



Development and Optimization of Sustainable Packaging System for Indian Cottage Cheese (Paneer)

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ABSTRACT

Background: Indian cottage cheese (Paneer), as a nutrient-dense food item, has a limited shelf life and is susceptible to microbial spoilage.

Methods: This study aimed to develop an active edible coating system utilizing the statistical methodology Response Surface Methodology (RSM). Within the framework of RSM, the study evaluated the dependent parameters (pH, porosity and titratable acidity) while investigating specific ranges for the independent variables (casein, glycerin, pectin and water). Subsequently, the optimized edible coating was fortified with antimicrobial agents, namely 1% Copper Nanoparticles (CNP) and 1% Eugenol Essential Oil (EO), to assess their effectiveness in monitoring changes in physicochemical characteristics and microbial proliferation over a 14-day storage period.

Result: The optimised concentrations of the independent variables were determined as follows: 8% casein, 4.6% glycerin, 0.65% pectin and 86% water, with the goal of preserving cottage cheese's quality attributes. Notably, on the 14th day of storage, a substantial reduction in microbial proliferation, averaging 88.6%, was observed in cottage cheese samples coated with CNP coatings. Conversely, EO coatings did not effectively limit microbial growth during storage. Consequently, it can be inferred that the casein-based coating incorporating CNP effectively retained moisture, maintained an optimal pH and ensured the safety and quality of cottage cheese.

Key words: Copper nanoparticles, Edible coating, Eugenol essential oil, Indian cottage cheese (Paneer), Response surface methodology.

INTRODUCTION

Indian cottage cheese commonly known as Paneer, is highly valued by vegetarians for its rich calcium, protein and healthy fat content. However, its high moisture content (up to 60%) limits its refrigerated shelf life to just 5 to 6 days, making it susceptible to microbial spoilage and quality degradation (Patil and Sonwane, 2022). Various traditional methods (e.g., preservative addition, heat sterilization, dehydration) and emerging techniques (e.g., high-pressure treatment, modified atmosphere packaging) have been explored to extend its shelf life (Prajapati *et al.*, 2023). However, these methods often negatively impact sensory and nutritional attributes, such as reducing whiteness, increasing titratable acidity and causing moisture loss (Kapoor *et al.*, 2021). This underscores the need for innovative techniques that can extend the shelf life of paneer while preserving its freshness and quality (Raju and Sasikala 2016).

Active edible coatings derived from biopolymers represent a cutting-edge approach to extending the shelf life of food products. Their direct application to food items significantly reduces the reliance on secondary packaging, thereby curbing waste generation and safeguarding food quality and safety (Quirós-Sauceda *et al.*, 2014). Extensive research has demonstrated the efficacy of incorporating essential oils (e.g., clove, oregano, cinnamon) into biopolymer-based coatings (e.g., sodium alginate, chito san) for prolonging the shelf life of Indian cottage cheese (paneer)

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(Jafarzadeh *et al.*, 2021; Seydim *et al.*, 2020). However, the application of nanoparticles, particularly copper nanopar-

tics (CNPs), in enhancing paneer's shelf life remains largely unexplored. CNPs, known for their potent antibacterial properties, have been effectively utilized in other food products; for instance, biopolymer films containing CNPs have successfully inhibited bacterial and fungal growth in stored yellow bell peppers, thereby enhancing preservation (Saravanakumar *et al.*, 2020).

In the current study, active antimicrobial edible coatings were formulated to enhance the preservation of Indian cottage cheese (paneer) at 15°C. This temperature was selected to evaluate the efficacy of antimicrobial agents in the coating, as it aligns with the conventional storage practices of Indian retailers for paneer. To the best of our knowledge, no prior research has been conducted to assess the shelf life of paneer at 15°C utilizing copper nanoparticles and Eugenol essential oil.

MATERIALS AND METHODS

Raw materials

Buffalo milk with 6% fat and 9% solid-not-fat (SNF) was sourced from Mother Dairy, Bindapur, New Delhi, India. Edible ingredients including casein and pectin were procured from Central Drug House Pvt. Ltd. (New Delhi, India), while eugenol essential oil was obtained from Loba Chemie. Analytical-grade chemical reagents such as oxalic acid, glycerin, copper chloride, copper nitrate, phenolphthalein indicator, sodium chloride, nutrient agar and sodium hydroxide were purchased from Hi Media Laboratories Pvt. Ltd. in Mumbai.

