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Mohd Mujeeb Ahmad
Department of Studies in
Microbiology, University of
Mysore, Manasa Gangothri
Mysore, Karnataka, India

Komal Chauhan
National Institute of Food
Technology Entrepreneurship
and Management (NIFTEM),
Sonapat, Haryana India

Asma Naz
Department of Plant Protection,
Aligarh Muslim University,
Aligarh, Uttar Pradesh, India

Mohammed Nayeem
National Institute of Food
Technology Entrepreneurship
and Management (NIFTEM),
Sonapat, Haryana India

Corresponding Author:
Komal Chauhan
National Institute of Food
Technology Entrepreneurship
and Management (NIFTEM),
Sonapat, Haryana India

Antimicrobial and antioxidant activity of impregnated pectin and alginate based bio composite packaging material for fresh produce safety

Mohd Mujeeb Ahmad, Komal Chauhan, Asma Naz and Mohammed Nayeem

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Abstract

The objective of the presented work was to develop, characterize and evaluate the biodegradable, proactive, protective, novel active packaging blend films of low methoxy pectin and sodium alginate and identify their antimicrobial and antioxidant ability with plant-based natural phytochemicals like cinnamaldehyde. Different blend films were prepared in different ratio like (75% pectin \pm 25% sodium alginate) (100% pure pectin), (100% pure sodium alginate), (100% Pectin+200 μ L cinnamaldehyde), 75% pectin+25 sodium alginate+700 μ L cinnamaldehyde), The antimicrobial activity was tested against foodborne pathogens *E. coli*O157:H7 (MTCC 90), and *Salmonella* Typhi (MTCC 733) and zone of inhibition were recorded. On the basis of linear correlation analysis (R^2), the free radical scavenging activity of 10% bio-composite incorporated with 0.30% cinnamaldehyde blends was 0.9235 for DPPH scavengers where citric acid were used as a positive control. The film was characterized by TGA, DSC, FTIR, and XRD analysis result was shown a significant effect on thermal behavior.

Keywords: Active packaging, antimicrobial activity, antioxidant activity, mechanical properties, structural properties

1. Introduction

Agri-commodities are a natural source for the growth of microorganisms as well as necessary for chemical reactions to take place. They are subjected to numerous physico-chemical changes during the pre and post harvesting of fresh produces. Supply chain is one of the major parts for fresh produce safety, quality like processing, handling, packaging, storage, distribution and transportation, in food chains and some climatic factors affect the quality of the fresh produces like time/temperature/relative humidity/water activity (Krasniewska *et al.* 2012) [18]. So, now a days packaging is one of the major concern for food industry to reduce the environmental impacts selection of eco-friendly bio based packaging materials may prevent food waste and food quality losses like chemical changes, enzymatic reactions, microbial spoilages, by providing them an outer layer barrier and protect from metabolic properties to retain food integrity (Laura *et al.* 2011) [14]. Different kinds of antimicrobial, antioxidant, anti-browning substances can also be incorporated into natural bio based biopolymers which may act as primary packaging material and improve application of bio packaging and replace the petroleum based plastic packaging. Active substances could be added in a limited amount as per the codex laws and can prevent microbial spoilage on food surfaces that stop chemical and microbial changes related to food borne outbreaks (Patricia *et al.* 2014) [20].

It is assumed that polysaccharide based films could be characterized by their hygroscopic nature, higher water vapor transmission rate, lower gas vapor transmission rate, highly adhesiveness, poor mechanical strength, good plasticity, good bioavailability, cohesiveness and nontoxic to the environment if compared to plastic packaging materials these properties are could be different, depending upon class of polymer (M.D Harun Rashid, *et al.* 2015) [15]. According to innovative materials science and nonfoods technology-based ideology researchers have focused on green-chemistry based active bio packaging materials with novel techniques to inhibit microbial growth and chemical reactions in the food system for maintain the quality, safety, freshness of the final product (Caballero *et al.* 2010) [12].

On the basis of their DE (degree of esterification) value, pectins can be classified into two categories i.e. HMP (high methoxy pectin) and LMP (Low methoxyl pectin). DE value for the former is more than 50% while that of latter is less than 50%. Baldwin *et al.* 1995 showed that

gelatinization properties and solubility of the pectin directly depends on its DE value. For this reason HMPs are used for the preparation of jams and jellies while LMPs are not because of its low gelation properties with sugar and acids. Pectin coatings can help in water retention from food by loosing dehydration of actual food. This also contributes towards retaining physical appeal of food items (Kester & Fennema 1986).

Like LMPs, alginates are ionic in nature and readily forms gel structures by reacting with suitable cations. Formation of alginate films is a complex multistep process wherein alginate solution is first dried up and then treated with calcium salts which results in quick cross linkage at interface. Permeability and the strength of the film depends on numerous factors such as cation concentration, rate of addition of cations, time taken for chemical reaction, composite constituents, temperature and pH (Kester & Fennema 1986).

Studies have shown that gelatinous coating obtained from alginate when applied on meat surfaces can significantly reduce moisture loss and prevents oxidation. Alginates may also find application in food encapsulation (Hambleton *et al.* 2009).

The anti-microbial, antioxidant natural substances such as flavonoids, carotenoids, terpenoids, vitamins and essential oils can be added to the bio based packaging materials like pectin, alginate, chitosan, arabicgum, xanthangum, carrageenan and CMC etc. Incorporation of phytochemicals such as cinnamaldehyde into pectin and sodium alginate bio based polymeric film increases antimicrobial and antioxidant activity and combination of both molecules affected through water binding capacity, thermal properties, mechanical properties and bio chemical properties of bio based packing materials (Caribe *et al.* 2015)^[6].

Bio-polymeric film can also be used as coating agent in fresh produce as a surface preservative that are susceptible to oxidation, moisture losses and improve food structure, aroma, and flavor (McHugh *et al.* 2014)^[22]. Polysaccharide films serve this purpose to further increase food quality, stability, functionality, and safety (Jian *et al.* 2015)^[16]. The natural phenomenon of oxidation has detrimental effect on various quality attributes of foods and thus use of antioxidants or its sources for producing packaging material is being explored in recent studies (Guçbilmez *et al.* 2007).

Active packaging of pectin with sodium alginate blend film were found more effective as investigated in this study we were develop pectin and sodium alginate blend films with or without incorporated cinnamaldehyde in a specific ratio to investigate the antimicrobial & antioxidant activity for fresh produce application (Jian-Hua *et al.* 2015)^[16].

The aim of this research was to prepare pectin and sodium alginate active blend films with or without phytochemical (cinnamaldehyde) and studied their effect for antimicrobial & antioxidant properties. The effectiveness of bio-composite incorporated cinnamaldehyde against two major food-borne pathogens, *E. coli* O157:H7 MTCC (90), *Salmonella typhii* MTCC (733), was evaluated at hot air oven temperature at 37 °C for 48 hours. Free radical scavenging activity of 10% biocomposite films was characterized by DPPH free radical scavenging activity assay at 525nm of wavelength antioxidant activity of bio based active packaging film with or without cinnamaldehyde. The free radical scavenging activity of Pectin + Sodium alginate and Bio-composite+0.30% Phytochemical were dependent on linear correlation analysis (R^2) which was 0.9235 for DPPH scavenging activity. The

lowest IC₅₀ values were 35 µg/mL and 25 µg/mL for Pectin + Sodium alginate and Bio-composite + 0.30% Phytochemical respectively at 40 µM of DPPH concentration.

