Biological Forum – An International Journal **15(5): 1310-1317(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Extension of Shelf Life and Enhancement of Antioxidant Activity in Paneer using a Casein based Coating with Clove Bud Essential Oil

Archana S.[1*], Divya K.B.[2] and Anjali M.K.[1]
[1]Teaching Assistant,
College of Dairy Science and Technology, Wayanad (Kerala), India.
[2]Assistant Professor, Dept of Dairy Technology,
College of Dairy Science and Technology (Kerala), India.

(Corresponding author: Archana S.)*
(Received: 07 March 2023; Revised: 04 April 2023; Accepted: 14 April 2023; Published: 20 May 2023)
(Published by Research Trend)

ABSTRACT: Although many studies have been conducted on the development of edible food packaging systems, there is limited research specifically focusing on coating paneer with a casein-based edible coating and its potential for extending the shelf life of the product. Casein-based edible coating incorporated with clove bud essential was applied to paneer in order to improve its limited shelf life. The coating solution was prepared by adding sodium caseinate, plasticizer (glycerol), pectin and clove bud essential oil at levels 13%, 3%, 1.25% and 0.3% respectively. The coated paneer along with control sample were tested for its sensory appeal, physico chemical qualities and microbial qualities during refrigerated storage which led to the conclusion that coated paneer showed longer shelf life (nine days) than the control sample (six days). Also, the coated paneer had good anti-oxidant property (IC 50- 5878 mg/Kg) initially when compared to the IC 50 value of control paneer (10340 mg/Kg) which kept decreasing on storage. Hence edible coating, apart from protecting the product from spoilage, has proven to be active agent of delivering anti-oxidants to the product, which is a relevant topic for further research.

Keywords: Sodium caseinate, Paneer, Clove bud essential oil, Shelf life, Anti-oxidant activity.

INTRODUCTION

As customer demand for minimally processed foods has grown in recent years, the packaging industry has also begun to investigate novel solutions. Although synthetic packaging material has advanced to reduce the mass transfer between the food product and the surroundings, it also has detrimental effects on the environment, including marine, air, and soil pollution (Kibria *et al.*, 2023). Edible films and coatings, based on biopolymers such as proteins, lipids, and carbohydrates are considered one of the solutions that can be used to replace plastic packaging materials fully or partially (Galus and Kadziska 2015). The significance of edible materials as value-added components and as protective agents in food systems has recently been the subject of numerous research.

Casein, a milk protein, can produce stable films that resist coagulation and denaturation even at high temperatures, making them suitable for a wide range of pH and temperature conditions. Caseinates, especially sodium caseinate and calcium caseinate, with their random coiled structure, readily form films from aqueous solutions, exhibiting strong intermolecular cohesion through the formation of hydrophobic, electrostatic, and hydrogen bonds (Khwaldia *et al.*, 2004). To enhance the mechanical properties and flexibility of casein-based films, plasticizers such as glycerol are commonly added to the coating solution (Hong and Krochta 2006). These films are produced through casting or extrusion methods for films and solution casting for coatings These coatings are typically applied to fruits and vegetables using either dipping or spraying techniques (Khan *et al.*, 2021). However, caseinate-based films exhibit poor water barrier properties, which can be improved by incorporating a lipid component into the film-forming solutions (Fabra *et al.*, 2008; Atarés *et al.*, 2010; Matsakidou *et al*., 2013).

Incorporating bioactive lipid components like essential oils rich in compounds such as cinnamamic aldehyde and eugenol into dairy products prevents oxidative damage, enhances antioxidant properties, and improves health benefits. To overcome the strong flavor and hydrophobic nature of essential oils, a compatible carrying system needs to be developed for their incorporation into dairy products without compromising sensory attributes. Clove bud essential oil integration into pectin-based biodegradable films affects mechanical, antioxidant, barrier, and antimicrobial properties (Nisar *et al.*, 2018). Essential oil incorporation in edible films and coatings allows for

sustained release of bioactive components during storage and are proven to be effective in increasing the shelf life of perishable dairy products like paneer (Karunamayi *et al.,* 2020; Lamdande *et al.,* 2012; Raju and Sasikala 2016; Azadbakht *et al.,* 2018; Issa *et al.,* 2017).

Paneer, a traditional milk product, is prepared by coagulating milk solids with organic acid, followed by curd pressing, and it has a limited refrigerated shelf life of approximately six days (Reeta *et al.,* 2012; Khatkar *et al*., 2017). The use of sodium caseinate edible films infused with plant essential oils, such as Laurel or oregano essential oil, has been demonstrated to effectively extend the shelf life of cheese. This highlights the efficacy of these films as carriers of active compounds, providing improved shelf life of the product (Alvarez *et al*., 2019).

In the present study, a casein-based edible coating infused with essential oil was utilized as an alternative approach to prolong the shelf life of paneer. The investigation involved assessing the physicochemical and microbiological changes that occurred during storage, as well as analyzing the antioxidant capacity of the coated paneer and monitoring its changes over time.