Manufacturing of Indian cottage cheese (Paneer)

Paneer samples were prepared in-house following a modified procedure based on Suthar *et al.*, (2018). Buffalo milk (1 liter) was pasteurized at 90°C for 5 minutes, then cooled to 70°C. 1% citric acid was added to induce curdling and the resulting coagulum was maintained above 63°C for 5 minutes. The curd was then pressed in a muslin cloth to remove excess whey and soaked in cold water (4°C) for 1 hour.

Synthesis of copper nano-particles (CNP)

CNP were prepared through the co-precipitation technique (Phiwdang *et al.*, 2013). Subsequently, the nanoparticle dimensions were assessed using dynamic light scattering (Litesizer DLS, Anton Paar, 82759629).

Experimental design

Response surface methodology (RSM) utilizing Design Expert Software version 8 (Statease Inc., Minneapolis, MN) was employed statistically to examine the effect of primary coating components (casein, glycerin and pectin) on porosity, titratable acidity and pH of coated cottage cheese samples. A central composite design (CCD) with four independent variables (casein, glycerin, pectin and water) was employed (Table 1). A quadratic equation (Eq. 1) was derived to evaluate interactions among these variables on pH (Y1), titratable acidity (Y2) and porosity (Y3) of the coated samples.

$$Y_i = \alpha_0 + \sum \alpha_i X_i + \sum \alpha_{ii} X_i^2 + \sum \alpha_{ij} X_i X_j \quad \dots \text{eq (1)}$$

Where

Y_i = Response function of the dependent variables, which include pH, titratable acidity and porosity.

α_0 , α_i , α_{ii} and α_{ij} = Constant terms, linear, quadratic and interactive coefficients, respectively.

X_i and X_j = Four independent variables under consideration.

To assess the model's efficacy, a regression analysis was performed, with the coefficient of determination (R^2) serving as the evaluation metric. Given the intricate nature of food products, an R^2 value exceeding 0.80 was considered indicative of a favourable model fit.

Edible coating of cottage cheese

Edible coatings for Indian cottage cheese (Paneer) were prepared by dissolving casein (8-12% w/v), glycerin (2-5% w/v) and pectin (0.5-1.5% w/v) in distilled water (80-90% w/v). The solutions were thoroughly homogenized for 30 minutes using a magnetic stirrer (Tarsons Spinint Ceramic Plate 4040). The edible coating solution was subsequently applied to the paneer cubes using a brushing technique, followed by air drying at ambient room temperature. Subsequently, the optimized edible coating solution was augmented with 1% (v/v) essential oil (EO) and copper nanoparticles (CNP) at 1% by weight of the polymer and compared against plain (without antimicrobial) and control samples to assess antimicrobial properties. The samples were packaged in zip-lock plastic pouches and stored under refrigeration conditions for shelf-life analysis.

Shelf-life studies

The coated samples underwent physicochemical and microbiological analyses over 2 weeks at 15°C to assess the antibacterial coating's efficacy. Shelf-life studies were conducted on days 0, 7 and 14 to evaluate coating effects on Indian cottage cheese (Paneer) quality.

Weight loss

The weight of coated samples was measured on the 0, 7th and 14th days of storage. Weight losses were calculated in triplicate using the formula presented in Eq 2.

% weight loss =

$$\frac{\text{Initial weight of sample} - \text{Final weight of sample}}{\text{Initial weight of sample}} \times 100 \quad \dots \text{eq (2)}$$

pH

Triplicate measurements were carried out to study the deflection in pH of the coated and control samples using a digital pH metre (LT-10, Labtronics, Panchkula, Haryana) (AOAC, 2003).

Water activity (aw)

The amount of free water present in Indian cottage cheese (Paneer) samples was quantified by measuring water activity in triplicate using a water activity metre (4 te-Aqualab, Maharashtra, India).

Titrateable acidity

The assessment of titrateable acidity, expressed as a percentage of lactic acid, in the Indian cottage cheese (Paneer) samples was conducted utilizing the standard titration method specified by the Bureau of Indian Standards (1983). The acidity of the sample was subsequently calculated using the formula presented in Eq. 3. All analyses were performed in triplicate.

