Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 4 Issue 7 July 2022

Research Article

Development and Evaluation of Lysozyme-Pectin Complexes for Antimicrobial Packaging of Chicken Patties

M Prasanna, E Naga Mallika*, K Sudheer and T Srinivasa Rao

Associate Professor, Department of Livestock Products Technology, Sri Venkateswara Veterinary University, Andhra Pradesh, India

***Corresponding Author:** E Naga Mallika, Associate Professor, Department of Livestock Products Technology, Sri Venkateswara Veterinary University, Andhra Pradesh, India.

DOI: 10.31080/ASVS.2022.04.0443

Received: June 15, 2022

Published: June 24, 2022

Abstract

Background: Edible films are made from biopolymers (pectin, starch, cellulose), gelatin, casein, and other proteins. Among these, pectin is used with no restriction and is considered to be widely accepted as healthy. Due to its constancy across a wide variety of pH and temperature ranges, lysozyme has a significant potential for food preservation. Therefore, this study aimed on development of pectin based edible films with lysozyme and to evaluate their effect on the quality of chicken meat patties.

Methods: Pectin films with lysozyme of different concentrations were developed and 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 to wrap the chicken meat patties. The product was evaluated for parameters like pH, percent cooking loss, 2-TBARS, microbial analysis and sensory evaluation.

Result: Results showed that the thickness, grammature, water vapour permeability, anti-oxidant activity, tensile strength and antimicrobial activity of T_3 film were significantly ($P \leq 0.05$) higher and lower water sorption compared to T_1, T_2 and control films. The pH, 2-TBARS, Percent cooking loss and microbial count values were lower in the chicken patties that were wrapped in T_3 film.

Keywords: Pectin; Lysozyme; Chicken Meat Patties

Introduction

Packaging satisfies the fundamental need to protect and preserve food quality and protection from initial processing to final consumption by avoiding unnecessary degradation in terms of chemical and biological changes. It helps to protect against harsh external environmental factors such as heat, light, presence or lack of moisture, pressure, microorganisms and gaseous emissions [4]. The customer is provided with greater ease of use and it saves time as the items are available in different sizes and shapes, along with the specifics of the label argument. Smart packaging systems, such as active and intelligent packaging concepts, help to understand the relationship between the packaging environment and the food to which active safety is needed [46].

Active packaging is an innovative approach for preserving or prolonging the shelf life of food items that ensure their consistency, protection and dignity. Active packaging systems can be divided

into absorbers (active scavenging systems) and emitters (active releasing systems). Absorbers extract undesired compounds from the product or its surrounding atmosphere such as moisture, carbon dioxide, oxygen, ethylene gas or odours from the food, while emitters add compounds to the packaged food or to the headspace such as antimicrobial compounds, carbon dioxide, additives, flavourings, ethylene or ethanol to avoid undesirable changes.

Antimicrobial films can provide an effective means of controlling foodborne pathogens and damaging microorganisms to improve food safety and reduce product spoilage. Antimicrobial films can be created by injecting chemical preservatives or antimicrobial agents into films that may be released into food to combat target microorganisms [31].

An increasing awareness among consumers about reluctantance to use chemical preservatives in meat products resulted in increased enthusiasm to implement more natural alternatives for improving food shelf life. This involve the use of natural antimicrobials derived from plant extracts such as essential oils and lysozyme, which can be used as alternatives to chemical additives [23] in antimicrobial packaging.

Biopolymers have been studied in relation to their film-forming properties for the development of edible films intended as food packaging [1]. Biopolymers (pectin, starch, cellulose), gelatin, casein and other proteins etc. are used in making edible films. Pectin is one of the key components of the plant cell wall chemically formed by polya1e4-galacturonic acids, which contributes to tissue integrity and rigidity and is known to be one of the most complex macromolecules in nature [19]. Depending on its degree of esterification with methanol, pectin may be graded as high methoxyl pectin or low methoxyl pectin.

Lysozyme is a lytic enzyme that has been found in many natural systems like chicken egg white, milk and body secreations, such as tears and saliva. Lysozyme has a high potential for food preservation due to its consistency over a wide range of pH and temperature levels [39]. Lysozyme was also incorporated into soy protein and corn zein [35], and alginate and carrageenan [9] based natural polymers.

With this context, the present work was planned to develop and standardize pectin-based edible antimicrobial films with immobilized lysozyme.

Materials and Methods

Immobilization of lysozyme

Lysozyme was immobilized following the method [2].

Lysozyme pectin complex formation

Lysozyme-pectin complexes were formed with lysozyme having 15000 specific units/mg and low methyl pectin. Low methyl pectin was taken at a constant level and the effective amount of lysozyme that can be immobilized on to pectin was determined by addition of lysozyme in sequential concentrations into pectin. The resultant suspensions were vortexed (REMI CM 101) for one minute and pH was maintained at 7. The developed turbidity due to immobilization of lysozyme was measured at 600 nm (25^0C) against imidazole-acetate buffer (5Mm, pH7) using a UV/Vis spectrophotometer (ThermoScientific nanodrop 2000c).

