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# Development of soybean bio-functional active packaging nanocomposite films using cellulose Nano Fibers and ZnO nano particles

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**Abstract**
In the current study, soybean water extract (SWE)-based nanocomposite film was developed by incorporating cellulose nanofiber (CNF) at various concentrations (0–3%) and ZnO nano particles. Effect of nano reinforcement on essential properties of the nanocomposite film such as barrier, mechanical, water affinity, and optical properties were evaluated. Homogeneous films with improved barrier and mechanical properties were observed until 3% CNF, beyond which considerable reduction in desirable properties was noticed due to nanoparticle's agglomeration effect. The physical, antioxidant and antibacterial properties of the developed bio-functional soy based active nano composite films were also investigated. It was observed that films prepared with incorporation in ZnO NPs were showing higher antioxidative (and antimicrobial properties as compared to the CNF incorporated films. Thick of CNF incorporated films was higher 71 to 72 µm as compared to ZnO NPs which as 62 to 64 µm. Mechanical properties of both the nano materials incorporated films were increased as compared to control.

**Keywords:** Bio-functional, active, nanocomposite, cellulose nano fibers, ZnO nano particles

## Introduction

A current trend in food packaging development is that, where ever possible, packaging films should not only be natural and environmentally friendly but also functional and cost-effective. Single-use plastics pose threat not only to the environment but also to terrestrial and aquatic life (Thompson *et al*., 2009) [11]. This has necessitated the development of active edible films made from natural biodegradable materials. Edible films are generally defined as continuous structures that can be prepared from edible materials, such as proteins, polysaccharides, and lipids with incorporation of nano materials. They can be used as coatings on food or between food components. They provide barrier and protection while improving the quality and safety of food products (Thompson *et al*., 2019) [11].

The primary role of active films is controlling the moisture loss and reducing the adverse chemical reaction rates to enhance the quality and safety of a wide range of processed as well as fresh foods (Debeaufort *et al*., 1998) [3]. In addition, the incorporation of various food additives such as antimicrobials, antioxidants, flavors, and colors into the edible film matrix further extends their applications (Tavassoli-Kafrani *et al*., 2016) [10]. However, the permeability and mechanical properties of the edible film are not on par with conventionally used synthetic plastic films (Murrieta-Martínez *et al*., 2018). Hence, the present research contributions were geared towards these property enhancements.

This study focuses on enhancing the mechanical and barrier properties of a soy water extract based active nano composite films made from a multicomponent system consisting of cellulose nano fibres and ZnO nanoparticles. Dispersion of nano materials into the soy water extract matrix formed a continuous and cohesive network stabilized by hydrogen bonds and intermolecular electrostatic interactions. Then, we developed active antimicrobial films by adding ZnO NPs to the CNF film and evaluated the anti-oxidative and anti-microbial properties of the films. Nanocomposite films made from protein rich source and polysaccharides have been studied the least mainly because mixtures of proteins and polysaccharides are unstable due to the large molecular size of alginate molecules and weak intermolecular interactions between protein and alginate. Phase separation is the typical phenomenon observed in protein-polysaccharide mixtures because of thermodynamic incompatibility between two macromolecules (Doublier *et al*., 2000) [4].

Such problems can be overcome by modifying structures of film-forming materials like polysaccharides (eg. forming nano-sized particles) for better compatibility.

## Materials and Methods

### Materials

Cellulose nanofibers and ZnO NP powder collected from Chemical & Biochemical Processing Division (CBPD), ICAR- Central Institute for Research on Cotton Technology (India). Tween 80 were bought from Sigma Aldrich, Germany. Glycerol and bee wax was obtained from a local manufacturer. Soy water extracts prepared and collected from Centre of Excellence on Soybean Processing & Utilization, ICAR-Central Institute of Agricultural Engineering (India). All the media, chemicals and reagents were purchased form himedia. Pathogenic culture were collected form standard sources.

