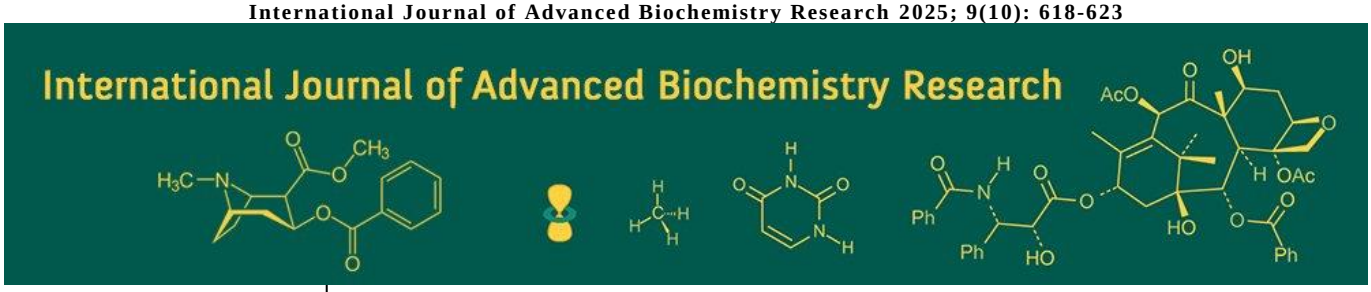

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Storage stability of paneer wrapped with chitosan based microencapsulated oregano essential oil edible films

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Abstract
The functional value of Paneer was improved by wrapping it with chitosan based edible films incorporated with different levels of microencapsulated oregano essential oil (OEO) powder. The paneer samples-control I (Raw), control II (LDPE-packed) and treatments T_1 (0.5%), T_2 (1%), T_3 (1.5%) and T_4 (2%) oregano essential oil films were stored at 4±1 °C to study their quality behaviour for a period of 15 days. The pH, water activity, moisture, DPPH and ABTS showed significant decrease with advancement of storage while as the protein, fat and TBARS showed significant increase with advancement of storage. The standard plate count and yeast and mold count showed significant increase from day 0 to day 15. Coliforms were not detected throughout the entire storage period. However, the values of microbial counts were within acceptable limits upto day 10 of storage period. Although the sensory scores decreased with storage period for all attributes, but remained acceptable upto day 10. Thus, it was concluded that chitosan based oregano essential oil films efficiently improved the functional value of paneer without affecting its storage stability for atleast 10 days at refrigeration temperature.

Keywords: TBARS, paneer, oregano essential oil, chitosan, microencapsulated, edible film

Introduction
India has seen a phenomenal rise in milk production over the past few decades. Products like paneer, yoghurt, cheese, butter, ghee, khoya and malai offer cheap, nutritious food to a large population. Paneer, a soft cheese made by acid coagulation of milk, has a short shelf life of 1-2 days at room temperature and 5-6 days under refrigeration (Raju and Sasikala, 2016) [12]. Microbial growth is the main cause of spoilage, favoured by pH of paneer (4.5-4.7). As per FSSAI, paneer should have ≤ 70% moisture and ≥ 50% fat on dry matter basis. It is also rich in protein and fat soluble vitamins (Raju and Sasikala, 2016) [12].

To improve shelf life, techniques like preservatives, packaging, temperature/light barriers, modified atmosphere, irradiation, antimicrobial films/coatings and biopreservatives have been studied. Biopreservatives-natural or controlled microflora or antimicrobials are effective. Bacteriocins, antimicrobial peptides from bacteria are used for food preservation (Lamdande *et al.*, 2012) [9].

Edible films/coatings made from proteins, polysaccharides and lipids protect food from physical, chemical and microbial deterioration. They act as moisture/gas barriers, preserve texture and colour and extend shelf life. Biopolymers like starch, cellulose and gelatin are biodegradable but have limited barrier properties which can be improved with additives like plasticizers like glycerol and sorbitol (Saklani *et al.*, 2019) [16].

Active edible packaging with antimicrobials and antioxidants reduces microbial growth and oxidation, enhancing taste and shelf life. Multilayer packaging improves barrier properties. Microencapsulation enhances antimicrobial strength and nutrient release. It protects active compounds from oxygen, water and light (Leclercq *et al.*, 2009) [10]. Chitosan, derived from chitin via deacetylation, is a biodegradable polymer with antimicrobial properties.

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It forms thin coatings that slow spoilage and allow gas/water permeability. Maltodextrin and Gum arabic are used for film formation and emulsification. Plant based antimicrobials in coatings help control spoilage. Antioxidants delay oxidation and extend shelf life.

