Research Articles

Journal of Meat Science
Year 2025 (June), Volume-20, Issue-1
ISSN 0975-5209 (Print)
ISSN 2581-6616 (Online)

Creation and assessment of lysozyme–alginate complexes for antimicrobial packaging of chicken patties

M.Prasanna[1], E. Naga Mallika[2], K.Sudheer[3]

[1]M.V.Sc scholar, Department of Livestock Products Technology, Sri Venkateswara Veterinary University, Andhra Pradesh-521102, India
[2]Associate Professor, Department of Livestock Products Technology, Sri Venkateswara Veterinary University, Andhra Pradesh-521102, India
[3]Assistant Professor, Department of Livestock Products Technology, Sri Venkateswara Veterinary University, Andhra Pradesh-521102, India

ARTICLE INFO

*Corresponding author:
E-mailaddress: 7731976798d@gmail.com
(*Dr.M.Prasanna)
Received 2024-07-16 ; Accepted 2025-05-21

doi10.48165/jms.2025.20.01.4

ABSTRACT

Edible films are made from biopolymers like alginate, pectin, starch, cellulose etc. Among these alginate is regarded as healthful and is taken without limitation. Lysozyme has a broad range of pH and temperature stability, making it a good preservation agent. This study aimed on development of alginate based edible films with lysozyme and to evaluate their efficacy on the quality of chicken meat patties. Alginate films with lysozyme of different concentrations were developed i.e., T1 (alginate with 1.62 $\times 10^6$ specific units of lysozyme), T2(alginate with 3.24 $\times 10^6$ specific units of lysozyme) and T3 (alginate with 8.1 $\times 10^6$ specific units of lysozyme). One best film was selected based on parameters like thickness, grammature, water vapour permeability, anti-oxidant activity, tensile strength and antimicrobial activity along with control. The product was evaluated for parameters like pH, percent cooking loss, 2-TBARS, microbial analysis and sensory evaluation, Results showed that the thickness, grammature, water vapour permeability, anti-oxidant activity, tensile strength and antimicrobial activity of T3 film were significantly ($P \leq 0.05$) higher and lower water sorption compared to T1, T2 and control films. The pH, 2-TBARS, Percent cooking loss and microbial count values were lower in the chicken patties that were wrapped in T3 film.

Keywords: Alginate, lysozyme, chicken, meat patties,

INTRODUCTION

Packaging is an integral aspect of ensuring the safety and quality of food products, acting as a protective barrier from initial processing to the point of consumption. Its role extends beyond mere containment, encompassing the prevention of undesired chemical and biological changes in the products. Shielding against external environmental factors such as heat, light, moisture, pressure, microorganisms, and gaseous emissions is a critical function of packaging (Bilska 2011). Moreover, packaging provides consumers with convenience by offering various sizes and shapes of food items, accompanied by detailed labelling information.

In the evolving landscape of smart packaging, concepts like active and intelligent packaging add a dynamic dimension to the relationship between packaging and food safety (Yadav et al. 2015). Active packaging, as an innovative approach, focuses on preserving and extending the shelf life of food products, ensuring their integrity and quality. This system comprises absorbers, eliminating undesirable compounds from the product or its surroundings, and emitters, introducing substances to prevent unwanted changes in the packaged food.

One notable aspect of active packaging is the use of antimicrobial films, which effectively control foodborne pathogens and spoilage microorganisms, thus enhancing food safety. These films can incorporate chemical preservatives or antimicrobial agents that target specific microorganisms

(Moraes et al. 2007).

Responding to the increasing consumer awareness of the drawbacks of chemical preservatives in meat products, there is a growing interest in natural alternatives to improve food shelf life. Natural antimicrobials derived from plant extracts, such as essential oils and lysozyme, are gaining prominence as substitutes for chemical additives (Brody 1997) in antimicrobial packaging.

Biopolymers, including Alginate, starch, cellulose, gelatin, and other proteins, have been extensively researched for their film-forming properties in the development of edible films for food packaging (Azeredo et al. 2009). Alginate, a natural polysaccharide derived from brown seaweeds, is particularly noteworthy for its versatility and unique properties, making it a preferred material in the creation of edible packaging and films.

Lysozyme, a lytic enzyme found in various natural sources like chicken egg white, milk, tears, and saliva, exhibits exceptional potential for food preservation due to its stability across a wide range of pH and temperature conditions (Proctor and Cunningham, 1988). Lysozyme has been successfully incorporated into natural polymers such as soy protein, corn zein (Padgett et al. 1998), as well as alginate and carrageenan (Cha et al. 2002).

In light of these considerations, this study aims to contribute to the development and standardization of Alginate-based edible antimicrobial films with immobilized lysozyme, providing a promising avenue for enhancing food safety and shelf life.

MATERIALS AND METHODS

IMMOBILIZATION OF LYSOZYME

The immobilization of lysozyme was carried out in accordance with the procedure outlined in reference (Bayarri et al.2014). Formation of Lysozyme-Alginate Complexes Lysozyme-Alginate complexes were prepared using lysozyme with a specific activity of 15000 units per milligram and alginate. A constant quantity of alginate was used, and the optimal amount of lysozyme for immobilization onto was determined by gradually adding lysozyme in incremental concentrations to the Alginate. The resulting mixtures were vigorously vortexed for one minute while maintaining a pH of 7. The turbidity generated as a result of lysozyme immobilization was quantified at 600 nm (at 25°C) against an imidazole-acetate buffer (5mM, pH 7) using a UV/Vis spectrophotometer (ThermoScientific Nanodrop 2000c).

ASSESSMENT OF LYSOZYME ACTIVITY

The activity of lysozyme was evaluated following the methodology outlined in reference (Bayarri et al.2014).

LYSOZYME ACTIVITY ASSAY

Lysozyme activity was determined by monitoring the reduction in absorbance at 450 nm, which was caused by the lysis of M. lysodeikticus cells at 25°C. In a 1 cm cuvette, 2.9 ml of the M. lysodeikticus suspension (with an OD450 of 1) in a 5 mM phosphate buffer adjusted to the appropriate pH, and 0.1 ml of the enzyme solution (prepared at the same pH) were rapidly mixed. The decrease in absorbance was recorded using a UV/Vis spectrophotometer (Thermo Scientific Nanodrop 2000c) until a plateau was reached. Lysozyme activity was calculated from the slope of the initial linear portion of the absorbance versus time curve. The hydrolytic activity of the lysozyme solution can be determined using the following formula: Activity (units per milliliter) = S / 0.001 × V

"In our study, we quantified enzyme activity, with one unit representing a change of 0.001 in absorbance per minute. This activity (referred to as 'Activity') was calculated using the slope (S) of the initial linear segment of the absorbance versus time curve, in conjunction with the volume (V) of the lysozyme solution.

