# Antimicrobial and antioxidant activity pectin and sodium alginate bio composite packaging material for fresh produce.

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## Abstract

**The main aim of this research work was to prepare, characterize and evaluate the bio degradable, 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 ± 25% sodium alginate) (100% pure pectin), (100% pure sodium alginate), (100% Pectin+200 µ L cinnamaldehyde), 75% pectin+25 sodium alginate+700 µ L cinnamldehyde). The antimicrobial activity were tested againstfood borne pathogens *E. coli* O157:H7 (MTCC 90), and Salmonella typhii (MTCC 733) and zone of inhibition were recorded. The free radical scavenging activity of 10% bio-composite incorporated cinnamaldehyde 0.30% blends based on linear correlation analysis (R2) which was 0.9235 for DPPH scavengers and citric acid were used as positive control. The films were characterized by TGA, DSC, ATR-FTIR, XRD analysis results showed a significant effect on thermal behavior of polymeric structures.**

**Keywords:** Active packaging, Antimicrobial activity, Antioxidant activity, Mechanical properties, Structural properties, Cinnamaldehyde.

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## Introduction

Agri-commodities are a natural source for the growth of microorganisms as well as necessary for chemical reactions to take place. They may undergo physical, chemical and microbiological 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. 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 [1]. 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 [2].

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. 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 [3,4].

Pectin (21CFR184.1588) are water-soluble anionic polymers composed mainly of (1 → 4) –α -D-galactopyranosyluronic acid units. Pectins with a degree of esterification (DE) above 50% are labeled high-methoxyl pectin (HMP) and below 50% are termed Low-Methoxyl Pectin (LMP). The differences in methyl ester content and DE affect solubility and gelation properties of pectin. HMP forms gels with sugar and acid, especially in jams and jellies [5]. LMP, produced from chemical de-esterification, forms gels in the presence of divalent cations. Calcium cations bridge adjacent LMP chains via ionic interactions and withinterchain H-bonding yield a 3D-gel network. Edible pectin film can be formed by evaporating water from pectin gel. Although pectinate coatings are not adequate moisture barriers, they can retard water loss from enrobed food by acting as a sacrificing agent when moisture evaporates from their gel matrix rather than dehydrating the food significantly. Pectin coatings have been investigated for their ability to retard moisture loss and lipid migration and improve handling and appearance of foods [6].

Alginate (21CFR184.1011). Alginates are salts of alginic acid that is a linear (1 → 4) linked polyuronic acid containing three types of block structures: poly- β -D-manopyranosyluronic acid (M) block, poly -α -L-gulopyranosyluronic acid (G) blocks, and MG blocks containing both polyuronic acids. These highly anionic polymers have the ability to form instantaneous gel structures by reacting with divalent or trivalent cations, without

heating or cooling, similar to LMP. Alginate films can be formed from evaporating solvent from alginate gel or by a two-step procedure that involves drying of alginate solution followed by treatment with a calcium salt solution to induce instantaneous cross-linking at the interface. Film strength and permeability can be altered by the concentration of polyvalent cations, the rate of its addition and time of exposure, pH, temperature, and the presence of composite constituents [7].

Gelatinous alginate coatings effectively lessen desiccation in enrobed meats by acting as a sacrificing agent. Owing to their good $O_2$ barrier properties, alginate coatings can protect foods against oxidation. Alginates also have versatile uses in encapsulation.

The anti-microbial, antioxidant natural substances such as flavonoids, carotenoids, terpenoids, vitamins and essential oils can be incorporated into the bio based packaging materials such as 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.

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. Polysaccharide films serve this purpose to further increase food quality, stability, functionality, and safety. Incorporation of antioxidants into packaging material has also become very popular since oxidation is one of the big problems which are influencing the food quality and stability [8].

Active packaging of pectin with sodium alginate blend film were find 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 and antioxidant activity for fresh produce application [9].

The main aim of this research work was to prepare pectin and sodium alginate active blend films with or without phytochemical (cinnamaldehyde) and studied their effect for antimicrobial and 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℃ for 48 hours. Free radical scavenging activity of 10% biocomposite films was characterized by DPPH free *radical scavenging activity assay at 525 nm 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 (R2) which was 0.9235 for DPPH scavenging activity [10]. 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 M of DPPH concentration.

## Materials and Methods

### *Materials*

Pectin and sodium alginate, Glycerol, acetic acid, d-Sorbitol, NaBr, tween 40, cinnamaldehyde all chemicals were food grade and purchased from local market.