Acidity (% lactic acidity) =

$$\frac{10 \times \text{Volume of 0.1N HCl used} \times 0.9}{\text{Mass of paneer in gram}} \quad \dots \text{eq (3)}$$

Microbiological assay

Paneer samples stored for 14 days underwent bacterial load analysis using the Standard Plate Count method. One gram of sample was diluted with saline (0.85% NaCl) in a 1:10 ratio and the solutions were inoculated on nutrient agar (NA). Subsequently, microbial colonies were enumerated after incubation at 37°C. All analyses were performed in triplicate.

RESULTS AND DISCUSSION**Fitting the response surface models**

By fitting the quadratic design in the experimental model, 27 experiment runs were obtained and performed in duplicate. Porosity, titrateable acidity and pH were the studied responses for the design. The goodness of the fitted model is determined by the high value of the coefficient of determination (R^2) for the studied responses (Table 2).

Effect of independent variables on edible coated cottage cheese Sample

The study demonstrated the combined effect of concentrations of casein, pectin, glycerin and water on the porosity, titrateable acidity and pH of the edible-coated paneer samples.

Porosity

The influence of independent factors on porosity was evaluated using response surfaces, with interaction factors having $p < 0.05$ considered. Fig 1a illustrates that water content in the edible coating positively affects porosity of Indian cottage cheese (paneer), crucial for preserving its porous structure. Additionally, the analysis suggests that lower concentrations of glycerin and casein are favored, as reduced levels improve porosity. In contrast, higher amounts of plasticizer (glycerin) result in a more porous and hydrophilic polymer structure, which is prone to water vapor permeation due to the reorganization of the protein network and the increase in free volume (Khan *et al.*, 2021). Additionally, higher casein concentrations in the coating adversely impact porosity of paneer, potentially leading to denser and firmer textures due to occupying interstitial spaces between particles.

Acidity

The response surface analysis, illustrated in Fig 1c, reveals the interacting effects of independent variables the acidity of Indian cottage cheese (paneer). Among these, glycerin and casein demonstrated statistically significant impacts ($p < 0.05$), highlighting their role in acidity. The response surface graph depicts a gradual curve incline with increa-

Table 1: Concentration of independent variables used in RSM.

Symbols	Independent variables	Levels		
		Low (-α)	Center	High (+α)
X_1	Casein (%)	8	10	12
X_2	Glycerin (%)	2	3.5	5
X_3	Pectin (%)	0.5	1.0	1.5
X_4	Water (%)	80	85	95

Table 2: Final equation of response variables and their respective R^2 .

Variables	Equation	R^2
Porosity	$+18.91 + 0.079X_1 - 0.57X_2 - 0.44X_3 + 0.49X_4 + 0.13X_1X_2 + 0.25X_1X_3 + 0.81X_1X_4 - 0.55X_2X_3 + 0.77X_2X_4 + 0.23X_3X_4 + 0.087X_{12} + 0.23X_{22} - 0.55X_{32} - 1.06X_{42}$	0.84
Titrateable acidity	$+1.13 - 0.011X_1 + 0.049X_2 + 0.11X_3 + 5.958E-003X_4 + 0.11X_1X_2 - 0.054X_1X_3 - 0.030X_1X_4 + 0.011X_2X_3 + 0.033X_2X_4 + 0.035X_3X_4 - 0.11X_{12} - 0.091X_{22} + 0.012X_{32} - 0.050X_{42}$	0.81
pH	$+6.89 + 0.88X_1 + 0.037X_2 + 0.12X_3 - 0.093X_4 - 0.072X_1X_2 - 0.12X_1X_3 - 0.011X_1X_4 + 0.18X_2X_3 - 0.10X_2X_4 - 1.875E-003X_3X_4 - 0.18X_{12} + 0.23X_{22} + 0.48X_{32} + 0.32X_{42}$	0.82

sing concentrations of casein and glycerin, yet reaching optimal levels results in diminishing effects. These findings underscore the importance of optimal glycerin and casein concentrations to maintain paneer acidity below 0.50%. Glycerin, as a plasticizer, affects film permeability, potentially altering its composition, especially moisture content, thus impacting lactic acid bacteria proliferation and acidity (Wang *et al.*, 2020).

pH

pH is crucial for assessing the quality of Indian cottage cheese (paneer). The interaction of examined factors did not significantly influence it. Thus, the investigation focused on independent factors affecting pH. Fig 1d shows the impact of casein concentration on the pH of paneer, indicating a positive correlation. Variations in casein concentration indirectly impact pH by modulating water equilibrium and buffering capacity, owing to the diverse chemical composition of proteins and minerals. Consequently, the pH may experience a shift toward increased acidity (Bonnaillie *et al.*, 2014).