MATERIAL AND METHODS

Raw materials. The current research was conducted at the College of Dairy Science and Technology in Mannuthy, Thrissur, at the Dairy Technology Department. The University Dairy Plant, KVASU, Thrissur, supplied milk for the study. For sodium caseinate preparation, Sagar brand (Amul) skimmed milk powder was utilised. CKS Products in Ernakulam, Kerala, delivered glycerol and pectin required for the study. Synthite Private Ltd, Ernakulam were kind enough to supply Essential oils required for the study free of cost. Merck India Pvt Ltd. and Sigma Aldrich provided the chemicals and Himedia, Mumbai supplied the microbiological media. The packaging material for the storage studies was procured from the local market in Thrissur.

Application of edible coating on paneer. Paneer was made as per the procedure given by Bhattacharya *et al.* (1971). Sodium caseinate was prepared from skim milk as per the procedure given by Sarode *et al.* (2016), and the edible coating was prepared as per the procedure by Bonnaillie *et al*. (2014) with minor modifications.

The level of ingredients for edible coating was optimized during Response Surface Methodology using Design expert software and the optimum combination obtained was glycerol (3 percent), 3% pectin solution (1.25 percent), and clove bud essential oil (0.3 percent). (Archana, 2021). The sodium caseinate solution with added ingredients was stirred, and paneer cubes were dipped, dried, and packed in sterilized LLDPE pouches. Samples were stored at refrigerated temperature (7±1°C) and examined for sensory, chemical, and microbiological parameters in 4 replications at 3 days intervals at intervals.

Shelf-life analysis. The coated paneer samples under refrigeration (7±1°C) were subjected to sensory, physico-chemical and microbiological examination in 4 replications at 3 days intervals.

Sensory evaluation. The paneer samples were evaluated organoleptically for different quality attributes like flavor, body and texture, color and appearance and overall acceptability by a selected panel of judges comprising of five members. The paneer was evaluated in raw form. A hedonic scale score card (9-point) was used for evaluation (Annexure).

Physico-chemical analysis. The initial compositional analysis of the coated paneer along with control was done. The pH of the paneer sample was determined by blending 10g of paneer with 10 ml of distilled water and dipping the pH electrode (Eutech, Model- EC510). The titratable acidity was determined by the method recommended by AOAC (1990) for cheese. The water activity of the sample was measured using a water activity meter (AQUA LAB). The paneer coated with casein based edible coating was subjected to Thiobarbituric Acid (TBArase) value test according to the method recommended by Sidwell *et al.* (1955), Free Fatty Acid test by extraction titration method suggested by Deeth (1975) and Tyrosine value test as per the modified method of Juffs (1973).

Anti-oxidant activity. The antioxidant activity of coated paneer during refrigerated storage was measured using the method suggested by Brand *et al.* (1995). The free radical scavenging activity of the sample was determined based on the stable free radical (DPPH) with an anti-oxidant component in the media resulting in the bleaching of the DPPH due to its quenching by the interaction with the analytes. The decrease in absorbance of DPPH compared to blank measured spectrophotometrically at 516nm related to the concentration of anti-oxidant in the test solution.

Microbiological evaluation. Total viable count of each paneer sample was estimated by pour plate technique, as described by Mortan (2001) while the Coliform and yeast and mould count of each paneer sample was estimated by IS: 1224, 1981.

Experimental design. Each experiment was repeated in 3 days in four replications and the results are expressed as the means with standard errors. The comparison between the periods within each sample was done using Repeated measures ANOVA while the comparison between the samples in each period was done using Independent t-test. The statistical analysis were performed using IBM SPSS STATISTICS (version 26).

RESULTS AND DISCUSSION

Shelf life analysis

Changes in sensory quality of coated paneer during refrigerated storage (7 ± 1°C). The sensory scores (Table 1) of coated and control paneer declined progressively during refrigerated storage (7±1°C).

Flavor. The flavor score for control paneer declined from an initial mean value of 8.58 to 6.23 at the end of the 6th day of storage. However, coated paneer showed a similar decline only after 9 days of refrigeration. Statistical analysis revealed significant differences in

flavor scores between the control group and the coated paneer group at multiple time points. Specifically, the control group exhibited significant differences ($p>0.05$) in flavor scores between the 0th and 3rd day, and between the 3rd and 6th day. Meanwhile, the coated paneer showed significant differences ($p>0.05$) in flavor scores between the 0th and 3rd day, the 3rd and 6th day, and the 6th and 9th day.

Color and appearance. The color and appearance scores of both the coated and control paneer declined during refrigerated storage (7±1°C). Specifically, the score of control paneer decreased from 8.50 to 6.29 by the end of the 6th day, with a significant difference ($p<0.05$) observed between the 0th and 3rd day of storage. In the case of the coated paneer, the initial sensory score of 7.95 decreased to 6.59 after 9 days of refrigerated storage, and significant differences ($p<0.05$) were observed between scores on the 3rd and 6th day, as well as between the 6th and 9th day of storage.