Fig. 1 ***Micrococcus lysodeikticus*** **on nutrient agar**

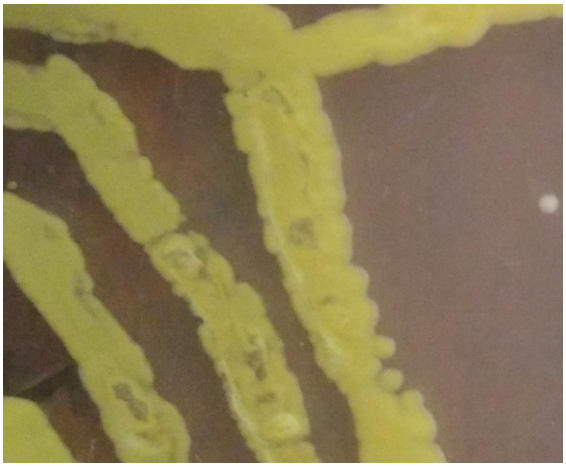
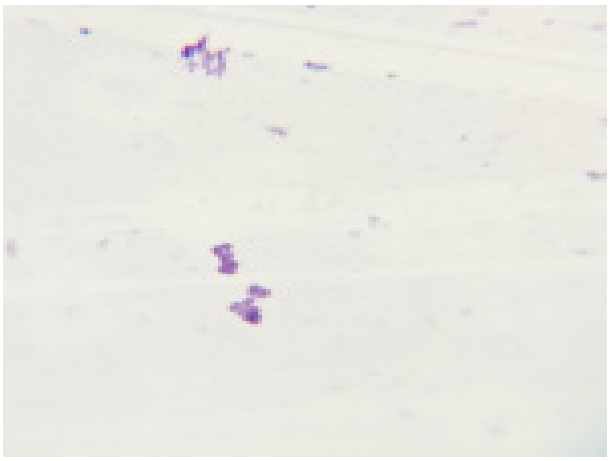


Fig. 2 ***Micrococcus lysodeikticus*** **under microscope**



PREPARATION OF FILMS

To prepare Alginate films with immobilized lysozyme, the immobilized lysozyme was incorporated into Alginate films at various concentrations. We formulated film-forming solutions by dissolving Alginate at a concentration of 3% (w/v) in distilled water and then heating it to 80°C to allow for gelatinization. To enhance the film's flexibility, we introduced glycerol as a plasticizer at a concentration of 5%, at a temperature of 70°C. After cooling the solution to 50°C, different volumes of the lysozyme solution were added to achieve varying lysozyme concentrations. The total solution volume was adjusted by modifying the level of distilled water. This resulted in the creation of three distinct film-forming solutions, in addition to a control solution.

The four solutions were cast onto separate petri plates, each with a 10-centimeter diameter, and then left to dry at 59°C for 24 hours in a hot air oven. This process led to the formation of four different types of films, designated as C (control), T1 (with 1.62 x 10^6 specific units of lysozyme), T2 (with 3.24 x 10^6 specific units of lysozyme), and T3 (with 8.1 x 10^6 specific units of lysozyme). Subsequently, these dried films were carefully removed from the petri plates and stored in desiccators for future analysis.

The films were subjected to comprehensive evaluation, focusing on various parameters.

Fig. 3&4 Preparation of alginate films using petri plates

Fig. 5 Control film

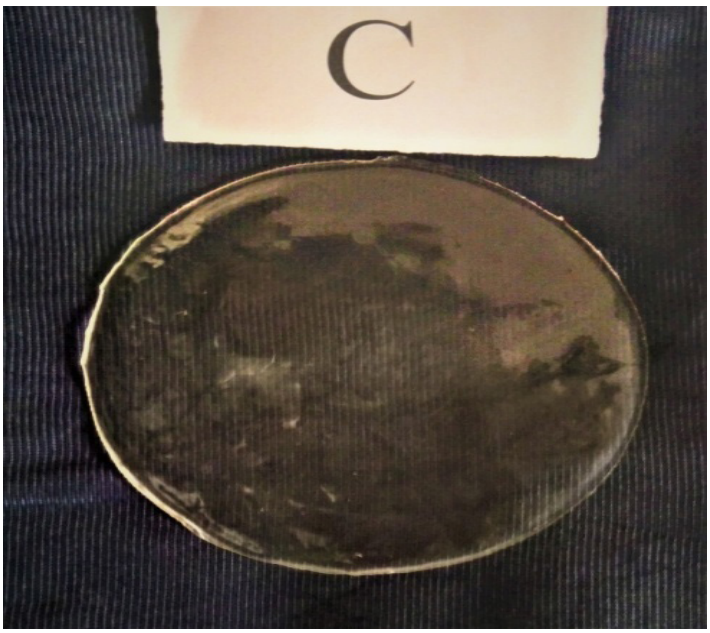
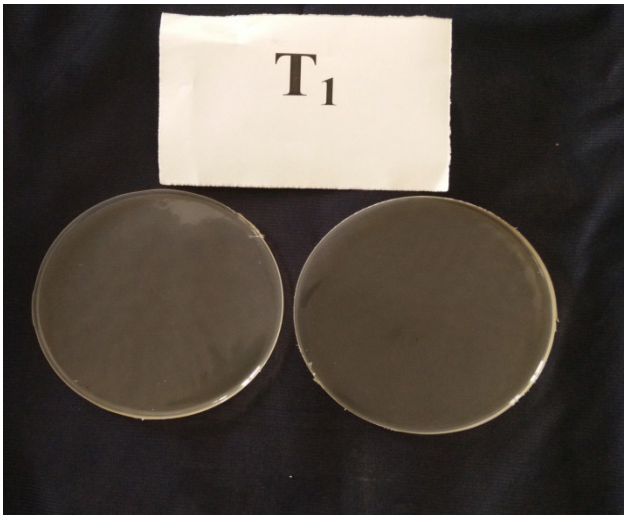
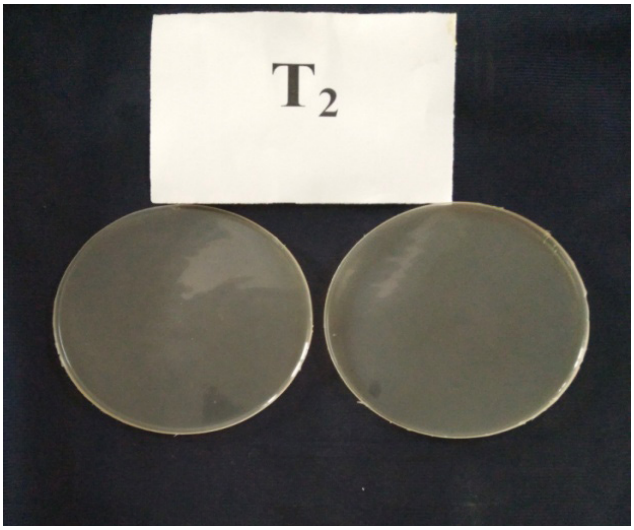
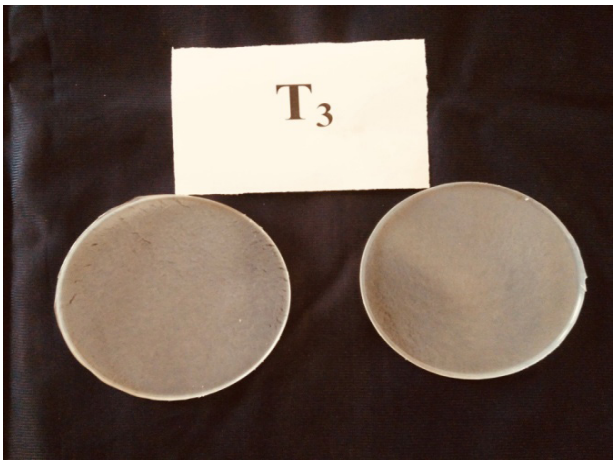


Fig. 6 Alginate films obtained with different concentrations of immobilized lysozyme







FILM CHARACTERISTICS

FILM THICKNESS: The thickness of the films was measured in microns using a digital micrometer. The average of at least 10 random measurements was recorded as the film thickness.