Optimization of edible coating

A numerical and graphical optimization approach, employing a maximum desirability index, was utilized to determine the optimal concentrations of independent factors. Selection criteria were based on achieving maximum porosity while minimizing titratable acidity. The optimized combination of coating solution, yielding the highest desirability, comprised 8% casein, 4.6% glycerin, 0.65% pectin and 86% water. Utilizing these concentrations, predicted properties of the response variables for the

optimized coating solution were determined: porosity at 19%, titratable acidity at 0.52% and pH at 6.

Indian cottage cheese (Paneer) treated with the optimized coating solution, both with and without antimicrobial compounds, underwent comprehensive analysis to assess the influence of the edible coating on physicochemical and microbiological parameters.

Characterization of CNP

The diameter and zeta potential of the CNP, measured through Dynamic Light Scattering, were determined to be 178 nm and -3.0 mV, respectively. Additionally, the polydispersity index was found to be 27.9%. CNP larger than 100 nm exhibit reduced ion release, thereby minimizing the generation of copper ions responsible for cellular toxicity (Sabella *et al.*, 2014). Additionally, their larger size significantly limits cellular uptake, as these particles are less likely to penetrate cell membranes. In the context of edible coatings, only trace amounts-less than 8mg-of CNP were absorbed by 100 grams of paneer, further ensuring food safety.

Shelf life studies

Moisture loss

During storage, moisture loss from Indian cottage cheese (paneer) results from continuous water migration to the surroundings, causing weight reduction. Untreated samples exhibited the highest weight loss, up to 55%, followed by plain and EO samples. In contrast, CNP-coated samples demonstrated superior moisture retention, as shown in Fig 2a, with an average moisture loss of 40% over the study period. This underscores the importance of casein-

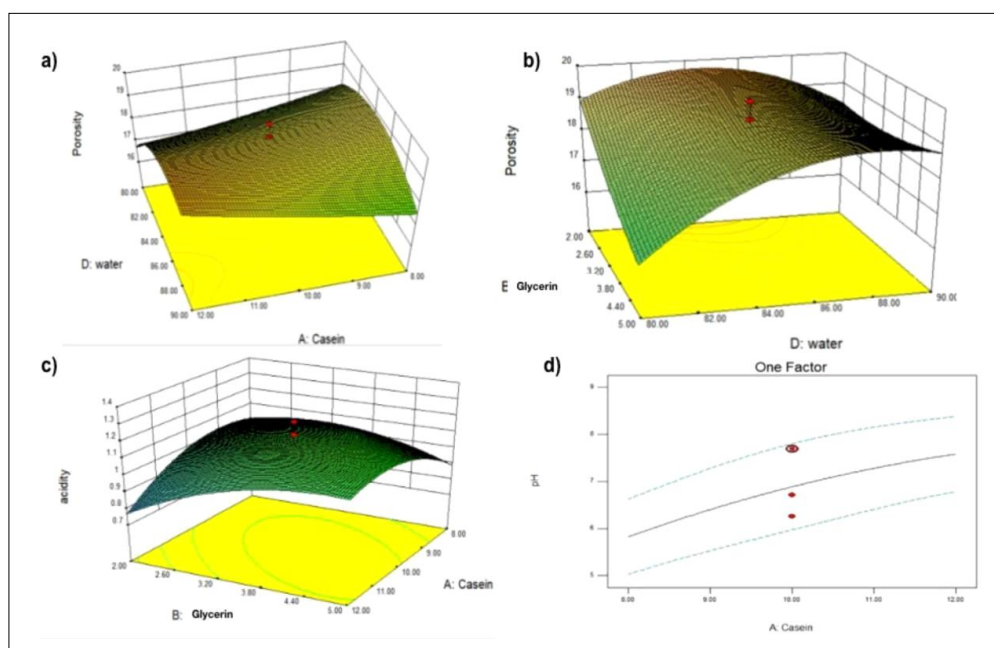


Fig 1: a, b) Response surface graph of interaction factor on porosity, 1c: Response surface graph of interaction factor on Titratable acidity 1d: Effect of concentration of casein on pH.

pectin-based coatings in moisture preservation. Similar results of CNP were also suggested by Youssef *et al.*, 2020 in case of processed cheese. This might be due to the interaction between casein, pectin and glycerol, enhancing the tactile properties and water resistance of casein films. Pectin acts as a linear-chain crosslinker, forming robust networks by binding negatively charged OH and COO groups on pectin chains with positively charged casein groups like -NH, effectively resisting water migration and enhancing moisture retention in coated cottage cheese samples.