Oregano essential oil, rich in thymol and carvacrol has antioxidant, antimicrobial and anti-inflammatory properties. It is effective against both Gram-positive and Gram-negative bacteria.

This study is one attempt in that direction wherein the storage stability of paneer is being evaluated (Ruberto and Baratta, 2000) [13].

Materials and Methods

Active edible films were prepared by following the protocol as described by Zehra *et al.* (2022) [20] with slight modifications. The films were prepared by dissolving 1 g of chitosan in 100 ml of distilled water followed by the addition of 1% acetic acid. To this mixture, 1% glycerol was added as a plasticizer and 2-3 drops of Tween 80 as an emulsifier. The mixture was heated until completely dissolved. Different concentrations of microencapsulated oregano oil powders (0.5%, 1%, 1.5% and 2%) were added to these solutions. The solutions were casted on polypropylene petri dishes and dried at 40±5 °C for 42 hours in an incubator. The dried films obtained were peeled off manually and stored in a humidity chamber at 50% relative humidity and 23±2 °C.

Paneer was prepared by following the protocol as described by Raju and Sasikala (2016) [12]. The milk was heated at 82 °C followed by cooling to 70 °C. 1% citric acid heated to 70° was added slowly till coagulum separated out. The coagulum was poured in hoops and pressed by weights (3-5) kg to drain the whey. The pressed paneer block was dipped in chilled water at 4-6 °C for 1-2 hours. The paneer blocks were packaged with Chitosan-Oregano edible film, stored at 4 °C temperature and evaluated for storage parameters.

Analytical procedures: The pH of the cooked samples was determined as per the method of Trout *et al.* (1992) by using a digital pH meter (Model CP 901, Century Instruments Ltd., and India). The percentage moisture, protein and fat of the product samples were evaluated as per the standard procedure of AOAC (2019) [2]. The water activity was estimated as per standard procedure of AOAC, 2019 [2] by using Pre Aqua Water activity analyzer. The instrument was first calibrated with 1.00 a_w primary standard reading and 0.760 a_w secondary standard reading. The different samples were placed in different sample cups and the final readings were displayed on the screen after the instrument beeped. Antioxidant activity was assessed using DPPH-RSA and ABTS-RSA assays as per Brand-Williams *et al.* (1995) [5] and Re *et al.* (1999) [14]. The estimation of TBARS value was done by following the method of Witte *et al.* (1970) [18] with slight modifications. 5g aliquot of the sample was taken and triturated with 25 ml of pre-cooled 20% trichloroacetic acid (TCA). The contents were then quantitatively transferred into a beaker by rinsing with 25 ml of chilled distilled water, well mixed and filtered through ash less Whatman filter paper No. 1 (GE Healthcare, U.K). A quantity of 5 ml of TCA extract (filtrate) was mixed with 5 ml of TBA reagent (0.01M) in duplicate test tubes and placed in hot air oven for 30 minutes until colour develops. A blank sample was made by mixing 3 ml of 10% TCA and 3 ml of the TBA reagent. Absorbance (O. D.) was measured, at fixed wavelength of 532 nm using UV-VIS spectrophotometer (HITACHI, UV-Spectrophotometer U-1800, Japan). The TBA value was calculated as mg malonaldehyde per kg of sample by multiplying O.D. value with k factor 5.2.

Microbiological analysis: The samples of paneer were subjected to microbiological analysis for standard plate count, coliform count and yeast and mold count as per the method described by APHA (1984) [1]. The cfu/g was calculated by taking average number of colonies which was multiplied by reciprocal of the dilution factor and expressed as $\log_{10}$cfu/g of sample.

Sensory evaluation: The paneer samples were presented to a group of semi-trained panelists consisting of the scientists and post graduate students of the Division of Livestock Products Technology, FVSc & AH, SKUAST-Kashmir. The samples were evaluated for various sensory parameters, viz. Appearance, Flavour, Body and texture and Overall acceptability as per the modified score card which was prepared on the basis of scores as adopted by Peryam and Pilgrim (1957) [11] based on a 9-point descriptive scale, where 9 = like extremely and 1 = dislike extremely. A score of six corresponding to desirable sensory characteristics of the products was taken as the minimum score for the acceptability of the product.

Statistical Analysis: The data generated was analysed statistically using the software of Statistical Package for Social Sciences (SPSS-Base 20.0). Analysis of variance was computed and significance of mean was tested at 5% level of significance.

