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Properties and antimicrobial activity of fish protein isolate/fish skin gelatin film containing basil leaf essential oil and zinc oxide nanoparticles

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ABSTRACT

Composite films based on fish protein isolate (FPI) and fish skin gelatin (FSG) blend incorporated with 50 and 100% (w/w, protein) basil leaf essential oil (BEO) in the absence and presence of 3% (w/w, protein) ZnO nanoparticles (ZnONP) were prepared and characterised. Tensile strength (TS) decreased, whilst elongation at break (EAB) increased as BEO level increased ($p < 0.05$). However, ZnONP addition resulted in higher TS but lower EAB ($p < 0.05$). The lowest water vapour permeability (WVP) was observed for the film incorporated with 100% BEO and 3% ZnONP ($p < 0.05$). BEO and ZnONP incorporation decreased transparency of FPI/FSG films ($p < 0.05$). FTIR spectra indicated that films added with BEO exhibited higher hydrophobicity. Both BEO and ZnONP had a marked impact on thermal stability of the films. Microstructural study revealed that the presence of ZnONP prevented bilayer formation of film containing 100% BEO. FPI/FSG films incorporated with 100% BEO, especially in combination with ZnONP, exhibited strong antibacterial activity against foodborne pathogenic and spoilage bacteria and thus could be used as an active food packaging material to ensure safety and to extend the shelf-life of packaged foods.

1. Introduction

Biopolymers have been widely paid attention over the last decades due to their advantages and potential applications in food industries (Kanmani & Rhim, 2014; Rhim & Ng, 2007). Biopolymer films are the excellent vehicles for incorporating a wide variety of additives, such as antimicrobials, antioxidants, antifungal agents, colourants, and other nutrients, thus improving food quality and extending shelf-life of foods and products (Rhim & Ng, 2007). Amongst biopolymers, proteins from different sources have been impressively used for the development of biodegradable films due to their relative abundance and good film-forming ability (Gennadios, Weller, Hanna, & Froning, 1996; Prodpran, Benjakul, & Artharn, 2007).

Fish protein isolate (FPI) prepared by alkaline solubilisation was reported as the promising starting material for preparation of films with negligible yellow discolouration (Tongnuanchan, Benjakul, Prodpran, & Songtipya, 2011). However, FPI based film is rigid, thus requiring the addition of high amount of hydrophilic plasticiser (Tongnuanchan et al., 2011). Plasticised-FPI films have the poor water vapour barrier property, owing to hydrophilicity of amino acids in protein molecules and to the significant amounts of hydrophilic plasticisers required for film flexibility (Prodpran et al., 2007). Recently, properties of FPI films from yellow stripe trevally, an abundant trash dark muscle fish, could be modified by blending with fish skin gelatin (FSG) at a ratio of 5:5 with lower glycerol content (30%) (Arfat, Benjakul, Prodpran, & Osako, 2014). FPI/FSG blend films showed the improved mechanical and water vapour barrier properties, compared with FPI films. However, FPI/FSG blend films still have poorer mechanical as well as water vapour barrier properties, in comparison with synthetic films.

Nanoparticles with the filler property has been implemented in biopolymer films for improving mechanical, thermal and water vapour barrier properties (Kovacevic, Vrsaljko, Blagojevic, & Leskovac, 2008). Amongst nano-fillers, ZnO nanoparticles (ZnONP) have the excellent ability for nano-scale dispersion and interfacial interactions in protein matrix due to their large specific surface area and high surface energy (Rouhi, Mahmud, Naderi, Ooi,

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& Mahmood, 2013). Recently, the incorporation of ZnONP as functional filler into the biopolymer films such as starch based films has been reported to improve mechanical and water vapour barrier properties (Alebooyeh, Nafchi, & Jokar, 2012; Yu, Yang, Liu, & Ma, 2009). ZnO is currently listed as a generally recognised as safe (GRAS) material by the Food and Drug Administration (21CFR182.8991) and has previously shown strong *in vitro* antimicrobial activity against foodborne pathogens and spoilage bacteria (Espitia et al., 2013; Zhang, Ding, Povey, & York, 2008).

Antimicrobial biodegradable films have received increasing attention because of their potential to delay microbial spoilage of foods (Emiroglu, Yemis, Coskun, & Candogan, 2010; Rhim & Ng, 2007). Essential oils from aromatic and medicinal plants have been known to be biologically active, mainly possessing antibacterial and antioxidant properties (Ahmad, Benjakul, Prodpran, & Agustini, 2012). Although most essential oils are classified as Generally Recognised as Safe, their use as food preservatives is often limited due to negative organoleptic effects when added in sufficient amounts to provide an antimicrobial effect. The advantage of applying such essential oils through the use of films, instead of applying them directly on foods, is that low diffusion rate of the active compounds allows to attain the desired antimicrobial effect with lower oil concentrations, thus limiting unwanted flavours and odours to the food (Sánchez-González et al., 2011). Additionally, essential oils are hydrophobic in nature and the incorporation of essential oils could improve the water vapour barrier property and impart flexibility of protein films (Tongnuanchan, Benjakul, & Prodpran, 2013). The use of essential oil exhibited the increased antimicrobial activity, when combined together with various nanoparticles (Allahverdiyev, Kon, Abamor, Bagirova, & Rafailovich, 2011). To the best of our knowledge, no information regarding the combined effect of essential oils and ZnONP on properties of protein based films has been reported. The present study aimed to investigate the combined effects of ZnONP and basil leaf essential oil (BEO) on physico-mechanical and thermal properties of FPI/FSG blend films. Antimicrobial activity of films against *Listeria monocytogenes* (foodborne pathogen) and *Pseudomonas aureginosa* (food spoilage bacteria) was also examined.

