



# Development of new active packaging film made from a soluble soybean polysaccharide incorporating ZnO nanoparticles

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## ABSTRACT

This study aimed to develop a soluble soybean polysaccharide (SSPS) nanocomposite incorporating ZnO nanoparticles. The nanocomposites were prepared using the solvent-casting method. SEM, AFM, DSC and X-ray diffraction methods were applied to characterize the resulting films. Furthermore, the antibacterial and anti-mold activities of SSPS/ZnO films were assessed against the selected microorganisms. The results indicated that incorporating ZnO into the SSPS film affected the tensile strength and elongation at break significantly. In addition, the antibacterial, antifungal and yeasticidal activities of ZnO/SSPS films have been approved. XRD results showed a crystal plane of hexagonal ZnO, while SEM showed that there was not a good affinity between ZnO and SSPS. Mono-dispersed particles with clearly spherical morphology and with no voids on the surface were observed using AFM. Fluctuation in  $T_g$  and  $T_m$  resulted from incorporating ZnO. In summary, the potential of ZnO as a functional filler in SSPS film has been demonstrated.

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## 1. Introduction

The use of plastics in food packaging is growing at an accelerated rate. The reason for this increase is the lower cost of packaging materials. This is due to the technological innovations and the properties of plastic films, which make them very suitable for food-packaging applications. However, excessive consumption of synthetic plastic materials, as well as their poor degradation, will cause a massive accumulation of plastic waste disposed in the environment (Cha & Chinnann, 2004). Consumer demand for healthy and additive-free foods, as well as environmental concerns during the past decades, has led to the development of new packaging materials. The incorporation of antimicrobials in packaging material is one of the most promising avenues for developing active packaging, as this can improve the safety of food by inhibiting pathogenic

bacteria or controlling spoilage flora (Xia, Ghasemlou, Rubino, Auras, & Baghdachi, 2015). A number of naturally derived polymers such as polysaccharides, proteins and lipids have been widely explored to develop active packaging films. Among them, polysaccharide-based packaging films are particularly attractive due to their better film-forming property and moderate oxygen and moisture permeability.

According to a recent report (Centers for Disease Control and Prevention), food-borne diseases account for 76 million cases annually in the United States alone, with 325,000 hospitalizations and 5000 deaths. As a rough estimate, one out of four persons may experience a food-borne disease each year. The resulting medical costs and productivity losses are in the range of US\$ 6.6 billion–37.1 billion (Johnson, Hayes, Brown, Hoo, & Ethier, 2014). Additionally, because of several food-related incidents and reported outbreaks worldwide, consumer confidence is no longer absolute. Because the application of this technology can improve food safety by inhibiting pathogenic bacteria, it offers a promising alternative to conventional packaging, while simultaneously reducing the environmental impact of both food waste and packaging waste (Salarbashi et al., 2014a,b).

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Poor mechanical and water–vapor barrier properties have been the main challenges in using soluble soybean polysaccharide (SSPS) (Tajik et al., 2013); however, it offers suitable visual properties and low price. By adding plasticizers in different ratios, it is possible to diminish the drawbacks of SSPS. In this study, different essential oils were investigated as antimicrobial agents and additives to improve physical properties. Although some studies have shown that adding essential oils can improve some of the drawbacks, there are also some concerns about the stability of natural antimicrobial agents (Salarbashi et al., 2013, 2014a,b). The results of these studies were encouraging enough for us to investigate alternative strategies to develop SSPS materials that could be considered suitable for food-packaging applications. We concluded that such materials still lacked some of the desired physical and mechanical properties, despite the incorporation of essential oil. Therefore, we needed to further improve their physical and mechanical properties. Currently, one of the most effective alternatives for improving the barrier and mechanical properties of packaging materials is the formation of nanocomposites (Shahabi-Ghahfarrokhi, Khodaiyan, Mousavi, & Yousefi, 2015b).

Nanotechnology has opened up new directions to explore novel materials in a wide range of industrial sections, and has brought significant changes to both producers and consumers (Roco, Mirkin, & Hersam, 2011). This multidisciplinary approach to manipulating the nanostructure of films has begun to receive much attention, particularly focused on the improvement of material performance such as mechanical and barrier properties. In addition, the unlikelihood that nanoparticles will be released from the polymers has been shown to minimize the amount of harmful nanocomponents in the food, thereby confirming the safety of nanocomposites for food-contact purposes (Xia, Rubino, & Auras, 2014). Compared to the instability of organic antibacterial materials, the experimental use of nano metal or metal oxides as reinforcing filler in polymers has become much more common over the past decade due to their high thermal stability and functions such as strong antimicrobial activity. Nanoparticles of one such metal oxide, zinc oxide (ZnO), have emerged as a new class of important materials that are increasingly being developed for use in research and health-related applications. This is due to their low cost, accessibility and white appearance (Shahabi-Ghahfarrokhi, Khodaiyan, Mousavi, & Yousefi, 2015c). Nanocomposites incorporating ZnO (ZNs) are generally recognized as safe substance approved by the US Food and Drug Administration and could be considered as a nano filler for various polymers, as they offer properties such as antibacterial effect or intensive ultraviolet absorption (Shankar, Teng, Li, & Rhim, 2015; Xie, He, Irwin, Jin, & Shi, 2011). Although over the last few years several reports have addressed the antimicrobial activity of Zn, quantitative data to aid an understanding of the antimicrobial activity of ZnO in polymer systems is still lacking.