### Preparation of nanocomposite films

**Soybean biofunctional active packaging film using cellulose nano fibers:** The soy water extract was cooked for 30 mins than 1.5ml glycerol was added. In this solution tween 80 and beewax was added. Prepared solution was mixed at mechanical stirrer at 85 °C. ZnO nano particles powder was added @ 0.1% and solution was kept in sonicator for degassing. It was cooled at room temperature. The mixtures were cast onto flat leveled, non-stick plates to set. Once set, the plates were held overnight at 55°C for 10 hr unbroken into the dryer and then cooled to an ambient temperature before peeling the films off the plates. The film samples were deposited in plastic bags and held in desiccators and made in triplicate at 60% RH for further testing.

The soy water extract was cooked for 30 mins than 1.5ml glycerol was added. In this solution tween 80 and beewax was added. Prepared solution was mixed at mechanical stirrer at 85 °C. cellulose nano fibers were added @ of different concentrations and solution was kept in sonicator for degassing. It was cooled at room temperature. The mixtures were cast onto flat leveled, non-stick plates to set. Once set, the plates were held overnight at 55°C for 10 hr unbroken into the dryer and then cooled to an ambient temperature before peeling the films off the plates. The film samples were deposited in plastic bags and held in desiccators and made in triplicate at 60% RH for further testing.

### Film Thickness

The thickness of the films was measured with an Outside Micrometer 3203-25A, Insize, India at 5 random positions of the film with 0.01 mm accuracy. Water Vapor Permeability and mechanical properties of obtained films were calculated based on film thickness.

### Optical properties

The L, a,b values of obtained films were measured using the Hunter lab colorimeter. The light transparency of the films was measured in a UV-Vis Spectrophotometer (Shimadzu, 1800, Japan). In this test, rectangular strips of film specimens (3 X 3 cm) were placed in the test cell of the spectrophotometer, and transmittance was recorded at wavelengths 200nm-800nm. All tests were conducted in triplicates.

### Mechanical Properties

The tensile strength and elongation at break of the soybean bifunctional active packaging films were tested according to the method mentioned in STAS ASTM D882-02(Standard Test Method for Tensile Properties of Thin Plastic Sheeting) ASTM D882-02, Standard Test Method. Tests were performed at an ambient temperature of 25:C. In this method, three replicates of strips of dimensions 100 mm x 10 mm were cut for testing. The rate of grip separation was 10mm/minute. The parameters determined were maximum load at break (MPa) and extension of length at break (%)The tensile strength (TS) and elongation at break (E%) of each film were calculated according to equations 2 &3.

TS(MPa) =Maximum force at break Film surface area
E% = Extension of length at break * 100 Initial length

### Water Vapor Permeability (WVP) and Water Vapor Transmission Rate (WVTR)

The WVP of prepared films was measured according to the gravimetric method at 25:±1:C following the ASTM method E96 (2000). This method was described in detail by Rachtanapun *et al*., 2011 [9]. In this method, a sample of the edible film was firmly fixed on top of a container filled with silica gel. The sealed containers were then placed in desiccators previously saturated with sodium chloride at a temperature of 25:C. The films were weighed daily for 10 days and the WVP of films was calculated according to the formula.

WVP = W x T
t*a*P *(R1 –R2)

Where 'WVP' is Water Vapor Permeability (gH2O mm.), 'W/t' is the constant rate of weight change, 'T' is the average thickness of the film (mm), 'a' is the permeation area(cm2), P is the partial pressure of water vapor at 25: C(3.159 kPa) and(R1-R2) is the relative humidity difference between two sides of the film. The WVTR of the obtained films was determined from the slope of the straight line (g/day) divided by permeation area(m2). All measurements were done in triplicates.