Results and Discussion

Quality evaluation of Paneer wrapped with chitosan based oregano essential oil film during refrigerated storage (0-4 °C)

Physico-chemical properties

pH

During storage, an overall decrease in pH was observed in both controls as well as the treatments which might be due to the activity of lactic acid bacteria and other spoilage organisms, which ferment residual lactose and generate organic acids. In addition, oxidative changes in lipids may also contribute to increased acidity. The chitosan-oregano coatings, however, acted as antimicrobial barriers, suppressing microbial growth and thereby slowing down acid development. The results are in agreement with Karunamay *et al.* (2020) [8] who reported that the edible coatings incorporated with essential oils effectively delayed the reduction in pH of control and treated paneer samples. Archana *et al.* (2023) [3] also reported a decrease in pH of paneer with a casein based edible coating incorporated with clove bud essential oil.

Table 1: pH of Chitosan based Oregano Essential Oil films (Mean ± S.E.)

Treatments	0th	5th	10th	15th
C_1	5.96 ± 0.01^{dA}	5.61 ± 0.01^{cA}	5.41 ± 0.01^{bA}	4.90 ± 0.01^{aA}
C_2	5.95 ± 0.01^{dA}	5.71 ± 0.01^{cB}	5.51 ± 0.01^{bB}	5.31 ± 0.01^{aB}
T_1	5.95 ± 0.01^{dA}	5.72 ± 0.01^{cC}	5.61 ± 0.01^{bC}	5.41 ± 0.01^{aC}
T_2	5.95 ± 0.01^{dA}	5.73 ± 0.01^{cD}	5.64 ± 0.01^{bD}	5.51 ± 0.01^{aD}
T_3	5.95 ± 0.01^{dA}	5.81 ± 0.01^{cE}	5.74 ± 0.01^{bE}	5.63 ± 0.01^{aE}
T_4	5.95 ± 0.01^{dA}	5.83 ± 0.01^{cF}	5.76 ± 0.01^{bF}	5.66 ± 0.01^{aF}

Mean ± S.E with different superscripts differ significantly.
N = 6

Water activity

During storage, an overall decrease in water activity was observed in both controls as well as the treatments which can be attributed to the surface evaporation and redistribution of moisture within the matrix. Paneer wrapped with chitosan-oregano films exhibited lower aw than both controls throughout the storage period which might be due to increased WVTR that made films less effective as moisture barriers, thereby allowing greater water vapour

migration and a faster decline in water activity. The results are in agreement with Alizadeh-Sani *et al.* (2018) who reported that essential oil incorporation can create microstructural discontinuities in biopolymer films, thereby affecting their barrier properties. The results are in contrast with Archana *et al.* (2023) [3] who reported an increase in water activity of paneer with a casein based edible coating incorporated with clove bud essential oil.

Table 2: Water activity of Chitosan based Oregano Essential Oil films (Mean ± S.E.)

Treatments	0th	5th	10th	15th
C_1	0.99 ± 0.00^{dB}	0.98 ± 0.00^{cE}	0.96 ± 0.00^{bE}	0.95 ± 0.00^{aE}
C_2	0.98 ± 0.00^{dA}	0.97 ± 0.00^{cD}	0.96 ± 0.00^{bE}	0.95 ± 0.00^{aE}
T_1	0.98 ± 0.00^{dA}	0.96 ± 0.00^{cD}	0.95 ± 0.00^{bD}	0.94 ± 0.00^{aD}
T_2	0.98 ± 0.00^{dA}	0.94 ± 0.00^{cC}	0.93 ± 0.00^{bC}	0.92 ± 0.00^{aC}
T_3	0.98 ± 0.00^{dA}	0.93 ± 0.00^{cB}	0.92 ± 0.00^{bB}	0.90 ± 0.00^{aB}
T_4	0.98 ± 0.00^{dA}	0.92 ± 0.00^{cA}	0.90 ± 0.00^{bA}	0.89 ± 0.00^{aA}

Mean ± S.E with different superscripts differ significantly.
N = 6

Moisture Content

During storage, an overall decrease in moisture content was observed in both controls as well as the treatments which can be attributed to syneresis and water loss from the product surface. Paneer wrapped in chitosan-oregano films exhibited a greater reduction in moisture content than controls that can be directly associated with the higher water vapour transmission rate. Incorporation of oregano oil into the chitosan matrix likely reduced the compactness of the polymer structure, increasing porosity and thereby facilitating greater moisture migration. The results are in agreement with Souza *et al.* (2019) [15] who reported that essential oil incorporation compromised barrier properties by inducing phase separation or microstructural discontinuities. Archana *et al.* (2023) [3] also reported a decrease in moisture content of paneer with a casein based edible coating incorporated with clove bud essential oil.

Table 3: Moisture Content of Chitosan based Oregano Essential Oil films (Mean ± S.E.)