FILM GRAMMATURE: Grammature was determined according to the procedure described in reference (Geraldine et al.2008). It was calculated by dividing the film's weight in grams by its area in square meters.

TENSILE STRENGTH: The mechanical properties of the films were assessed by measuring the tensile strength (TS) and percent elongation at break (EAB). TS was determined following the method in reference (Berry and Stiffler 1981), using a texture profile analyzer (Tinius Olsen, Model H1KF, Redhill, RH1 5DZ, England).

PERCENT ELONGATION AT BREAK (EAB): Percent elongation at break was measured as per the method outlined in reference (Soni et al.2015), considering A as the initial length of the film and B as the final length of the film after stretching or at the time of break.

WATER VAPOUR PERMEABILITY: The water vapor permeability (WVP) of the films was measured gravimetrically, following the ASTM E96-92 method described in reference (Casariego et al.2009).

WATER SORPTION KINETICS: The water sorption of edible sodium alginate films was evaluated using the method in reference (Lavorgna et al.2010).

LIGHT TRANSMISSION AND FILM OPACITY: The transmission and opacity of the films were assessed according to the method described in reference (Tunc and Duman 2010).

ANTIOXIDANT ACTIVITY: Antioxidant activity was determined through the N, N-dimethyl-1,4-diaminobenzene (DMPD) free radical scavenging assay, as outlined in reference (Fogliano et al.1999).

ANTIMICROBIAL ACTIVITY: The antimicrobial activity of the films was evaluated against Staphylococcus aureus and E. coli using the agar well diffusion method described in reference (Pellissari et al.2009), with slight modifications. Aseptically, 6 to 8 mm diameter holes were punched in Muller Hinton agar plates, which were then spread with 0.1 ml of inoculum containing 105-106 CFU/ml of bacterial culture, standardized using the McFarland scale. The well floors were sealed with agarose to prevent solution diffusion beneath the agar. Using a sterile tip, 20 µl of each film forming solution formulation was introduced into the wells. The plates were incubated at 37 ± 1°C for 24 hours, and the diameter of the zone of inhibition around the wells was measured. It was then compared against an Antibiotic Sensitivity Test (ABST) zone of inhibition scale and standard antibiotic zones.

*In addition, antimicrobial activity was assessed through challenge studies with E. coli, following the method in reference (Naga Mallika et al.2008). E. coli was cultured on EMB agar, and specific films were placed on EMB agar. Reduction in E. coli counts was observed, and the counts were expressed as log 10 CFU/ml.

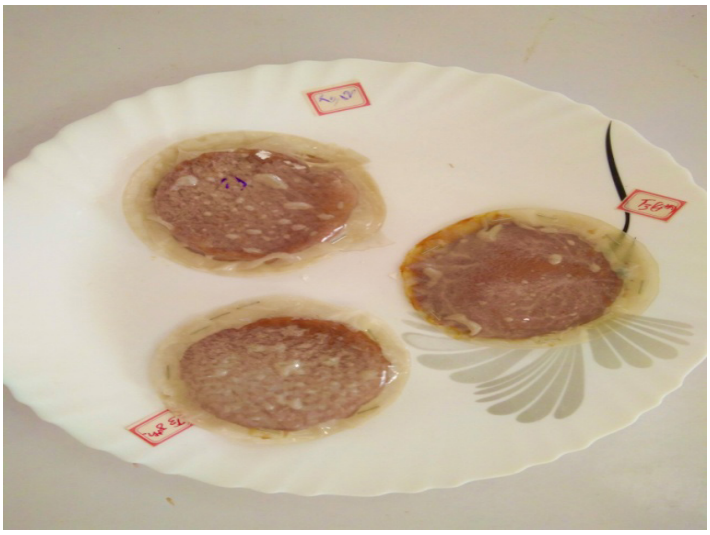
Chicken patties were prepared and they were wrapped in the films which are judged as the best film along with control.

pH: The pH of the preparation was determined using the method outlined in reference (Trout et al.1992).

2-Thiobarbituric Acid Reactive Substances (2-TBARS) Value: 2-TBARS values were determined following the method in reference (Tarladgis et al.1960).

Microbial Analysis: The microbial quality of the preparation was evaluated by estimating the Total Plate Count (TPC), Psychrophilic Bacterial Count (PBC), and Yeast and Mould Counts (YMC) using the pour plating technique, as per the standard procedure of the International Commission on Microbiological Specifications for Foods (ICMSF 1980).

Fig. 7: Chicken meat patties wrapped in Control and Treatment film (T_3)



STATISTICAL ANALYSIS: The results of the above parameters were analyzed using SPSS (version 17.0).

RESULTS AND DISCUSSION

Turbidity Differences in Lysozyme-Alginate Complexes:

SIGNIFICANCE OF LYSOZYME CONCENTRATION (P < 0.05): The observed significance in turbidity differences up to a lysozyme concentration of 2.88 mg per 15000 specific units of lysozyme underscores the sensitivity of the lysozyme-alginate complex formation process to variations in lysozyme concentration. This statistical significance highlights the intricate nature of the interaction, where subtle changes in lysozyme concentration significantly influence turbidity.

MAXIMUM TURBIDITY AT 2.88 MG LYSOZYME: The concentration of 2.88 mg of lysozyme resulting in significantly higher turbidity points to a critical juncture in the complex formation process. This observation suggests that, at this specific concentration, there is a notable augmentation in biopolymer aggregation. The intricate interplay between lysozyme and alginate at this point signifies the dynamic nature of the complexation phenomenon.

INITIAL TURBIDITY INCREASE: The initial increase in turbidity with rising lysozyme concentration signifies a progressive enhancement in biopolymer aggregation. This phenomenon aligns with the expectation that higher lysozyme concentrations would lead to increased complex formation. The significant increase observed up to 2.88 mg of lysozyme reflects a concentration-dependent effect on turbidity.

PLATEAU IN TURBIDITY: The absence of a further increase in turbidity beyond the concentration of 2.88 mg of lysozyme indicates a plateau in the complex formation process. This observation suggests that the biopolymer aggregation reaches a saturation point, potentially due to limitations in the bridging mechanism. Understanding this plateau is crucial for optimizing lysozyme-alginate complex formation.

MAXIMUM LOADING CAPACITY: Determining the maximum loading capacity of 144 mg of lysozyme per 1 gram of alginate without a subsequent increase in turbidity provides practical insights into the saturation limit of the complexation process. This finding is invaluable for designing formulations with controlled lysozyme concentrations to achieve desired properties without compromising turbidity.

