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Preparation and Characterization of Novel Bionanocomposite Based on Tapioca Starch/Gelatin/Nanorod-rich ZnO: Towards Finding Antimicrobial Coating for Nuts

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Abstract

The effects of zinc oxide nanorod on the barrier, sorption isotherm and antibacterial properties of tapioca starch/bovine gelatin Bionanocomposite films were investigated. The nanorod-rich ZnO (ZnO-N) was homogenized by sonication and incorporated into tapioca starch/bovine gelatin solutions at different concentrations (e.g. 0.5, 2, and 3.5% w/w dried solid). Incorporation of 3.5% of nanoparticles into tapioca starch/bovin gelatin films decreased the permeability to water vapor by 18%. The addition of low concentration ZnO-N to starch/gelatin solutions significantly decreased monolayer water content of the films. ZnO-N tapioca starch/bovin gelatin films exhibited excellent anti-microbial activity against *Staphylococcus aureus* and *Escherichia coli*. The results showed that the Bionanocomposite of ZnO-N has the potential to be an active packaging material in the pharmaceutical and food industries.

Keywords: Antimicrobial, Bionanocomposite, Sorption isotherm, Tapioca, Zinc oxide nanorod.

Introduction

The purpose of food packaging is to increase food shelf life by avoiding spoilage, bacteria, or the loss of food nutrients (Brody, 2003; Chaudhry *et al.,* 2008). The main types of plastics that are currently used in all applications are derived from non-renewable petroleum resources and non-biodegradable plastic materials (Zhao *et al.*, 2008). The use of a biopolymer such as starch can be an interesting solution because this polymer is quite cheap, abundant, biodegradable and edible. Starch is a renewable resource widely available and can be obtained from different by-products of harvesting and raw material industrialization. Edible and biodegradable films can offer great potential to enhance food quality, safety and stability (Garcia *et al.*, 2001). The unique advantages of edible films and coatings may lead to new product developments, such as individual packaging of particulate foods, carriers for different additives, and nutrient supplements (Vermeiren *et al.*, 1999). Nanotechnology is generally defined as the design, production, and application of structures, devices, and systems through control of the size and shape of the material at the 10^{-9} of a meter scale. Nanotechnology offers higher hopes in food packaging by promising longer shelf life, safer packaging, better traceability of food products, and healthier food. Oxygen inside food packaging is the main cause for food deterioration due to oxidation of fats and oils and growth of microorganisms. Also, oxygen accelerates the processes inside food packaging, leading to discoloration, changes in texture, rancidity and off-odor, and flavor problems. Nanotechnology can effectively produce oxygen scavengers for sliced

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processed meat, nuts, beverages, cooked pastas, and ready-to-eat snacks; moisture absorber sheets for fresh meat, poultry, and fish; and ethylene-scavenging bags for packaging of fruit and vegetables (Neethirajan and Jayas, 2010). Antimicrobial packaging is a type of active packaging which interacts with the product or the headspace to reduce, inhibit or retard the growth of microorganisms that may be present on food surfaces (Soares *et al.*, 2009). Bionanocomposites are a mixture of polymers with nano sized inorganic or organic fillers with particular size, geometry and surface chemistry properties (Giannelis, 1996). When polymers are combined with nano fillers, the resulting bio-nanocomposites exhibit significant improvements in mechanical properties, dimensional stability, and solvent or gas resistance with respect to the pristine polymer (Sinha Ray and Bousmina, 2005; Sinha Ray and Okamoto, 2003). ZnO, TiO_2, MgO, and CaO are among the inorganic materials that are particular interesting since they are both safe for animals and human and stable under harsh condition processes (Lin *et al.*, 2009). There are some reports about the improvements of biopolymer by incorporating nano particles, such as nano zinc oxide (Mohammadi Nafchi *et al.*, 2013; Alebooyeh *et al.*, 2012; Mohammadi Nafchi *et al.*, 2012).

In this study, ZnO nanorods were used as filler to prepare tapioca starch/bovin gelatin/ZnO-N Bionanocomposite. The films were characterized for their antibacterial, barrier, and sorption isotherm properties.