### *Preparation of film forming dispersions*

Film was prepared according to the casting method described. 6gm of pectin dissolved in 100 mL of distilled water, 2gm of sodium alginate dissolved in 30 mL of .15M acetic acid solution. Add 0.9 gm of food grade D-Sorbitol+glycerol, contained pectin film forming solution. Add 0.3 gm of D-sorbitol+glycerol contained sodium alginate film forming dispersion. Both mixture were warmed in hot plate at $45^0$C for 15 minutes with continuous stirring, temperature was maintained by thermometer, after all add cinnamaldehyde in range from 200 µl to 700 µl in biocomposite film forming dispersion, and add 1.2 mL of Tween 40 [11]. Homogenize the biocomposite film forming dispersion mixture by using ultra turrax blender at 21,600 rpm seed for two minutes.

### *Preparation of films*

Film forming solution were casted on plastic petri dishes [90 mm diameter] then dry under room temperature for 24-28 hrs. Film thickness was controlled by casting the amount of film solutions between 20-30 mL as mention in after that, dried films were peel off from the casting surface and preconditioned in desiccator at 22°C for two days over a saturated NaBr solution approx. RH 58%. It were seen that initial concentration of cinnamaldehyde in film forming dispersion was reduce at the time of film drying process due to the oil evaporation by heat treatment therefore, the losses of volatile compounds during the film drying process can range between 40% to 90% which were depend on the ratio of cinnamaldehyde incorporated bio-composite film.

### *Film thickness*

Films thickness was measured by hand-held digital micrometer. 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 [12].

### *Mechanical characterizations*

Tensile Strength (TS), elongation at break (E), and Elastic modulus (EM), stiffness, extension at maximum, percentage at break were measured by using (Instron Universal Testing Machine Model 5565) according to standard method (D9555-ASTM 2010). All film sample were conditioned for a maximum of 2 days in 25°C ± 2% RH over NaBr saturated solution. Then 3 cm width and 6 cm length cut of the films

tensile strength (MPa) were stretched at a cross-head speed of 100 mm/min$^{-1}$ and with a 0.1 kN load cell. Extension at maximum (mm) were determined by dividing the extension at the rupture of the film by the initial length of the film (50 mm) multiplied by 100. Elastic modulus of film samples (MPa) were determined from the slope of the linear portion of the stress strain curve, which corresponds to the stress divided by the strain of the film sample at least 16 replicates were performed for each film [13].

### *Structural characterizations*

**ATR- FTIR spectrum:** Fourier Transform Infrared (FTIR) Radiation sampling technique were used in this experiment (Perkin Elmer Spectrum). Bio-based material were analyzed in a spectrophotometer, equipped with an ATR accessory with an germanium crystal, IR light was passed through the sample with a frequency range of 4000-600 cm$^{-1}$ . The observation was taken in the wavelength range between 4000-600 cm$^{-1}$ at a resolution of 4 cm$^{-1}$ with a scan speed of 2 mm/sec for each sample and total 16 scans were co- added of each sample and data were analyzed.

**Thermo Gravimetric Analysis (TGA):** Thermo gravimetrical measurement were analyzed by using. Approximately, 4-5 mg (w/w) film samples were taken in standard aluminum cup and heating temperature range between 30°C to 600°C with heating rate of 10°C/min under a nitrogen flow of 50 cm$^3$/min. Empty aluminum cup were taken as a reference. Maximum thermal degradation temperature Tmax was evaluated [14].

**Differential Scanning Calorimeter (DSC):** Differential scanning calorimeter (DSC, Perkin Elmer) were analyzed by using 2-5 mg (w/w) measurements were carried out under 30 ml/min nitrogen flow rate in the temperature range from -25°C to 200°C. After a first heating step, cooling were performed at 10°C/min, and finally a second heating step at the same rate were recorded.

**X-ray diffraction pattern (XRD):** X-ray diffraction pattern of film were analyzed first, cutting thesquare pieces of films (3.0 to 3.0 cm) placed in aluminum slide (Powder XRD 100, Omni Scientific Instruments). The spectra were recorded using Cu-kα radiation (wavelength=1.54060 nm stick) source a nickel mono chromator filtering wave at 35 kV to 25 mA and diffraction data was collected from 2 θ=values of 40 C-20 C with a step size of 0.010C intensity of light was 0 to 7000 range with scanning speed of 0.4/min at room temperature were recorded. Crystalline index was calculated as CrI=[If-Is] × 100, Where If were stand for peak intensity of fundamental band at 2 θ=2.0-20 and where Is were stand for peak intensity of the second band at 2 θ=20.0 were analyzed.

