuum packaging	41.21± 0.10	41.21 ± 0.11	41.27± 0.13	41.42± 0.04	41.42± 0.04	spoiled	1.19^{ns}
t- value	0.00^{ns}	0.04^{ns}	0.48^{ns}	0.93^{ns}	1.72^{ns}	-	
D	CHANGE IN MOISTURE CONTENT DURING STORAGE (per cent)						
Atmospheric packaging	58.79 ± 0.10	58.77 ± 0.11	58.65 ± 0.10	58.39± 0.20	58.20 ± 0.21	spoiled	2.30^{ns}
Vacuum packaging	58.79 ± 0.11	58.78 ± 0.11	58.73± 0.13	58.58 ± 0.03	58.58 ± 0.06	spoiled	1.19^{ns}
t- value	0.00^{ns}	-0.04^{ns}	-0.48^{ns}	-0.93^{ns}	-1.72^{ns}		
E	CHANGE IN TBA VALUE DURING STORAGE (mg MDA / Kg)						
Atmospheric packaging	0.015± 0.002	0.015± 0.003	0.0157± 0.002	0.0203± 0.005	0.024± 0.003	spoiled	2.16^{ns}
Vacuum packaging	0.015 ± 0.002	0.015 ± 0.025	0.015± 0.002	0.015 ± 0.002	0.020 ± 0.004	spoiled	4.83^{ns}
t- value	0.00^{ns}	-0.09^{ns}	0.00^{ns}	0.85^{ns}	0.60^{ns}	-	
F	CHANGE IN TYROSINE DURING STORAGE (mg/100g)						
Atmospheric packaging	20.74 ± 0.07^{a}	20.97 ± 0.200^{a}	21.72 ± 0.233^{ab}	22.93 ± 0.29^{b}	24.35 ± 0.003^{c}	spoiled	59.51**
Vacuum packaging	20.74 ± 0.07^{a}	20.896 ± 0.11^{ab}	21.27± 0.133^{b}	22.10 ± 0.055^{c}	23.40 ± 0.005^{d}	spoiled	128.47**
t- value	0.00^{ns}	0.32^{ns}	1.69^{ns}	2.80^{ns}	142.00^{ns}		

^{ns}non significant ** significant at one percent level (p≤0.01)

*significant at five percent level (p≤0.05)

Figures are mean ± standard error of three replicates.

Means with different superscript (a, b, c and d) vary significantly within a row

Table 2: Changes in microbial attributes of coated paneer stored at room temperature

Sample	DAYS OF STORAGE						
	0	1	2	3	4	5	F value
A	CHANGE IN SPC (log_{10} cfu/g)						
Atmospheric packaging	2.20 ± 0.10^{a}	2.36± 0.058^{a}	2.63± 0.032^{b}	3.10± 0.10^{c}	3.72± 0.026^{d}	spoiled	161.39**
Vacuum packaging	2.10 ± 0.10^{a}	2.40 ± 0.099^{b}	2.89 ± 0.29^{abcd}	3.00 ± 0.00^{c}	3.66 ± 0.032^{d}	spoiled	22.67**
t- value	0.71^{ns}	-0.35^{ns}	-0.89^{ns}	1.00^{ns}	1.05^{ns}		
B	CHANGE IN COLIFROM (log_{10} cfu/g)						
Atmospheric packaging	nil	nil	nil	2.18 ± 0.066^{a}	2.63 ± 0.047^{b}	spoiled	319.74**
Vacuum packaging	nil	nil	nil	nil	nil	spoiled	-
t- value	-	-	-	-	-	-	-
C	CHANGE IN YEAST AND MOLD (log_{10} cfu/g)						
Atmospheric packaging	2.100 ± 0.10	2.10 ± 0.10	2.2± 0.10	2.3± 0.00	2.42± 0.058	spoiled	2.52^{ns}
Vacuum packaging	2.1 ± 0.10	2.0 ± 0.00	2.10± 0.10	2.100 ± 0.10	2.30 ± 0.00	spoiled	2.00^{ns}
t- value	0.00^{ns}	1.00^{ns}	0.71^{ns}	2.00^{ns}	2.00^{ns}		

^{ns} non significant ** significant at one percent level (p≤0.01) *significant at five percent level (p≤0.05)

Figures are mean±standard error of three replicates.

Means with different superscript (a, b, c and d) vary significantly within a row

acid when stored at refrigerated temperature for four days. The values for titratable acidity of paneer samples under study stored at room temperature were found to concur with titratable acidity values of paneer stored at refrigerated temperature. This strongly reveals the effectiveness of antimicrobial coating in retarding microbial fermentation of lactose at room temperature.