BRIDGING MECHANISM: Consistency with prior research on increased bridging effects up to a certain concentration, followed by a decrease, validates the study's findings. This observation supports the understanding that the lysozyme-alginate complexation involves a
nuanced bridging mechanism influenced by lysozyme concentration.

The results were partially in line with those of (Bayyari et al. 2014) and (Lian et al. 2012), where in there was an increased bridging till a particular concentration, thereafter a decrease was noticed.

ENZYME ACTIVITY DIFFERENCES IN IMMOBILIZED LYSOZYME:

SIGNIFICANCE OF LYSOZYME CONCENTRATION (P < 0.05): The significant difference in enzyme activity between immobilized lysozyme and free lysozyme regardless of concentration accentuates the transformative effect of immobilization on lysozyme functionality. This distinction highlights the altered behavior of lysozyme when integrated into the alginate matrix.

EFFECT OF LYSOZYME CONCENTRATION: The variation in lysozyme activity with different concentrations in the lysozyme-alginate complex underscores the nuanced impact of concentration on enzyme functionality. This observation introduces a critical consideration for optimizing lysozyme concentration in practical applications.

MINIMUM ACTIVITY AT 1.44 mg: The identified minimum in enzyme activity at a lysozyme-alginate concentration of 1.44 mg suggests a concentration-dependent effect on lysozyme functionality. This finding implies that excessively low or high concentrations may compromise lysozyme activity, reinforcing the need for a balanced approach.

IMMOBILIZATION EFFECT: The reduction in lysozyme activity post-immobilization highlights the consequences of restricted molecular flexibility within the complex. The study's focus on substrate diffusional obstacles provides a mechanistic understanding of the observed decrease in enzyme activity, offering insights for future optimization.

OPTIMAL CONCENTRATION: Identification of an optimal lysozyme concentration of 3.6 mg, where enzyme

activity is at its minimum, presents a key finding. The subsequent increase in activity with higher concentrations suggests a delicate balance between lysozyme concentration and its mobility within the alginate matrix.

ACTIVITY COMPARISON WITH FREE LYSOZYME: The observed differences in mobility between free and complexed lysozyme contribute to the understanding that while complexed lysozyme activity increases with concentration, it doesn't reach the level of free lysozyme. This distinction implies inherent differences in the dynamic behavior of free and immobilized lysozyme.

This study exhibited the intricate insights into the turbidity dynamics of lysozyme-alginate complexes and the nuanced variations in enzyme activity resulting from lysozyme immobilization. These findings provide a foundation for refining the formulation of alginate-based antimicrobial films, optimizing lysozyme concentrations, and enhancing their functional attributes for practical applications.

FILM THICKNESS:

The average film thickness of T2 and T3 exhibited a significant increase ($P < 0.05$) compared to both the other treatment films and the control film. However, the thickness of T1 showed a non-significant difference ($P < 0.05$) when compared to the control. The observed thickness enhancement could be attributed to the elevated viscosity of the solutions used in forming the films, distinguishing them from the remaining treatments.

GRAMMAGE:

The grammage of the T2 film was significantly higher, indicating its increased density. The distinctive colloidal properties of Alginate, such as thickening, suspending, and interactions between lysozyme and Alginate components, may contribute to the variations in film thickness (Galus and lenart et al. 2013).

TENSILE STRENGTH:

The average tensile strength of T3 was significantly higher ($P < 0.05$) compared to the other treatments and the control films. This could be attributed to the specific complexation between the polymer matrix of Alginate and lysozyme, leading to the formation of strong hydrogen bonds. The incorporation of lysozyme molecules in the film may have resulted in numerous individual complexes uniformly dispersed in the films, contributing to the increased tensile strength (da Silva et al. 2009).

PERCENT ELONGATION AT BREAK:

The average percent elongation at break for T3 was significantly higher ($P < 0.05$) than the other treatments and control films. This flexibility can be attributed to the materials used in film preparation, influenced by the interaction of protein polysaccharides and the plasticizing effect of glycerol. These factors may enhance the mobility of polymer chains, fostering higher molecular affinity between film components (Rodriguez et al. 2020). Interactions between materials also play a crucial role in determining the elastic properties of the material (Zhang et al. 2018).

WATER VAPOR PERMEABILITY:

The mean water vapor permeability values of T2 and T3 were significantly higher ($P < 0.05$) than those of T1 and the control films. The water vapor permeability of T1 showed a non-significant difference ($P < 0.05$) compared to the control. The increased water vapor permeability may be attributed to interactions involving hydrogen bonds, hydrophobic chains, and ionic bonds formed within the films (Rodriguez et al. 2020). Strong intermolecular interactions between amino hydroxyl and amino groups of proteins, as well as amylase and amylo-Alginate chains, could enhance the film's permeability.

WATER SORPTION KINETICS:

The mean water sorption value of T3 was significantly lower ($P < 0.05$) than that of the other treatments and control films.

MOISTURE SORPTION:

The reduction in moisture sorption can be attributed to the incorporation of lysozyme molecules into Alginate chains. This inclusion may alter the structural configuration of Alginate molecules, increasing interactions like hydrogen bonding and van der Waals forces with lysozyme molecules. Lysozyme, comprising both hydrophilic and hydrophobic amino acids, could form a hydrophobic core during film formation, with hydrophilic amino acid side chains protruding towards the aqueous film-forming solution. These hydrophobic interactions, crucial in lysozyme folding, could lead to increased hydrophobic side chains in the film matrix, resulting in decreased moisture content (Park et al. 2004).

LIGHT TRANSMISSION AND FILM OPACITY:

The opacity value of T3 was significantly higher ($P < 0.05$) than the other treatment and control films. This increase in opacity may be due to light-scattering micro-aggregates formed by immobilized lysozyme with Alginate in the solution, leading to reduced clarity compared to lysozyme-free control films (Bower et al. 2006).

ANTIOXIDANT ACTIVITY:

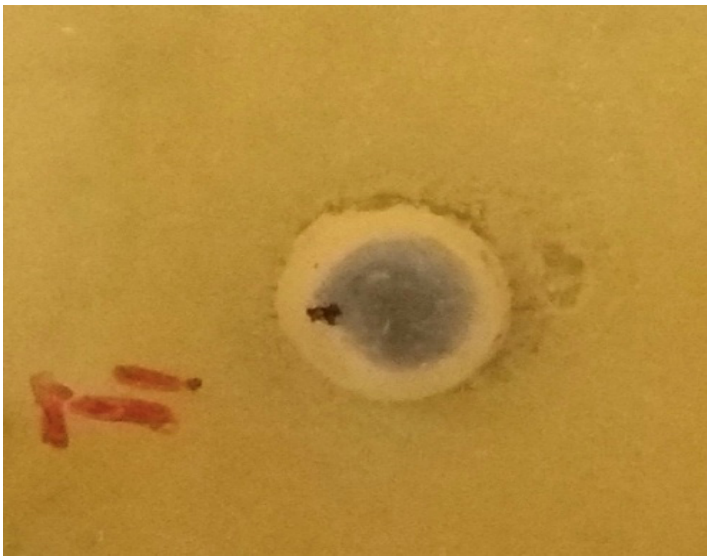
The antioxidant value of T3 was significantly higher ($P < 0.05$) than the other treatment and control films. This enhanced antioxidant activity may be attributed to the antioxidant properties of lysozyme. Lysozyme has been reported to generate peptides with high antioxidant activity, effectively inhibiting lipid peroxidation. Additionally, Alginate, with its galacturonic acid content, contributes various biological properties, including antioxidant capabilities (Carrilio et al. 2016), (Nasrulwathoni et al. 2019).