The activity of lysozyme was assessed following the method of [2].

Lysozyme activity evaluation

Lysozyme activity was assayed by monitoring the reduction of absorbance at 450 nm due to the lysis of *M. lysodeikticus* cells at 25^0C.

Briefly, in a 1 cm cuvette, 2.9 ml of the *M. lysodeikticus* suspension (OD450 = 1) in 5 mM phosphate buffer adjusted to the appropriate pH and 0.1 ml of the enzyme solution (prepared at the same pH) were mixed quickly, and the reduction in the absorbance was recorded using an UV/Vis spectrophotometer (Thermo Scientific nanodrop 2000c) until reaching a plateau. Lysozyme activity was calculated from the slope of the initial linear portion of absorbance vs. time curve. The hydrolytic activity of the lysozyme solution can be calculated using the below formula

$$\text{Activity (u/ml)} = S/0.001 \times V$$

One unit was 0.001 change in absorbance per minute. Activity: where S is the slope of the initial linear portion of absorbance vs. time curve and V is the volume of lysozyme solution.

Preparation of pectin films with immobilized lysozyme

The immobilized lysozyme formed as stated in 2.1.1 was incorporated in different concentrations into pectin films. Film forming

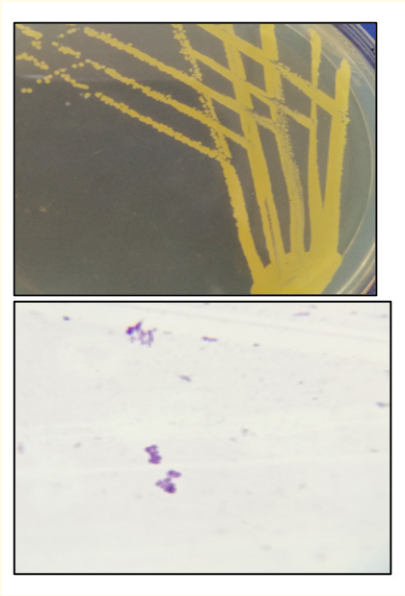


Figure 1: a: *Micrococcus lysodeikticus* on nutrient agar. *Micrococcus lysodeikticus* under microscope.

solutions were prepared by addition of pectin at 3 percent w/v level. The pectin was added into distilled water and heated to a temperature of 80^0C for allowing for gelatinization to occur. Glycerol at 5 percent level was added as a plasticizer to the solution at 70^0C. After bringing down the temperature of the solution to 50^0C, lysozyme solution was added in different volumes to possess different concentration of lysozyme. The total volume was adjusted by adjusting the level of distilled water. Three different film forming solutions were produced along with control.

- Pectin film forming solution with 1.62 x 10^6 specific units of Lysozyme (S1)
- Pectin film forming solution with 3.24 x 10^6 specific units of Lysozyme (S2)
- Pectin film forming solution with 8.1 x 10^6 specific units of Lysozyme (S3)
- Pectin film forming solution without Lysozyme (C).

The solutions were casted onto 4 different petri plates with a diameter of 10 centimetres and were kept at 59^0C for 24hours in a hot air oven to allow drying to form four different types of films viz., C, T_1, T_2, T_3, from C, S_1, S_2 and S_3, respectively. The dried films were then removed carefully from the petri plates and stored in desiccators until being used for further studies.

The films were evaluated for the following parameters.

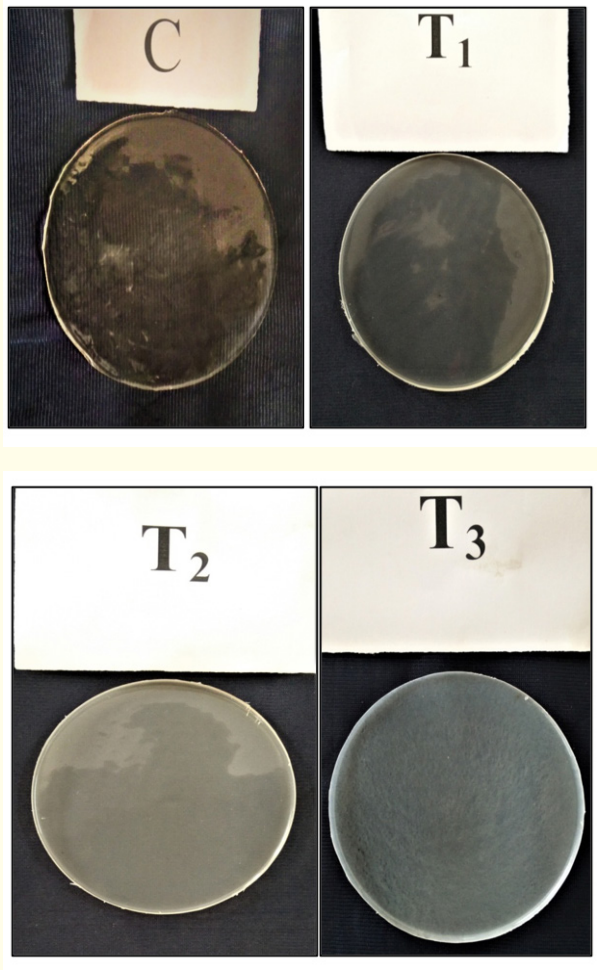


Figure 1b: Control film, pectin films obtained with different conc. of immobilized lysozyme.