Body and Texture. A progressive decline in body and texture scores for both the control and coated paneer was observed during refrigerated storage (7±1°C). In particular, the control paneer showed a drop from an initial score of 8.46 to 6.04 over the course of six days, with significant differences ($p <0.05$) found between the scores of 0th and 3rd day and the 3rd day and 6th day. The scores for coated paneer coating also exhibited a gradual decrease, decreasing from an initial score of 8.09 to 6.19 within a 9-day period of refrigerated storage. Statistical analysis indicated significant differences ($p<0.05$) in body and texture scores between the time periods of refrigerated storage, specifically between the 0th and 3rd day, 3rd and 6th day, and 6th and 9th day.

Overall acceptability. The overall acceptability scores revealed that the control paneer exhibited a decline in scores from 8.51 to 6.13 within 6 days of refrigerated storage at 7±1°C, with a significant difference ($p<0.05$) observed between the time periods of 0th and 3rd day, and 3rd day and 6th day. Similarly, the scores for coated paneer decreased from an initial score of 8.03 to 6.25 within 9 days of refrigerated storage at 7±1°C. The statistical analysis showed significant differences ($p<0.05$) between the time periods of 0th and 3rd day, 3rd and 6th day, and 6th and 9th day during refrigerated storage at 7±1°C.

From the sensory analysis, it can be concluded that the sensory scores for both the samples declined during refrigerated storage (7±1°C) however, the rate of decline in score was lesser for coated paneer when compared to the control. The decline in flavour scores for both coated and control paneer during refrigerated storage can be attributed to factors such as microbial growth which can cause off flavors, odors, and discoloration (Kumar *et al.*, 2015). Also, the breakdown of proteins and lipids in paneer can contribute to deleterious changes in flavor, color, and texture (Singh *et al.*, 2016). However, the longer acceptability of coated paneer over control could be attributed to adding clove bud essential oil, an antimicrobial agent to the coating (Kumar *et al*., 2015). The presence of this may have helped to prevent the spoilage organisms thereby improving its sensory properties. Similar observations in the sensory score were reported for paneer coated with whey protein-based edible coating with cinnamon oil up to the level of 1% by Punnagaiarasi *et al.* (2016).

Physico-chemical changes during storage. Changes in Moisture. A gradual decrease in the moisture content was seen in coated paneer as well as control during refrigerated storage (7±1°C) (Table 2). However, a significant difference ($p<0.05$) in moisture was observed between the samples only after the 6th day of storage. Throughout the refrigerated storage, the moisture content of the paneer consistently declined, which is likely due to moisture loss to the surrounding environment. Nevertheless, the presence of the coating on the paneer's surface acted as a physical barrier, potentially slowing down the rate of moisture expulsion when compared to the uncoated paneer (Singh *et al.*, 2021).

Changes in pH and Acidity. During refrigerated storage (7±1°C), significant differences ($p<0.05$) in pH and acidity were observed between coated paneer and the control, indicating that storage duration had a significant impact on both parameters. Makhal *et al.* (2014) found a similar decrease in pH for thymol-infused cottage cheese at a slower rate compared to the control under refrigerated conditions (4±1°C). Khatkar *et al.* (2017) observed a comparable trend in acidity for clove-treated paneer, aiming to extend its shelf life. Karunamay *et al.* (2020) found that using a starch-based film with clove and oregano essential oil on paneer resulted in slower pH changes and increased acidity. This was due to the antimicrobial effects of clove bud essential oil, which inhibited lactose fermenting organisms and slowed down the pH decline and acidity increase in the coated paneer.

Changes in Water Activity. Paneer is considered highly perishable due to its high water activity. Table 2 illustrates the findings regarding changes in the water activity of both coated paneer and the control sample during refrigerated storage (7±1°C). The water activity of control paneer increased from 0.98 to 0.99 significantly ($p<0.05$) at the end of 6 day of refrigerated storage. Coated paneer had initial water activity of 0.88 which increased to 0.94 significantly ($p<0.05$) from 3rd day of refrigerated storage. Water activity of control and coated paneer increased during storage but the rate of increase was found to be lesser for latter. This can be attributed to the protective barrier property of sodium caseinate based coating on it. Mishra *et al.* (2017) reported a similar increase in water activity during in paneer when stored at refrigerated temperature (7±1°C).

Changes in Thiobarbituric acid value. The TBA^{rase} value measures the extent of lipid oxidation in coated paneer during refrigerated storage (7±1°C) (Dorsey and Jones, 2017). The changes in TBA^{rase} value of coated paneer and control stored at refrigerated temperature (7±1°C) are given in Table 2.

Table 1: Effect of refrigerated storage (7 ± 1°C) on the sensory quality of paneer coated with casein based edible coating.