ANTIMICROBIAL ACTIVITY:

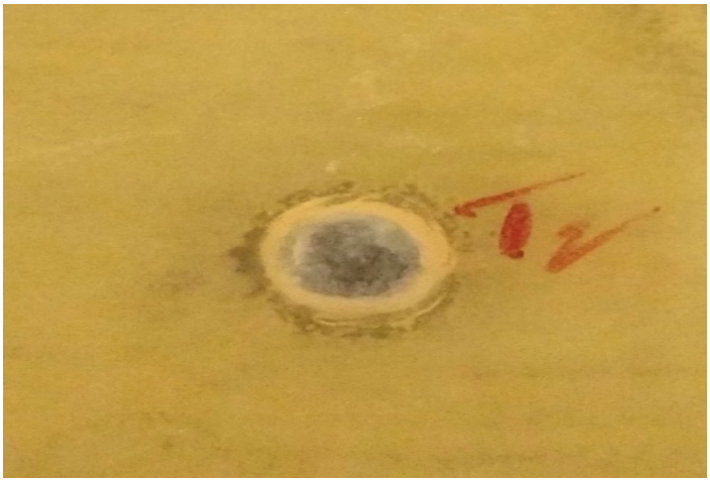
The diameter of inhibition zones of T3 against Staphylococcus aureus and Escherichia coli was significantly higher ($P < 0.05$) than the other treatment and control films. This suggests a lower diffusion rate at higher temperatures, indicating a more rigid matrix and slower protein release. The antimicrobial efficiency increased with prolonged exposure time, and the lysozyme retained its activity after 30 days of film storage. The antimicrobial activity of lysozyme was confirmed through log reduction measurements, with significantly lower log E. coli values in films incorporated with lysozyme compared to control films. The antimicrobial efficacy increased with higher lysozyme concentrations, with films containing 3.6 mg of lysozyme and 3 grams of Alginate showing notable efficiency. This release of lysozyme from Alginate films demonstrated antimicrobial activity comparable to lysozyme-chitosan composite films and lysozyme-immobilized polyvinyl alcohol films (Conte et al. 2006), (Huang et al.2012).

Fig. 8 Diameter of inhibition zones *(millimetres)* values of T_1, T_2 and T_3 against *Staphylococcus aureus.*

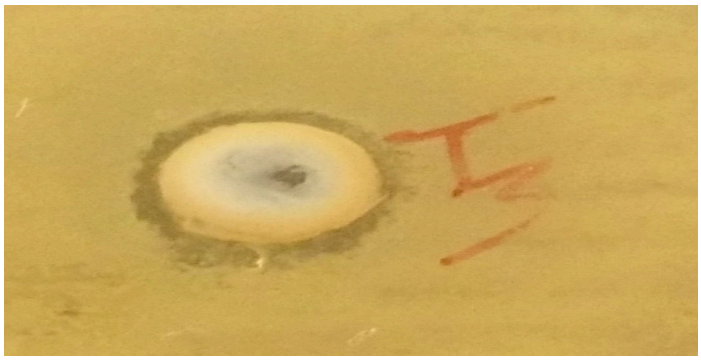
Treatment 1



Treatment 2



Treatment 3



QUALITY EVALUATION OF CHICKEN PATTIES WRAPPED IN FILMS:

pH:

The observed decrease in pH values of chicken meat patties wrapped in the treatment film is a significant finding. This acidity shift is attributed to the presence of lysozyme in the Alginate film. Lysozyme's antimicrobial properties are likely responsible for inhibiting bacterial activity in the meat, resulting in a lower pH. These results were in accordance with (Prathyusha et al.2016) who worked on alginate coatings enriched with grape seed extract for preservation of chicken nuggets and (jridi et al.2020). This effect not only contributes to the safety of the meat but also potentially enhances its shelf life by impeding microbial growth.

2-TBARS:

The lower 2-TBARS values in chicken meat patties wrapped in the treatment film suggest a potential reduction in lipid oxidation. This is attributed to the gradual migration of lysozyme from the Alginate film into the meat. Lysozyme, acting as an antioxidant, may play a role in slowing down oxidative processes. Moreover, alginate film's hindrance of

UV light penetration adds an additional layer of protection against lipid oxidation, ensuring the preservation of meat quality (Prathyusha et al.2016) and (kaewprachu et al. 2015).

PERCENT COOKING LOSS:

The significant reduction in percent cooking loss for chicken meat patties wrapped in the treatment film highlights the film's role in moisture retention during cooking. The lysozyme-Alginate complexes are thought to diminish interstitial spaces within the film network, resulting in lower evaporation and respiration rates which were similar to (Mallika et al. 2018) and (Rao et al.2019) who worked on starch coatings incorporated with Tannic acid for preservation of pork balls. This not only contributes to improved meat texture but also enhances the overall sensory experience for consumers.

TOTAL PLATE COUNT:

The lower total plate count values in chicken meat patties wrapped in the treatment film underscore the efficacy of lysozyme against microbial contamination. The hydrolysis of peptidoglycan in microbial cell walls by lysozyme leads to cell lysis, demonstrating its antimicrobial effect (Gill & Holley.,2000). This finding aligns with previous research on other food products and further establishes lysozyme as a valuable component for enhancing the microbial safety of packaged meats.