Film characteristics

Film thickness

The thickness of films was measured in *microns* with a digital micrometer. The average of at least 10 random measurements was represented as film thickness.

Film grammature

The grammature was estimated as per the procedure demonstrated in [15].

Grammature was determined by dividing film weight in grams with its area in square metres.

$$\text{Grammature}\left(\frac{gm}{m^2}\right) = \frac{\text{Weight of the film(gms)}}{\text{Area}(m^2)}$$

Tensile strength

The mechanical properties of the films were measured by means of its tensile strength (TS) and per cent elongation at break (EAB).

The TS value of the edible film was recorded as per the method in [3], with texture profile analyzer (Tinius Olsen, Model H1KF, Redhill, RH1 5DZ, England).

Per cent elongation at break (EAB)

Per cent elongation at break was measured according to the method in [41].

$$\text{Percent elongation at break} = \frac{B - A}{A} \times 100$$

Where,

A: Initial length of the film and

B: Final length of the film after stretching or at the time of break.

Water vapor permeability

The water vapour permeability (WVP) of the films was measured gravimetrically based on ASTM E96-92 method described in [8].

Water sorption kinetics

The water sorption of edible sodium alginate films was evaluated by following the method in [26].

Light transmission and film opacity

Transmission and opacity of the films were evaluated according to the method of [44].

Anti-oxidant activity

The antioxidant activity was determined by N, N-dimethyl-1, 4-diaminobenzene (DMPD) free radical scavenging assay as described in [13].

Anti-microbial activity

The anti-microbial activity of the films was assessed against *Staphylococcus aureus and E.coli* by agar well diffusion method of [37] with little modifications.

6 to 8 mm diameter hole was punched aseptically on Muller Hinton agar plates spreaded with 0.1 ml of inoculum with 10^5-10^6 CFU/ml of bacterial culture, standardised using McFarland scale. The floor of the well was sealed with agarose to avoid diffusion of solution beneath the agar. Using a sterile tip, a volume of 20 µl from each formulation of the film forming solution was introduced into the wells. The plates were incubated at 37 ± 1^0C for 24h. The diameter of the zone of inhibition around wells was measured and equated against an Antibiotic sensitivity test (ABST) zone of inhibition scale and compared with standard antibiotic zones.

The antimicrobial activity was also assessed through challenging studies with *E.coli* following the method in [33].

E.coli was grown on EMB agar, Specific films were placed on EMB agar and reduction in counts of *E.coli* was noticed. The counts were expressed as $\log_{10}$ CFU/ml.

pH: pH of the preparation was determined by following the method in [43].

2-Thiobarbituric acid reactive substances (2-TBARS) value

TBARS values were determined by the method in [42].

Microbial analysis

The microbial quality of preparation was evaluated by estimating the Total plate count (TPC), Psychrophilic Bacterial Count (PBC) and Yeast and Mould counts (YMC) following pour plating technique as per standard procedure of ICMSF (1980).

Statistical analysis

The results of the above parameters were analysed through SPSS (17.0).

Results and Discussion

Lysozyme pectin complex formation

Lysozyme and pectin complexes were formed and their turbidity was measured in order to estimate the extent of immobilization. The Mean ± SE values of turbidity due to immobilization of

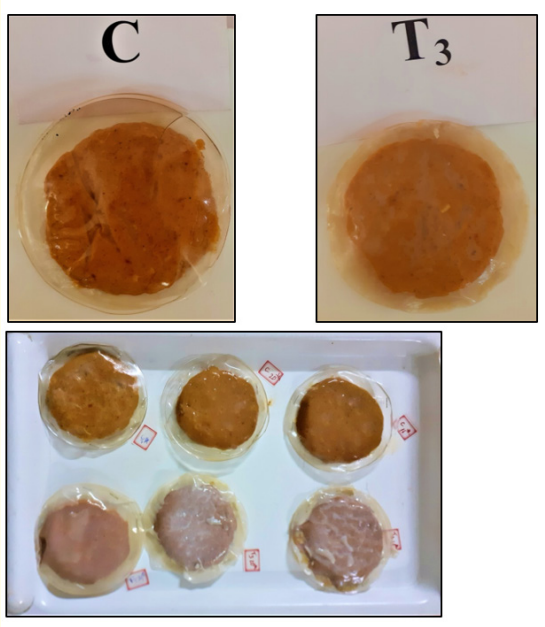


Figure 1c

lysozyme on pectin as influenced by different concentrations and different levels of immobilization of lysozyme were presented in table no. 1, figure 2 and analysis of variance in table 1.