2. Materials and methods

2.1. Chemicals

Zinc oxide nanoparticle (ZnONP) (particle size: 20–40 nm, specific surface area: 26.22 m^2/g) was purchased from Nano materials technology Co. Ltd. (Bangkok, Thailand). Sodium hydroxide and hydrochloric acid were obtained from Merck (Darmstadt, Germany). Commercial fish skin gelatin (FSG) from tilapia (~240 bloom) was purchased from Lapi Gelatine S.p.A (Empoli, Italy). Essential oil from the leaves of basil (*Ocimum basilicum*) was obtained from Botanicessence (Bangkok, Thailand). All chemicals were of analytical grade. *L. monocytogenes* DMST 1327 was obtained from Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand and *P. aeruginosa* TISTR 781 was obtained from Thailand Institute of Scientific and Technological Research (TISTR), Thailand.

2.2. Collection and preparation of fish sample

Fresh yellow stripe trevally (*Selaroides leptolepis*) with an average weight of 90–100 g/fish were purchased from a local market in Hat Yai, Songkhla province, Thailand. Fish were kept in ice with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University within 30 min. Upon the arrival, fish were immediately washed, filleted, and minced to uniformity by passing through a 5 mm screen using a Model HC 5000 mincer (Microfluidics, Massachusetts, USA).

2.3. Preparation of fish protein isolate

Prior to the isolation of fish protein, the prepared mince was subjected to washing as per the method of Toyohara, Sakata, Yamashita, and Shimizu (1990) with slight modifications. Fish mince was homogenised with 5 volumes of cold 0.05 M NaCl (2–4 °C) at a speed of 13,000 rpm for 2 min, using an IKA Labortechnik homogeniser (Selangor, Malaysia). The washed mince was filtered through two layers of cheese-cloth. The washing process was repeated twice. Washed mince obtained was stored on ice until used.

Washed mince was added with cold distilled water at the ratio of 1:9 (w/v), followed by homogenisation for 1 min at a speed of 13,000 rpm. The pH of homogenate was then adjusted to 11 using 2 M NaOH. The resulting mixture was centrifuged at 10,000 × g for 20 min at 4 °C using a refrigerated centrifuge (Avanti-JE Centrifuge, Beckman 163 Coulter Inc., Fullerton, CA, USA). The supernatant was collected and the pH was adjusted to 5.5 using 2 M HCl. The precipitate was then filtered through 4 layers of cheese-cloth. The retentate was dewatered by centrifugation at 12,000 × g for 20 min at 4 °C. The final pH of the sample was adjusted to pH 7.0 using 2 M NaOH. The sample was referred to as "fish protein isolate; FPI". FPI was used for film preparation.

2.4. Preparation of fish protein isolate/fish skin gelatin film added with BEO and ZnONP

Firstly, film-forming solution was prepared according to the method of Chinabhark, Benjakul, and Prodpran (2007). FPI was added with 3 volumes of distilled water and homogenised at 13,000 rpm for 1 min using a homogeniser. Subsequently, the pH of the mixture was adjusted to 3 using 1 N HCl, to solubilise the protein. The obtained solution was filtered through 2 layers of cheese-cloth to remove undissolved debris. The protein concentration of the filtrate determined by the Kjeldahl method (AOAC, 2000) was adjusted to 3% (w/v). Glycerol at 30% (w/w) of protein was used as a plasticiser. The mixtures were stirred gently for 30 min at room temperature and were used for preparing blend FFS.

Prior to blending, FSG powder was dissolved in distilled water to obtain the protein concentration of 3% (w/v). The pH of the mixture was adjusted to 3 using 1 N HCl. The solution was heated at 70 °C for 30 min. Then, glycerol (30%, w/w) was added as mentioned above. Thereafter, both FPI and FSG solutions were mixed at a ratio of 1:1 (v/v). The obtained solution was added without and with 3% ZnONP (w/w, protein content) in droplets. Before addition of ZnONP, ZnONP was suspended in distilled water and homogenised for 1 min at 5000 rpm (IKA Labortechnik homogeniser, Selangor, Malaysia). The obtained FPI/FSG/ZnONP suspension was stirred for 5 min and then homogenised for 30 s at the speed of 5000 rpm.

BEO previously mixed with Tween 20 at 25% (w/w, based on essential oil) was added to the FPI/FSG/ZnONP suspension at levels of 50% and 100% (w/w, protein content). Final volume was made up to 100 ml using distilled water previously adjusted to pH 3. To obtain the uniform distribution of BEO and ZnONP, the suspensions were homogenised with three passes through a high pressure homogeniser (Microfluidizer M-110EH, Microfluidics Corp., Newton, MA, USA) with an operating pressure of 1500 bars. Suspensions were gently stirred for 30 min at room temperature and were referred to as a film-forming suspension (FFS). Prior to casting, FFS samples were degassed for 10 min using the sonicating bath (Elmasonic S 30 H, Singen, Germany). To prepare the film, 4 g