


Physical and mechanical characterization and evaluation of antimicrobial potential of casein based edible film containing cinnamaldehyde extract

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Abstract : Studies produced composite films of sodium caseinate and cinnamaldehyde (0%, 1.5%, 2.5%, and 3.5%) using a reduced particle emulsion method. Variations in thickness, moisture content, water barrier (COBB test), tensile strength, and bursting strength were evaluated. The antimicrobial effect of each film was observed through the zone of clearance/inhibition against the *E. coli* K-12. There was a non-significant ($p>0.05$) and significant ($p<0.05$) increase in the thickness and moisture content between the control (0%) and cinnamaldehyde-concentrated films, respectively. COBB test values reflected better water barrier properties of cinnamaldehyde films than control. Film with 3.5% cinnamaldehyde concentration had the highest tensile strength but was non-significantly ($p>0.05$) different from 2.5% film. However, bursting strength was highest for the 2.5% film and was non-significantly ($p>0.05$) different from the control (0%) film. Antimicrobial testing demonstrated the highest zone of clearance for 2.5%, among all the three concentrations used. Further studies need to be conducted by applying the developed film on suitable dairy products and evaluating its shelf-life.

Keywords: Antimicrobial, Bio-preservation, Food safety, Smart packaging, Sustainability

Introduction

Food packaging is a crucial part of the food supply chain and assists in handling and protecting food products during

transportation (Singh et al. 2021). Food-grade plastic is the commonly used packaging material in the food sector, attributed to its lower cost and high performance (Jeevahan et al. 2017). This material is generally used for a single time and often dumped into the ocean or landfilled, contributing to numerous environmental concerns (Kumar et al. 2022). These concerns have driven the researchers to investigate innovative packaging techniques that can protect food products as well as are environmentally friendly. There has been considerable interest in edible films and coatings due to the elevation in health awareness among consumers (Mahcene et al. 2021). These are defined as the continuous matrices produced with edible materials and can be present either on the food surface or as a layer between the various layers of the food (Debeaufort and Voilley, 2009). As per Zhao et al. (2021) it is one of the most suitable sustainable packaging approaches as it can reduce carbon footprints. Consumers also find this packaging convenient as food products can be consumed without removing film (Barbosa et al. 2021).


According to Nair et al. (2023) protein-based edible films are known for their high resistance against atmospheric gases, including O_2 and CO_2 , fats, and aromas, and have relatively superior mechanical characteristics. Familiar protein sources from plants and animals are used as a base material of edible packaging material include gluten, peanut, bean, soy, milk protein (casein and whey), gelatin, and collagen (Kumar et al. 2022). Edible films made from milk proteins are usually transparent, pliable in nature, and have no taste (Mihalca et al. 2021). Their mechanical properties, like tensile strength, thickness, and barrier properties depend on the type of protein and the type and amount of plasticizer used (Wagh et al. 2014). Milk proteins are mainly classified as casein and whey protein, which are present in the ratio of 80:20 (Salunke et al. 2025; Joshi et al. 2024; Singh, 2023). Caseinate (used for the formation of edible film) usually stays in combination with calcium or sodium, the water-soluble form of casein. The films formed from casein are one of the clearest protein-based films known for their firmness, elasticity, and low oxygen permeability (Nair et al. 2023). Like all the protein-based films, casein films also have poor water vapor resistance. Further nutrient abundance may cause poor microbial resistance ability.

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Essential oils like carvacrol and cinnamaldehyde are agents that are known to show the antimicrobial properties against the numerous foodborne pathogenic microorganisms (Otoni et al. 2014 a, b). Hence, it can be conveniently used in edible films to improve the shelf life of the products. However, adding these substances can positively and negatively influence the film's properties. Literature studies have reported the effect on the physical and mechanical properties of films where variation was dependent on the type, percentage, physical state, and hydrophobicity of the oil used (Gallo et al. 2000). According to Otoni et al. (2014a) the preferable way to generate composite film is by dispersing the oil into film generating solution and then adding an emulsifier followed by homogenization to decrease the particle size in oil-in-water emulsion. Literature has reported the higher effectiveness of composite films when the films were formed by using this method (Otoni et al. 2014b).