Materials and Methods

Tapioca starch was purchased from SIM company Sdn. Bhd (Penang, Malaysia) and Bovine gelatin (Type B) was purchased from Sigma Chemical Co (St. Louis, MO, USA). Liquid sorbitol and glycerol were purchased from Liang Traco (Penang, Malaysia). All chemicals were of analytical grade. Zinc oxide nanorod (ZnO-N) was synthesized through the catalyst-free combust-oxidized mesh (CFCOM) process as described by Shahrom and Abdullah (2007). Zinc oxide nanorod was obtained from University Sains Malaysia (USM). Environmental scanning electron microscopy (ESEM) (Fig. 1) revealed that ZnO-N had a dimension in nanometer.

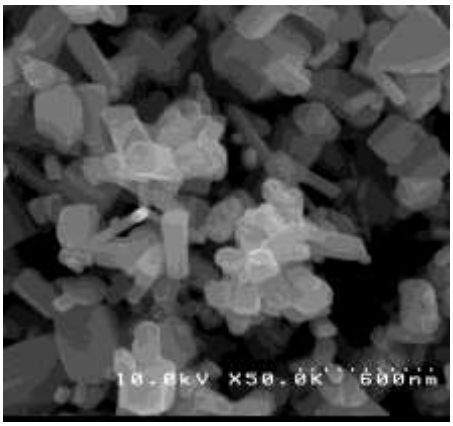

Fig.1. ESEM micrograph of nanorod-rich ZnO

Film preparation

ZnO-N was dispersed in water at different concentrations (0.5%, 2%, and 3.5%, w/w of total solid), stirred for one hour, and then sonicated in an ultrasonic bath (Marconi model, Unique USC 45 *kHz, Piracicaba, Brazil)* for 30 minutes to ensure that homogenization was completed. The solution was used to prepare the aqueous starch dispersion at 4% (w/w) and bovin gelatin %10 (w/w) of total starch. A mixture of sorbitol and glycerol (3:1) at 40% (w/w) of total solid was added as plasticizers in accordance with Mohammadi Nafchi and his colleagues (2011). Starch/gelatin nanocomposites were heated to 85 ±5°C and held for 45 min to allow for gelatinization. Then, the solution was cooled to room temperature. A portion (90 g) of the dispersion was cast on plates fitted with rims around the edge to yield a 16 × 16 cm^2 film-forming area. Films were dried under controlled conditions in a humidity chamber (30°C and 50% RH). Control films were prepared similarly but without addition of nanoparticles. Dried films were peeled and stored at 28±2 °C and 50±5% relative humidity (RH) until experimentation.

Sorption isotherm

The moisture sorption isotherm of the films at 25°C was studied using the method described by Bertuzzi *et al.*, (2007). Moisture content at equilibrium (g absorbed water/g dry film) was measured in triplicate for each relative humidity. Experimental sorption data were fitted using the GAB equation (van den Berg, 1984):

$$W=W_mCKa_w / (1-Ka_w) (1-Ka_w+CKa_w)$$

where W_m, K, and C are the GAB parameters, W is moisture content (dry basis), and a_w is water activity. To evaluate the accuracy of the GAB model for experimental sorption isotherm of the films, we calculated the percentage of mean relative deviation modulus (E) using the following formula:

$$E = \frac{100}{N}\sum_{i=1}^{N}\frac{|m_i - m_{pi}|}{m_i}$$

where N is the number of experimental data and m_i and m_{pi} are the experimental predicted values, respectively. A modulus (E) value below 10% indicates a good fit (Masclaux *et al.*, 2010).

Antimicrobial assay

An antimicrobial activity test on the films was carried out using the agar diffusion method according to Maizura *et al.*, (2007). First, Mueller Hinton agar (Merk, Darmstadt, Germany) plates were seeded with 1 mL of inoculums containing approximately10^5–10^6 CFU/mL of *E. coli* and *S. aureus*. Then, film disks were placed on the plates. The plates were incubated at 37°C for 24 h. Next, the plates were examined for "zone of inhibition" of the film discs. Antimicrobial effects of the films were determined by calculating inhibition zone against *E. coli* and *S. aureus* on solid media. The area of the whole zone was calculated and then subtracted from the film disk area, and this difference area was reported as zone of inhibition against *E. coli* and *S. aureus*.