S. no	Concentration of immobilized lysozyme (in mg with 15000 specificunits/mg of lysozyme)	Turbidity
1	0.72	0.0022 ± 0.00[a]
2	1.44	0.0029 ± 0.00[b]
3	2.16	0.0031 ± 0.00[b]
4	2.88	0.0040 ± 0.00[c]
5	3.60	0.0040 ± 0.00[c]
6	4.32	0.0040 ± 0.00[c]

Table 1: Turbidity of lysozyme pectin complexes as influenced by different concentrations of lysozyme.

The Mean ± SE values of turbidity of lysozyme- pectin complexes (LP Complexes) with a concentration of 0.72, 1.44, 2.16, 2.88, 3.60 and 4.32 (in mg with 15000 specific units of lysozyme) immobilized on to pectin were 0.0022 ± 0.00, 0.0029 ± 0.00, 0.0031 ± 0.00, 0.0040 ± 0.00, 0.0040 ± 0.00 and 0.0040 ± 0.00, respectively. The turbidity of lysozyme-pectin complexes with different concentrations of lysozyme differed significantly ($P < 0.05$) till a concentration of 2.88 in mg with 15000 specific units of lysozyme and thereafter no significant ($P < 0.05$) difference, was noticed. The concentration of 2.88 mg with 15000 specific units per mg of lysozyme showed significant ($P < 0.05$) higher turbidity than the rest of the lower concentrations.

The turbidity values for increasing concentrations of lysozyme adsorbed on to a specific amount of pectin were increased initially and there after no change in the turbidity was evident.

The concentration of 2.88 mg of lysozyme had significantly higher turbidity than the rest of the lower concentrations. This indicated the extensive biopolymer aggregation at intermediate lysozyme-pectin concentration.

At concentrations lower than 2.88 mg of lysozyme, the biopolymer was slightly aggregated and had an average size which was responsible for the particular turbidity. Then the increase in lysozyme concentration had resulted in a widening of the combination of individual complexes with each other and there by apparently increasing the mean size of the complex, thus increasing the turbidity.

Specifically 1 gram of pectin powder could load 144 mg of lysozyme at maximum in this present study. From this concentrations turbidity was not increased. This could be due to the inability of biopolymer to aggregate beyond a concentration through a restricted bridging mechanism.

The results were partially in line with those of [2,27], where in there was an increased bridging till a particular concentration, thereafter a decrease was noticed.

Lysozyme activity evaluation

The activity of lysozyme in free and immobilized state was presented in table 2, figure 3 and analysis of variance in table 1

The Mean ± SE values of the enzyme activity in (U/ml) for different concentrations of lysozyme at 0.72, 0.72, 1.44, 2.16, 2.88, 3.60, and 4.32 (in mg with 15000 specific units/mg concentrations)

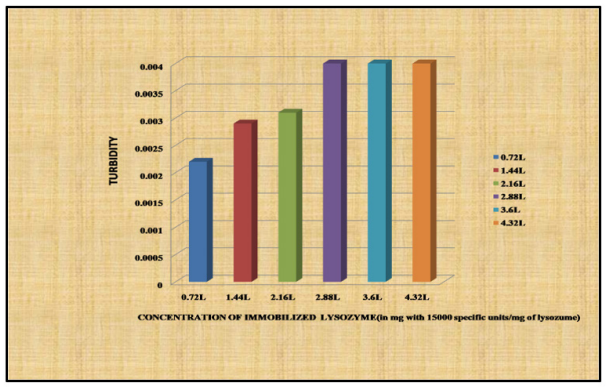


Figure 2: Turbidity of lysozyme pectin complexes as influenced by different levels of lysozyme.

S. no	Concentration of lysozyme (In mg with 15000 specific units/ mg of lysozyme)	Activity of lysozyme (Units/ml)
1.	0.72 (without immobilization)	979.33 ± 1.65^{e}
2	0.72 (Immobilized)	903.83 ± 0.60^{b}
3	1.44 (Immobilized)	897.16 ± 0.70^{a}
4	2.16 (Immobilized)	909.66 ± 1.02^{c}
5	2.88 (Immobilized)	920.50 ± 0.76^{d}
6	3.60 (Immobilized)	903.50 ± 1.52^{b}
7	4.32 (Immobilized)	1125.66 ± 4.17^{f}

Table 2: Activity of lysozyme - pectin complexes as influenced by different levels of immobilization of lysozyme.

were 979.33 ± 1.65, 903.83 ± 0.60, 897.16 ± 0.70, 909.66 ± 1.02, 920.50 ± 0.76, 903.50 ± 1.52 and 1125.66 ± 4.17, respectively. The activity of immobilized lysozyme irrespective of concentration had differed significantly ($P < 0.05$) with free lysozyme. The lysozyme immobilized on pectin at a concentration of 1.44 in mg with 15000 specific units of lysozyme had recorded significantly ($P < 0.05$) lower activity than the rest of the test concentrations. According to the results obtained in the study and figure 3, the lysozyme activity was decreased and reached a minimum when the lysozyme-pectin concentration was 1.44 mg. Then lysozyme activity was increased again and remained so at a concentration of 4.32 mg.