2. Materials and Methods

2.2 Preparation of film forming dispersions

Film was prepared according to the casting method described by (Fakhreddin *et al.* 2015)^[23]. 6gm of pectin was dissolved in 100mL of distilled water; 2gm of sodium alginate was dissolved in 30mL of 0.15M acetic acid solution. Added 0.9gm of food grade D-Sorbitol+ glycerol, to pectin film forming solution. Added 0.3gm of D-sorbitol+ glycerol contained sodium alginate film forming dispersion. The two mixtures were heated on a hot plate at 45 °C for 15 minutes with continuous stirring. Added cinnamaldehyde in range from 200µl to 700µl in biocomposite film forming dispersion, and then added 1.2mL of Tween 40. Homogenized the biocomposite film forming dispersion mixture by using ultra turrax blender at 21,600 rpm for two minutes (Shiv *et al.* 2015)^[21].

2.3 Preparation of films

Film forming solutions were casted on plastic petri dishes [90 mm diameter], dried at room temperature for 24-28 hrs. Film thickness was controlled by taking the volume between 20-30 mL (Figure 1). The films were peeled off from the casting surface, preconditioned in desiccator at 22 °C for 48hours over a saturated NaBr solution at 58%RH (Laura *et al.* 2011)^[19]. The initial concentration of cinnamaldehyde in film forming dispersion is reduced at the time of film drying process due to the oil evaporation by heat treatment as reported earlier (Otoni *et al.* 2014)^[11]. The volatile compounds lost during drying process ranged between 40% to 90% as shown in Table 1 and was dependent on the ratio of cinnamaldehyde incorporated bio-composite film (Zhang *et al.* 2015)^[16].

2.4 Film thickness

Hand-held digital micrometer (7031 Mitutoyo Corporation, Kanogawa Japan) was used to measure the thickness of the developed films. The average values of three random positions measurement were taken to the nearest 0.001 mm then all the films were cut into rectangular strips (3 cm width and 6 cm length) for thickness measurements (Shankar Shiv.*et al.* 2015)^[21].

2.5 Mechanical Characterizations

Mechanical characterization namely tensile strength (TS), elongation at break (E), and elastic modulus (EM), stiffness, extension at maximum, percentage at breaking point were measured using Instron Universal Testing Machine (Model 5565) according to standard method (D9555-ASTM 2010). The film samples were conditioned for a maximum of 48hours days at 25 °C ± 2% RH over NaBr saturated solution. Thereafter, the films of 3cm width and 6 cm length were cut and the tensile strength (MPa) was stretched at a cross-head speed of 100 mm/min⁻¹ with a 0.1 kN load cell. Extension at maximum (mm) was calculated and expressed as percentage by dividing the extension at the rupture by the initial length of the film (50 mm). The slope of the linear portion of the stress strain curve, corresponding to the ratio of stress to strain of the film was used to calculate Elastic modulus of film (MPa) (Zhang *et al.* 2014)^[36].

2.6 Structural characterizations

2.6.1 ATR- FTIR spectrum

Fourier Transform Infrared (FTIR) was used in this experiment (Perkin Elmer Spectrum). Bio-based material were analyzed in a spectrophotometer, equipped with an ATR accessory with a germanium crystal. IR light was transmitted through the sample with a frequency range of 4000-600 cm^{-1} . The observation was read at the wavelength ranging between 4000-600 cm^{-1} at a resolution of 4 cm^{-1} with a scan speed of 2mm/sec for each sample and a total 16 scans were co-added for each sample (Syed *et al.* 2010) [30].

2.6.2 Thermo gravimetric analysis (TGA)

Thermo gravimetric measurement was analyzed using TGA, (PerkinElmer 800). 4-5 mg (w/w) of film samples were taken in standard aluminum cup and heated to a temperature ranging from 30°C to 600°C with heating rate of 10°C/min under a nitrogen flow of 50 cm^3/min . Empty aluminum cup served as reference. Maximum thermal degradation temperature T. max was evaluated (Marina Ramos *et al.* 2014) [24].

2.6.3 Differential scanning calorimeter (DSC)

Differential scanning calorimeter (DSC, Perkin Elmer) was used for analyzing 2-5 mg (w/w) of the sample under 30ml/min nitrogen flow rate with temperature ranging from -25°C to 200°C was analysed. The sample was cooled at 10°C/min. The process was repeated and the readings were recorded (Patricia *et al.* 2014) [20].

2.6.4 X-ray diffraction pattern (XRD)

X-ray diffraction pattern of film was analyzed. The cut square pieces of films (3.0 to 3.0 cm) were placed in aluminum slide (Powder XRD 100, Omni Scientific Instruments). The spectra were recorded using Cu- α radiation (wavelength = 1.54060 nm stick) source a nickel mono chromator filtering wave at 35 kV to 25 mA. The diffraction data was collected from 2θ =values of 4°C and -20 °C with a step size of 0.01°C. The intensity of light was varied from 0 to 7000 with scanning speed of 0.4/min at room temperature (Nowzari *et al.* 2013) [26]. Crystalline index was calculated as

$$\text{CrI} = \frac{I_f - I_s}{I_f} \times 100$$

Where

I_f stands for peak intensity of fundamental band at $2\theta=2.0-20$

I_s stands for peak intensity of the second band at $2\theta=20.0$.

2.7 Antioxidant activity

The antioxidant activity of 10% bio composite film was determined by using DPPH assay (Joshi *et al.* 2013) [17]. This assay is based on the principle of color change with reduction of the odd electron in the DPPH radical by receiving one hydrogen atom from antioxidants (Marina Ramos *et al.* 2014) [24]. 100 mg/mL or 10% (w/w) bio composite film sample was dissolved in 1ml (v/v) of hot de-ionized water at 45°C. 0.8 mg (w/w) DPPH was mixed with 5ml (v/v) of methanol solution (200 μl of 0.6 mM). The solution was left to stand for 15 min in dark (Subramanian *et al.* 2013) [31]. Thereafter, the sample were loaded as 10 μl of film with 50 μl of DPPH and 140 μl methanol in 96-micro titer well plate. The absorbance was read at 525 nm against citric acid in UV-vis spectrophotometer. All determinations were performed in triplicates and the percentage scavenging effects were

calculated as

$$\% \text{ Inhibition} = \left[\frac{A_{1\text{Blank}} - A_{2\text{Sample}}}{A_{\text{Blank}}} \right] \times 100\%$$

Where

A_1 = Absorbance of the control without sample

A_2 = Absorbance of the sample

A= Absorbance of sample without DPPH free radical.

The scavenging ability of 10% biocomposite film samples were expressed as IC value, which was the effective concentration at which 50% of DPPH radicals were scavenged. The IC₅₀ values were calculated from the relationship curve of scavenging activities (%) versus that were compared and adjusted to McFarland 0.5 concentrations of respective sample (Pengfei *et al.* 2009) [28].

2.8 Antimicrobial Activity

Antimicrobial activity were done by nutrient agar direct plating assay stock culture of *E. coli* O157:H7 (MTCC 90), *Salmonella typhi* MTCC733), were kept frozen at -25°C in nutrient agar the culture was then refrigerated by transferring one loop of each bacterium into 10mL of nutrient broth incubated overnight at 37 °C. 10 mL of aliquot from each overnight culture was again transferred to a 10 mL nutrient broth and left at 37°C to attain maximum growth (Dutta P. K *et al.* 2009). Inoculations on agar plate were performed with appropriately diluted culture to get the target inoculums. Nutrient agar acts as model for solid food item. Aliquot of nutrient agar (20 ml) were solidified in petri-dishes. Thereafter the inoculations of each strain of selected microbes were performed on solidified culture media. For next 1 to 2 days of storage, films were then cutted in 1cm diameter incorporated into petri-plates film were placed in inoculated surface plates were then covered with thin polythene to prevent dehydration (Laura *et al.* 2011) [19].