Attributes	Sample	Days of Storage					
		0	3	6	9	12	Chi square value
Flavor	Control	8.58 ± 0.153^{xa}	7.33 ± 0.166^{xab}	6.225 ± 0.103^{xb}	SPOILED	SPOILED	12**
	Coated paneer	8 ± 0.170^{ya}	7.74 ± 0.108^{xab}	7.29 ± 0.100^{yb}	6.175 ± 0.090^{yb}	SPOILED	14.63**
	Z value	-2.163*	-1.686 ^{ns}	-2.934 **			
Color and appearance	Control	8.5 ± 0.129^{xa}	8.00 ± 0.105^{xa}	6.29 ± 0.100^{xb}	SPOILED	SPOILED	11.56**
	Coated paneer	7.95 ± 0.100^{ya}	7.88 ± 0.083^{xa}	7.33 ± 0.083^{yab}	6.59 ± 0.152^{yb}	SPOILED	16.44**
	Z value	-2.539*	-0.172 ^{ns}	-2.923 **			
Body and Texture	Coated paneer	8.09 ± 0.100^{xa}	7.77 ± 0.103^{xab}	7.20 ± 0.100^{yb}	6.19 ± 0.098^{yb}	SPOILED	17.58**
	Control	8.46 ± 0.181^{xa}	7.93 ± 0.092^{xab}	6.04 ± 0.041^{xb}	SPOILED	SPOILED	11.27**
	Z value	-1.639 ^{ns}	-0.948 ^{ns}	-3.017**			
Overall Acceptability	Control	8.51 ± 0.129^{xa}	8.04 ± 0.041^{xab}	6.125 ± 0.055^{xb}	SPOILED	SPOILED	11.56**
	Coated paneer	8.025±0.078^{ya}	7.83 ± 0.105^{xa}	7.25 ± 0.091^{xab}	6.25 ± 0.091^{yb}	SPOILED	16.93**
	Z value	-2.351*	-1.687 ^{ns}	-2.939 **			

Figures are mean ± standard error of three replications, **-Significant at one per cent level (p<0.01), a-b - Means with different superscript vary significantly within a row, x-y Mean with different superscripts vary significantly within a column.

Table 2: Effect of refrigerated storage (7 ± 1°C) on the physico chemical properties of paneer coated with casein based edible coating.

Days	DAYS OF STORAGE					F value
	0	3	6	9	12	
A	CHANGE IN MOISTURE DURING STORAGE (percent)					
Control	55.82 ±0.168^{xa}	55.39 ± 0.155^{xa}	54.94 ±0.228^{xb}	SPOILED	SPOILED	8.72**
Coated paneer	55.93 ± 0.233^{xa}	55.645 ± 0.229^{xa}	55.435 ±0.148^{ya}	54.882 ±0.283^{yab}	SPOILED	4.87*
t – value	0.41^{ns}	1.41^{ns}	2.56*			
C	CHANGE IN pH DURING STORAGE					
Control	5.75±0.014^{xa}	5.65±0.013^{xb}	5.48±0.024^{xc}	SPOILED	SPOILED	76.60**
Coated paneer	5.78±0.01^{xa}	5.69±0.015^{yb}	5.61±0.019^{yc}	5.54±0.020^{yd}	SPOILED	75.32**
t – value	1.26^{ns}	3.005*	4.079**			
D	CHANGE IN ACIDITY DURING STORAGE (per cent LACTIC ACID)					
Control	0.22±0.006^{xa}	0.26±0.002^{xb}	0.31±0.004^{xc}	SPOILED	SPOILED	86.59**
Coated paneer	0.23±0.008^{xa}	0.26±0.002^{xb}	0.28±0.002^{yc}	0.29±0.002^{yd}	SPOILED	32.19**
t- value	1.115^{ns}	-0.961^{ns}	-6.325**			

Figures are mean ± standard error of three replications, *-Significant at five per cent level (p<0.05), **-Significant at one per cent level (p<0.01), $^{a-d-}$ Means with different superscript vary significantly within a row, $^{x-y}$Mean with different superscripts vary significantly within a column

Days	DAYS OF STORAGE					F value
	0	3	6	9	12	
E	CHANGE IN WATER ACTIVITY VALUE DURING STORAGE					
Control	0.98±0.002^{xa}	0.98±0.002^{xa}	0.992±0.001^{xab}	SPOILED	SPOILED	4.62*
Coated paneer	0.88±0.003^{ya}	0.895±0.002^{ya}	0.916±0.003^{yb}	0.945±0.002^{yc}	SPOILED	89.24**
t- value	-26.372**	-38.616**	-21.767**			
F	CHANGE IN TBA^{rase}VALUE DURING STORAGE (per cent)					
Control	0.012±0.000^{xa}	0.021±0.001^{xb}	0.023±0.0010^{xb}	SPOILED	SPOILED	26.57**
Coated paneer	0.011±0.0004^{xa}	0.015±0.001^{yb}	0.020±0.000^{xc}	0.025±0.000^{yd}	SPOILED	103.16**
t- value	-0.96 ^{ns}	-3.53**	-2.22^{ns}			
A	CHANGE IN FFA VALUE DURING STORAGE ((µeq/g))					
Control	0.547±0.009^{xa}	0.65±0.018^{xb}	0.761±0.007^{xc}	SPOILED	SPOILED	109.37**
Coated paneer	0.56±0.0146^{xa}	0.59±0.003^{ya}	0.669±0.008^{yb}	0.703±0.008^{yc}	SPOILED	91.09**
t value	1.039^{ns}	-5.511**	-7.94**			
B	CHANGE IN TYROSINE DURING STORAGE (mg/100ml)					
Control	22.23±0.539^{xa}	27.195±0.217^{xb}	33.313±0.48^{xc}	SPOILED	SPOILED	137.54**
Coated paneer	22.18±0.276^{xa}	25.08±0.166^{yb}	28.938±0.534^{yc}	31.37±0.418^{yd}	SPOILED	154.6**
t value	0.815^{ns}	-12.19**	-6.07**			