Complexation with pectin was basically immobilization of lysozyme that may incite a reduction in the mobility of the lysozyme. This mobility is important in light of the fact that the lysozyme activity was assessed by studying the initial degradation kinetics of the *Micrococcus lysodeikticus* cell wall. The reduction in lysozyme activity after immobilization, in the similar manner could be credited to the substrate diffusional obstacles coming about as a result of the diminished molecular flexibility of the compound [24].

In this present study, the lysozyme activity was minimum at 3.6 mg concentration and when the lysozyme concentration was increased above this concentration, activity was also increased. This might be due to the fact that the amount of lysozyme in the solution was sufficient to form small individual complexes with a higher mobility and hence a higher activity. But the activity could not reach to a level as that of free lysozyme due to the differentiations in mobility between free and complexed lysozyme.

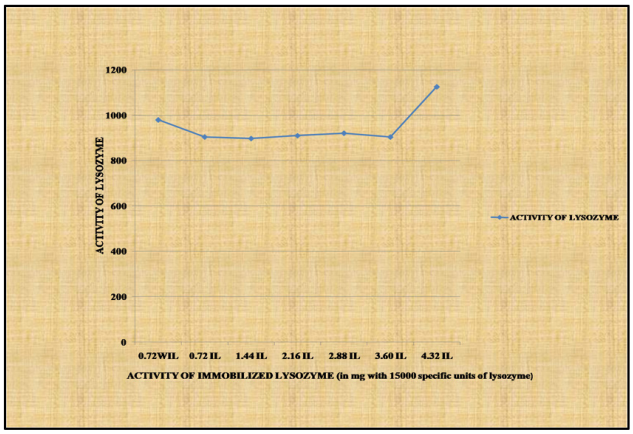


Figure 3a: Activity curve of lysozyme-pectin complexes as influenced by different levels of immobilization of lysozyme.

Film thickness and grammature

The mean film thickness of T_2 and T_3 were significantly ($P < 0.05$) higher than the rest of the treatment and control films. The thickness of T_1 differed non-significantly ($P < 0.05$) with control. The increase in thickness might be due to the increased viscosity of film forming solutions when compared to the others.

The grammature was significantly higher for T_2 film indicating its higher density. The unique colloidal properties of pectin including, thickening, suspending and interaction between the lysozyme and pectin components might be responsible for the varied thickness of the films [14].

Tensile strength

The mean tensile strength of T_3 was significantly ($P < 0.05$) higher than the rest of the treatment, and control films. This might be due to the interaction between polymer matrix of pectin and lysozyme in specific complexation at particular concentration which might have resulted in the formation of strong hydrogen bonds, further coupled with the fact that the lysozyme molecules incorporated in the film could be associated with pectin chains forming many individual complexes which were homogeneously dispersed in these films which might have resulted in increased tensile strength [11].

Per cent elongation at break

The mean per cent elongation at break of T_3 was significantly ($P < 0.05$) higher than the rest of the treatment, and control films. This could be attributed to the greater flexibility of the materials utilized for preparation of these films, due to protein polysaccharides interaction coupled with the plasticizing effect of glycerol which might have increased the mobility of polymer chains leading to possible higher molecular affinity between the components of the film [40]. The interactions between the materials also play an important role on the elastic properties of the material [47].

Water vapour permeability

The mean water vapour permeability value of T_2 and T_3 was significantly ($P < 0.05$) higher than the T_1 and control films. The water vapour permeability of T_1 differed non- significantly ($P < 0.05$) with control. increase in the water vapour permeability could be due to the interaction of hydrogen bonds, hydrophobic chains with ionic bonds which were generated on films [40]. In this way strong intermolecular interaction between amino hydroxyl and amino groups of proteins and amylase and amylo-pectin chains could improve the permeability of film.

Water sorption kinetics

The mean water sorption value of T_3 was significantly ($P < 0.05$) lower than the rest of treatment and control films. This decrease in the moisture sorption might be due to the addition of lysozyme molecules into pectin chains. This might alter the structural configuration of pectin molecules by increasing interactions between pectin and lysozyme molecules, such as hydrogen bonding and vanderwaals interactions. Meanwhile, lysozyme contains both hydrophilic and hydrophobic amino acids. During film formation, the lysozyme hydrophobic core may be formed with the hydrophilic amino acid side chains protruding towards the aqueous film forming solution by the hydrophobic interactions that play an important role in the folding of lysozyme [38]. The increased hydrophobic side chains in the film matrix by the addition of lysozyme may be responsible for the decrease in moisture content of the films [36].

Light transmission and film opacity

The mean opacity value of T_3 was significantly ($P < 0.05$) higher than the rest of treatment and control films. which could be due to reflectance of the presence of light-scattering micro aggregates of immobilized lysozyme with pectin formed within the solution resulting in reduced clarities when compared to the lysozyme-free control films [5].