Treatments	0th	5th	10th	15th
C_1	53.30 ± 0.03^{dA}	52.85 ± 0.03^{cE}	51.85 ± 0.03^{bF}	50.84 ± 0.03^{aF}
C_2	53.30 ± 0.03^{dA}	52.75 ± 0.03^{cE}	51.75 ± 0.03^{bE}	50.75 ± 0.03^{aE}
T_1	53.30 ± 0.03^{dA}	52.65 ± 0.03^{cD}	51.65 ± 0.03^{bD}	50.65 ± 0.03^{aD}
T_2	53.30 ± 0.03^{dA}	52.55 ± 0.03^{cC}	51.55 ± 0.03^{bC}	50.55 ± 0.03^{aC}
T_3	53.30 ± 0.03^{dA}	52.35 ± 0.03^{cB}	51.35 ± 0.03^{bB}	50.35 ± 0.03^{aB}
T_4	53.30 ± 0.03^{dA}	52.15 ± 0.03^{cA}	51.15 ± 0.03^{bA}	50.15 ± 0.03^{aA}

Mean ± S.E with different superscripts differ significantly.
N = 6

Protein content

During storage, an overall increase in protein content was observed in both controls as well as the treatments which can be attributed to moisture loss which leads to a relative concentration of solids including proteins. The treatments containing chitosan-oregano essential oil films maintained higher protein levels compared to the control groups that is due to the protective barrier properties of chitosan films which reduce microbial proteolysis and enzymatic degradation of proteins. Oregano essential oil, being rich in phenolic compounds such as carvacrol and thymol may have further contributed to the inhibition of proteolytic bacteria thereby lowering protein breakdown. The results are in agreement with Yangilar, (2015) who reported that chitosan whey protein edible films reduced proteolysis and helped maintain protein levels in Gobek Kashar Cheese.

Table 4: Protein Content of Chitosan based Oregano Essential Oil films (Mean ± S.E.)

Treatments	0th	5th	10th	15th
C_1	19.15 ± 0.03^{aA}	19.45 ± 0.03^{bA}	19.70 ± 0.03^{cA}	19.96 ± 0.03^{dA}
C_2	19.15 ± 0.03^{aA}	19.55 ± 0.03^{bB}	19.80 ± 0.03^{cAB}	20.05 ± 0.03^{dB}
T_1	19.15 ± 0.02^{aA}	19.65 ± 0.02^{bC}	19.83 ± 0.02^{cB}	20.15 ± 0.02^{dC}
T_2	19.15 ± 0.02^{aA}	19.75 ± 0.02^{bD}	19.95 ± 0.02^{cC}	21.05 ± 0.02^{dD}
T_3	19.15 ± 0.03^{aA}	19.75 ± 0.03^{bD}	20.00 ± 0.03^{cCD}	21.15 ± 0.03^{dE}
T_4	19.15 ± 0.03^{aA}	19.85 ± 0.03^{bE}	20.10 ± 0.03^{cD}	21.35 ± 0.03^{dF}

Mean ± S.E with different superscripts differ significantly.
N = 6

Fat content

During storage, an overall increase in fat content was observed in both controls as well as the treatments which can be attributed to moisture loss which leads to a relative concentration of solids including fats. The treatments containing chitosan-oregano essential oil films maintained higher fat levels compared to the control groups which suggests that the films not only restricted moisture migration but also provided oxidative stability to the fat fraction. The results are in agreement with Yangilar, (2015) who reported that the fat content in coated kashar cheese samples increased during ripening and coatings helped preserve fats.

Table 5: Fat Content of Chitosan based Oregano Essential Oil films (Mean ± S.E.)

Treatments	0th	5th	10th	15th
C_1	22.20 ± 0.03^{aA}	22.25 ± 0.03^{bA}	22.75 ± 0.03^{cA}	23.12 ± 0.04^{dF}
C_2	22.20 ± 0.03^{aA}	22.35 ± 0.03^{bB}	22.85 ± 0.03^{cB}	23.35 ± 0.03^{dE}
T_1	22.20 ± 0.03^{aA}	22.43 ± 0.03^{bC}	22.87 ± 0.03^{cB}	23.37 ± 0.03^{dD}
T_2	22.20 ± 0.04^{aA}	22.55 ± 0.04^{bD}	23.30 ± 0.04^{cC}	23.55 ± 0.04^{dC}
T_3	22.20 ± 0.03^{aA}	22.65 ± 0.03^{bE}	23.45 ± 0.03^{cD}	23.65 ± 0.03^{dB}
T_4	22.20 ± 0.03^{aA}	22.75 ± 0.03^{bF}	23.55 ± 0.03^{cE}	23.85 ± 0.03^{dA}

Mean ± S.E with different superscripts differ significantly.
N = 6

Antioxidant properties

TBARS

The TBARS values increased significantly ($p<0.05$) in both controls as well as the treatments indicating progressive lipid oxidation. Control samples exhibited the highest TBARS levels reflecting higher susceptibility of lipids to oxidative rancidity in the absence of active packaging. Conversely, treatments with oregano essential oil based chitosan films showed markedly lower TBARS value which can be attributed to the strong antioxidant activity of oregano essential oil that effectively scavenges free radicals and suppresses lipid peroxidation. The results are in agreement with Karunamay *et al.* (2020) [8] who reported significant ($p<0.05$) increase in the TBARS values of paneer wrapped with oregano oil based packaging film. Archana *et al.* (2023) [3] also reported an increase in the TBARS values of paneer with a casein based edible coating incorporated with clove bud essential oil.