Figures are mean ± standard error of three replications, *-Significant at five per cent level (p<0.05), **-Significant at one per cent level (p<0.01), $^{a-d-}$ Means with different superscript vary significantly within a row, $^{x-y}$Mean with different superscripts vary significantly within a column

The significant ($p<0.05$) increase in TBArase value over the storage period for both the control and coated paneer suggests lipid oxidation occurred. The increase in TBA value during storage, suggests that a possible reduction in the protective effect of the coating has occured. In line with the findings, which indicated an increase in TBA value during storage, a study by Wanjari *et al.* (2019) found that different levels of garlic extract did not significantly impact the rate of lipid oxidation in paneer. This further supports the notion that the protective effect of the coating may have diminished over time, allowing increased lipid oxidation.

Changes in Free Fatty acids (FFA). The FFA (Free Fatty Acid) value is a measure of the degree of lipolysis, or the breakdown of fats, in food during refrigerated storage (7±1°C) (Dalvandi *et al*., 2020). Table 2 displays the changes in FFA (Free Fatty Acid) value for coated paneer and the control during refrigerated storage (7±1°C). Both samples showed significant increases ($p<0.05$) in FFA value, indicating lipolysis. However, the rate of increase was slower in the coated paneer, indicating reduced lipolysis compared to the control. On the 3rd and 6th day, significant differences ($p<0.05$) were observed between the samples, highlighting the protective effect of the coating in limiting lipolysis. The coating likely acted as a barrier, reducing fat hydrolysis and the release of free fatty acids. Gokhale *et al*. (2016) reported a similar increase in FFA value during refrigerated storage for partially dried paneer treated with citric acid, vinegar and lactic acid. The results obtained are also consistent with those reported by Khatkar *et al.* (2017).

Changes in Tyrosine Value. The tyrosine content is a measure of proteolysis in products high in protein during refrigerated storage (7±1°C) (Biswas *et al.*, 2017). During storage, both the control and coated paneer showed a significant increase ($p<0.05$) in tyrosine content, indicating proteolysis. However, the coated paneer had a lesser extent of increase compared to the control, which suggests reduced proteolysis in the treated product. Similar results were observed in studies with paneer incorporated with garlic extract (Wanjari *et al.*, 2019) and paneer coated with an edible film containing clove and oregano essential oil (Karunamay *et al*., 2020) during refrigerated storage. The reduction in proteolysis in the coated paneer could be attributed to the presence of bioactive compounds or inhibitory effects on proteolytic enzymes.

Changes in Microbial quality during storage. Significant differences ($p<0.05$) were observed in the Standard Plate Count (SPC), coliform count (CC), and yeast and mould count (YMC) of the coated paneer and the control throughout the refrigerated storage period (7±1°C), as shown in Table 3. However, the rate of increase in these microbial counts was lower for the coated paneer compared to the control.

Table 3: Effect of refrigerated storage (7 ± 1°C) on microbiological quality of paneer coated with casein based edible coating.

Days	DAYS OF STORAGE					
	0	3	6	9	12	F value
A	CHANGE IN SPC (log10cfu/g)					
Control	4.37 ± 0.077^{xa}	4.59 ± 0.036^{xb}	4.78 ± 0.015^{xc}	SPOILED	SPOILED	19.11**
Coated paneer	4.33 ± 0.063^{xa}	4.47 ± 0.031^{yb}	4.59 ± 0.019^{yc}	4.73 ± 0.025^{yd}	SPOILED	70.24**
t – value	-0.38^{ns}	-3.57**	-7.86**			
B	CHANGE IN COLIFROM COUNT (log10cfu/g)					
Control	0.47 ± 0.028^{xa}	0.53 ± 0.026^{xb}	0.606 ± 0.008^{xb}	SPOILED	SPOILED	10.87**
Coated paneer	0.42 ± 0.034^{xa}	0.47 ± 0.022^{xb}	0.504 ± 0.006^{yb}	0.636 ± 0.038^{yc}	SPOILED	9.94**
t – value	-0.28^{ns}	-1.82^{ns}	-9.70**			
C	CHANGE IN YEAST AND MOULD COUNT (log10cfu/g)					
Control	1.28 ± 0.024^{xa}	1.48 ± 0.027^{xb}	1.76 ± 0.019^{xc}	SPOILED	SPOILED	33.83**
Coated paneer	1.26 ± 0.011^{xa}	1.39 ± 0.023^{xb}	1.43 ± 0.034^{yb}	1.64 ± 0.022^{yc}	SPOILED	80.60**
t – value	0.81^{ns}	-2.55^{ns}	-8.31**			