The present study aims to explore the potential use of Zn in SSPS-based films as nano filler with a dual function; i.e., simultaneously modifying the films' functional and antimicrobial properties. The study analyzes the morphology and the thermal, mechanical, and physical properties of the produced films, and finds the optimal concentration of Zn to maximize the films' effectiveness against selected microorganisms. Ultimately, the results of this study will contribute to the development of a packaging system that supports an extended shelf life for food applications.

## 2. Materials and methods

### 2.1. Materials

SSPS containing 5% protein, 5.4% moisture, 8.6% ash and 81% carbohydrate was purchased from Fuji Oil Co. (Osaka,

Japan). Zn (100 nm; based on the manufacturer) was purchased from US Research Nanomaterials Inc. (Houston, USA). Glycerol, Mueller–Hinton Agar and Mueller–Hinton Broth (MHB) were bought from Merck Co. (Darmstadt, Germany). Tryptone Soy Agar (TSA), Tryptone Soy Broth (TSB) and Sabouraud Dextrose Agar (SDA) were obtained from Himedia (Mumbai, India). Gentamicin, Amikacin, Nysatin and Clotrimazole were obtained from Sigma-Aldrich. All other chemicals used were of analytical grade.

### 2.2. Microorganisms

*Escherichia coli* O157:H7, *Bacillus cereus* PTCC1247, *Staphylococcus aureus* PTCC 1112, *Candida albicans* ATCC 10231 and *Penicillium expansum* ATCC 7861 were provided by the Persian type-culture collection (Tehran, Iran) and the American type-culture collection (Rockville, MD, USA). The bacteria were stored in Microbank™ vials at  $-80^{\circ}\text{C}$  in MHB supplemented with 20% (v/v) sterile glycerol. The stock cultures of the studied bacteria were grown in MHB, and were incubated ( $37^{\circ}\text{C}$ ; 24 h) before each experiment. The stock culture of the fungi was propagated ( $25^{\circ}\text{C}$ ; 48 h) on SDA to ensure viability and purity.

### 2.3. Preparation of films

To prepare the suspension, different ratios of Zn (5%, 10% and 15% w/v) were dissolved in hot water and stirred ( $80^{\circ}\text{C}$ ; 2 h) completely using a magnetic stirrer. A sodium caseinate solution (20% w/v) was mixed into the suspension. SSPS/Zn nanocomposite films were prepared by gradually adding and stirring (1 h) 2.4 g of SSPS powder into the Zn suspensions until completely dissolved. The glycerol was added as a plasticizer (50% w/w of the polysaccharide) and mixed ( $80^{\circ}\text{C}$ ; another 20 min). The concentration of plasticizer was determined according to our previous research (Salarbashi et al., 2013). The solution was then sonicated in an ultrasonic bath (30 min;  $80^{\circ}\text{C}$ ). The control film was prepared using the described procedure without Zn. The films were cast by pouring the mixture onto polystyrene Petri dishes (14 cm in diameter) placed on a leveled surface and allowing them to dry (48 h; at room temperature and relative humidity). The dried films were peeled from the casting surface and conditioned inside desiccators. To obtain a relative humidity of 55%, desiccators contained saturated magnesium nitrate solution at  $25 \pm 1^{\circ}\text{C}$ .

### 2.4. Film-thickness measurement

The thickness of the film specimens was determined using a hand-held digital micrometer (Mitutoyo No. 293-766, Tokyo, Japan). Thickness was measured precisely ( $\pm 0.001$  mm) at 10 random points, and the average was used to calculate the permeability and mechanical properties.

### 2.5. Film solubility in water

Solubility in water (SW) was defined as the ratio of the water-soluble dry matter of film dissolved in distilled water (Shahabi-Ghahfarrokhi, Khodaiyan, Mousavi, & Yousefi, 2015a). A  $20 \times 20 \text{ mm}^2$  specimen, dried at  $105 \pm 1^{\circ}\text{C}$  to constant weight in a laboratory oven (Shimaz Co., Iran), was cut from each film and weighed to determine the initial dry weight ( $m_1$ ). The SW of the specimen was measured by immersion in 50 mL distilled water with periodic stirring ( $25^{\circ}\text{C}$ ; 6 h). Then, the remaining pieces of films were taken out and dried at  $105 \pm 1^{\circ}\text{C}$  until constant weight

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