Table 6: TBARS of Chitosan based Oregano Essential Oil films (Mean ± S.E.)

Treatments	0th	5th	10th	15th
C_1	0.26 ± 0.00^{aA}	0.49 ± 0.00^{bE}	0.69 ± 0.00^{cF}	1.11 ± 0.00^{dF}
C_2	0.26 ± 0.00^{aA}	0.48 ± 0.00^{bE}	0.67 ± 0.00^{cE}	1.00 ± 0.00^{dE}
T_1	0.26 ± 0.00^{aA}	0.37 ± 0.00^{bD}	0.55 ± 0.00^{cD}	0.69 ± 0.00^{dD}
T_2	0.26 ± 0.00^{aA}	0.35 ± 0.00^{bC}	0.53 ± 0.00^{cC}	0.69 ± 0.00^{dC}
T_3	0.26 ± 0.00^{aA}	0.34 ± 0.00^{bB}	0.50 ± 0.00^{cB}	0.67 ± 0.00^{dB}
T_4	0.26 ± 0.00^{aA}	0.32 ± 0.00^{bA}	0.49 ± 0.00^{cA}	0.66 ± 0.00^{dA}

Mean ± S.E with different superscripts differ significantly.
N = 6

DPPH-RSA (1, 1-diphenyl 2-picryl-hydrazyl radical scavenging activity)

The DPPH-RSA values decreased significantly (p<0.05) in both controls as well as the treatments which can be attributed to the gradual depletion of antioxidant compounds and progressive lipid oxidation with time. Paneer samples wrapped in oregano essential oil incorporated chitosan films retained significantly (p<0.05) higher DPPH values throughout storage which indicates that the concentration of oregano essential oil in the film played a dose dependent role in sustaining antioxidant activity. The results are in agreement with Al-Hijazeen *et al.* (2016) [6] who reported that oregano essential oil significantly (p<0.05) improved DPPH scavenging capacity and inhibited oxidation in ground chicken breast meat.

Table 7: DPPH of Chitosan based Oregano Essential Oil films (Mean ± S.E.)

Treatments	0th	5th	10th	15th
C_1	22.48 ± 0.00^{dA}	21.35 ± 0.00^{cA}	20.86 ± 0.00^{bA}	19.21 ± 0.00^{aA}
C_2	22.48 ± 0.00^{dA}	21.36 ± 0.00^{cB}	20.87 ± 0.00^{bB}	19.22 ± 0.00^{aB}
T_1	22.48 ± 0.00^{dA}	21.41 ± 0.00^{cC}	20.89 ± 0.00^{bC}	19.25 ± 0.00^{aC}
T_2	22.48 ± 0.00^{dA}	21.58 ± 0.00^{cD}	20.93 ± 0.00^{bD}	19.59 ± 0.00^{aD}
T_3	22.48 ± 0.00^{dA}	21.79 ± 0.00^{cE}	20.99 ± 0.00^{bE}	19.93 ± 0.00^{aE}
T_4	22.48 ± 0.00^{dA}	21.91 ± 0.00^{cF}	21.32 ± 0.00^{bF}	20.99 ± 0.00^{aF}

Mean ± S.E with different superscripts differ significantly.
N = 6

ABTS-RSA (2, 2-azino-bis-3 ethylbenthiazoline-6-sulphonic acid radical scavenging activity)

The ABTS-RSA values decreased significantly (p<0.05) in both controls as well as the treatments which can be attributed to the gradual depletion of antioxidant compounds and progressive lipid oxidation with time. Paneer samples wrapped in oregano essential oil incorporated chitosan films retained significantly (p<0.05) higher DPPH values throughout storage which indicates that the concentration of oregano essential oil in the film played a dose dependent role in sustaining antioxidant activity. The results are in agreement with Al-Hijazeen *et al.* (2016) [6] who reported that oregano essential oil significantly (p<0.05) improved scavenging capacity and inhibited oxidation in ground chicken breast meat.

Table 8: ABTS of Chitosan based Oregano Essential Oil films (Mean ± S.E.)