3. Results and Discussion

Bio polymeric film forming dispersion, images after making the bio polymeric films of different range by using solution casting method (Figure 1) and composition of bio polymeric film forming dispersions (Table 1).

3.1 Antimicrobial activity

Anti-microbial properties of Pure pectin, pectin+200 μl cinnamaldehyde, PP+PSA, biocomposite+700 μl cinnamaldehyde film (Figure 2) were tested by nutrient agar plating assay against food borne pathogens: *Salmonella typhi*, *Xanthomonas comprestis*, *Xanthomonas oryzae*, *E. coli*, *Streptococcus pyogenes*, *Bacillus Cereus* (McHugh *et al.* 2011). The antimicrobial activity of carvacrol and oregano oil against *E.coli* O157: H7 in pectin-alginate films and film forming solutions was found to be higher than the activity of cinnamaldehyde (A. Maria *et al.* 2007). Pectin+200 μl cinnamaldehyde and pectin+ sodium alginate composite film showed the highest ($p < 0.05$) antimicrobial properties due to greater inhibition zone observed. (G. Caio Otoni *et al.* 2014) [11].

Antimicrobial effects compared to other films sodium alginate film were found highly positive inhibitor against *Salmonella typhi*, *Xanthomonas oryzae*, *E. coli* *Xanthomonas* (Pereda *et al.* 2011) [27]. In pectin+200 μl cinnamaldehyde *Streptococcus pyogenes*, *Bacillus cereus*, *E. coli* were showed less inhibition effect, and bio composite of pectin + sodium alginate was

found positive against the tested organisms (Figure 2) (Maria B *et al.* 2009) [25]. The empty zones around the films were understood as antimicrobial diffusion which causes growth inhibition of the targeted microorganism (A. Maria *et al.* 2007) [3].

Films have the ability to retard or inhibit of microbial growth to stop their multiplication or to delay of microbial kinetics mechanisms (Gitonga *et al.* 2015) [13] and their nutrient uptakes. The enhanced antimicrobial activity of small size droplets in the films increases the ability of the active compound to move from films and penetrate microbial cells (Huang *et al.* 2010) [10]. 12µl to 45µl were showed minimum inhibitor concentration of cinnamaldehyde. Sodium alginate already showed antimicrobial properties in it as showed in past research work we can use this films in food industry as anti-microbial coating (Gomez *et al.* 2010) [10].

3.2 Antioxidant activity

The free radical scavenging activities of different formulations of biopolymer and phytochemicals supplemented bio composites were analyzed using citric acid as positive control by DPPH method (Dicastillo *et al.* 2015) [7]. The free radical scavenging activity of pectin + sodium alginate and biocomposite+0.30%. Phytochemical were dependent on linear correlation analysis (R^2) which was 0.9235 for DPPH scavenging (Figure 3).

The lowest IC_{50} values were 35 µg/ml and 25 µg/ml for pectin + sodium alginate and biocomposite+0.30% Phytochemical, respectively at 40 µM of DPPH concentration (Table 2). The free radical scavenging activity was known to increase with presence of hydroxyl ions and existence of double bonds (Kang *et al.* 2010). Flavones substituted with methoxy groups in the B ring of compound instead of hydroxyl group also reported as a major determinant for lipid peroxidation inhibition (Joshi *et al.* 2013) [17]. Therefore the DPPH free radical scavenging activity showed by synthesized biopolymers was significant for IC_{50} value and suggests that could be used in food industry.

3.3 Thickness measurements

Thickness of pectin and sodium alginate films was altered significantly ($p < 0.001$) from 268 to 174.20 µm, on the basis of composition of mixture used for developing the films as shown in Figure 4. Phytochemical incorporated films thickness were 166.2 to 481.4 µm (Galus Sabina, Lenart Andrzej, *et al.* 2013) [10]. These values are lower when compared to data published in the other research work for pectin + sodium alginate based bio polymeric films (Vargas *et al.* 2011). Pure sodium alginate films were thicker compared to pure pectin films. This property is related to compound rheology; molecular bonding interaction while thickness of pectin and sodium alginate composite was higher due to the cinnamaldehyde entered into the polymer rings increase the structural behavior of polymer surfaces which may increasing the thickness of film solution (Galus Sabina *et al.* 2013) [10]. This is attributed to the colloidal properties of the compound including thickness, suspension and interactions among the components (Silva *et al.* 2009). Sodium alginate composite films incorporating lactose as developed by Bajdik *et al.* 2009 and with whey protein isolate and gelatin as prepared by Wang *et al.* 2010 gave similar results. Figure 4 shows the thickness, young modulus, of PP, PSA, PP+PSA, pectin 200 µl + phytochemical, and bio composite 700 µl added.

3.4 Mechanical analysis

Tensile strength, thickness, stiffness, young modulus, tensile strength, extension at maximum and percentage of break investigated at room temperature data of interpretation are presented in Figure 5. Tensile properties decrease as the concentration of anti-microbial substance increases in blend films (Wang *et al.* 2012) [35]. Elongation at break is the measure of film's plasticity i.e. the extent to which the film can be stretched before it breaks while the tensile strength of the film is the mechanical resistant offered by the bonds between the chains (Gonzalez *et al.* 2011) [19]. Altenhofen da Silva *et al.* 2009 showed that the tensile strength of the films and elongation at break are inversely proportional to each other. Value of stiffness significant ($p < 0.001$) 3935.5 to 7224.2 V/m, with highest value for pure pectin film and the lowest value for pectin + sodium alginate composite film (75-25%) for young modulus significant ($p < 0.001$) 30.32 to 78.915 µPa highest value for pure pectin and the lowest value for biocomposite+0.7% phytochemical (cinnamaldehyde). $SD \pm 0.001 >$ significance, values were given as mean \pm standard deviation. Figure 5 shows tensile strength, extension at maximum, % strain at break of PP, PSA, PP+PSA, pectin 200 µl+ phytochemical, and bio composite 700 µl added.

Values with common superscripts in a column are not significantly different ($p < 0.001$) Values of tensile strength were found significant ($p < 0.001$) from 0.4912 to 3.9187 MPa with the least value was found for pectin based bio composite blend film and the highest for pure pectin film (Figure 5). Elongation at break for different samples were found between 5.9% to 14.9% and the highest values was obtained for mixed films. Values of pure pectin film are not similar to what was shown by Kang *et al.* 2005 who demonstrated tensile strength of 193 MPa and elongation at break of 2.6%.