Water activity

Fig 2b presents the fluctuations in water activity (aw) of edible-coated paneer samples throughout the storage period. By the 14th day, the control samples exhibited the lowest water activity, likely attributed to the depletion of monolayer moisture, a phenomenon absent in the coated samples. The aw values demonstrated a consistent decline over time, aligning with the findings reported by Sen *et al.*, (2023). In the control group, the aw values were recorded as 0.98, 0.84 and 0.79 on days 0, 7 and 14, respectively. Among the coated samples, the CNP-coated paneer displayed the lowest aw *i.e.* 0.84 after 14 days, signifying its superior ability to maintain optimal water activity while curbing microbial proliferation during the storage period.

Titrateable acidity

Fig 2c illustrates the variations in titrateable acidity during the storage of Indian cottage cheese (paneer) at 15°C. The control samples demonstrated a significant increase in

acidity, rising from 0.31% on day 0 to 0.78% by day 14, likely due to microbial proliferation, exceeding the FSSAI limit of less than 50%. All samples initially remained within this limit for the first seven days, but subsequently showed a gradual rise in acidity, potentially due to peptization and bacterial fermentation. Among the samples, CNP-coated paneer exhibited the smallest increase in acidity, followed by EO-coated samples, highlighting CNP's efficacy in suppressing the growth of lactic acid bacteria during storage (Rajeshkumar *et al.*, 2019).

pH

Fig 2d illustrates a gradual decrease in pH of the cottage cheese sample over storage time. Paneer samples displayed minimal pH fluctuation over the 14-day storage period, implying a robust buffering capacity that restrains pH alterations. Except for the control sample, the pH of the samples remained within the range (5.7-6.0) reported by other researchers.

Antibacterial activity

The control samples initially showed a standard plate count (SPC) of 4.97×10^5 CFU/g, which increased to 63.5×10^6 CFU/g by day 14. In comparison, EO-coated samples reached 10.55×10^7 CFU/g on day 14, approximately 40 times higher than the control. In contrast, the CNP-incorporated edible coating achieved an 88.6% reduction in microbial load by day 14, primarily due to the cytotoxic effects of copper. The high surface area-to-volume ratio of CNPs enhanced their binding to bacterial cell membranes, leading to genetic damage, oxidative stress