Treatments	0th	5th	10th	15th
C_1	20.24 ± 0.00^{dA}	19.22 ± 0.00^{cA}	18.19 ± 0.00^{bA}	17.21 ± 0.00^{aA}
C_2	20.24 ± 0.00^{dA}	19.22 ± 0.00^{cA}	18.20 ± 0.00^{bB}	17.22 ± 0.00^{aA}
T_1	20.24 ± 0.00^{dA}	19.29 ± 0.00^{cA}	19.19 ± 0.00^{bC}	18.25 ± 0.00^{aB}
T_2	20.24 ± 0.00^{dA}	20.12 ± 0.00^{bB}	20.19 ± 0.00^{cD}	19.25 ± 0.00^{aC}
T_3	20.24 ± 0.00^{cA}	20.16 ± 0.00^{bB}	20.36 ± 0.00^{dE}	19.38 ± 0.00^{aD}
T_4	20.24 ± 0.11^{bA}	20.72 ± 0.11^{cC}	20.19 ± 0.11^{bD}	19.79 ± 0.11^{aE}

Mean ± S.E with different superscripts differ significantly.
N = 6

Microbiological Quality

Standard plate count

The SPC values increased significantly (p<0.05) in both controls as well as the treatments reflecting general microbial proliferation over time. However, the SPC values were consistently lower in paneer wrapped with chitosan-oregano essential oil films compared to controls which indicates that the antimicrobial action of chitosan combined with oregano essential oil effectively inhibited bacterial growth. The active compounds in oregano essential oil, mainly carvacrol and thymol disrupt bacterial cell membranes, while chitosan exerts antimicrobial effects through chelation of essential metals and disruption of cell walls, resulting in extended microbial stability of paneer. The results are in agreement with Karunamay *et al.* (2020) [8] who reported that oregano essential oil significantly (p<0.05) decreased the standard plate count values as compared to control in paneer wrapped with oregano essential oil edible packaging film. Archana *et al.* (2023) [3] also reported an increase in SPC of paneer with a casein based edible coating incorporated with clove bud essential oil.

Table 9: SPC of Chitosan based Oregano Essential Oil films (Mean ± S.E.)

Treatments	0th	5th	10th	15th
C_1	1.17 ± 0.23^{aA}	3.19 ± 0.23^{bD}	5.88 ± 0.23^{cC}	6.26 ± 0.23^{cB}
C_2	1.17 ± 0.23^{aA}	3.13 ± 0.23^{bCD}	5.83 ± 0.23^{cC}	6.25 ± 0.23^{cB}
T_1	1.17 ± 0.23^{aA}	3.03 ± 0.23^{bC}	4.80 ± 0.23^{cB}	5.18 ± 0.23^{cA}
T_2	1.17 ± 0.23^{aA}	3.02 ± 0.23^{bBC}	4.77 ± 0.23^{cAB}	5.18 ± 0.23^{cA}
T_3	1.17 ± 0.23^{aA}	2.90 ± 0.23^{bB}	4.73 ± 0.23^{cAB}	5.17 ± 0.23^{cA}
T_4	1.17 ± 0.23^{aA}	2.73 ± 0.23^{bA}	4.70 ± 0.23^{cA}	5.15 ± 0.23^{cA}

Mean ± S.E with different superscripts differ significantly. N = 6

Coliform count

The coliforms were not detected in any of the paneer samples throughout the 15 day storage period. This indicates excellent hygienic quality of the paneer and effective microbial control during processing and packaging. The absence of coliforms can be attributed to proper pasteurization and good manufacturing practices followed during paneer production. The results are in agreement with Karunamay *et al.* (2020) [8] who reported that edible films containing oregano essential oil successfully inhibited coliform growth in paneer for the entire storage period, ensuring product safety. The results are in contrast with Archana *et al.* (2023) [3] who reported an increase in coliform count of paneer with a casein based edible coating incorporated with clove bud essential oil.

Table 10: Coliform count of Chitosan based Oregano Essential Oil films (Mean ± S.E.)

Treatments		0th	5th	10th	15th
C_1		ND	ND	ND	ND
C_2		ND	ND	ND	ND
T_1		ND	ND	ND	ND
T_2		ND	ND	ND	ND
T_3		ND	ND	ND	ND
T_4		ND	ND	ND	ND

Mean ± S.E with different superscripts differ significantly. N = 6

Yeast and mold count

The yeast and mold counts increased during storage in both controls and treatments, showing the natural growth tendency of fungi in dairy products. However, paneer wrapped in chitosan-oregano essential oil films exhibited no growth on fifth day and lower counts for the tenth and fifteenth day as compared to controls. The antifungal activity is attributed to the bioactive phenolic compounds in oregano essential oil, which interfere with fungal cell metabolism and membrane integrity, while chitosan further limits fungal growth by forming a protective barrier and

altering the microenvironment of the product. The results are in agreement with Karunamay *et al.* (2020) [8] who reported that edible films containing oregano essential oil successfully inhibited yeast and mold counts in paneer for the entire storage period, ensuring product safety. Archana *et al.* (2023) [3] also reported an increase in yeast and mold count of paneer with a casein based edible coating incorporated with clove bud essential oil.