3.5 Structural properties

(ATR-FTIR) spectrum

ATR-FTIR spectroscopy works on the principle of on the of absorption bands recognition which are generated due to vibrations of functional groups at a particular wavelength. In the pure pectin film, compound spectra for pure pectin film showed strong alkyne \equiv C-H stretch at 3305.72nm, a strong alkanes C-H stretch at 2950 nm, a strong alkyls - C-H stretch at 1463.11nm, a characteristic band due to the high methyl chain present in the pectin structure, and a secondary alcoholic m-s, C-O stretch at 1111.65nm and an ester aliphatic -O=C-O-C stretch at 1195.18nm. A characteristic band was observed present of intra-molecular bond (Zhang *et al.* 2015) [37]. In the pectin film+200µl cinnamaldehyde, an amides w-m stretch at 3252.54 nm and a aromatic compounds m-s ring C=C stretch at 1467.20nm two or four sharp bends, due to presence of phenol ring into the pectin matrix, an amine v-s N-H stretch at 1580.40nm, a alkyl s, C-H stretch were found at 1450.69nm, and a small alkenes C-Hm+s stretch at 921.61nm (Harun M.D, Rashid *et al.* 2015) [15]. In the pure sodium alginate film amides w-m N-H symmetric stretch at 3252.54nm were observed, an esters s- C=O stretch at 1735.29nm was identified another stretch was found at 1590.20nm, and an alcoholic stretch was found at 1094.37nm (Sahiner, Mehtap *et al.* 2015) [32]. In the bio composite film 700µl+cinnamaldehyde film a weak characteristics amines N-H band was found at 3400.20nm, an alkanes strong C-H stretch was found at 2930.10nm, another sharp, very strong anhydrides C=O symmetric band were observed at

1810.50nm then an m-s secondary alcoholic stretch found at 1200.55nm, after that an m-s ethers =C-O-C symmetric stretch was found at 1020.80nm. All peaks were studied the understand chain interactions in the blend films combined spectra as shown in Figure 6.

It was investigated that blends film showed antimicrobial & antioxidant properties more in comparison to pectin film. Due to inter or intramolecular H-bonds breaking, the percentage of transmission was always higher (Bitencourt *et al.* 2014) [4]. Film incorporated by cinnamaldehyde that completely changed the physical and chemical structure of the natural polymers or polymer blends constituents with altered chemical bonds it was the ability of bio polymeric materials. As showed in this work, bio composites have more antimicrobial or antioxidant activity in comparison with single polymeric film. The novel approach of this research work was that the free radical scavenging activity detected in both composite blends, as well as cinnamaldehyde present and absent blended films, so it can be used to stop the oxidation reaction of different food commodities and act as an anti-oxidative bio polymeric material.

3.6 TGA

TGA is a widely used methodology for analyzing the polymer's thermal stability. The weight loss (TG) and (DTG) curves for TGA tests of pure pectin film, pure sodium alginate film, pectin + sodium alginate film, pectin + sodium alginate+ composite film+700 μ l are reported in Figure 7. Significant weight loss stage was observed in the TG curve of pure pectin film, and pure sodium alginate films. The weight loss in pure pectin films was observed at 110-250 $^{\circ}$ C (delta Y=1.180 mg), and at 48.93-198.93 $^{\circ}$ C(delta Y=3.075mg) in pure sodium alginate films. The reported and initial weight for pure pectin and pure sodium alginate film is 3.003mg and 5.499mg respectively. Weight loss in the films could be the result of losses of bound moisture as suggested in previous studies (Lewandowska *et al.* 2009).

Temperature effects on biocomposite blend pectin + sodium alginate and pectin + sodium alginate 700 μ l+cinnamaldehyde blend characterized in all the stages. The first major weight loss of pectin + sodium alginate blend film at 100-220 $^{\circ}$ C (delta Y=2.397 mg) was due to the moisture evaporation of both polymers (Lewandowska *et al.* 2009) while the second minor weight loss step at 291-36 $^{\circ}$ C (delta Y=0.456 mg) was due to thermal degradation of pectin and sodium alginate blend films. The blend of biocomposite pectin + sodium alginate/700 μ l incorporated cinnamaldehyde film were tested first minor weight loss occurs at 35-53 $^{\circ}$ C (11.589%) due to the breaking of H₂O bond, moisture loss occurs. The second major weight loss occurs was at 143-250 $^{\circ}$ C (39.975%) which is the result of thermal degradation of polymeric molecules, and the third minor weight loss occurs at 333-410 $^{\circ}$ C was the result of degradation of byproducts generated by pectin and sodium alginate during the thermal degradation process (Chen *et al.* 2009). Thermal degradation leads to formation of aldehydes and alkenes groups in liquid state (Holland and Hey, 2001). At 250-350 $^{\circ}$ C, pure pectin film showed more thermal stability than sodium alginate film (lower weight loss). With the gradual increase in pectin, the residual mass increases from 20% to 40%. The temperature at the maximum decomposition rate (T_{max}) are shown in Table 5 and it was found that higher pectin content in the blend resulted in higher value of T_{max} which proves that addition of pectin contributes

to thermal stability of these film. This is in accordance with the studies shown by Peesan *et al.* 2003 and Lewandowska 2009.

3.7 DSC thermograph

Differential screening calorimeter data analysis was showed the peak height, delta H, peak temperature, endothermic and exothermic graph analysis. The glass transition temperature (T_g) was taken as the inflection point of the specific heat increment at the glass-rubber transition, while the melting temperature (T_m) and the crystallization temperature (T_c) were taken as the peak temperature of the endothermic and exothermic during the cooling and the heating. Glass transition temperature (T_g) of pure pectin samples were at 150.34 $^{\circ}$ C, pure sodium alginate at 165.75 $^{\circ}$ C, pectin sodium alginate bio composite at 124.17 $^{\circ}$ C and bio composite incorporated photochemical at 173.67 $^{\circ}$ C. These samples were analyzed using a differential scanning calorimeter (DSC-department of studies in physics, university of Mysore) as identified by (Basu *et al.*2007). The study of glass transition involves recording of three temperatures i.e. at the start, at mid-point and at the end of transition steps. The temperature at mid-point is known as transition temperature (T_g) which was recorded during the cooling as well as second heating steps (Figure 8).

3.8 X-rays diffraction

X-ray diffraction patterns of pectin, sodium alginate and cinnamaldehyde films are shown in Figure 9. Pectin and sodium alginate showed amorphous characters, and more noise also occurs in the crystal lettuce of polymers peaks and intense x-rays beam at different angles in the polymer surfaces (Sangeetha *et al.* 2011). The peak was found to be very weak, and the intensity of incident light was more in the d-spacing of the molecules (Figure 9).

4. Conclusion

The developed films were analysed by ATR-FTIR spectroscopy, DSC, TGA and XRD. The results showed that in the absence of cinnamaldehyde, antioxidant and antimicrobial properties were in pectin, sodium alginate, and bio composite film. Films incorporated with cinnamaldehyde showed higher antimicrobial inhibition compared to films without added cinnamaldehyde. Films prepared without cinnamaldehyde showed brittleness, higher water absorption capacity, greater water solubility and lower mechanical properties. The results showed that in the presence of cinnamaldehyde, bio composite blends showed higher IC₅₀ values and DPPH scavenging activity than films without adding cinnamaldehyde. This shows that tested films have the potential to act as a food surfaces coating in different fresh agricultural commodities and could act as an active bio-packaging. These films can be used in antimicrobial packaging or protective packaging materials. Further research is required to verify the beneficial effect of the developed film for fresh produce as a surface decontaminant for fresh produce.

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6. Conflict of Interest

The authors declare that there is no conflict of interest.

7. Authors' Contributions

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

8. Funding: None

9. Data Availability

All datasets generated or analyzed during this study are included in the manuscript and/or the Supplementary Files.

10. Ethics Statement

This article does not contain any studies with human participants or animals performed by any of the authors.