Figures are mean ± standard error of three replications, **-Significant at one per cent level ($p<0.01$), $^{a-d}$Means with different superscript vary significantly within a row, $^{x-y}$ Mean with different superscripts vary significantly within a column

Table 4: Anti-oxidant activity IC 50 value of Coated paneer and control during refrigerated storage period (7±1°C).

Days	IC 50 VALUE (mg/Kg)					F value
	0	3	6	9	12	
Control	10340 ± 0.447^{a}	20980 ± 0.307^{b}	28422 ± 0.365^{c}	SPOILED	SPOILED	501120716.8**
Coated paneer	5878 ± 0.302^{a}	6964 ± 0.175^{a}	7150 ± 0.263^{a}	8230 ± 0.124^{a}	SPOILED	6590072.5**
t value	5734**	38384**	33633**			

Figures are mean ± standard error of three replications, **-Significant at one per cent level ($p<0.01$), $^{a-c}$Means with different superscript vary significantly within a row, $^{x-y}$Mean with different superscripts vary significantly within a column.

This improvement in microbiological quality of the coated paneer in comparison to control, can be attributed to the antimicrobial properties of clove bud essential oil, as reported by Khatkar *et al*. (2017). Similar findings were reported by Raju and Sasikala (2016) for paneer with a sodium alginate edible film incorporating cinnamon essential oil, and by Lamdande *et al*. (2012) for whey protein concentrate film coated

paneer, stored in LDPE packaging at refrigeration temperature (5 ± 1°C). Makhal *et al.* (2012) also observed increased microbial counts in cottage cheese incorporated with thymol during refrigerated storage (5 ± 1°C).

Changes in anti-oxidant potency during storage. The antioxidant activity of casein-based edible coated paneer during refrigerated storage (7 ± 1°C) is shown in Table 4. The antioxidant capacity of the sample was assessed using the IC50 value, which represents the concentration needed to inhibit a biological function by half. The IC50 values increased from 10340 mg/Kg to 28422 mg/Kg for the control and from 5878 mg/Kg to 7150 mg/Kg for the coated paneer over 6 and 9 days, respectively. Significant differences ($p<0.05$) in antioxidant potency were observed between the control and coated paneer throughout the storage period.

The results indicate that the coated paneer exhibited higher antioxidant activity compared to the control paneer throughout the storage period. This is supported by previous studies on clove oil, which demonstrated high antioxidant activity with a DPPH free radical scavenging activity (EC50) of 21.50 mg/Kg (Gulcin *et al*., 2010). Other studies have also highlighted the phenolic compounds present in clove bud essential oil, such as eugenol, ß-caryophyllene, and acettaugenol, which contribute to its antioxidant properties (Dashipoor *et al.,* 2014). Additionally, clove bud essential oil has shown significant inhibition of linoleic acid autoxidation (Binsi *et al*., 2017), and its incorporation in cakes has exhibited antioxidant activity during storage (Ibrahium *et al.,* 2013).

Overall, the findings suggest that the casein-based edible coating, particularly when incorporating clove bud essential oil, enhances the antioxidant activity of paneer, contributing to its oxidative stability during refrigerated storage.

CONCLUSIONS

The present study aimed to investigate the efficacy of a casein-based edible coating incorporated with clove bud essential oil in extending the shelf life of paneer, a traditional dairy product. The coated paneer samples were stored under refrigeration (7 ± 1°C), and an analysis of sensory evaluation, physicochemical characterization, antioxidant activity assessment, and microbiological evaluation was conducted at regular intervals throughout the storage period. The findings revealed that the paneer coated with the novel edible coating exhibited significantly slower sensory deterioration, including improved flavor, color, appearance, and texture attributes, compared to the uncoated control samples. Moreover, the coated paneer showed reduced moisture loss, decreased pH and acidity levels, and maintained lower water activity values during storage, thus effectively mitigating quality deterioration. Importantly, the incorporation of clove bud essential oil in the edible coating imparted remarkable antimicrobial properties, as indicated by the lower microbial counts in the coated samples. Consequently, the results highlight the potential of the casein-based edible coating with clove bud essential oil as an efficient approach for enhancing the shelf life of paneer while maintaining its sensory and nutritional attributes.