**Antioxidant activity:** The antioxidant activity of 10% bio composite film were determine by using the stable radical (DPPH). This method was based on color decay when the odd electron of the nitrogen atom in the DPPH radical was reduced by receiving one hydrogen atom from antioxidant compounds. 100 mg/mL or 10% (w/w) bio composite film sample were dissolved in 1ml (v/v) of hot de-ionized water at 45°C. 0.8 mg (w/w) DPPH radical mixed with 5ml (v/v) of methanol solution (200 µl 0.6 mM) were left to stand for 15 min in dark, after that Sample were loaded as 10 µl of film+50 µl DPPH+140 µl methanol in 96-micro titer well plate then identify the absorbance measurement at 525 nm against citric acid blank or positive control in UV-vis spectrophotometer. All determinations were performed in duplicates and the percentage scavenging effects were calculated as % Inhibition=[A1Blank – A2Sample / A Blank] × 100%Where:-

A1=Absorbance of the control without sample

A2=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.

**Antimicrobial activity:** Antimicrobial activity were done by nutrient agar direct platingassay stock culture of *E. coli* O157:H7 (MTCC 90), Salmonella typhiiMTCC733), supplied (Microbial type culturecollection and gene bank, institute of microbial technology, Chandigarh India) were kept frozen at –25°C in nutrient agar the culture was then refrigerated by transferring a loopful of each bacterium into 10mL of nutrient broth incubated at 37°C overnight. A 10 mL of aliquot from each overnight culture was again transferred into 10 mL of nutrient broth and grown at 37°C to the end of the exponential phase of growth. These appropriately diluted cultures were then used for the inoculation of the agar plates in order to obtain target inoculumnutrient agar was used as a model solidified food system aliquot of nutrient agar (20 mL) were poured into petri-dishes. After the culture medium were solidified, properly diluted overnight cultures from each strain were inoculated on thesurface then films were cut in 1cm diameter incorporated into petri-plates film were placed in inoculated surface plates were then covered with thin polythene to avoid dehydration andstored for 1 to 2 days.

## Results and Discussion

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

### *Antimicrobial activity*

Anti-microbial properties of Pure pectin, pectin+200 µl cinnamaldehyde, PP+PSA, biocomposite+700 µl cinnamaldehyde film were tested by nutrient agar plating assay against food borne pathogens: Salmonella typhii, Xanthomonascomprestris, Xanthomonasoryzae, *E.coli*, Streptococcus pyogenes, Bacillus Cereus. The antimicrobial activity of oregano essential oil and carvacrol in pectin-alginate edible films and film forming solutions against *E.coli* O157: H7 was significantly greater than the activity of cinnamaldehyde. Pectin+200 µl cinnamaldehyde and pectin+ sodium alginate composite film showed the highest ($p<0.05$) antimicrobial properties indicated by greater inhibition zone.

Antimicrobial effects compared to other films sodium alginate film were found highly positive inhibitor against Salmonella typhii, Xanthomonas oryzae, *E. coli* Xanthomonas. 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. A clear zone surrounding the films were explained as antimicrobial diffusion from the film and this diffusion of the film causes an inhibition of growth of target microorganism [15].

Films have the ability to retard or inhibit of microbial growth to stop their multiplication or to delay of microbial kinetics mechanisms and their nutrient uptakes and the improved antimicrobial activities of smaller droplets found in the films to a greater ability of the active compound to migrate from films and penetrate microbial cells. 12 µl to 45 µl 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.

### *Antioxidant activity*

The free radical scavenging activity of different formulations of biopolymer and phytochemicals supplemented bio composites were analyzed using citric acid as positive control by DPPH method. The free radical scavenging activity of pectin+sodium alginate and biocomposite+0.30%. Phytochemical were dependent on linear correlation analysis (R2) which was 0.9235 for DPPH scavenging.