Changes in moisture and total solids

The moisture values changed from initial mean value of 58.79 per cent to 58.20 per cent percent for atmospheric packaged samples and from 58.79 per cent to 58.58 per cent for vacuum packaged samples over period of four days. The total solids content changed from 41.21 to 41.79 per cent for atmospheric packaged samples and from 41.21 to 41.42 per cent for vacuum packaged samples. No significant differences for F values of moisture and total solids were found in atmospheric and vacuum packaged samples. The findings suggest that the samples coated with the edible coating under study did not experience significant moisture loss. This indicates that the coatings assist in reducing moisture loss in paneer. Khwaldia *et al.* (2004) observed that whey protein films most effectively limit the water vapor condensation in fruit and vegetable packaging, thereby, restricting microbial spoilage.

Changes in TBA Value

The degree of lipid oxidation during storage is measured by using the TBA value. The TBA values for atmospheric packaged samples showed an increasing trend from the second day of storage while the same was observed for vacuum packaged sample on the fourth day. The TBA values for atmospheric and vacuum packaged samples changed from initial mean value of 0.015 to 0.024 mgMDA/kg and from 0.015 to 0.020 mgMDA/kg respectively on the fourth day of storage at ambient conditions. These results are consistent with the findings of

Table 3: Changes in sensory characteristics of coated paneer stored at room temperature

Sample	DAYS OF STORAGE						
	0	1	2	3	4	5	Chi square value
A	CHANGE IN FLAVOUR						
Atmospheric packaging	8.33 ± 0.166^{a}	8.33± 0.166^{abc}	7.916 ± 0.08^{abc}	7.50 ± 0.38^{bc}	7.166 ± 0.166^{c}	spoiled	10.32*
Vacuum packaging	8.583± 0.08^{a}	8.583 ± 0.083^{abc}	8.08± 0.22^{abc}	7.75 ± 0.144^{bc}	7.33± 0.166^{c}	spoiled	11.09*
Z- value	-1.29^{ns}	-1.29^{ns}	-0.48^{ns}	-0.23^{ns}	-0.75^{ns}		
B	CHANGE IN BODY AND TEXTURE						
Atmospheric packaging	8.25± 0.26^{a}	8.25 ± 0.25^{a}	7.91 ± 0.08^{ab}	7.58± 0.08a^{b}	7.166 ± 0.166^{b}	spoiled	10.44*
Vacuum packaging	8.33 ± 0.166^{a}	8.33 ± 0.17^{a}	7.92 ± 0.08^{ab}	7.66 ± 0.08^{b}	7.33± 0.166^{b}	spoiled	11.21*
Z- value	-0.26^{ns}	-0.25^{ns}	0.00^{ns}	-0.75^{ns}	-0.75^{ns}		
C	CHANGE IN COLOUR AND APPEARANCE						
Atmospheric packaging	8.16± 0.166^{a}	8.16± 0.166^{a}	7.916 ± 0.08^{ab}	7.416 ± 0.33^{ab}	6.58 ± 0.08^{b}	spoiled	11.25*
Vacuum packaging	8.33 ± 0.166 ^{a}	8.17± 0.17^{a}	8.08± 0.22^{ab}	7.66 ± 0.08^{ab}	6.75 ± 0.144^{b}	spoiled	10.94*
Z- value	-0.75^{ns}	0.00^{ns}	-0.47^{ns}	-0.26^{ns}	-0.94^{ns}		
D	CHANGE IN OVERALL ACCEPTABILITY						
Atmospheric packaging	8.25 ± 0.25^{a}	7.91± 0.36^{a}	7.66± 0.33^{a}	7.50 ± 0.25^{a}	7.25 ± 0.25^{a}	spoiled	7.93^{ns}
Vacuum packaging	8.33 ± 0.17^{a}	8.17 ± 0.17^{a}	7.92± 0.08^{ab}	7.67 ± 0.08^{ab}	7.33 ± 0.30^{b}	spoiled	10.50*
Z- value	-0.26^{ns}	-0.47^{ns}	-0.26^{ns}	-0.26^{ns}	-0.47^{ns}		

^{ns} non significant ** significant at one percent level (p≤0.01)

*significant at five percent level (p≤0.05)

Figures are mean±standard error of three replicates.

Means with different superscript (a, b, c) vary significantly within a row

Bukhari *et al*. (2012) who also reported a similar increase in TBA value of Kaladhi (hard cheese variety), during storage for 28 days.

Changes in Tyrosine values

The extent of proteolysis during storage was measured using tyrosine value. The tyrosine value showed an increasing trend over the four days of storage. The values for atmospheric packaged samples increased from initial mean value of 20.74 to 24.35 mg/100ml while that for vacuum packaged increased from 20.74 to 23.40 mg/100ml during the storage period. F values show significant difference (p≤0.01) on four days of storage for atmospheric and vacuum packaged samples. However independent t test shows no significant difference between the values obtained for atmospheric packaged and vacuum packaged samples. The increase in tyrosine value noted during storage mainly results from autolysis within the paneer structure and, to a lesser extent, bacterial activity. The results are in agreement with the observations for changes in tyrosine content of paneer tikka during storage

by Ahuja *et. al*. (2013).