Our studies have used the reduced particle-sized emulsion formation method to generate sodium caseinate-based edible films containing cinnamaldehyde at 0% (control), 1.5%, 2.5%, and 3.5% concentration. Spices are the inherent part of Indians diet hence our film can impart shelf stability as well as can deliver a spicy tinge to the products (Shinoj and Mathur, 2006). Glycerol and Tween 60 were used as a plasticizer and emulsifier, respectively. All the factors and concentrations of material used for film formation were kept consistent except for the amount of cinnamaldehyde. Therefore, it was hypothesized that all the alterations in the film properties were due to the variation in the cinnamaldehyde extract concentration. Thickness, moisture content and mechanical properties (tensile and bursting strength) of the developed films were evaluated and compared to each other. The zone of inhibition of each film was assessed against *E. coli* K-12, a common microbial contaminant in milk and milk products (Soomro et al. 2002). While recent literature has examined the impact of incorporating essential oils extract such as orange oil oregano and sage into casein based edible films, there is a scarcity of research regarding the influence of cinnamaldehyde incorporation on these film properties (Bhatia et al. 2023, 2024). Hence this study aims to address this knowledge gap, offering valuable insights into how varying levels of cinnamaldehyde content can affect the various characteristics of the film.

Materials and Methods

Film formation

A film solution containing 8 g of sodium caseinate (concentration) was prepared by dissolving it in 88 mL of distilled water under continuous stirring at 60°C. Following the complete dissolution,

4 g of food grade anhydrous glycerol (Merck Specialties Pvt Ltd, Mumbai, Maharashtra, India, 400018) as subsequently introduced. Cinnamaldehyde (e"98% concentrated, Himedia Laboratories Pvt. Ltd., Mumbai, Maharashtra, India, 400086) was added in the continuous stirred solution at 0% (control samples), 1.5%, 2.5%, and 3.5% w/w. The concentration levels were determined based on a review of existing literature on the development of edible films incorporating essential oils (Seydim and Sarikus, 2006; Ravishankar et al. 2012). The solution was continuously stirred for 2 h. Tween 60 (polyoxyethylene (20) sorbitan monostearate) was added as a surfactant at 2.5% w/v of the solution. The solutions were then homogenized for 15 min with an ultra-turrax at a rotational speed of 10,000 rpm. The resulting solutions underwent a 2 h deaeration process before being casted into plates. Each plate held 20 g of the solution. The finished samples were dried overnight in an incubator set to 37 . Following that, the samples were properly peeled and subjected to a 48 h conditioning process in an incubator set at a relative humidity of 50 ± 2%. The final film formed has been shown in Figure 1.

Thickness and moisture content evaluation

The film thickness was measured using a digital micrometer with a sensitivity of 0.01 mm (M/S Engineering Corporation, Uttar Pradesh, India). The films were cut into 10 cm 2.5 cm segments, and the mean thickness was calculated by taking ten random measurements. The moisture content of the films was determined by introducing the film samples measuring 10 cm 10 cm into a digital moisture analyzer (Delmhorst Instrument Co., RDM-3P, Towaco NJ 07082, USA, with calibration services provided by Calibration Services-92). The average moisture content of each film sample was determined by taking ten random readings.



Fig. 1 Pictorial representation of the film formed

Mechanical properties evaluation

Tensile Strength

To assess the tensile strength, the films were cut into strips measuring 10 cm × 2.5 cm. These strips were then placed onto a tensile strength tester (Test Techno Consultants, Vadodara, Gujarat, India, 391350).

Bursting Strength

To measure the bursting strength, the films were inserted into the bursting strength tester (Presto Stantest Pvt Ltd., Faridabad, India). The peak load was recorded for both inward and outward directions, and the average peak load was determined based on ten data points.