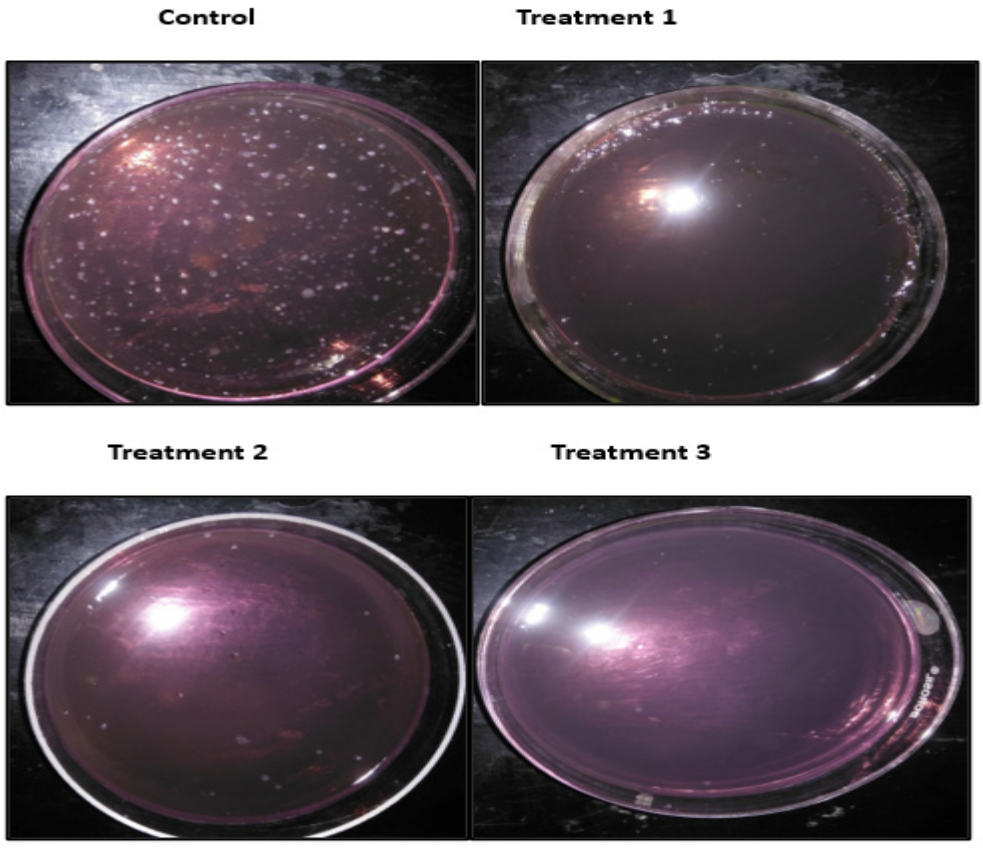
The reduced yeast and mold count values in the treatment film provide additional evidence of the antifungal activity of lysozyme. The disruption of microbial membranes by lysozyme contributes to this effect, reinforcing the film's role in preventing spoilage and extending the freshness of the packaged meat (Min et al. 2005).

SENSORY SCORES:

The decline in sensory scores over refrigerated storage time is a natural trend observed in both treatment and control groups. However, the slower rate of decline in sensory scores for patties wrapped with the T3 film is a noteworthy observation. This suggests that the antimicrobial and antioxidant effects of lysozyme in the Alginate film contribute to maintaining the sensory qualities of the meat for a more extended period.

The incorporation of lysozyme into Alginate films proves to be a multifunctional approach in enhancing the quality and safety of packaged chicken meat. From microbial control to lipid oxidation prevention and improved cooking attributes, these findings provide valuable insights into the potential applications of lysozyme-incorporated films in the food packaging industry. This results are in correlation with (Liu et al. 2007).

Fig. 9 Reduction in log CFU/ml count values of C, T_1, T_2 and T_3 films against E.coli.



CONCLUSION:

In this context, it can be concluded that, lysozyme can be effectively immobilized on to alginate, using physical absorption method. It was clear that immobilization might improve the enzyme's stability. The usability of lysozyme was preserved for longer time. The immobilized enzyme had good antimicrobial activity and was synergistic with alginate base. This indicates the potential use of lysozyme and alginate combination for food packaging manufacture.

ACKNOWLEDGEMENTS

Authors are very much thankful to Sri Venkateswara Veterinary University, Andhra Pradesh, India for providing necessary facilities and budget to carry out this original research work.

Table 1: FILM PARAMETERS OF T_1, T_2 ND T_3 EDIBLE ALGINATE FILMS AS INFLUENCED BY DIFFERENT CONCENTRATION OF IMMOBILIZED LYSOZYME

FILM CHARACTERISTICS	TREATMENT GROUPS			
	CONTROL	T_1	T_2	T_3
1.FILM THICKNESS (microns)	432.50±11.66^{a}	510.00±28.54^{a}	1954.00±7.50^{b}	1954.17±6.85^{b}
2.GRAMMATURE (gm/m^2)	488.82±0.44^{a}	526.98±0.65^{c}	553.59±0.23^{d}	425.04±0.53^{b}
3.TENSILE STRENGTH (N/cm^2)	5.11±0.61^{a}	14.14±1.83^{b}	14.94±1.68^{b}	19.87±2.13^{c}
4.ELONGATION AT BREAK (%)	16.20±0.55^{a}	14.44±0.67^{b}	12.76±0.41^{c}	25.21±0.31^{d}
5.WATER VAPOUR PERMEABILITY (×10^{-10}g m^{-1} s^{-1} Pa^{-1})	2.12±0.20^{a}	2.65±0.19^{a}	7.32±0.57^{b}	8.61±0.88^{b}
6.WATER SORPTION (%)	38.94±1.02^{d}	36.79±3.06^{c}	31.82±4.72^{ab}	23.02±4.00^{a}
7.FILM OPACITY	0.006±0.00^{a}	0.014±0.00^{a}	0.015±0.00^{a}	0.046±0.00^{b}
8.ANTI OXIDANT ACTIVITY (µg/ml of trolox equivalent)	3.86 ±1.72^{a}	9.87±1.84^{b}	9.92±1.76^{b}	16.30±1.29^{c}
9.ANTIMCROBIAL ACTIVITY AGAINST S.aureus (diameter in mm)	0±00^{a}	7.02±0.15^{b}	6.09±0.20^{c}	11.03±0.10^{d}
10.ANTIMCROBIAL ACTIVITY AGAINST E.coli (diameter in mm)	0±00^{a}	5.01±0.11^{b}	9.05±0.08^{c}	11.99±0.11^{d}
11.ANTIMICROBIAL ACTIVITY against E.coli (log CFU/ml.)	3.838±0.07^{a}	1.643±0.10^{a}	0.208±0.02^{b}	0.127±0.01^{c}

Note: Means bearing common superscript in each row do not differ significantly.
Significance: ($P<0.05$)

Table 2: PRODUCT PARAMETERS OF CONTROL AND T_3 EDIBLE ALGINATE FILMS AS INFLUENCED BY DIFFERENT CONCENTRATION OF IMMOBILIZED LYSOZYME