Changes in Free Fatty Acids value (FFA)

Lipolysis associated with the product during storage was measured by using FFA values. The FFA values showed an increase from initial mean value of 1.433 to 1.87µeq/g for atmospheric packaged samples and an increase from 1.43 to 1.77µeq/g for vacuum packaged samples during its storage period of four days at room temperature. Significant difference ($p \leq 0.05$) for atmospheric packaged samples was noticed. F value vacuum packaged samples also showed significant difference ($p \leq 0.01$). There was no significant difference between the atmospheric packaged and vacuum packaged samples on analysis using independent t test. Similar observations were made Bukhari *et al*. (2012) in Kaladhi.

Changes in microbiological attributes

The standard plate count (SPC), coliform count and yeast and mold count showed an increasing trend over the course of storage at room temperature. The SPC count increased from 2.20 to 3.72$\log_{10}$cfu/g during four days of storage for the atmospheric packaged samples. For the vacuum packaged samples the increase was from 2.10 to 3.66 $\log_{10}$cfu/g. The values obtained during four days showed significant difference ($p \leq 0.01$) for both atmospheric packaged and vacuum packaged samples. But no significant difference between the values obtained for atmospheric and vacuum packaged samples were observed. The coliform count was absent for atmospheric packaged samples until the second day of storage. However, the count increased from 2.18$\log_{10}$ cfu/g on the third day to 2.63$\log_{10}$cfu/g on the fourth day. The coliform count was absent in vacuum packaged samples for three days of storage but the samples showed unsatisfactory count on the fourth day. The yeast and mold count also displayed an increasing trend. The counts for atmospheric packaged samples increased from initial mean value of 2.10$\log_{10}$cfu/g to 2.42$\log_{10}$cfu/g at the end of fourth day. The counts for vacuum packaged samples increased from initial mean value of 2.10$\log_{10}$cfu/g to 2.30$\log_{10}$cfu/g during the storage period. An increase SPC count from 3.9$\log_{10}$cfu/g to 4.39$\log_{10}$cfu/g was reported by Khatkar et al. (2017) for paneer samples stored at refrigerated temperature over three days. In the present study the SPC count of paneer sample was found to be comparatively lower even when stored at room temperature. This strongly indicates that the antimicrobial edible coating hinders the growth and spread of microorganisms.

Changes in sensory attributes during storage

The values for flavour, body and texture, colour and appearance and overall acceptability showed significant difference ($p \leq 0.05$) for both atmospheric and vacuum packaged samples. The flavour scores reduced from initial mean value of 8.33 to 7.166 on the fourth day of storage at ambient temperature for atmospheric packaged samples. For the vacuum packaged samples, the flavour scores reduced from initial mean value of 8.583 to 7.33 during its storage period. The scores for body and texture decreased from initial mean value of 8.25 to 7.166 for atmospheric packaged samples and from initial mean value of 8.33 to 7.33 for vacuum packaged samples on the fourth day of storage. Colour and appearance score reduced from initial mean value of 8.16 to 6.58 for atmospheric packaged samples and from initial mean value of 8.33 to 6.75 for vacuum packaged samples on the fourth day of storage. Overall acceptability values on the initial day were 8.25 and 8.33 for atmospheric and vacuum packaged samples respectively. However, this reduced to 7.25 and 7.33 respectively on the fourth day of room temperature storage. Dwivedi *et al.* (2014) had reported similar decreasing trend in sensory scores of paneer samples during storage.

Conclusion

Microbiological analysis revealed that paneer coated with the optimized coating maintained satisfactory microbial counts (total viable count, coliforms, yeast, and mold) within FSSAI guidelines for four days at room temperature. Subsequently, the coated paneer samples were packaged under atmospheric and vacuum conditions and stored at room temperature. Storage studies included

monitoring changes in physicochemical characteristics (pH, moisture content, total solids, TBA value, free fatty acids, and tyrosine), microbiological attributes (total viable count, coliforms, yeast, and mold), and sensory characteristics (flavor, body and texture, color and appearance, and overall acceptability). Based on the observed changes in physicochemical, microbiological, and sensory attributes during storage at room temperature, it was concluded that the coated paneer samples were well preserved for four days. By the fifth day, microbial counts exceeded FSSAI limits, and a slightly objectionable odor developed. Therefore, applying whey-based edible coatings to freshly prepared paneer samples is recommended to significantly extend their shelf life. Although samples packaged under vacuum conditions showed lower microbial counts compared to those packaged atmospherically, this packaging technique did not extend the product's shelf life beyond four days at room temperature.

Conflict of interest

The authors declare that they have no conflict of interest.

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