### Film solubility in water

Film solubility was determined using a method from (Jutaporn *et al*., 2011) [5]. In this method, film portions were cut measuring 1×3 cm2 were cut were dried at 110˚C in a vacuum oven for 24 hrs. Portions were weighed to the nearest 0.0001 g for the initial dry weight. Films were then immersed in a 100 ml round bottom flask containing 50 ml of distilled water and shaken gently in a rotary shaker at 25 ˚C for 24 h. The solution obtained was then filtered through Whatman No. 1 filter paper to recover the remaining undissolved film. The remaining pieces of film portions after dissolution were dried at 110 $^0$C to obtain a constant weight (Final dry weight) [36]. Tests for each type of film were carried out in three replicates. Solubility in water (%) was calculated by using Equation:

Solubility %= Initial dry weight –Final dry weight * 100
Initial dry weight

## Biofunctional properties

### Antioxidant property

The radical scavenging activity of obtained films was determined using DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay [35]. The stable free radical DPPH exhibits a deep violet color in solutions and shows strong absorption at 517 nm. The deep violet color disappears when an electron is paired off by an antioxidant. The decrease in absorption is a measure of the antioxidant activity. In this method, 3 ml of edible film solution was mixed with 1mL of 1 mM of DPPH dissolved in methanol. The mixture was mixed vigorously in a vortex shaker and left in the dark at ambient temperature for 30 minutes. The absorbance was then measured at 517 nm. The sample solution acts as a hydrogen atom donor and on reaction with the DPPH solution forms a stable non-radical form of DPPH with simultaneous change of the solution from violet color to pale yellow Control samples were prepared using the same method without ZnO nano-particles. The percentage of DPPH free radical quenching activity was determined using the following equation 6: DPPH scavenging effect, % =(AbsDPPH-AbsExtract) *100 AbsDPPH where AbsDPPH is the absorbance value at 517 nm of the methanolic solution of DPPH and AbsExtract is the absorbance value at 517 nm for the sample extracts. Each sample was assayed at least five times.

### Evaluation of Antimicrobial activity

Edible films must be microbiologically safe for human consumption. For this purpose, the antimicrobial activity of all the film-forming solutions was tested against enterobacteria, Escherichia coli, Staphylococcus aureus etc. on the agar well diffusion method reported by Ngo *et al*., 2018 [7].

### Statistical Evaluation

All the experiments were conducted in triplicates and the results were expressed as mean value ± standard deviation. All the experiments were conducted in triplicates and the results were expressed as mean value ± standard deviation. Comparisons among multiple groups were determined using a one-way analysis of variance. (ANOVA).

## Results and Discussion

### Colour and optical characteristics of films

The color and transparency of films are important factors for consumers acceptance. The results of the color evaluation of the obtained films are shown in Table 1. The a* value shows the color of the films difference in the films were not significant. An increase in b* indicated films becoming more yellow probably due to flavonoids present in soy water extract. The L* parameter was used to describe the brightness of the films ranging from 0 to 100. The L* values show that the concentration ZnO nano particles had a negligible effect on the brightness of films as values were not significantly different.

### Visual characteristics

Films were transparent, shiny in appearance, with no visible pores or crevices. All obtained films had regular edges with no particle deposition observed on surfaces. UV-Visible light barrier performance Photochemical degradation of food products can lead to oxidation, nutrient losses, and the development of off-flavors Verduin *et al*., 2020 [12]. UV-visible light barrier performance is thus an important parameter of edible films to pack food products that are sensitive to light.

**Table 1:** Colour and optical characteristics of soybean bio-functional active packaging nanocomposite films

| Treatments | L | a | B |
|---|---|---|---|
| CONTROL | 82.53 | -4.19 | 35.19 |
| EF_CNF (A) | 79.99 | -4.89 | 34.79 |
| EF_CNF (B) | 81.41 | -5.90 | 34.61 |
| EF_CNF (C) | 78.51 | -3.44 | 27.91 |
| EF_NP (A) | 81.52 | -4.58 | 34.65 |
| EF_NP (B) | 80.62 | -4.92 | 35.50 |
| EF_NP (C) | 79.86 | -5.23 | 34.56 |
| EF_NP (D) | 80.88 | -5.83 | 33.45 |