Water absorptivity

For the assessment of water absorption ability, COBB test was conducted using a COBB tester (Test Techno Consultants, Vadodara, Gujarat, India, 391350). The films were appropriately trimmed to match the dimensions of the COBB well sheet and subsequently weighed. 100 mL of distilled water was introduced into the well. The film was placed into the apparatus and the well was maintained in an inverted orientation for a duration of 60 sec. It was then unloaded and carefully positioned between two sheets of blotting paper. A 10 kg force (kgf) was exerted against the object by means of a rolling motion, first in a forward direction and subsequently in a backward direction. Following this procedure, a specimen was extracted from the space enclosed by the blotting papers and subjected to measurement of its weight. The process of transferring the samples from the loading area to the weighing stage was successfully executed within a time frame of less than 10 sec. The COBB index was determined by using equation 1.

$$\text{COBB Index} = (\text{Final weight} - \text{Initial weight}) \times 100$$

Evaluation of antimicrobial efficiency

The antimicrobial effect of cinnamaldehyde was assessed against *E. coli* K-12 using agar-well assay. The experimental procedure involved the formation of wells on solidified MacConkey agar plates (Himedia Laboratories Pvt. Ltd., Mumbai, Maharashtra, India, 400086) swabbed with *E. coli*, followed by the introduction

of the extract into these wells. The plates were thereafter placed in an incubator set at a temperature of 37 °C for 24 h. The measurement of the inhibition zone was conducted to confirm the antimicrobial properties of the cinnamaldehyde extract, using 95% ethanol as positive control.

The antimicrobial effectiveness of developed films (0%, 1.5%, 2.5%, and 3.5% cinnamaldehyde) were then assessed by spot-assay method by measuring the zone of inhibition. The films were fragmented into 1 cm × 1 cm pieces using sterile forceps. *E. coli* K12 culture (0.5 McFarland Standard) was swabbed onto the MacConkey agar plates and the film fragments were carefully positioned onto the streaked area and the petri dishes were subjected to incubation at a temperature of 37 °C / 24 h. Relative inhibition zone measurement of films was reported as compared to the inhibition zone of cinnamaldehyde extract.

Statistical Analysis

All the tests were conducted in replicates where each sample was assigned independently and randomly, and the mean values were reported. To evaluate the effect of different concentration of cinnamaldehyde, on thickness, moisture content, tensile, bursting strength, and water absorptivity, one-way ANOVA was carried out and the significant differences among the treatments were determined using Tukey HSD at 5% level of significance ($p < 0.05$).

Results and Discussion

Thickness and Moisture Content

Table 1 demonstrates the thickness and moisture percentage of the different caseinate films formed with varying concentrations of cinnamaldehyde. Thickness is classified as the edible film's physical property, which directly affects the permeation rate of gas, water vapors, and other volatile components. In our case, there was a non-significant ($p > 0.05$) increase in the thickness values as the concentration of the cinnamaldehyde increased from 0% to 3.5%. This might be attributed to the elevation of the solid components of the film, increased bonding between oil (cinnamaldehyde) and the casein matrix and the microdroplets formation of oil components. Our results were in agreement with the literature (Bhatia et al. 2022, 2023). Further, it was observed that the moisture content increased significantly ($p < 0.05$) with

Table 1: Variation in the thickness, moisture content and water barrier ability (COBB Index) of the developed caseinate film with variation in the percentage of cinnamaldehyde concentration

Film	Thickness (mm)	Moisture %	COBB Index (g/m ² /min ²)
0% Conc. (control)	0.21 ± 0.02 ^a	9.08 ± 0.19 ^a	43.56 ± 2.02 ^a
1.5% Conc.	0.22 ± 0.03 ^a	9.94 ± 0.13 ^b	39.33 ± 2.67 ^b
2.5% Conc.	0.23 ± 0.02 ^a	9.94 ± 0.30 ^b	38.95 ± 0.54 ^b
3.5% conc.	0.24 ± 0.03 ^a	10.05 ± 0.13 ^b	38.36 ± 2.19 ^b

the addition of the cinnamaldehyde extract. The elevated moisture might be part of the water content of the extract added. However, the increase was non-significant ($p>0.05$) upon variation in concentration.