Table 11: Yeast and Mold Count of Chitosan based Oregano Essential Oil films (Mean ± S.E.)

Treatments	0th	5th	10th	15th
C_1	0.00 ± 0.04^{a}	2.87 ± 0.04^{bC}	4.08 ± 0.04^{cC}	5.17 ± 0.04^{dD}
C_2	0.00 ± 0.05^{a}	2.90 ± 0.05^{bC}	3.98 ± 0.05^{cC}	5.07 ± 0.05^{dCD}
T_1	0.00 ± 0.11^{a}	0.50 ± 0.11^{bB}	3.92 ± 0.11^{cC}	5.05 ± 0.11^{dCD}
T_2	0.00 ± 0.04^{a}	0.00 ± 0.04^{aA}	3.75 ± 0.04^{bB}	4.95 ± 0.04^{cBC}
T_3	0.00 ± 0.03^{a}	0.00 ± 0.03^{aA}	3.68 ± 0.03^{bAB}	4.83 ± 0.03^{cB}
T_4	0.00 ± 0.04^{a}	0.00 ± 0.04^{aA}	3.57 ± 0.04^{bA}	4.68 ± 0.04^{cA}

Mean ± S.E with different superscripts differ significantly.
N = 6

Sensory attributes

The sensory attributes were affected during 15 days of refrigerated storage. The general appearance scores of paneer decreased gradually in both controls and treatments. The control samples showed a sharper decline in scores, whereas the treated samples maintained relatively higher scores. The slower decline in treated samples suggests that chitosan-oregano essential oil films helped preserve the surface characteristics. The results are in agreement with Karunamay *et al.* (2020) [8] who reported that antimicrobial and barrier films maintain the visual quality of dairy products during storage. Archana *et al.* (2023) [3] also reported an increase in appearance of paneer with a casein based edible coating incorporated with clove bud essential oil. Flavour scores decreased progressively during storage in all samples. The control samples experienced a greater reduction, indicating noticeable off-flavours likely due to microbial growth and oxidation. Paneer wrapped with chitosan-oregano films retained higher flavour scores, reflecting the ability of the films to inhibit microbial proliferation and reduce lipid oxidation which helps preserve natural flavour. The results are in agreement with Karunamay *et al.* (2020) [8] who reported that antimicrobial and barrier films maintain the flavour of dairy products during storage. Archana *et al.* (2023) [3] also reported an increase in flavour of paneer with a casein based edible coating incorporated with clove bud essential oil. Body and texture scores also decreased during storage. Controls showed more pronounced deterioration likely due to moisture loss, syneresis and microbial activity causing textural softening. Treatments maintained better body and texture indicating that chitosan films acted as a moisture barrier and helped retain firmness, smoothness and cohesiveness of paneer during storage. The results are in agreement with Karunamay *et al.* (2020) [8] who reported that antimicrobial and barrier films maintain the body and texture of dairy products during storage. Archana *et al.* (2023) [3] also reported an increase in body and texture scores of paneer with a casein based edible coating incorporated with clove bud essential oil. The overall acceptability scores reflected the combined effect of appearance, flavour and texture. The control samples experienced a marked decline over 15 days, whereas, treated samples retained higher OA scores, indicating better consumer perceived quality. The improved acceptability in treated paneer can be attributed to the antimicrobial and antioxidative properties of chitosan-oregano films, which slowed spoilage and maintained desirable sensory characteristics throughout storage. The results are in agreement with Khatkar *et al.* (2017) [7] who reported decrease in overall acceptability of paneer treated with clove essential oil. Archana *et al.* (2023) [3] also reported decrease in overall acceptability of paneer with a casein based edible coating incorporated with clove bud essential oil.

Table 12: General Appearance of Chitosan based Oregano Essential Oil films (Mean ± S.E.)