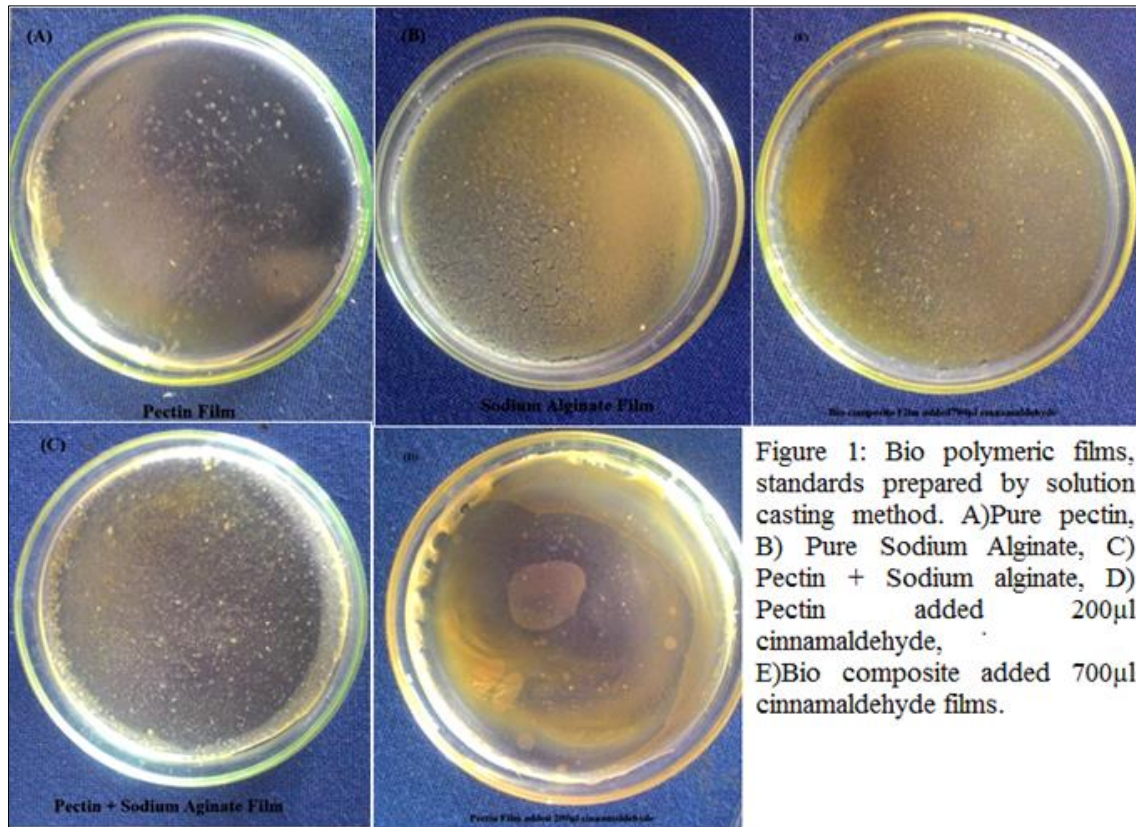


Fig 1: Bio polymeric films, standards prepared by solution casting method A) Pure pectin, B) Pure sodium alginate, C) Pectin + sodium alginate, D) Pectin added 200µl cinnamaldehyde, E) Bio composite added 700µl cinnamaldehyde films.

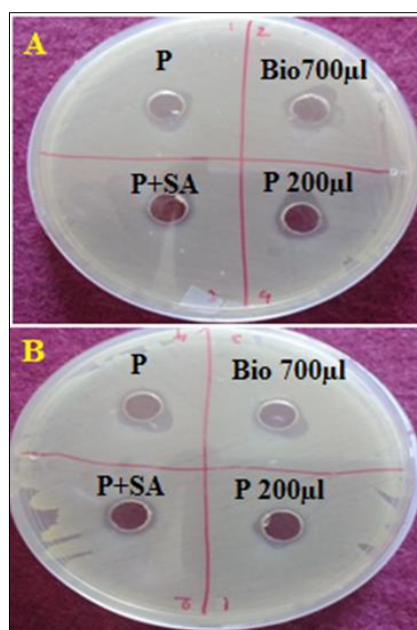


Fig 2: Anti-microbial activity test results showed different bio polymeric films against the two different strain of A) *E. coli* (MTCC 90) B) *Salmonella typhi* (MTCC 733). a clear zone of inhibition were observed of the films or cinnamaldehyde.

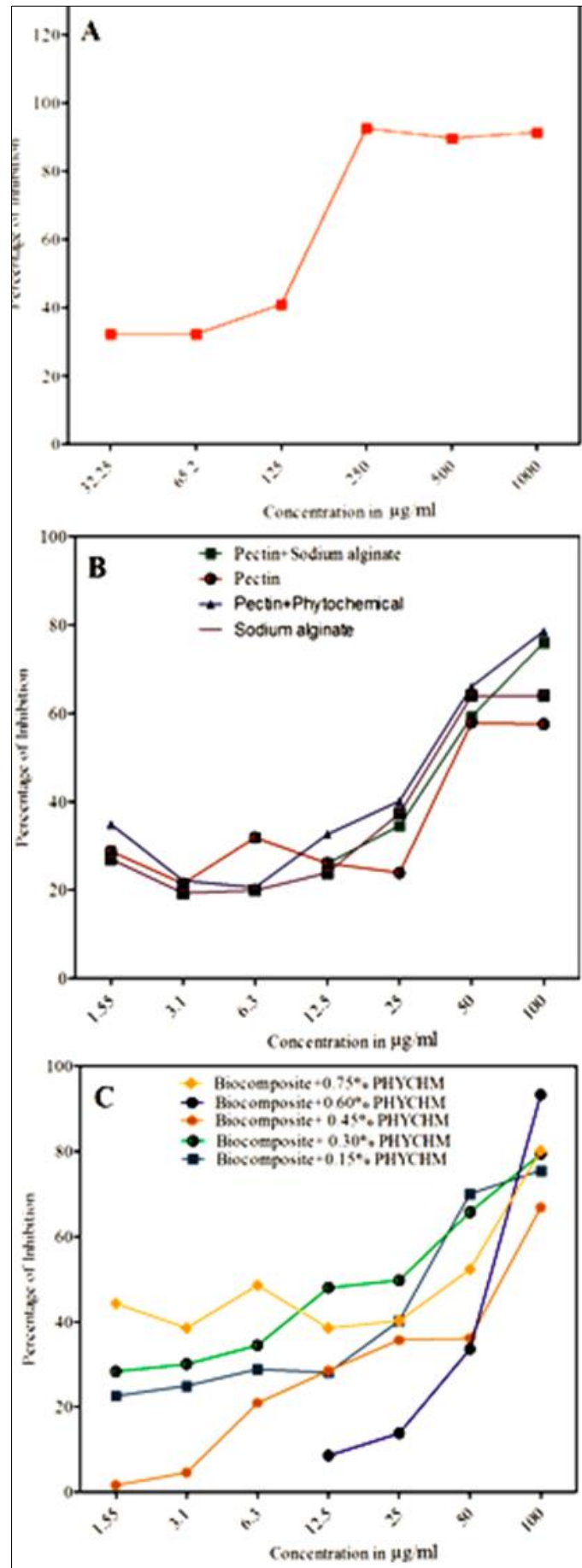


Fig 3: DPPH radical scavenging activity of bio polymeric films, A) Anti-oxidant activity of citric acid was used as positive control B) Anti-oxidant activity of different range of bio polymeric films PP, PSA, PP+PSA, PP + Phytochemical C) Anti-oxidant activity of bio composite films containing different concentration of phytochemical.