Tensile Strength and Bursting Strength

Mechanical characteristics of any packaging film are the crucial parameters that demonstrate its durability and are correlated to the molecular bonding, components and processing technique used for its development (Ulyarti et al. 2019). Our studies evaluated the tensile strength and bursting strength of the films consisting of cinnamaldehyde in varying proportions (Figure 2). The tensile strength of the edible film is highly dependent on the

bonds between its components. It refers to the maximum amount of stress that edible film can bear during stretching prior to breaking (Ghiasi et al. 2020). The film’s protein content (protein-protein interactions) significantly affects its tensile strength (Jooyandeh, 2011). Interestingly, the chemical structure of essential oils can also influence the film’s tensile strength. In our case, protein content was constant in all the films. However, variation can be seen with the change in cinnamaldehyde concentration. The tensile strength values obtained for 0% (control), 1.5%, 2.5%, and 3.5% concentrated films were 1.5 ± 0.07^a , 2.23 ± 0.07^b , 2.40 ± 0.10^{bc} and 2.56 ± 0.12^c kgf, respectively. There was a significant ($p<0.05$) increase in the film’s tensile strength upon incorporating cinnamaldehyde. The increase was non-significant ($p>0.05$) between the 1.5% and 2.5% film and

Table 2: Zone of clearance/inhibition of the various caseinate films produced and the cinnamaldehyde blank against the *E. coli* K 12

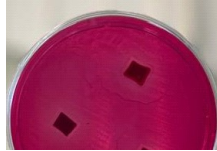




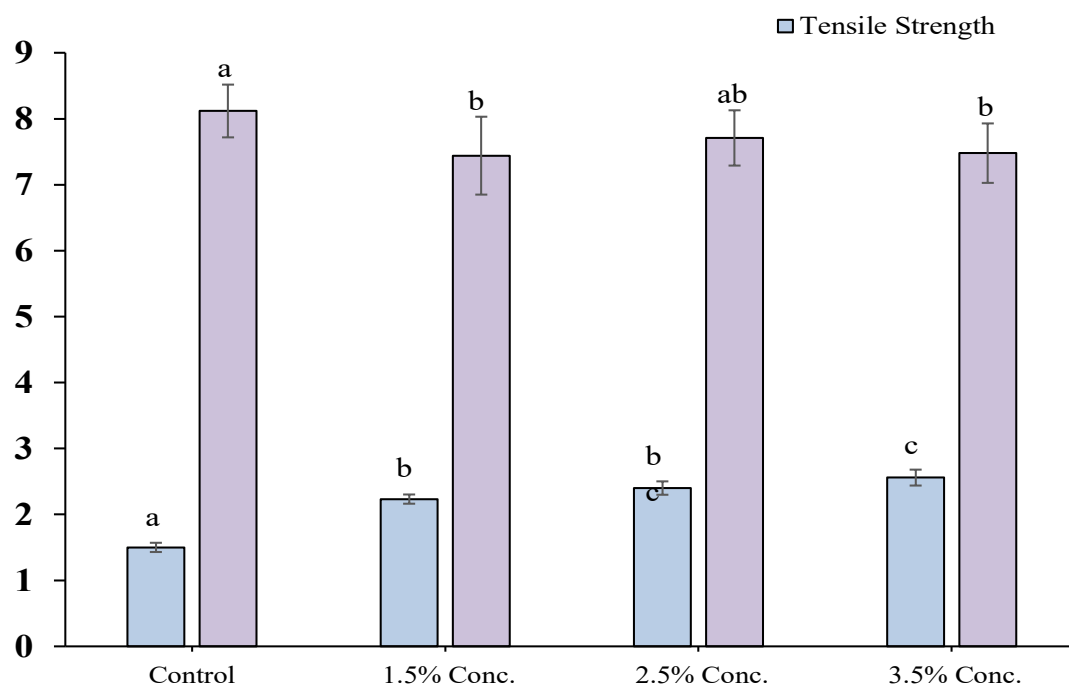
Sample	Zone of clearance/inhibition	Picture
Control	No clear zone	
Cinnamaldehyde control	++++	
1.5% concentrated film	+	
2.5% concentrated film	+++	
3.5% concentrated film	++	