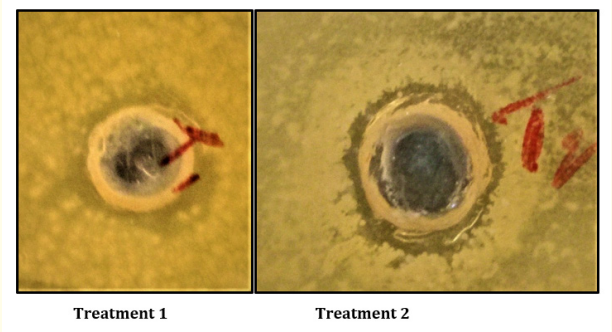
Anti-oxidant activity

The mean antioxidant value of T_3 was significantly ($P < 0.05$) higher than the rest of treatment and control films. This could be due to the antioxidant properties of lysozyme. Lysozyme was reported to produce peptides with high antioxidant activity that were found to efficiently inhibit lipid peroxidation [7], pectin was also reported to possess various biological properties such as antioxidant, anti-tumour and anti-inflammatory activities with it's galacturonic acid content [34].

Anti-microbial activity

The mean diameter of inhibition zones of T_3 against *Staphylococcus aureus* and *Escherichia coli* was significantly ($P < 0.05$) higher than the rest of the treatment and control films. This result showed that at these temperatures a lower diffusion rate corresponded, since the matrix is more rigid and the release of the protein is slower. The lysis halo was increased with increase in the time allowed. After few days, the inhibition zones covered the whole surface of the petri dish, indicating a slow release of lysozyme. To test if the lysozyme entrapped in the film could keep its activity during the storage of the film at refrigerated temperature,

the test was repeated after maintaining an aliquot for 30 days. The obtained results did not differ from the previous. This second microbiological test was carried out to assess the activity of lysozyme after its release from the film. In order to verify if the molecule maintained its activity after being entrapped in the film, its activity was measured by measuring the log reduction of *E. coli* on EMB agar. The log *E. Coli* values for all films incorporated with lysozyme were significantly ($P < 0.05$) lower than the control films. The reduction efficiency was increased with increasing concentration of lysozyme. High efficiency was noted in the films incorporated with 3.6 mg of lysozyme immobilized with 3 grams of pectin. These films were able to reduce the counts from 15.56 ± 0.59 log CFU to 0.11 ± 0.01 log CFU. Comparing the results, it was confirmed that lysozyme in antimicrobial form was successfully released from the pectin films and exhibited effective antimicrobial activity. The results were similar to those of lysozyme-chitosan composite films with 60 per cent lysozyme incorporation [36], and lysozyme immobilized polyvinylalcohol films [10].



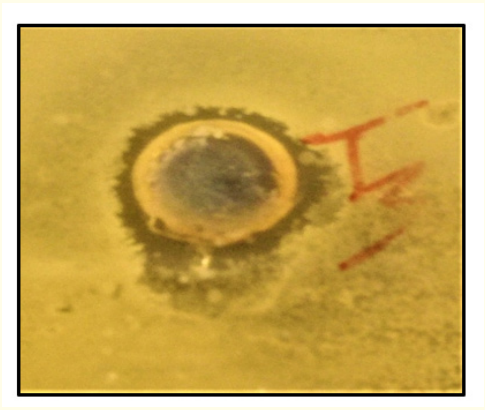
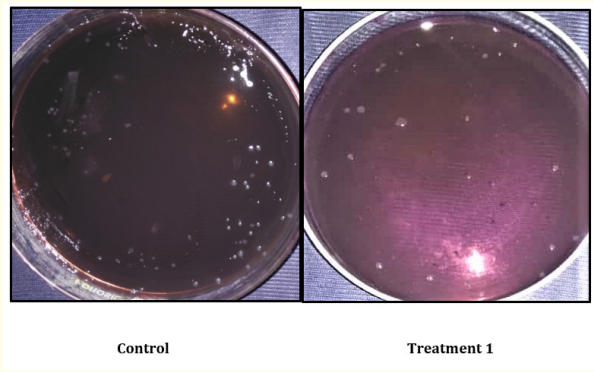


Figure 3b: Diameter of inhibition zones *(millimetres)* values of T_1, T_2 and T_3 against *Staphylococcus aureus.*



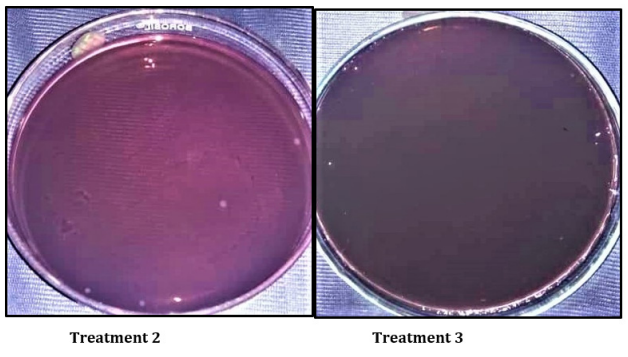


Figure 3c: Reduction in log CFU/ml count values of C, T1, T2 and T3 films against *E.coli*.

pH

The mean pH values of chicken meat patties wrapped in treatment film were significantly ($P < 0.05$) lower. This may be due to reduction in bacterial activity by the presence of lysozyme in the pectin film [20].