FUTURE SCOPE

In the future, further research can be conducted to explore the long-term stability and effectiveness of the casein-based edible coating with clove bud essential oil in extending the shelf life of paneer under different storage conditions, such as varying temperatures and packaging methods. Additionally, investigating the impact of the edible coating on the nutritional profile of paneer and conducting consumer acceptance studies would provide valuable insights for commercial implementation. Furthermore, exploring the potential application of this edible coating in extending the shelf life of other dairy products would broaden its practicality and market potential in the food industry.

Author contributions. All authors contributed substantially to the successful completion of the work. S. Archana: Investigation, methodology, data analysis, and writing of the original draft; K B Divya: Resources, Conceptualization, Supervision, Writing - Review and editing.

Conflict of Interest. None.

ANNEXURE

Score card for organoleptic evaluation of paneer with casein based edible coating. Please evaluate the samples on a 9-point Hedonic scale, for their sensory attributes of flavor, body and texture, color and appearance and overall acceptability

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Characteristics	**Sample number**			
Flavor	**1**	**2**	**3**	**4**
Body and Texture				
Color and appearance				
Overall acceptability				

Remarks

Signature: Name:

REFERENCES

Alvarez, B. S., Damiani, N., Czerner, M., Martucci, J. F. & Gende, L. B. (2019). Application of active and edible films of sodium caseinate to extend the shelf-life of argentinepategrás cheese.

AOAC (Association of Official Analytical Chemists) (1990). Official methods of analysis.

Archana, S. (2021). Development of casein based edible coating incorporated with essential oil for paneer Kerala Veterinary and Animal Sciences University, Kerala.

Atarés, L., Bonilla, J. and Chiralt, A. (2010). Characterization of sodium caseinatebased edible films incorporated with cinnamon or ginger essential oils. *Journal of Food Engineering, 100* (4), 678-687.

Azadbakht, E., Maghsoudlou, Y., Khomiri, M., &Kashiri, M. (2018). Development and structural characterization of chitosan films containing Eucalyptus globulus essential oil: Potential as an antimicrobial carrier for packaging of sliced sausage. *Food packaging and shelf life, 17*, 65-72.

Binsi, P.K., Ninan, G. and Ravishankar, C. N. (2017). Effect of curry leaf and clove bud essential oils on textural and oxidative stability of chill stored sutchi catfish fillets. *J. Texture Stud., 48*(3), 258-266.

Biswas, O., Talwar, N. A., Bhattacharya, D., Das, S. K., & Chaudhary, S. (2017). Effect of refrigeration on quality and stability of fresh fish and poultry muscles. *Indian J Anim Health, 56*(1), 65-76.

Bonnaillie, L. M., Zhang, H., Akkurt, S., Yam, K. L. & Tomasula, P. M. (2014). Casein films: the effects of formulation, environmental conditions and the addition of citric pectin on the structure and mechanical properties. *Polymers. 6*, 2018-2036

Brand-Williams, W., Cuvelier, M. E. & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology, 28(1), 25-30.

Dalvandi, F., Almasi, H., Ghanbarzadeh, B., Hosseini, H. & Karimian Khosroshahi, N. (2020). Effect of vacuum packaging and edible coating containing black pepper seeds and turmeric extracts on shelf life extension of chicken breast fillets. *Journal of Food and Bioprocess Engineering, 3*(1), 69-78.

Dashipoor, A., Khaksar, R., Hosseini, H., Shojaee, A. S. and Ghanati, K. (2014). Physical, antioxidant and antimicrobial characteristics of carboxymethyl cellulose edible film cooperated with clove essential oil. Zahedan. *J. Res. Med. Sci., 16*(8), 34-42.

Deeth, H. C. (2011). Milk lipids| lipolysis and hydrolytic rancidity.

Dorsey, B. M. & Jones, M. A. (2017). Healthy components of coffee processing by-products. In Handbook of coffee processing by-products (pp. 27-62). Academic Press.

Fabra, M. J., Talens, P. and Chiralt, A. (2008). Tensile properties and water vapor permeability of sodium caseinate films containing oleic acid–beeswax mixtures. *J. Food Eng. 85*, 393–400.

Galus, S. & Kadzińska, J. (2016). Moisture sensitivity, optical, mechanical and structural properties of whey protein-based edible films incorporated with rapeseed oil. *Food Technology and Biotechnology* (1), 78-89.

Gokhale, A. J. & Pandya, A. J. (2009). Enhancement of shelf life of paneer by adopting hurdle technology. In XXXVII Dairy industry conference, Goa, Feb (pp. 7-9).

Gülçin, İ., Elmastaş, M. & Aboul-Enein, H. Y. (2012). Antioxidant activity of clove oil–A powerful antioxidant source. *Arabian Journal of chemistry, 5*(4), 489-499.

Hong, S. I. and Krochta, J. M. (2006). Oxygen barrier performance of whey-proteincoated plastic films as affected by temperature, relative humidity, base film and protein type. *J. Food Eng., 77*(3), 739-745.

Ibrahium, M.I., Abd El-Ghany, M.E. and Ammar, M. S. (2013). Effect of clove essential oil as antioxidant and antimicrobial agent on cake shelf life. *World J. Dairy & Food Sci., 8*(2), 40-146.