Fig. 2 Variation in the tensile strength (kgf) and bursting strength (kg/cm²) of the caseinate films produced with different concentrations of cinnamaldehyde



2.5% and 3.5% film. However, a significant ($p < 0.05$) improvement between 1.5% and 3.5% film was observed. These results contrasted with the reports by Zhao et al. (2021) and Bhatia et al. (2023). However, Otoni et al. (2014b) reported similar or increased tensile strength of pectin-based edible films on the addition of cinnamaldehyde (at 2% w/v), which was in agreement with our studies (Otoni et al. 2014b). Zhao et al. (2021) have mentioned that the decreased tensile strength of the film on the addition of cinnamaldehyde could be due to a heterogeneous matrix of the film forming solution that produced the film with discontinuous bond. Further, the declined strength was more pronounced in the films with cinnamaldehyde concentration in the range of 4-10%. This necessitates optimizing essential oil concentration to obtain the maximum antimicrobial effect with minimal effect on the mechanical properties. Bhatia et al. (2022) reported the declined tensile strength in their case was due to the weak bonding between polymer and oil. The deficient bonding may have arisen from the absence of the emulsifier in their film. However, in our case the incorporation of Tween 60 might have resulted in stable emulsion and fostered the bonding of oil and casein which might have resulted in the improved tensile strength.

Bursting strength reflects the ability of the film to bear the pneumatic load. According to Bhatia et al. (2022) the bursting or puncture strength of edible films is a crucial mechanical property of the edible film that evaluates the susceptibility to fragility, brittleness, and tolerance against the physical handling. In our studies, none of the developed films ruptured at the initial stage of bursting strength testing which indicates that the incorporated concentrations had not exceeded the level where weakened interactions between the film network would render it fragile or

brittle. The observed values for 0% (control), 1.5%, 2.5%, and 3.5% films were 8.12 ± 0.40^a , 7.44 ± 0.59^b , 7.71 ± 0.42^{ab} and 7.48 ± 0.45^a . The decline in the bursting of the film was observed on the incorporation of cinnamaldehyde, this might be attributed to the increased thickness as Bhatia et al. (2022) has reported the inverse correlation of puncture strength with the thickness of the film. However, the alteration in bursting strength in our case had an asymmetrical order where 1.5% film had significant decline as compared to control while 2.5% and 3.5% cinnamaldehyde had non-significant decline as compared to control film. Overall, among the cinnamaldehyde films, 2.5% concentrated film demonstrated the highest bursting strength.

COBB Test

Hydrophilic components of edible films warrant the need of testing their ability to resist water (Bamdad et al. 2006). COBB test has been extensively used for the evaluation of water resistance of paper, paperboard, and porous films (Samyn et al. 2010). Addition of cinnamaldehyde (oil) could deliver the porous structure to the casein based edible film (Soomro et al. 2002), that potentially makes it suitable to be tested with COBB test for the assessment of water barrier ability. A lower COBB index value signifies lower water absorption hence the higher water resistance (Anthony et al. 2015). Results revealed that there was a significant ($p < 0.05$) decrease in COBB value ($\sim 9.7\%$) with the addition of cinnamaldehyde extract to the film. It was attributed to the increased hydrophobic characteristics of the film solutions (Acevedo-Fani et al. 2015). However, with the elevation in the concentration of cinnamaldehyde from 1.5% to 3.5%, the decline in COBB index values were non-significant ($p > 0.05$, 1-5-2.5%).