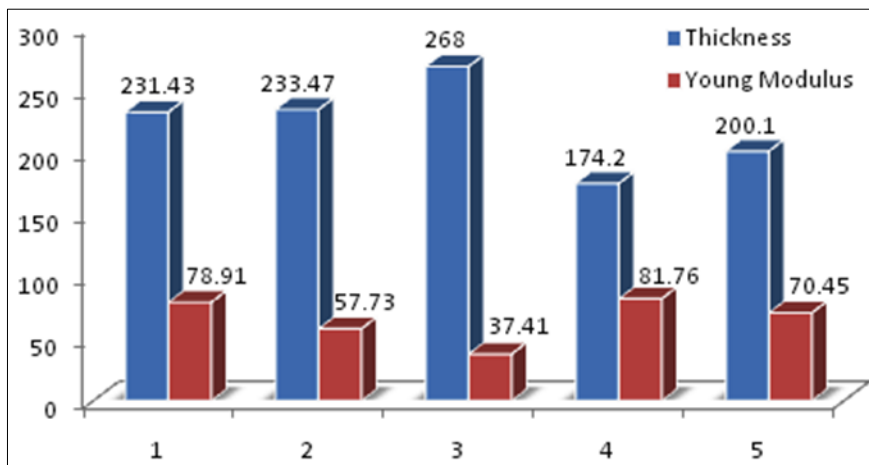


Fig 4: Analysis of thickness and young modulus, of 1) pure pectin film, 2) pure sodium alginate film, 3) pectin+ sodium alginate film, 4) pectin incorporated 200 pl cinnamaldehyde film; 5) Bio composite blend film incorporated 00 pl cinnamaldehyde).

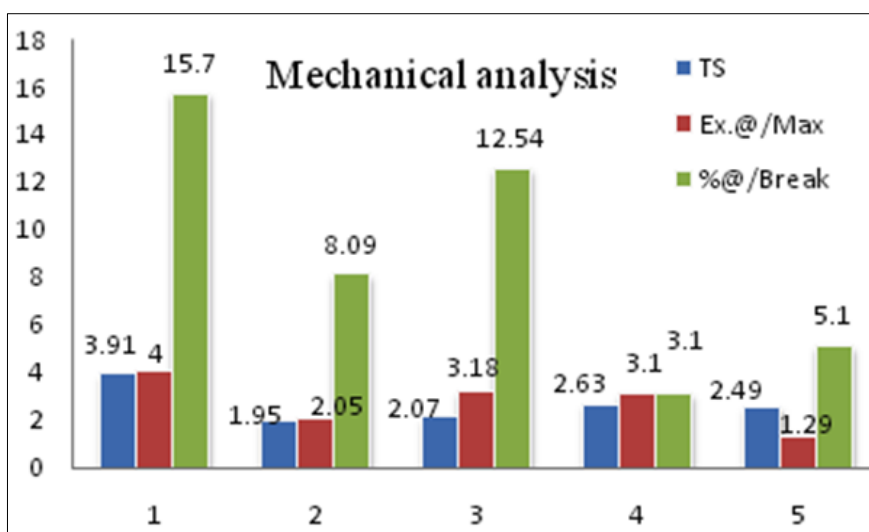


Fig 5: The measurements of the tensile strength, extension at maximum and percentage strain at break of 1) pure pectin film, 2) pure sodium alginate film, 3) pectin + sodium alginate film, 4) pectin incorporated 200W rkmarnaldehyde film, 5) Bio composite incorporated 700W cinnamaldehyde film.

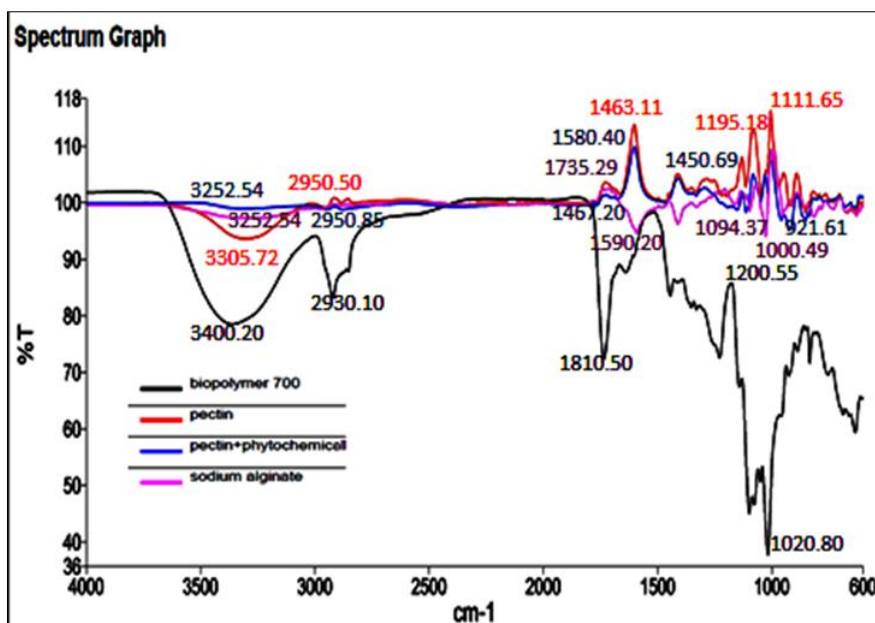


Fig 6: ATR-FTIR functional groups spectra analysis of the pure pectin film, pure sodium alginate film, pectin incorporated 200W cinnamaldehyde film and bio composite incorporated 7000 cinnamaldehyde blend film.

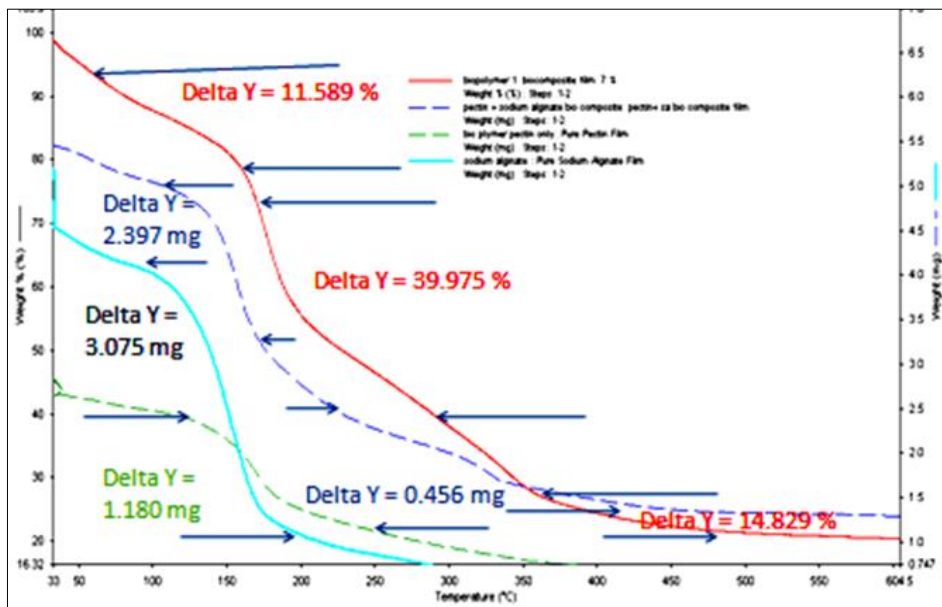


Fig 7: Analysis of thermal weight losses vs. temperature of different bio polymeric films curve showed the glass transition stages of bio polymeric materials at a their specified temperature ranges, 1) pure pectin fib, 2) pure sodium alginate Sam, 3) pectin + sodium alginate blend film, 4) bio composite blend incorporated 700 ail cinnamaldehyde SIm