The lowest IC50values were 35 µg/ml and 25 µg/ml for pectin +sodium alginate and biocomposite+0.30% Phytochemical, respectively at 40 µM of DPPH concentration. The free radical scavenging activity was known to increase with presence of hydroxyl ions and existence of double bonds reported as Isorhamnetin-a compound with hydroxyl groups had been reported to exhibit a high DPPH scavenging activity in the order of isorhamnetin>ascorbic acid>BHT. However, 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. Therefore the DPPH free radical scavenging activity showed by synthesized biopolymers was significant for IC50 value and suggests that could be used in food industry.

### *Thickness measurements*

Thickness of pectin and sodium alginate films changed significantly ($p<0.001$) from 268 to 174.20 µm, depending on composition of the film forming mixture. Phytochemical incorporated films thickness were 166.2 to 481.4 µm. Those values are lower than showed in the other research work for pectin+sodium alginate based bio polymeric films based on polysaccharides. 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. It is associated with the compound's unique colloidal properties including thickness and suspending, and interaction between components. Similar results were presented for composite films based on sodium alginate with lactose or with whey protein isolate and gelatin.

### *Mechanical analysis*

Tensile strength, thickness, stiffness, young modulus, tensile strength, extension at maximum and percentage of break investigated at room temperature data of interpretation. The tensile properties decrease as the concentration of anti-microbial substance increases in blend films. Tensile strength accounts for film's mechanical resistance due to cohesion forces between chains, while elongation at break measures its plasticity, which is capacity of a film to extend before breaking. Due to the structural nature of those attributes, usually films with high tensile strength show low elongation at break so that both properties should be analyzed simultaneously. 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 µ Pa highest value for pure pectin and the lowest value for biocomposite+0.7% phytochemical (cinnamaldehyde). SD ± 0.001>significance, values were given as mean ± standard deviation. Extension at maximum, % strain at break of PP, PSA, PP+PSA, pectin 200 µl+phytochemical, and bio composite 700 µl added.

Values with the same superscript letters within a column are not significantly different ($p<0.001$) Values of tensile strength ranged significantly ($p<0.001$) from 0.4912 to 3.9187 MPa with the lowest value for pectin based bio composite blend film .7%+phytochemical, and the highest for pure pectin film and for extension at maximum. Elongation at break ranged from 5.9% to 14.9% with the highest values for mixed films values of pure pectin film are different compared to those obtained by who demonstrated tensile strength of 193 MPa and elongation at break of 2.6% Similarly, values for pure alginate film were also different from those presented.

### *Structural properties*

**(ATR-FTIR) spectrum:** ATR-FTIR spectroscopy is based on the identification of absorption bands concerned with the vibrations of functional groups at a particular wavelength present. In the pure pectin film, compound spectra for pure pectin film showed strong alkyne C-H stretch at 3305.72 nm, a strong alkanes C-H stretch at 2950 nm, a strong alkyls -C-H stretch at1463.11 nm, a characteristic band due to the high methyl chain present in the pectin structure, and a secondary alcoholic m-s, C-O stretchat 1111.65 nm and an ester aliphatic -O=C-O-C stretch at 1195.18 nm. A characteristic band was observed present of intra-molecular bond. In the pectin film+200 µl cinnamaldehyde, an amides w-m stretch at 3252.54 nm and a aromatic compounds m-s ring C=C stretch at 1467.20 nm two or four sharp bends, due to presence of phenol ring into the pectin matrix, an amine v-s N-H stretch at 1580.40 nm, a alkyls, C-H stretch were found at 1450.69 nm, and a small alkenes C-Hm+s stretch at 921.61 nm. In the pure sodium alginate film amides w-m N-H symmetric stretch at 3252.54 nm were observed, an esters s- C=O stretch at 1735.29 nm was identifiedanother stretch was found at 1590.20 nm, and an alcoholic stretch was found at 1094.37 nm. In the bio composite film 700 µl +cinnamaldehyde film a week characteristics amines N- H band was found at 3400.20 nm, an alkanes strong C-H stretch was found at 2930.10 nm, another sharp, very strong anhydrides C=O symmetric band were observed at 1810.50 nm then an m-s secondary alcoholic stretch found at 1200.55 nm, after that an m-s ethers =C-O-C symmetric stretch was found at 1020.80 nm. All the peaks were used to analyze the chain interactions in the blend films combined spectra.

It was investigated that blends film showed antimicrobial and antioxidant properties more in comparison to pectin film. Due to inter or intramolecular H-bonds breaking, the percentage of transmission was always higher. 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.