Treatments	DAYS OF STORAGE PERIOD								
DAY 0*	**DAY 4***	**DAY 8***	**DAY 12***	**DAY 16***	**DAY 20***	**DAY24***		**DAY28***	**OVER-ALL-MEAN**
		Colour							
C	8.56±0.01	8.24±0.02	7.86±0.02	7.39±0.01	6.83±0.05^x	6.20±0.02^x	5.81±0.05^x		5.35±0.04^x	**7.03±0.02^x**
T_3	8.68±0.04	8.42±0.009	8.12±0.01	7.81±0.03	7.12±0.02	6.76±0.03	6.23±0.01		5.85±0.07	**7.37±0.02^y**
OVERALL MEAN	**8.62±0.02^a**	**8.33±0.01^b**	**7.99±0.01^c**	**7.60±0.02^d**	**6.97±0.03^e**	**6.40±0.02^f**	**6.02±0.03^g**		**5.60±0.05^h**	
		Flavour							
C	8.66±0.02	8.38±0.02	7.90±0.02	7.55±0.01	6.82±0.06^x	6.27±0.04^x	5.88±0.04^x		5.35±0.05^x	**7.10±0.03^x**
T_3	8.77±0.01	8.45±0.00	8.12±0.01	7.90±0.01	7.53±0.01	7.04±0.05	6.57±0.02		6.22±0.01	**7.57±0.01^y**
Overall mean	**8.71±0.01^a**	**8.41±0.01^b**	**8.01±0.01^c**	**7.72±0.01^d**	**7.1±0.03^e**	**6.65±0.045^f**	**6.22±0.00^g**		**5.78±0.03^h**	
		Tenderness							
C	8.76±0.03	8.40±0.01	7.87±0.02	7.61±0.02	7.26±0.01^x	6.85±0.03^x	6.20±0.01^x	5.54±0.02^x	**7.31±0.01^x**	
T_3	8.80±0.01	8.44±0.02	8.02±0.02	7.80±0.01	7.30±0.04	6.89±0.03	6.57±0.03	6.49±0.01	**7.5±0. 0^y**	
OVER-ALL MEAN	**8.78±0.02^a**	**8.42±0.015^b**	**7.94±0.02^c**	**7.70±0.01^d**	**7.28±0.02^e**	**6.87±0.03^f**	**6.38±0.02^g**	**6.01±0.01^h**		
		Juiciness							
C	8.59±0.03	8.32±0.01	8.06±0.07	7.78±0.03	6.98±0.04^x	6.09±0.05^x	5.74±0.03^x	4.98±0.04^x	**7.06±0.03^x**	
T_3	8.73±0.04	8.47±0.02	8.10±0.05	7.91±0.01	7.51±0.05	6.47±0.02	5.87±0.03	5.29±0.05	**7.29±0.03^y**	
OVER-ALL MEAN	**8.66±0.03^a**	**8.39±0.01^b**	**8.08±0.06^c**	**7.84±0.02^d**	**7.24±0.04^e**	**6.28±0.03^f**	**5.80±0.03^g**	**5.13±0.04^h**		
		Overall Acceptability							
C	8.43±0.02	8.21±0.01	7.62±0.01	7.25±0.01	6.91±0.02^x	6.28±0.01^x	5.89±0.03^x	5.30±0.04^x	**6.98±0.01^x**	
T_3	8.51±0.01	8.28±0.01	7.86±0.01	7.57±0.01	7.03±0.06	6.84±0.03	6.17±0.06	5.86±0.02	**7.26±0.02^y**	
OVER-ALL MEAN	**8.47±0.01^a**	**8.24±0.01^b**	**7.74±0.01^c**	**7.41±0.01^d**	**6.97±0.04^e**	**6.56±0.02^f**	**6.03±0.04^g**	**5.58±0.03^h**		

REFERENCE:

Azeredo, H. M. C., Mattoso, L. H. C., Wood, D., Williams, T. G., Avena-Bustillos, R. J., & McHugh, T. H. (2009). Nanocomposite edible films from mango puree reinforced with cellulose nanofibers. *Journal of Food Science, 74*(5), N31–N35. https://doi.org/10.1111/j.1750-3841.2009.01196.x

Bayarri, M., Oulahal, N., Degraeve, P., & Gharsallaoui, A. (2014). Properties of lysozyme/low methoxyl (LM) pectin complexes for antimicrobial edible food packaging. *Journal of Food Engineering, 131*, 18–25. https://doi.org/10.1016/j.jfoodeng.2014.01.013

Berry, B. W., & Stiffler, D. M. (1981). Effect of electric stimulation, boning temperature, formulation and rate of freezing on sensory, cooking, chemical and physical properties of ground beef patties. *Journal of Food Science, 46*(4), 1103–1106. https://doi.org/10.1111/j.1365-2621.1981.tb03028.x

Bilska, A. (2011). Packaging systems for animal origin food. *Log Forum, 7*(1), 1–4.

Bower, C. K., Avena-Bustillos, R. J., Olsen, C. W., McHugh, T. H., & Bechtel, P. J. (2006). Characterization of fish-skin gelatin gels and films containing the antimicrobial enzyme lysozyme. *Journal of Food Science, 71*(5), M141–M145. https://doi.org/10.1111/j.1750-3841.2006.00058.x

Brody, A. L. (1997). Packaging of food. In A. L. Brody & K. S. Marsh (Eds.), *The Wiley encyclopedia of packaging* (2nd ed., pp. 699–704). Wiley.

Carrillo, W., Gómez-Ruiz, J. A., Miralles, B., Ramos, M., Barrio, D., & Recio, I. (2016). Identification of antioxidant peptides of hen egg-white lysozyme and evaluation of inhibition of lipid peroxidation and cytotoxicity in the Zebrafish model. *European Food Research and Technology, 242*(10), 1777–1785. https://doi.org/10.1007/s00217-016-2662-2

Casariego, A., Souza, B. W. S., Cerqueira, M. A., Teixeira, J. A., Cruz, L., Díaz, R., & Vicente, A. A. (2009). Chitosan/clay films properties as affected by biopolymer and clay micro/nanoparticles concentrations. *Food Hydrocolloids, 23*(7), 1895–1902. https://doi.org/10.1016/j.foodhyd.2009.01.002

Cha, D. S., Choi, J. H., Chinnan, M. S., & Park, H. J. (2002). Antimicrobial films based on Na-alginate and κ-carrageenan. *Lebensmittel-Wissenschaft & Technologie, 35*(7), 715–719. https://doi.org/10.1006/fstl.2002.0925

Conte, A., Buonocore, G. G., Bevilacqua, A., Sinigaglia, M., & Del Nobile, M. A. (2006). Immobilization of lysozyme on polyvinyl alcohol films for active packaging applications. *Journal of Food Protection, 69*(4), 866–870. https://doi.org/10.4315/0362-028X-69.4.866

Da Silva, M. A., Bierhalz, A. C. K., & Kieckbusch, T. G. (2009). Alginate and pectin composite films crosslinked with Ca^{2} ions: Effect of the plasticizer concentration. *Carbohydrate Polymers, 77*(4), 736–742. https://doi.org/10.1016/j.carbpol.2009.02.014

De Souza, P. M., Fernandez, A., Lopez-Carballo, G., Gavara, R., & Hernandez-Muñoz, P. (2010). Modified sodium caseinate films as releasing carriers of lysozyme. *Food Hydrocolloids, 24*(4), 300–306. https://doi.org/10.1016/j.foodhyd.2009.11.002

Fogliano, V., Verde, V., Randazzo, G., & Ritieni, A. (1999). Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. *Journal of Agricultural and Food Chemistry, 47*(3), 1035–1040. https://doi.org/10.1021/jf980496s

Galus, S., & Lenart, A. (2013). Development and characterization of composite edible films based on sodium alginate and pectin. *Journal of Food Engineering, 115*(4), 459–465. https://doi.org/10.1016/j.jfoodeng.2012.03.006

Geraldine, R. M., Soares, N., Botrel, D., & Goncalves, L. (2008). Characterization and effect of edible coatings on minimally processed garlic quality. *Carbohydrate Polymers, 72*(3), 403–409. https://doi.org/10.1016/j.carbpol.2007.09.010

Gill, A. O., & Holley, R. A. (2000). Inhibition of bacterial growth on ham and bologna by lysozyme, nisin and EDTA. *Food Research International, 33*(1), 83–90. https://doi.org/10.1016/S0963-9969(00)00011-6