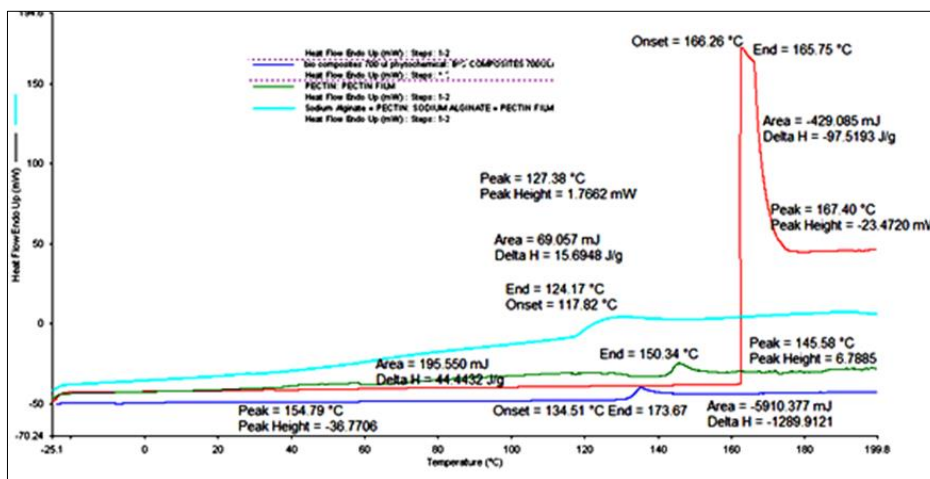


Fig 8: Differential scanning thermograph of 1) pure pectin film, 2) pure sodium alginate film, 3) pectin + sodium alginate composite film, 4) bio composite incorporated 700p1 rinnamaldehyde blend film were identified by using the standard method

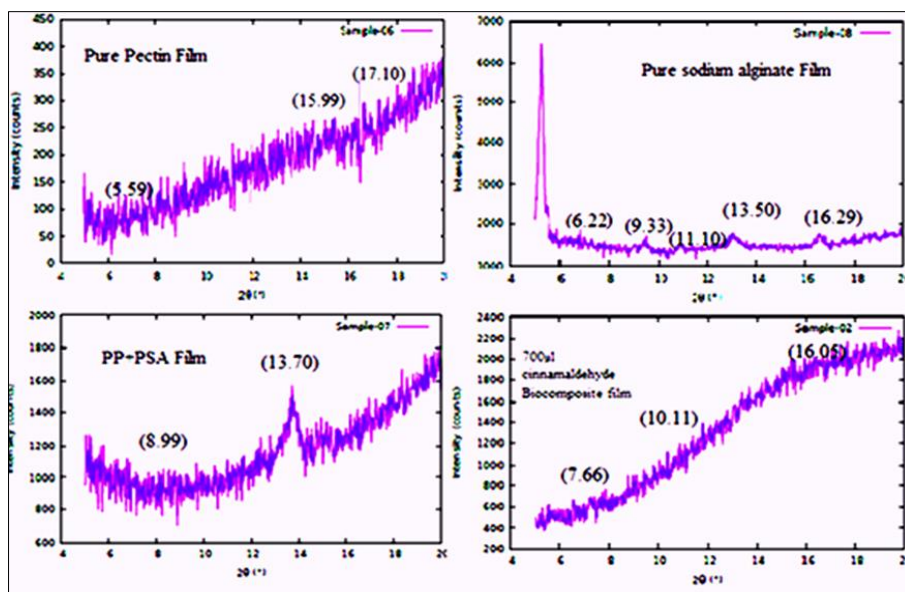


Fig 9: X-ray diffraction pattern of bio polymeric films 1) pure pectin film, 2) pure sodium alginate film, 3) pectin + sodium alginate blend films, 4) bio composite incorporated 700W •firmarnaldehyde film were determine of their crystallographic peaks at 26=4-20 angles.

Table 1: Composition of the different components in film forming dispersions (Hill)

Sample	Phytochemical (Cinnamaldehyde %)	Acetic acid (N/A)	d-Sorbitol & glycerol (w/w)	Tween 40 (v/v)
PP	-	-	0.9gm	1.2ml
PSA	-	30ml .15M	0.9gm	1.2ml
PP+SA	-	30ml .15M	0.9gm	1.2ml
Bio composite	.7%	30ml .15M	0.9gm	1.2ml
Pectin	.2%	30ml .15M	0.9gm	1.2ml

a) Pure pectin film, b) Pure sodium alginate film, c) Pectin + sodium alginate composite film d) Bio composite, Pectin + sodium alginate + 7000 cinnamaldehyde added film e) Pectin + 2000 cinnamaldehyde added film.

Table 2: IC₅₀ values of bio polymeric films using antioxidant activity by DPPH method

Types of Biopolymers	IC ₅₀ values (it Wml)
Pectin+ Sodium alginate	35
Pectin	40
Pectin+ Phytochemical	25
Sodium alginate	28
Bio-composite+ 0.15% Phytochemical	50
Bio-composite+ 0.30% Phytochemical	25
Bio-composite+ 0.45% Phytochemical	75
Bio-composite+ 0.60% Phytochemical	50
Bio-composite+ 0.75% Phytochemical	45
Citric acid (positive control)	140

P+SA, Pectin+phyto, Bio composite + 0.30% Phytochemical showed significance effect of free radical scavenging properties in blends.