2-TBARS

The mean 2-TBARS values of chicken meat patties wrapped in treatment film were significantly ($P < 0.05$) lower than the patties that were wrapped in control film. This may be due to slow migration of lysozyme that was incorporated in the developed film which could retard the oxidation and further the effect was potentiated by the inhibition of UV light penetration through the film into the meat samples [23].

Percent cooking loss

The mean per cent cooking loss values of chicken meat patties wrapped in treatment film were significantly ($P < 0.05$) lower than the patties that were wrapped in control film. This might be associated with lower evaporation rate and respiration rate due to wrapping in films [28]. The complexes formed between lysozyme and pectin might have reduced the interstitial space within the film network and consequently decreased the evaporation [12].

Total plate count

The mean total plate count values of chicken meat patties wrapped in treatment film were significantly ($P < 0.05$) lower than the patties that were wrapped in control film. This might be due to the antimicrobial effect of lysozyme that could be related to the hydrolysis of peptidoglycan of microbial cell wall resulting in the lysis of microbial cells [16]. The results were well in agreement with [45] in beef patties with use of zein films incorporated with partially purified lysozyme. The mean yeast and mould count values of chicken meat patties wrapped in treatment film were significantly ($P < 0.05$) lower than the patties that were wrapped in control film. This might be due to membrane disruption by amphipathic helix stretches in the C-terminus of lysozyme resulting in antifungal activity [29]. It is obvious that the sensory scores decreased significantly ($P < 0.0.5$) in both treatment and control groups with increasing refrigerated storage time. However, the rate of decrease in T_3 film wrapped patty was slower. This might be due to antimicrobial effect, anti oxidant effect of lysozyme in the pectin films.

Treatments	**Days of storage period**								
Day 0*	**Day 4***	**Day 8***	**Day 12***	**Day 16***	**Day 20***	**Day24***		**Day28***	**Overall 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}
Overall 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}
Overall 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}
Overall 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}	

Table 3

Conclusion

In this context, it can be concluded that, lysozyme can be effectively immobilized on to pectin, using physical absorption method. It was evident that the stability of the enzyme could be increased by immobilization. The usability of lysozyme was preserved for longer time. The immobilized enzyme had good antimicrobial activity and was synergistic with pectin base. This indicates the potential use of lysozyme and pectin 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.

Conflict of Interest

There is no conflict of interest.

Bibliography

1. Azeredo HMC., *et al.* "Nanocomposite edible films from mango puree reinforced with cellulose nanofibers". *Journal of Food Science* 74.5 (2009): N31eN35.
2. Bayarri M., *et al.* "Properties of lysozyme/low methoxyl (LM) pectin complexes for antimicrobial edible food packaging". *Journal of Food Engineering* 131 (2014): 18-25.
3. Berry BW and Stiffler DM. "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 (1981): 1103-1106.
4. Bilska A. "Packaging systems for animal origin food". *Log Forum* 7 (2011): 1-4.
5. Bower CK., *et al.* "Characterization of fish-skin gelatin gels and films containing the antimicrobial enzyme lysozyme". *Journal of Food Science* 71.5 (2006): M141-M145.
6. Brody AL. "Packaging of food. In A. L. Brody and K. S. Marsh (Eds.). "The Wiley encyclopedia of packaging 2nd edition". New York: Wiley (1997): 699e704.
7. Carrillo W., *et al.* "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 (2016): 1777-1785.
8. Casariego A., *et al.* "Chitosan/clay films properties as affected by biopolymer and claymicro/nanoparticles concentrations". *Food Hydrocolloids* 23.7 (2009): 1895-1902.
9. Cha DS., *et al.* "Antimicrobial films based on Naalginate and/kappa/-carrageenan". *LebensmWiss Technol* 35 (2002): 715-719.

10. Conte A., *et al.* "Immobilization of lysozyme on polyvinylalcohol films for active packaging applications". *Journal of Food Protection* 69.4 (2006): 866-870.

11. Da Silva MA., *et al.* "Alginate and pectin composite films crosslinked with Ca^{2+} ions: Effect of the plasticizer concentration". *Carbohydrate Polymers* 77.4 (2009): 736-742.

12. Di pierro., *et al.* "Effect of transglutaminase on the mechanical and barrier properties of whey protein pectin films prepared by complexation pH". *Journal of Agricultural and Food Chemistry* 61 (2013): 4593-4598.

13. Fogliano V., *et al.* "Method for Measuring Antioxidant Activity and Its Application to Monitoring the Antioxidant Capacity of Wines". *Journal of Agricultural and Food Chemistry* 47 (1999): 1035-1040.

14. Galus S and Lenart A. "Development and characterization of composite edible films based on sodium alginate and pectin". *Journal of Food Engineering* 115.4 (2013): 459-465.

15. Geraldine RM., *et al.* "Characterization and effect of edible coatings on minimally processed garlic quality". *Carbohydrate Polymers* 72 (2008): 403-409.

16. Gill AO and Holley RA. "Inhibition of bacterial growth on ham and bologna by lysozyme, nisin and EDTA". *Food Research International* 33 (2000): 83-90.

17. Grunert KG and Valli C. "Designer-made meat and dairy products: Consumer-led product development". *Livestock Production Science* 72 (2001): 8398.