Our results were in agreement with the literature studies. Ghani et al. (2018) also observed improved water vapor resistance by adding cinnamon essential oil in the polysaccharide film. This effect can be seen up to a specific limit. After that, the high bond between the essential oil's nanoemulsion and film base material weakens the polymer network. Otoni et al. (2014b) also observed the amelioration in the water barrier properties of the pectin-based edible film upon incorporation of cinnamaldehyde (2% w/v).

Evaluation of antimicrobial effect

Otoni et al. (2014a) reported that the antimicrobial activity of cinnamaldehyde was more pronounced for Gram-positive bacteria than for Gram-negative ones. It was because the lipopolysaccharide components of the Gram-negative's hydrophilic membrane are resistive to hydrophobic components (cinnamaldehyde). At the same time, its absence in Gram-positive bacteria provides efficient penetration of cinnamaldehyde inside the cell. Our studies evaluated the efficiency of produced films against Gram-negative bacteria, i.e., *E. coli* K-12. Cinnamaldehyde is known for deprivation of the DNA synthesis in *E. coli* species by breaking the cellular homeostasis through cell membrane action (Pereira et al. 2021). *E. coli* is a multi-faceted bacterium, that plays a variety of roles in milk and milk products ranging from faecal indicators to causing spoilages and many of its strains acting as pathogens (Soomro et al. 2002). Therefore, its presence in milk has always been a concern for the dairy industry. The zone of clearance/inhibition observed for different films has been given in Table 2. As anticipated, the blank sample had no clear zone, however, the tested sample of cinnamaldehyde clearly reflected the effective inhibitory effect against the targeted microorganism. This demonstrates that the antimicrobial effect of the developed film was attributed to the added cinnamaldehyde content. Zhu et al. (2020) have also reported that essential oils' active components play a crucial role in delivering antimicrobial activity.

In the case of cinnamaldehyde, a positive zone of clearance was obtained for all the concentrations tested (1.5%, 2.5% and 3.5%). However, among the three, 2.5% film demonstrated the highest (+++) and closest to the cinnamaldehyde control (++++) zone of inhibition. Studies have reported a similar antimicrobial effect of cinnamaldehyde incorporated in the edible film of different base materials (apple, carrot, hibiscus, pectin, etc.) on foodborne pathogens like *L. monocytogenes*, *S. aureus*, *S. enterica*, *E. coli* O157: H7 (Zhu et al. 2014, 2020) In these studies, log reduction was significantly dependent on the concentration of cinnamaldehyde used. However, in our studies, cinnamaldehyde was used because of its possible acceptance with *paneer*, cottage cheese, etc. in our future studies.

Conclusion

A study was carried out to analyze the antimicrobial effect of the various concentration of cinnamaldehyde extract incorporated in the casein-based edible films, alongside the assessment of variation (at $p < 0.05$) in the thickness, moisture content, mechanical properties (tensile and bursting strength), and water barrier properties. The results demonstrated that as compared to control cinnamaldehyde incorporated film had lower bursting strength while the higher thickness, higher moisture content, improved tensile strength, water barrier ability and antimicrobial effect. Among the three concentrations used (at 1.5%, 2.5% and 3.5%), 2.5% film demonstrated the highest bursting strength, antimicrobial effect (zone of inhibition against *E. coli* K-12). The tensile strength was highest for 3.5% film, however, was non-significantly ($p > 0.05$) different from 2.5% film. Therefore, based on the results obtained, 2.5% was the most suitable concentration to incorporate into the produced film. Future studies will be focused on applying the developed films *paneer*, cottage cheese, etc. and evaluation of its shelf-life extension, physio-chemical, sensory, and nutritional properties.

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