Water vapor permeability

Water vapor permeability (WVP) tests of the films were carried out following the modified method (Yu *et al.*, 2009) of ASTM standard E96-05 (ASTM, 2005). The test cups were filled with water up to 1.5 cm below the film. A plot of gained weight versus time was used to determine the WVTR. The slope of the linear portion of this plot represented the steady state amount of water vapour transmission through the film per unit time (g/h). Three samples per treatment were tested. The regression coefficients should be 0.99 or greater. The WVP of film was calculated by multiplying the steady WVTR by the film thickness and dividing that by the water vapour pressure difference across the film.

Statistical analysis

ANOVA and Duncan's Post Hoc tests were used to compare means of physicochemical properties of Bionanocomposite films at the 5% significance level. Statistical analysis was conducted using GraphPad Prism 6 (GraphPad Software Inc., 2236 Avenida de la Playa, La Jolla, CA 92037, USA).

Results

Sorption isotherm

The theoretical sorption isotherm curves fitted with the GAB equation and experimental data for tapioca starch/bovin gelatin films at 25°C are presented in Fig. 2. The GAB parameters are presented in Table 1. The percentage of mean relative deviation modulus (E) for starch/gelatin bionanocomposite films was lower than 10% (Table 1) (Masclaux *et al.*, 2010). The sum of squares of error (SSE) for films was about 10^{-4} (Table 1), indicating that the GAB model gave a good fit for the sorption isotherm. Based on the Brunauer, Emmett, and Teller classification, films with $0 \leq K \leq 1$ and $C_G \geq 2$ are type II and those with $0 \leq K \leq 1$ and $0 \leq C_G \leq 2$ are type III (Blahovec, 2004); therefore, the tapioca starch/bovin gelatin nanocomposite films can be classified as type II. In all ranges of a_w (0.1–0.9), the ZnO-N incorporated films exhibited less equilibrium water content compared with control films.

Table 1. GAB parameters for sorption isotherm of tapioca starch / bovin gelatin / ZnO-N composite film at 25°C.

ZnO nanorod (%)	M_m	C_G	K	E (%)	SSE
0	0.0908	10.5896	0.8844	4.7136	0.0004
0.5	0.0881	8.0744	0.8901	4.8379	0.0003
2	0.0751	8.7905	0.9153	4.6859	0.0003
3.5	0.0714	8.0061	0.8961	3.9588	0.0001

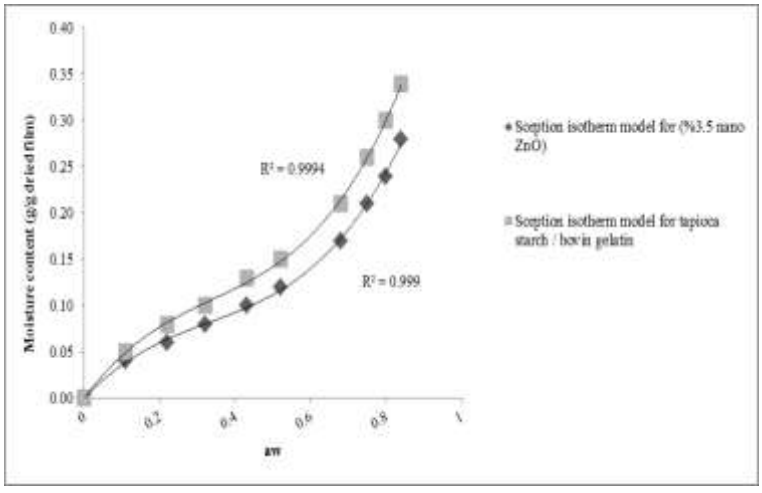

Fig. 2. Sorption isotherm for tapioca starch / bovin gelatin films and 3.5% ZnO-N incorporated tapioca starch / bovin gelatin films at 25°C.

Antimicrobial assay

Effects of tapioca starch / bovin gelatin film reinforced with ZnO-N on the growth of *S. aureus* and *E. coli* were investigated. The inhibition zone of nano-incorporated films was significantly

increased by increasing ZnO-N contents, suggesting (Fig. 3) that tapioca starch / bovin gelatin film incorporated with ZnO-N can act as an active film against microorganisms. *E. coli* has shown higher susceptibility to ZnO nanoparticles compared to *S. aureus*.