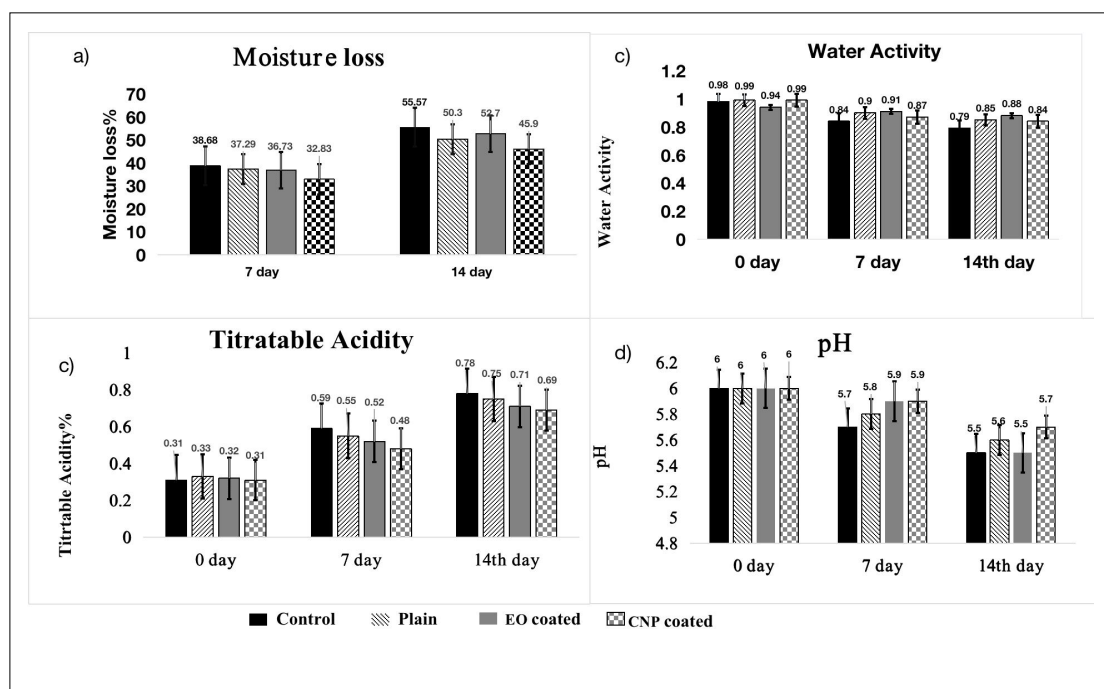


Fig 2: Effect of antimicrobial incorporated edible coating on quality attributes of stored cottage cheese samples a) moisture loss, b) water activity, c) pH, d) Titrateable acidity.

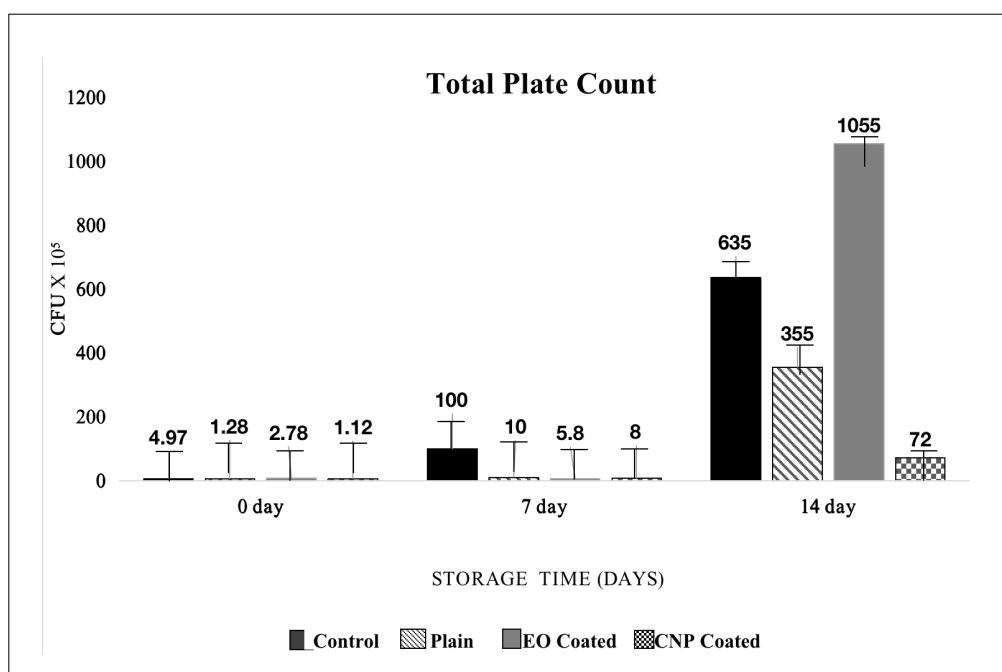


Fig 3: Effect of antimicrobial incorporated edible coating on microbial count of stored cottage cheese samples.

and ultimately bacterial cell death (Rajeshkumar *et al.*, 2019). Despite these effects, all samples exceeded the FSSAI microbial count standard of less than 3.5×10^5 CFU/g after 5 days as shown in Fig 3 (FSSAI, 2016). Additionally, after 14 days, control and plain-coating samples displayed lower bacterial densities compared to EO-coated samples, potentially influenced by evolving physical parameters during storage. EO-coated samples on day 14 exhibited the highest water activity, likely facilitating bacterial proliferation, in line with findings by Wang *et al.*, (2022). This observation aligns with the notion that lipophilic nature of EO might be less effective in environments rich in protein and fat, thus failing to adequately inhibit bacterial growth.

CONCLUSION

The study utilized Response Surface Methodology (RSM) to optimize an edible coating formulation for Indian cottage cheese (paneer), aiming to extend its shelf life. The results demonstrated that a casein-pectin-based coating, enriched with copper nanoparticles, effectively retained moisture and inhibited bacterial growth, prolonging the shelf life to 5 days at 15°C , compared to the typical 2-day shelf life. Future research should explore a wider range of antimicrobial coatings to further refine and enhance preservation techniques for paneer.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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