Huang, W., Xu, H., Xue, Y., Huang, R., Deng, H., & Pan, S. (2012). Layer-by-layer immobilization of lysozyme–chitosan–organic rectorite composites on electrospun nanofibrous mats for pork preservation. *Food Research International, 48*(2), 784–791. https://doi.org/10.1016/j.foodres.2012.06.022

Jridi, M., Abdelhedi, O., Salem, A., Kechaou, H., Nasri, M., & Menchari, Y. (2020). Physicochemical, antioxidant and antibacterial properties of fish gelatin-based edible films enriched with orange peel pectin: Wrapping application. *Food Hydrocolloids, 103*, 105688. https://doi.org/10.1016/j.foodhyd.2020.105688

Kaewprachu, P., Osako, K., Benjakul, S., & Rawdkuen, S. (2015). Quality attributes of minced pork wrapped with catechin–lysozyme incorporated gelatin film. *Food Packaging and Shelf Life, 3*, 88–96. https://doi.org/10.1016/j.fpsl.2015.01.001

Lavorgna, M., Piscitelli, F., Mangiacapra, P., & Buonocore, G. G. (2010). Study of the combined effect of both clay and glycerol plasticizer on the properties of chitosan films. *Carbohydrate Polymers, 82*(2), 291–298. https://doi.org/10.1016/j.carbpol.2010.04.069

Lian, Z. X., Ma, Z. S., Wei, J., & Liu, H. (2012). Preparation and characterization of immobilized lysozyme and evaluation of its application in edible coatings. *Process Biochemistry, 47*(2), 201–208. https://doi.org/10.1016/j.procbio.2011.10.035

Liu, L., Kerry, J. F., & Kerry, J. P. (2007). Application and assessment of extruded edible casings manufactured from pectin and gelatin/sodium alginate blends for use with breakfast pork sausage. *Meat Science, 75*(2), 196–202. https://doi.org/10.1016/j.meatsci.2006.06.021

Mallika, E. N., Rani, M. S., & Rao, B. E. (2018). Influence of calcium alginate coating on quality of pork patties. *Internation-*

al Journal of Current Microbiology and Applied Sciences, 7(6), 824–827. https://doi.org/10.20546/ijcmas.2018.706.098

Min, S., Harris, L. J., Han, J. H., & Krochta, J. M. (2005). Listeria monocytogenes inhibition by whey protein films and coatings incorporating lysozyme. *Journal of Food Protection, 68*(11), 2317–2325. https://doi.org/10.4315/0362-028X-68.11.2317

Moraes, A. R., Gouvêa, L. R., Soares, N. F., Santos, M. D., & Gonçalves, M. C. (2007). Development and evaluation of antimicrobial film on butter conservation. *Ciência e Tecnologia de Alimentos, 27*(1), 33–36. https://doi.org/10.1590/S0101-20612007000100006

Naga Mallika, E., Masthan Reddy, P., Prabhakar Reddy, K., Rama Prasad, J., Sreenivasulu, D., & Prabhakar, K. (2008). Studies on the development of low fat functional pork storage and their shelf life using optimized hurdle technology [Unpublished doctoral thesis]. SVVU.

Nasrulwathoni, N., Shan, C. Y., Shan, W. Y., Rostinawati, T., Indradi, R. B., Pratiwi, R., & Muchtaridi, M. (2019). Characterization and antioxidant activity of pectin from Indonesian mangosteen (*Garcinia mangostana* L.) rind. *Heliyon, 5*(8), e02299. https://doi.org/10.1016/j.heliyon.2019.e02299

Padgett, T., Han, I. Y., & Dawson, P. L. (1998). Incorporation of food-grade antimicrobial compounds into biodegradable packaging films. *Journal of Food Protection, 61*(10), 1330–1335. https://doi.org/10.4315/0362-028X-61.10.1330

Park, S. I., Daeschel, M. A., & Zhao, Y. (2004). Functional properties of antimicrobial lysozyme–chitosan composite films. *Journal of Food Science, 69*(8), M215–M221. https://doi.org/10.1111/j.1365-2621.2004.tb09883.x

Pellissari, F. M., Grossmann, M. V. E., Yamashita, F., & Pineda, E. A. G. (2009). Antimicrobial, mechanical, and barrier properties of cassava starch–chitosan films incorporated with oregano essential oil. *Journal of Agricultural and Food Chemistry, 57*(16), 7499–7504. https://doi.org/10.1021/jf9002363

Prathyusha, K., Mallika, E. N., Rao, B. E., & Rao, T. S. (2016). Evaluation of edible polymer coatings enriched with grape seed extract on chicken meat nuggets. *Meat Science, 11*(2), 40–44.

Proctor, V. A., & Cunningham, F. E. (1988). The chemistry of lysozyme and its use as a food preservative and a pharmaceutical. *Critical Reviews in Food Science and Nutrition, 26*(4), 359–395. https://doi.org/10.1080/10408398809527473

Rao, V. G., Mallika, E. N., & Rao, B. E. (2019). Effect of application of natural polyphenol containing polymer coatings on quality of pork balls. *Journal of Meat Science, 14*(2), 6–11.

Rodriguez-Misael, C., Villegas-Yepez, C., Gil Gonzalez, J. H., Rodriguez, P. E., & Ortega-Toro, R. (2020). Development and evaluation of edible films based on cassava starch, whey protein, and beeswax. *Heliyon, 6*(9), e04972. https://doi.org/10.1016/j.heliyon.2020.e04972

Soni, A., Kandeepan, G., Mendiratta, S. K., Shukla, V., & Kumar, A. (2015). Development and characterization of essential oils incorporated carrageenan based edible film for packaging of chicken patties. *Nutrition & Food Science, 46*(1), 82–95. https://doi.org/10.1108/NFS-04-2015-0040

Tarladgis, B. G., Watts, B. M., Younathan, M. T., & Dugan, L. R. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of the American Oil Chemists' Society, 37*(1), 44–48. https://doi.org/10.1007/BF02630824

Trout, E. S., Hunt, M. C., Johnson, D. E., Claus, J. R., Kastner, C. L., & Kropf, D. H. (1992). Chemical, physical and sensory characterization of ground beef containing 5 to 30% fat. *Journal of Food Science, 57*(1), 25–29. https://doi.org/10.1111/j.1365-2621.1992.tb05433.x

Tunc, S., & Duman, O. (2010). Preparation and characterization of biodegradable methyl cellulose/montmorillonite nanocomposite films. *Applied Clay Science, 48*(3), 414–424. https://doi.org/10.1016/j.clay.2010.01.016

Yadav, K. S., Prabha, R., & Renuka, K. (2015). Active packaging: Concepts and applications. *International Journal of Food and Nutritional Sciences, 4*(2), 194–200.

Zhang, Y., Simpson, B. K., & Dumont, M. J. (2018). Effect of beeswax and carnauba wax addition on properties of gelatin films: A comparative study. *Food Bioscience, 26*, 88–95. https://doi.org/10.1016/j.fbio.2018.10.005