5. References

- Arantzazu Vald Cristina, Ramos Marina, Burgos Nuria, Garrigos Maria Del Carmen, Jimenez Alfonso *et al.* Active edible films: Current state and future trends. *J Appl. Polym. Sci* 2015;132:42631.
- Theeranun Janjarasskul, John Krochta M *et al.* Edible packaging materials annual review of food science and technology- Annual Review of Food Science and Technology 2010;1:415-48.
- Maria Rojas-Grau, Roberto Avena-Bustillos J, Olsen Carl, Friedman Mendel, Henika Philip R, Belloso Olga Martin *et al.* Effects of plant essential oils and oil compounds on mechanical, barrier and antimicrobial properties of alginate-apple puree edible films. *Journal of Food Engineering* 2007;81:634-641.
- Bitencourt CM, Favaro CS, Sobral PJA, Carvalho RA, *et al.* Gelatin-based films additivated with curcuma ethanol extract: Antioxidant activity and physical properties of films *Food Hydrocolloids* 2014;40:145-152.
- Caballero Lopez ME, Gomez-Estaca J, Lopez De Lacey A *et al.* Biodegradable gelatin chitosane films incorporated with essential oils as antimicrobial agents for fish preservation"-*Food Microbiology* 2010;27:889-896.
- Caribe Leticia, Reis Batista, Oliveira Carolina *et al.* Active biocomposites of cassava starch, the effect of yerba mate extract and mango pulp as antioxidant additives on properties and the stability of a packed products food and bio products processing 2015;94:382-391.
- Dicastillo Carlo De Lopez, Navarro Rosa, Guarda Abel, Galotto Maria Jose *et al.* Development of Biocomposites with Antioxidant Activity Based on Red Onion Extract and Acetate Cellulose *journal/antioxidants* 2015. ISSN 2076-3921.
- Dutta PK, Tripathia Shipra, Mehrotraa GK, Dutta Joydeep *et al.* Perspectives for chitosane based antimicrobial films in food applications. *Food Chemistry* 2009;114:1173-1182.
- Devlieghere F, Vermeulen A, Debevere J *et al.* Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. *Food Microbiology* 2004;21:703-714.
- Galus Sabina, Lenart Andrzej *et al.* Development and characterization of composite edible films based on sodium alginate and pectin. *Journal of Food Engineering* 2013;115:459-465.
- Caio Otoni G, Marcia R, Fauze Aouada A, Geany Canilloto P, Renato S, Marcos Lorevice V *et al.* Antimicrobial and physical-mechanical properties of pectin/papaya puree/ cinnamaldehyde nano emulsion edible composite films. *Food Hydrocolloids* 2014;41:188-194.
- Gómez Estaca J, López de Lacey A, López-Caballero ME, Gómez-Guillén MC, Montero P *et al.* Biodegradable gelatin-chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. *Food Microbiology* 2010;27:889-896.
- Gitonga Stephen Giteru, Coorey Ranil, Bertolatti Dean, Elizabeth Watkin, Stuart Johnson, Zhongxiang Fang *et al.* Physicochemical and antimicrobial properties of citral and quercetin incorporated kafirin-based bioactive films. *Food Chemistry* 2015;168:341-347.
- Gonzalez Laura Sanchez, Amparo Chiralt, Chelo González-Martínez, Maite Cháfer *et al.* Effect of essential oils on properties of film forming emulsions and films based on hydroxypropyl methylcellulose and chitosan *Journal of Food Engineering* 2011;105:246-253.
- Harun MD or Rashid, Rahaman Saifur MD, Kabir Shariff Enamul, Khan Mubarak A *et al.* Effect of hydrochloric acid on the properties of biodegradable packaging materials of carboxy methylcellulose/poly(vinyl alcohol) blends" *applied polymer science* 2015, 133.
- Jian-Hua Li, Jing Miao, Jiu-Lin Wu, Shan-Fei Chen, Qi-Qing Zhang, *et al.* Preparation and characterization of active gelatin-based films incorporated with natural antioxidants"- *Food Chemistry* 2015;168:341-34.
- Joshi AJ, Gadhwal MK, Joshi UJD, Mello P, Sinha R, Govil G *et al.* Synthesis of B-ring substituted flavones and evaluation of their antitumor and antioxidant activities. *Med. Chem. Res* 2013. DOI 10.1007-00044-012-0423-1.
- Krasniewska krolina, Gniewosz Malgortaza *et al.* Substances with Antibacterial Activity in Edible Films. *Food Nutrition Science* 2012;62(4):199-206.
- Laura Sanchez, Gonzaleza Maite Chafera, Manuel Hernandezb, Amparo Chiralta, Chelo Gonzalez-Martinez *et al.* Antimicrobial activity of polysaccharide films containing of essential oils. *Food Control* 2011;22:1302-1310.
- Patricia Rijo, Diogo Matias *et al.* Antimicrobial Plant Extracts Encapsulated into Polymeric Beads for Potential Application on the Skin. *Polymers* 2014;6:479-490.

21. Shankar Shiv, Teng Xinnan, Li Gaobin, Rhim Jong Whan *et al.* Preparation, characterization, and antimicrobial activity of gelatine / ZnO nano composite films. *Food Hydrocolloids* 2015;45:264-271.
22. McHugh Tara H, Du1 Wen-Xian, Bustillos Roberto Avena J, Sui Sheng Hua T *et al.* Antimicrobial volatile essential oils in edible films for food safety 2011, 2014;162:149-155.
23. Seyed Fakhreddin Hosseini, Masoud Rezaei, Mojgan Zandi, Farhid Farahmand Ghavi *et al.* Development of bioactive fish gelatin /chitosan nano particles composite films with antimicrobial properties. *food chemistry* 2015.
24. Marina Ramos, Alfonso Jiménez, Mercedes Peltzer *et al.* Development of novel nano-biocomposite antioxidant films based on poly (lactic acid) and thymol for active packaging. *Food Chemistry* 2014;162:149-155.
25. Maria B, Vasconez Silvia Flores K, Carmen Campos A, Juan Alvarado, Lia Gerschenson N *et al.* Antimicrobial activity and physical properties of chitosan–tapioca starch based edible films and coatings. *Food Research International* 2009;42:762-769.
26. Nowzari Fariba, Shabanpour Bahareh, Ojagh Seyed Mahdi *et al.* Comparison of chitosan–gelatine composite and bilayer coating and films effect on the quality of refrigerated rainbow trout. *Food Chemistry* 2013;141:1667-1672.
27. Pereda M, Ponce AG, Marcovich NE, Ruseckaite RA, Martucci JF *et al.* Chitosan-gelatin composites and bilayer films with potential antimicrobial activity *Food Hydrocolloids* 2011;25:1372-1381.
28. Pengfei L, Tiansheng D, Xianglin H, Jianguo W *et al.* Antioxidant Properties of Isolated Isorhamnetin from the Sea Buckthorn Marc. *Plant Foods Hum. Nutr* 2009;64:141-145.
29. Huang Q, Yu H, Ru Q *et al.* Bioavailability and delivery on nutraceuticals using nanotechnology, *Journal of Food Science* 2010;75:R50-R57.
30. Seyed Mahdi Ojagha, Masoud Rezaeia, Hadi Razavib Seyed *et al.* Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. *Food Chemistry* 2010;120:193-198.
31. Subramanian Ramasamy, Palanivel Subbramanyan, Vairamuthu Raj, *et al.* Antioxidant activity of the stem bark of Shorearoxburghii and its silver reducing power. *Springer Plus* 2013;2:28.
32. Sahiner Mehtap, Sagbas Selin, Oral Behzat Bitlisli *et al.* p(AAm/TA)-based IPN hydrogel films with antimicrobial and antioxidant properties for biomedical applications. *J of applied polymer science* 2015. DOI: 10.1002/APP.41876.
33. Solak O, Ayten Dyankoya, Syetla M *et al.* Composite Films from Sodium Alginate and High Methoxyl Pectin - Physicochemical Properties and Biodegradation in Soil” *Ecological Balkanica* 2014;6(2):25-34.
34. Shaaban Hamdy A, Hamdy Osman F, Amer M *et al.* Antimicrobial Activity of New Edible Bio nanocomposite Prepared By Pectin And Cinnamon Essential Oil Nanoemulsions. *Middle East Journal of Applied Sciences* 2014;4(2):336-344. ISSN 2077-4613.
35. Wang Sunan, Marccone Massimo F, Barbut Shai, Tak lim Loong *et al.* Fortification of dietary biopolymers-based packaging material with bioactive plant extracts”-*Food Research International* 2012;49:80-91.
36. Zhang Qi-Qing, Jian-Hua Li, Jing Miao, Jiu-Lin Wu, Shan-FeiChen *et al.* Preparation and characterization of active gelatin-based films incorporated with natural antioxidants. *Food Hydrocolloids* 2014;37:166-173.
37. Zhang Hongcai, Zhao Yanyun *et al.* Preparation, characterization and evaluation of tea polyphenoleZn complex loaded b-chitosan nanoparticles *Food Hydrocolloids* 2015;48:260-273.
38. Daniel Karp, Kate Scow, Jeffery McGarvey *et al.* Towards a decision-support tool for identifying and mitigating on-farm risks to food safety 2019.