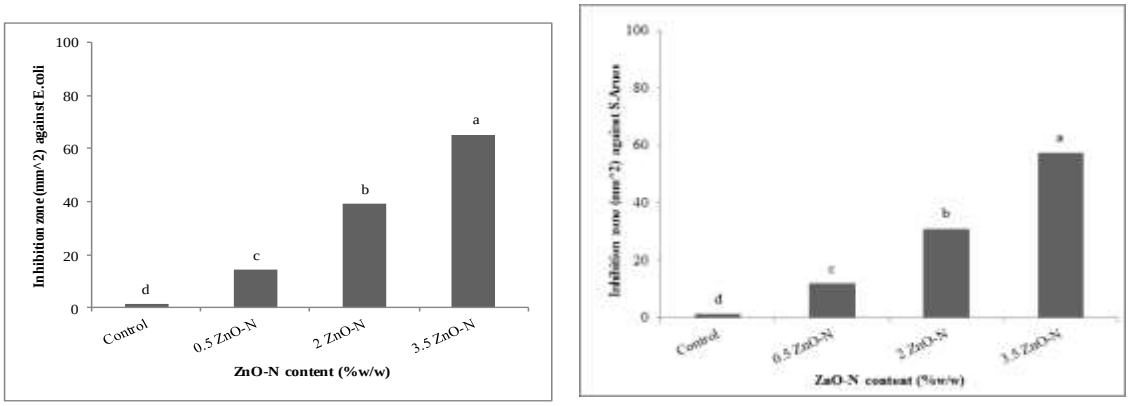

Fig. 3. Effects of ZnO nanorod contents on antimicrobial activity of tapioca starch / bovin gelatin nanocomposite films. Inhibition zone = total inhibition area − total film area.The bars show mean (n = 3) ± SD. Different letters on the bars represent the significant difference at 5% level of probability.

Permeability to water vapor

Water vapor permeability of the film is an important property in packaging industry. The results of water vapor permeability are presented in Table 2. Water vapor permeability significantly decreased by addition of ZnO-nr. Incorporation of 3.5% of nanoparticles to tapioca starch/bovin gelatin films decreased the permeability to water vapor by 18%.

Table 2. Water vapor permeability (WVP) of tapioca starch / bovine gelatin nanocomposites.

ZnON (%)	WVP×10^{-7} [g/ m Pa h]
0	4.20 ± 0.01 a
0.5	4.15 ± 0.01 b
2	4.01 ± 0.02 c
3.5	3.42 ± 0.02 d

Values are mean (n=3) ± SD. Different letters in WVP column values represent significant difference at 5% level of probability among tapioca starch / bovine gelatin films.

Discussion

Sorption isotherm

The GAB equation is a three-parameter equation with k, C, and W_m as constants. W_m in the GAB equation is the mass fraction of water in the material equivalent to a unimolecular layer of water covering the surface of each particle. C is a constant at constant temperature and is related to the heat of adsorption of water on the particles. k is a third parameter that corrects for the difference in properties of adsorbed water relative to liquid water and permits the GAB equation to hold over a wider range of moisture content than the BET.

Both k and C are temperature-dependent. According to Muller *et al.* (2011), an increased K value for bionanocomposites in comparison with C_G indicates a reduction in sorption energy for the absolute values of the multilayers. This observation may be attributed to the interaction between plasticizer, biopolymer matrix, and ZnO-N, leading to reduced hydroxyl group availability to interact with water and, consequently, a less hygroscopic matrix. Muller *et al.*, (2011) reported that ion–dipole interactions occur between ZnO,

water, and/or plasticizer; specifically between the zinc and the hydroxyl groups of the plasticizer and water. The monolayer factor (m_o) for the films in this study is consistent with those of previous researches, since the addition of nanoparticles decreased the hydrophilic behavior of the biopolymer films (Blahovec, 2004; Muller *et al.,* 2011; Zeppa *et al.,* 2009). The incorporation of ZnO-nr to tapioca starch/gelatin films decreased the free water; consequently, the reduction of disposal capable water for enzymatic and chemical reaction increased food shelf life.