Treatments	0th	5th	10th	15th
C_1	8.70 ± 0.16^{dBC}	7.50 ± 0.16^{cB}	6.40 ± 0.16^{bA}	5.60 ± 0.16^{aA}
C_2	8.40 ± 0.14^{dB}	7.80 ± 0.14^{cBC}	6.80 ± 0.14^{bA}	6.20 ± 0.14^{aB}
T_1	8.90 ± 0.12^{dC}	7.90 ± 0.12^{cC}	7.50 ± 0.12^{bB}	6.90 ± 0.12^{aC}
T_2	8.90 ± 0.13^{dC}	7.90 ± 0.13^{cC}	7.50 ± 0.13^{bB}	6.70 ± 0.13^{aC}
T_3	7.90 ± 0.12^{dA}	7.00 ± 0.12^{cA}	6.60 ± 0.12^{bA}	6.20 ± 0.12^{aB}
T_4	7.90 ± 0.13^{cA}	6.70 ± 0.13^{bA}	6.50 ± 0.13^{bA}	6.10 ± 0.13^{aB}

Mean ± S.E with different superscripts differ significantly.
N = 6

Table 13: Flavour of Chitosan based Oregano Essential Oil films (Mean ± S.E.)

Treatments	0th	5th	10th	15th
C_1	9.00 ± 0.14^{dC}	7.80 ± 0.14^{cB}	6.50 ± 0.14^{bA}	5.50 ± 0.14^{aA}
C_2	9.00 ± 0.08^{dC}	8.00 ± 0.08^{cB}	6.90 ± 0.08^{bAB}	5.80 ± 0.08^{aAB}
T_1	8.70 ± 0.16^{cBC}	7.70 ± 0.16^{bB}	7.50 ± 0.16^{bCD}	6.70 ± 0.16^{aD}
T_2	8.70 ± 0.14^{cBC}	7.80 ± 0.14^{bB}	7.70 ± 0.14^{bD}	6.80 ± 0.14^{aD}
T_3	8.40 ± 0.15^{cAB}	7.30 ± 0.15^{bA}	7.20 ± 0.15^{bBC}	6.40 ± 0.15^{aCD}
T_4	8.30 ± 0.13^{dA}	7.00 ± 0.13^{cA}	6.60 ± 0.13^{bA}	6.20 ± 0.13^{aBC}

Mean ± S.E with different superscripts differ significantly.
N = 6

Table 14: Body and Texture of Chitosan based Oregano Essential Oil films (Mean ± S.E.)

Treatments	0th	5th	10th	15th
C_1	8.80 ± 0.14^{dA}	7.80 ± 0.14^{cAB}	6.80 ± 0.14^{bA}	4.50 ± 0.14^{aA}
C_2	8.80 ± 0.12^{dA}	8.00 ± 0.12^{cB}	7.30 ± 0.12^{bB}	5.20 ± 0.12^{aB}
T_1	8.60 ± 0.15^{cA}	8.00 ± 0.15^{bB}	7.20 ± 0.15^{aAB}	6.80 ± 0.15^{aD}
T_2	8.60 ± 0.14^{dA}	8.10 ± 0.14^{cB}	7.40 ± 0.14^{bB}	6.90 ± 0.14^{aD}
T_3	8.50 ± 0.16^{cA}	7.50 ± 0.16^{bA}	6.80 ± 0.16^{aA}	6.70 ± 0.16^{aD}
T_4	8.50 ± 0.14^{dA}	7.50 ± 0.14^{cA}	6.80 ± 0.14^{bA}	6.00 ± 0.14^{aC}

Mean ± S.E with different superscripts differ significantly.
N = 6

Table 15: Overall Acceptability of Chitosan based Oregano Essential Oil films (Mean ± S.E.)

Treatments	0th	5th	10th	15th
C_1	8.90 ± 0.14^{dCD}	7.40 ± 0.14^{cA}	6.30 ± 0.14^{bA}	4.30 ± 0.14^{aA}
C_2	9.00 ± 0.11^{dD}	7.80 ± 0.11^{cA}	6.90 ± 0.11^{bB}	5.70 ± 0.11^{aB}
T_1	8.60 ± 0.16^{cBC}	7.70 ± 0.16^{bA}	7.60 ± 0.16^{bC}	6.70 ± 0.16^{aD}
T_2	8.80 ± 0.15^{cCD}	7.60 ± 0.15^{bA}	7.70 ± 0.15^{bC}	6.80 ± 0.15^{aD}
T_3	8.30 ± 0.16^{cAB}	7.50 ± 0.16^{bA}	7.20 ± 0.16^{bB}	6.50 ± 0.16^{aCD}
T_4	8.20 ± 0.13^{cA}	7.40 ± 0.13^{bA}	7.10 ± 0.13^{bB}	6.20 ± 0.13^{aC}

Mean ± S.E with different superscripts differ significantly.
N = 6

Conclusion

Overall, oregano oil based chitosan films present a natural and effective packaging option to extend paneer shelf life. Paneer wrapped in these films had better oxidative stability, higher antioxidant retention and controlled microbial growth.

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