### Film solubility in water

Table 2 reveals the findings pertaining to the solubility of the films within water. In the composite samples, no obvious changes in were observed with the addition of up to 5% CNFs ($p > .05$), meaning that the gelatin film was left largely unaffected in terms of water resistance. However, increasing the concentration of CNFs up to 7.5% led to decreased WS from 59.83 ± 1.11% to 57.45 ± 0.44% in comparison with the neat gelatin film ($p < .05$). These results con-firm those of previous studies on alginate and other nano-composite films (Abdollahi, Alboofetileh *et al*., 2013; Alizadeh-Sani *et al*., 2018) [1].

### Thickness and Mechanical properties

The thickness of a film is influenced by the incorporation of fillers into its matrix. Table 2 reveals that the thickness of the cellulose nanofiber films were observed between 71.2 µm to 72.7 µm where as not much difference was observed in case of ZnO nano particle films as compared to control. The thickness of a film is influenced by the incorporation of fillers into its matrix.

The obtained films were soft, smooth, shiny, and flexible without visible pores or cracks in the structure. The films had no odor but a sweet taste. The films showed low adhesion to the silicone surface with regular edges. The thickness and mechanical properties of the obtained films are shown in Table 2.

**Table 2:** Thickness and mechanical properties of the soy based CNF and ZnO nano particle films

| Treatments | WVP (x $10^{-10}$ g.m/m2.Pa.S) | TS(MPa) | EAB (%) | Moisture content (%) | Solubility (%) | Density (g/cm3) | Thickness, µm |
|---|---|---|---|---|---|---|---|
| CONTROL | 6.12 | 2.28 | 121.5 | 33.90 | 24.65 | 1.28 | 61.2 |
| EF_CNF (A) | 4.64 | 3.41 | 103.5 | 30.46 | 23.85 | 1.30 | 71.2 |
| EF_CNF (B) | 3.51 | 3.98 | 90.52 | 28.12 | 23.25 | 1.31 | 72.7 |
| EF_CNF (C) | 2.72 | 4.52 | 85.03 | 27.23 | 23.56 | 1.32 | 71.8 |
| EF_NP (A) | 5.42 | 3.25 | 113.52 | 29.45 | 24.14 | 1.28 | 63.2 |
| EF_NP (B) | 3.75 | 4.40 | 106.75 | 29.05 | 23.69 | 1.29 | 64.3 |
| EF_NP (C) | 2.52 | 4.60 | 102.84 | 28.45 | 23.44 | 1.30 | 65.5 |
| EF_NP (D) | 1.52 | 5.82 | 94.36 | 28.12 | 22.82 | 1.32 | 63.8 |

## Bifunctional properties
### Antioxidant property
The antioxidant activity is an important parameter of edible films to prevent oxidative degradation, such as lipid peroxidation in foods Krinsky *et al*., 1992 An antioxidant can reduce the stable radical DPPH to yellow colored diphenylpicrylhydrazine(DPPH). Mainly flavonoids present in licorice roots is responsible for the radical scavenging activity in glycyrrhizin. Figure 1 reveals the radical scavenging percentage of each sample. Tocopherol and trolox were used as standards. Sample EF_NP (D) reported the maximum scavenging ability at 48.5 ± 0.9%, while sample SP3 and SP4 had comparable radical scavenging ability as shown in the table 1. No color change was reported in Control film, while. This shows obtained films can prevent oxidation of its contents making them an ideal packaging material for extending shelf life of foods and beverages.