**TGA:** TGA is considered the most important method for studying the thermal stability of polymers. The weight loss (TG) and (DTG) curves for the TGA tests of pure pectin film, pure sodium alginate film, pectin+sodium alginate film, pectin+sodium alginate+composite film+700 µl were reported. One significantweight loss stage was observed in the TG curve of pure pectin film, and pure sodium alginate films. Respectively weight loss in the films was probably due to the losses of adsorbed bound water reported by several authors.

The thermal behavior of biocomposite blend pectin+sodium alginate and pectin+sodium algiante 700 µl +cinnamaldehyde blend characterized in all the stages. The first major weight loss of pectin+sodium alginate blend film at 100-220 (delta Y=2.397 mg) was due to the moisture evaporation of both polymers. The second minor weight loss step at 291-36°C (delta Y=0.456 mg) was due to the thermal degradation of pectin and sodium alginate blend films. The blend of biocomposite pectin+sodium ccurs e/700 µl incorporated cinnamaldehyde film were tested first minor weight loss ccurs at 35-53°C (11.589%) due to the breaking of $H_2O$ bond, moisture loss occurs. The second major weight loss occurs was at 143-250°C (39.975%) due to the thermal degradation of polymeric molecules, and the third minor weight loss occurs at 333-410°C was due to the degradation of byproducts generated by pectin and sodium alginate during the thermal degradation process. According to Holland and Hey, thermal degradation resulted in the formation of aldehyde and alkenes groups in the molten state. In the range between 250- 350°C, pure pectin film showed more thermal stability than sodium alginate film (lower weight loss). As pectin ratio increased in the blend the residual mass increased from 20% to 40%. The temperature at the maximum decomposition rate (Tmax) was determined, and it was found that pectin addition resulted in a Tmax increase of the blends, which indicates that the bio composite film showed higher thermal stability due to the pectin addition. The thermal behavior of pectin and glass transition temperature (Tg) decreased with an increase in moisture content of pectin. The source of extraction methods also strongly influences glass transition.

**DSC thermograph:** Glass transition temperature (Tg) of pure pectin samples were at 150.34°C , pure sodium alginate at 165.75°C, pectin sodium alginate bio composite at 124.17°C and bio composite incorporated photochemical at 173.67°C. These samples were analyzed using a Differential Scanning Calorimeter (DSC- department of studies in physics, university of Mysore) as identified. The analysis of glass transition reports the onset, the mid-point, and the end temperatures of the step once the start and stop points of the transition are provided, and the midpoint of the step is considered as Tg data were recorded both during the cooling and second heating steps. The glass transition temperature (Tg).

**X-rays diffraction:** X-ray diffraction patterns of pectin, sodium alginate and cinnamaldehyde films. Pectin and sodium alginate showed amorphous characters, and more noise alsooccurs in the crystal lettuce of polymers peaks and intense x- rays beam at different angles in the polymer surfaces (Tables 1 and 2).

***Table 1.*** *Compositon of the different componentsin Film Forming Disperions (FFD).*

| Sample | Phytochemical (Cinnamm aldehyde %) | Acetic acid(v/v) | d-Sorbitol and glycerol (w/w) | Tween 40(v/v) |
|---|---|---|---|---|
| PP | - | - | 0.9 gm | 1.2 ml |
| PSA | - | 30 ml .15 M | 0.9 gm | 1.2 ml |
| PP+SA | - | 30 ml .15 M | 0.9 gm | 1.2 ml |
| Bio composite | 7% | 30 ml .15 M | 0.9 gm | 1.2 ml |
| Pectin | 2% | 30 ml .15 M | 0.9 gm | 1.2 ml |
| Pure pectin film, b) Pure sodium alginate film, c) Pectin+sodium alginate composite film d) Bio composite, pectine+sodium alginate+700ul cinnamaldehyde added film e) pectin+200ul cinnamaldehyde added film | | | | |

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

| Types of Biopolymers | IC50 values (ug/ml) |
|---|---|
| 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+0.3% Phytochemical showed significance effect of free radical scavenging properties in blends. | |

## Conclusion

Differential screening colorimeter data analysis was showedthe peak height, delta H, peak temperature, endothermic and exothermic graph analysis. The glass transition temperature (Tg) was taken as the inflection point of the specific heat increment at the glass–rubber transition, while the melting temperature (Tm) and the crystallization temperature (Tc) were taken as the peak temperature of the endothermic andexothermic during the cooling and the heating.

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