18. Jayasena DD and Jo C. "Essential oils as potential antimicrobial agents in meat and meat products: A review". *Trends in Food Science and Technology* 34 (2013): 96e108.

19. Jolie RP., *et al.* "Pectin methylesterase and its proteinaceous inhibitor: A Review". *Carbohydrate Research* 345.18 (2010): 2583e2595.

20. Jridi M., *et al.* "Physicochemical, antioxidant and antibacterial properties of fish gelatin-based edible films enriched with orange peel pectin: Wrapping application". *Food Hydrocolloids* 103 (2020): 105688.

21. Kaewprachu P., *et al.* "Quality attributes of minced pork wrapped with catechin-lysozyme incorporated gelatin film". *Food Packaging and Shelf Life* 3 (2015): 88-96.

22. Kapetanakou AE and Skandamis PN. "Applications of active packaging for increasing microbial stability in foods: Natural volatile antimicrobial compounds". *Current Opinion in Food Science* 12 (2016): 1e12.

23. Kim J., *et al.* "Nanostructures for enzyme stabilization". *Chemical Engineering Science* 61 (2006): 1017-1026.

24. Laurent MA and Boulenguer P. "Stabilization mechanism of acid dairy drinks (ADD) induced by pectin". *Food Hydrocolloids* 17.4 (2003): 445e454.

25. Lavorgna M., *et al.* "Study of the combined effect of both clay and glycerol plasticizer on the properties of chitosan films". *Carbohydrate Polymers* 82 (2010): 291-298.

26. Lian Z X., *et al.* "Preparation and characterization of immobilized lysozyme and evaluation of its application in edible coatings". *Process Biochemistry* 47 (2012): 201-208.

27. Mallika EN., *et al.* "Influence of calcium alginate coating on quality of pork patties". *International Journal of Current Microbiology and Applied Sciences* 7.6 (2018): 824-827.

28. Min S., *et al.* "Listeria monocytogenes inhibition by whey protein films and coatings incorporating lysozyme". *Journal of Food Protection* 68.11 (2005): 2317-2325.

29. Mondry H. "Packaging systems for processed meat. In S. A. Taylor, A. Raimundo, M. Severini, and F J M Smulders (Eds), Meat quality and meat packaging". Utrecht, Holland: ECCEAMST (1996): 323-333.

30. Moraes AR., *et al.* "Development and evaluation of antimicrobial film on butter conservation". *CiênciaeTecnologia de Alimento* 27 (2007): 33-36.

31. NagaMallika E., *et al.* "Studies on the development of low-fat functional pork storage and their shelf life using optimized hurdle technology". Thesis submitted to SVVU (2008): 97-100.

32. Nasrulwathoni., *et al.* "Characterization and antioxidant activity of pectin from Indonesian mangosteen (*Garciniamangostana* L.) rind". Heliyon 5.8 (2019).

33. Padgett T., *et al.* "Incorporation of food-grade antimicrobial compounds into biodegradable packaging films". *Journal of Food Protection* 61 (1998): 1330-1335.

34. Park SI., *et al.* "Functional properties of antimicrobial lysozyme-chitosan composite films". *Journal of Food Science* 69.8 (2004): M215-M221.

35. Pellissari FM., *et al.* "Antimicrobial, mechanical, and barrier properties of cassava starch-chitosan films incorporated with oregano essential oil". *Journal of Agricultural and Food Chemistry* 57 (2009): 7499-7504.

36. Proctor VA and Cunningham FE. "The chemistry of lysozyme and its use as a food preservative and a pharmaceutical". *CRC Critical Reviews in Environmental Science and Technology* 26 (1988): 359-395.

37. Rodriguez-Misael Cortes., *et al.* "Development and evaluation of edible films based on cassava starch, whey protein, and bees wax". *Heliyon* 6.9 (2020).

38. Soni A., *et al.* "Development and characterization of essential oils incorporated carrageenan based edible film for packaging of chickenpatties". *Nutrition and Food Science* 46.1 (2015): 82-95.

39. Tarladgis BG., *et al.* "A distillation method for the quantitative determination of malonaldehyde in rancid foods". *Journal of American Oil Chemists Society* 37 (1960): 44-48.

40. Trout ES., *et al.* "Chemical, physical and sensory characterization of ground beef containing 5 to 30% fat". *Journal of Food Science* 57 (1992): 25-29.

41. Tunc S and Duman O. "Preparation and characterization of biodegradable methyl cellulose/montmorillonitenanocomposite films". *Applied Clay Science* 48.3 (2010): 414-424.

42. Unalan IU., *et al.* "Active packaging of ground beef patties by edible zein films incorporated with partially purified lysozyme and Na2EDTA". *International Journal of Food Science and Technology* 46.6 (2011): 1289-1295.

43. Yadav KS., *et al.* "Active packaging: concepts and applications". *International Journal of Food and Nutritional Sciences* 4 (2015): 194-200.

44. Zhang Y., *et al.* "Effect of beeswax and carnauba wax addition on properties of gelatin films: a comparative study". *Food Bioscience* 26 (2018): 88-95.