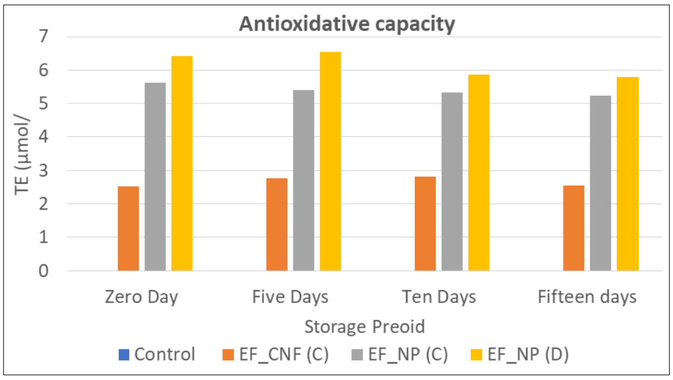


**Fig 1:** Anti-oxidative properties of soybean bio-functional active packaging nanocomposite films

### Antimicrobial activity of film forming solution
The antimicrobial action of the soybean bio-functional active packaging nano composite film forming solution were tested against selected pathogens like *E. coli* family and *S. aureus*, *B. cereus* and *S. typhi*. The zone of inhibition of the film-forming solutions against the growth of selected micro-organisms are shown in Figure 2 (3). The diameter of zone of inhibition indicates the antimicrobial activity of film-forming solutions. It was observed that film SP1 did not show any inhibitory effect against the four tested micro-organisms, hence it has not been showed in the graph. The observed values show

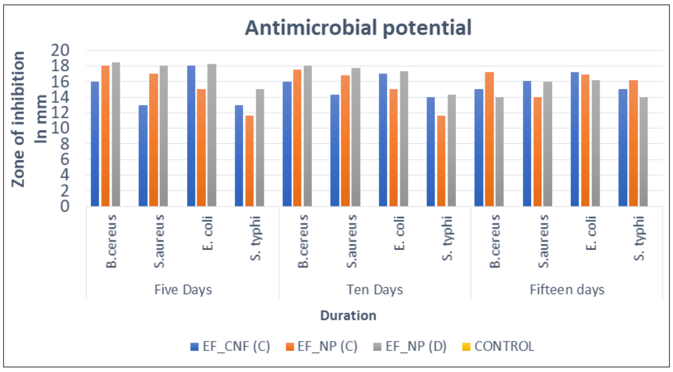


**Fig 2:** Anti-microbial properties of soybean bio-functional active packaging nanocomposite films

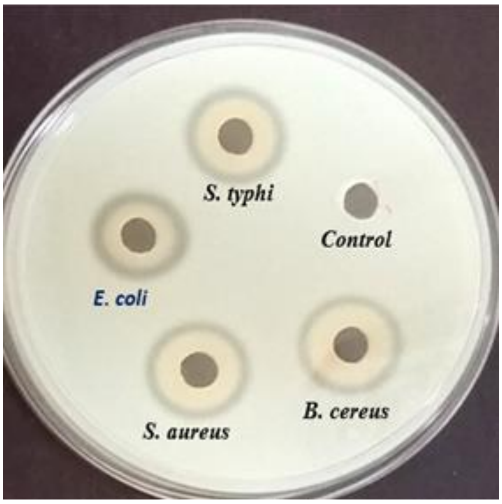


**Fig 3:** Zone of clearance in EF_NP (D) sample

## Conclusions
Soybean bio-functional active packaging nanocomposite films made from soy water extract + cellulose nanofiber and soy water extract and ZnO nano particles have replaced conventional plastic based packaging. In this work, films composed of soy water extract enriched with nano materials were obtained. The incorporation of nano materials in film forming solution led to an improvement of moisture barrier properties, hydration properties and mechanical properties. The edible material acts as a substitute to films made from sugar which can be beneficial to patients suffering from diabetes. Additionally, the films exhibited radical scavenging activity which can be used to prevent lipid oxidation of its contents thereby extending shelf life of foods. Besides, its physiological benefits, the obtained films have been observed to modify flavor and texture of its contents which is an important parameter for designing functional foods. The

material obtained from mixing soy water extract and nano materials had optimal characteristics for use as a packaging material: low solubility, homogeneity, regular cuts and margins, low roughness, good tensile strength, good barrier properties and elasticity. The material thus obtained can easily be reproduced on an industrial scale as the process involves use of cheap, natural, biodegradable materials and without use of rigorous automation.

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