Antimicrobial assay

The antimicrobial activity of ZnO nanoparticles has been tested against *E. coli* and *S. aureus* which have presented sensitivity to these nanoparticles. These results are consistent with previous reports on nanobiocomposites (Adams *et al.,* 2006; Sawai, 2003; Xie *et al.,* 2011). The exact mechanism of action of ZnO nanoparticles is still unknown. However, the antimicrobial activity of these nanoparticles can be attributed to several mechanisms, including the release of antimicrobial ions (Kasemets *et al.,* 2009). interaction of nanoparticles with microorganisms, subsequently damaging the integrity of bacterial cell (Zhang *et al.,* 2008) and the formation of ROS by the effect of light radiation (Jalal *et al.,*2010). In addition to the aforementioned mechanisms, Padmavathy and Vijayaraghavan (2008) suggest that the antibacterial effect of ZnO can also be the result of mechanical damage to the cell membrane caused by the abrasive surface of nanoparticles, since ZnO nanoparticles have been considered to be abrasive due to surface defects such as edges and corners (Espitia *et al.,* 2002). The nano size of ZnO is more effective than micro size due to its easy penetration through cell wall of microorganisms (Zhang *et al.,* 2010). The higher resistance of *S. aureus* to ZnO nanoparticles can be explained by the differences between these two bacteria related to the intracellular antioxidant content such as carotenoid pigments in the interior of *S. aureus*, which promote a greater oxidant resistance as well as the presence of potent detoxification agents such as antioxidant enzymes, particularly catalase (Applerot *et al.,* 2009).

Permeability to water vapor

The significant decrease in WVP after the addition of ZnO-N can be attributed to the greater water resistance of ZnO-N compared to the bio composite matrix. The incorporation of these nanorods to the matrix introduced a tortuous pathway for water vapor molecules to pass through (Yu *et al.,* 2009). Permeability reduction in ZnO-N incorporated tapioca starch/bovin gelatin films can be described based on the Nielsen (1967) simple model of tortuosity. This model proposes that each layer of a filler particle is perpendicularly oriented to the diffusion pathway, indicating that water vapor should travel in a longer diffusive path for the permeability coefficient to decrease. The improvement in the barrier property is explained by a decrease in the area available for diffusion, which resulted from one effect: impermeable nanoparticles replacing permeable spaces of the polymer (Lepot *et al*., 2011). Yu *et al.* (2009) used ZnO nanoparticles in combination with pea starch and found that water vapor permeability significantly decreased with the addition of nanoparticles. Walnut kernels generally contained about 60 % oil (Prasad, 1994). Most of nuts were rich in oleic acid while walnuts were also high in two polyunsaturated fatty acids linoleic acid and α-linolenic acids. The major fatty acids found in walnut oil included oleic, linoleic and linolenic acids (Savage, 2001). Acyl lipid constituents, such as oleic, linoleic and linolenic acids, had one or more allyl groups within the fatty acid molecule and thus were readily oxidized to hydroperoxides. Storage capabilities were also dependent on the polyunsaturated fatty acid (PUFA) levels because PUFAs were more susceptible to oxidative degradation (Hsieh and Kinsella, 1989).

Incorporation of ZnO-nr to tapioca starch/gelatin films decreased the permeability to gases and may have also partially reduced hydroperoxides compounds.

Conclusions

In this study, the ZnO nanoparticles were incorporated into the tapioca starch/bovin gelatin films at different concentrations (0.5% to 3.5%, w/w total solid). After incorporation of nano filler, we observed significant differences in film properties, especially in WVP, and Sorption isotherm. Bionanocomposite films decreased the permeability to gases; consequently. There was a reduction in polyunsaturated fatty acids oxidation such as Walnut kernels. Monolayer of water and free water increased nut shelf life.

The films displayed an excellent antimicrobial activity against the *E. coli* and *S. aureus*. On the other hand, zinc is one of essential trace element for human body that is under strict regulation; the bionanocomposites based on ZnO-N have an excellent potential application in the food packaging and nut coating.

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