Issa, A., Ibrahim, S. A. & Tahergorabi, R. (2017). Impact of sweet potato starch-based nanocomposite films activated with thyme essential oil on the shelf-life of baby spinach leaves. *Foods, 6*(6), 43.

Juffs, H. S. (1973). Proteolysis detection in milk: I.Interpretation of tyrosine value data for raw milk supplies in relation to natural variation, bacterial counts and other factors. *Journal of Dairy Research, 40*(3), 371-381.

Karunamay, S., Badhe, S. R., Shulka, V. & Pawar, P. A. (2020). Effect of Edible Packaging Film Treated with Admixture of Clove and Oregano Essential Oil in Extending the Shelf Life of Paneer. *Journal of Food Research, 8*(01), 01-04.

Khan, M. R., Volpe, S., Valentino, M., Miele, N. A., Cavella, S. & Torrieri, E. (2021). Active casein coatings and films for perishable foods: structural properties and shelf-life extension. *Coatings, 11*(8), 899.

Khwaldia, K., Perez, C. and Banon, S. (2004). Milk proteins for edible films and coatings. *Crit Rev Food Sci Nutr. 44*(4), 239–251.

Kibria, M. G., Masuk, N. I., Safayet, R., Nguyen, H. Q. & Mourshed, M. (2023). Plastic Waste: Challenges and Opportunities to Mitigate Pollution and Effective Management. *International Journal of Environmental Research, 17*(1), 20.

Kumar, Y., Yadav, D. N., Ahmad, T. & Narsaiah, K. (2015). Recent trends in the use of natural antioxidants for meat and meat products. *Comprehensive Reviews in Food Science and Food Safety, 14*(6), 796-812.

Lamdande, A. G., Garud, S. R. & Kumar, A. (2012). Impact of coating, packaging materials and temperatures on acidity of paneer. *Bioinfolet-A Quarterly Journal of Life Sciences, 9*(4), 445-447.

Makhal, S., Kanawjia, S. K. & Giri, A. (2014). Effectiveness of thymol in extending keeping quality of cottage cheese. *Journal of food science and technology, 51*, 2022-2029.

Matsakidou, A., Biliaderis, C.G. and Kiosseoglou, V. (2013). Preparation and characterization of composite sodium caseinate edible films incorporating naturally emulsified oil bodies. *Food Hydrocolloids, 30*(1), 232-240.

Mishra, D., Rao, K. J., Bhardwaj, R., Deshmukh, G. P., Kumar, R. & Eknath, K. P. (2017). Effect of water activity on sensory, textural properties and shelf life of paneer. *Int J Pure Appl Biosci., 5*(4), 238-245.

Morton, R. D. (2001). Aerobic plate count. Compendium of methods for the microbiological examination of foods, *4*, 63-67.

Nisar, T., Wang, Z. C., Yang, X., Tian, Y., Iqbal, M. & Guo, Y. (2018). Characterization of citrus pectin films integrated with clove bud essential oil: Physical, thermal, barrier, antioxidant and antibacterial properties. *International journal of biological macromolecules, 106*, 670-680.

Punnagaiarasi, A., Elango, A., Kalaikannan, A. and Selvaraju, M. (2016). Evaluation of sensory quality of edible

coated paneer using cinnamon oil. *International Journal for Science and Nature*, *7*(3), 591-593.

Raju, A. and Sasikala, M. S. (2016). Natural Antimicrobial Edible Film for Preservation of Paneer. Biosci. Biotech. Res. *Asia*. *13*(2), 1083-1088.

Reeta, A. & Kumar, B. K. (2012). Kumbhar, Study of sensory and textural properties of paneer using edible coating. *Sci. Rep.*, *1*(12), 1-5.

Sarode, A. R., Sawale, P. D., Khedkar, C. D., Kalyankar, S. D. & Pawshe, R. D. (2016). Casein and caseinate: methods of manufacture.

Sidwell, C. G., Salwin, H. & Mitchell Jr, J. H. (1955). Measurement of oxidation in dried milk products with thiobarbituric acid. *Journal of the American Oil Chemists Society*, *32*(1), 13-16.

Singh, G., Singh, S., Kumar, B. & Gaikwad, K. K. (2021). Active barrier chitosan films containing gallic acid based oxygen scavenger. *Journal of Food Measurement and Characterization*, *15*, 585-593.

Wanjari, T. P, Badhe, S. R, Shukla, S, Yadav, S. R. and Vidhya, V. M (2019). Evaluation of garlic extract in extending shelf life of paneer. *Int. J. Livest. Res.*, *9*(7), 100-110.

How to cite this article: Archana S., Divya K.B. and Anjali M.K. (2023). Extension of Shelf Life and Enhancement of Antioxidant Activity in Paneer using a Casein based Coating with Clove Bud Essential Oil. *Biological Forum – An International Journal, 15*(5): 1310-1317.