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Improvement of fish protein film with nanoclay and transglutaminase for food packaging

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ABSTRACT

Montmorillonite (MMT) nanoclay and microbial transglutaminase (MTGase) were incorporated into Fish myofibrillar protein (FMP) film to improve its functional properties. The MMT and MTGase weight percent relative to protein was varied from 1 to 5 and 1 to 3, respectively, and their impact on physical, mechanical, and barrier properties of the FMP films was investigated. Microstructure of FMP nanocomposites was characterized through; scanning electron microscopy, X-ray diffraction and Fourier transform infrared spectroscopy. The results showed that incorporating MMT and MTGase into film improves significantly water gain, water vapor permeability, and solubility of the FMP film. It was also shown that the combined effect of clay and MTGase improves significantly the tensile strength and elongation of nanocomposites ($p < 0.05$). The SEM, XRD and FTIR results confirmed that the improvements are related to the MMT exfoliation and good interaction between fish myofibrillar protein and MTGase in the presence of MMT.

1. Introduction

Packaging is used to protect food from secondary contaminations after processing and during storage to maintain the food product quality. Many materials are employed for preparation of packages but the majority of them are made of plastics. Synthetic plastic packages have come into wide-spread use thanks to its good mechanical properties and effectiveness as a barrier to oxygen and water. However, synthetic films represent a serious ecological problem due to their non-biodegradability. As a consequence, in recent years, packaging research has focused more on biodegradable and/or edible films made from natural polymers. Such polymers may be protein, lipid or polysaccharide-based and their chemical nature determines the physical properties of the resulting films. Among these materials, proteins from different sources have been extensively employed because of their relative abundance, film forming ability and nutritional qualities (Pires et al., 2011).

Proteins biopolymers are capable of forming film and their properties can be varied with source of it. Myofibrillar and sarcoplasmic proteins from fish muscle have been widely used as film forming material (Tongnuanchan et al., 2011). During processing of sea foods, a substantial amount of by-products which are good source of protein, is accessible. As a consequent, producing biofilms from by-products leads to upgrading fish processing industry (Pires et al., 2011).

The most important characteristics of films and coatings are to retard the migration of moisture, oxygen, carbon dioxide, microbes or solutes, as well as to prevent collapse of products (Artharn et al., 2007). These characteristics in biofilms cannot compete with synthetic packages.

In order to optimize mechanical properties of biofilms, some methods and technologies have been applied by now. Recently, polymer–clay nanocomposites have received significant attention as an alternative to conventional filled polymers, because of their ability for nano-scale dispersion, which brings significant improvement in mechanical and physical properties compared to micro-scale polymer composites. Several studies have reported amelioration of mechanical properties (Lavorgna et al., 2010; Xu et al., 2006), thermal stability (Darder et al., 2003), functional properties (Rhim et al., 2006), barrier properties (Casariego et al., 2009; Rhim et al., 2006), and water solubility (Casariego et al., 2009) of chitosan films via incorporation of nanoclay into chitosan in the range of 1–5 wt.%. Montmorillonite is the most widely studied type of clay which is a hydrated alumina-silicate layered clay consisting of an edge-shared

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octahedral sheet of aluminum hydroxide between two silica tetrahedral layers. The imbalance of the surface negative charges is compensated by exchangeable cations (typically Na+ and Ca2+) (Azeredo et al., 2010).

chemical cross-linking agents, such as glutaraldehyde, formaldehyde, and glyoxal (Bigi et al., 2004; Cuq et al., 1997) or enzymes, such as microbial transglutaminase (Yi et al., 2006), have been also used to improve the properties of biofilms. Chemical cross-linkers usually are toxic, which limits their use in food systems (Tseng et al., 1990). Therefore, enzymes as cross-linking agents can be a better alternative for food packaging. Microbial transglutaminases can catalyze formation of ε-(γ-glutamyl) lysine isopeptide bonds which leads to improvement of physical and textural properties of many food proteins, such as tofu, boiled fish paste, and sausage (Benjakul et al., 2004; Nonaka et al., 1996; Seguro et al., 1995; Sharma et al., 2002; Shimba et al., 2002; Soeda et al., 1995).

Considering rare investigations about combination of nano-clay and enzymatic treatments on properties of fish based biofilms, in this study, effect of montmorillonite nanoclay and MTGase treatments on physico-chemical properties of fish myofibrillar protein (FMP) composite films have been investigated.

2. Methods

2.1. Chemical compounds

Microbial transglutaminase (MTGase) from Streptoverticillium mobaraense was supplied by Ajinomoto, Japan. Na+ montmorillonite (MMT) from Southern Clay Products, USA were used as received. Glacial acetic acid and NaCl from Merck, Germany

2.2. Preparation of fish myofibrillar protein (FMP)

Fresh Silver carp (*Hypophthalmichtis molitrix*) was captured and kept on ice (1 h) untill delivery to the laboratory. Upon the arrival, washed with tap water, filleted and manually chopped. The film-forming solution was prepared according to the method of Limpan (Pires et al., 2011). Briefly, the fish mince was mixed with three volumes of cold distilled water and homogenized at 13,000 rpm for 2 min, followed by filtering through a layer of nylon cloth. The mince was mixed with five volumes of 50 mM NaCl for 5 min and filtrated through a layer of nylon cloth. The washing process was repeated twice. Then, washed mince obtained were stored on ice until used for film preparation.

2.3. Preparation of film-forming solutions (FFS) and film casting

Nanocomposite samples were prepared according to methods reported by Xu et al. (2006). Selected amounts of clay (1, 3, and 5 wt% on solid protein) were dispersed in 100 mL of distilled water and vigorously stirred for 24 h. To prepare FMP–FFS, washed mince was added with distilled water to obtain the final protein concentrations of 2% (w/v). The mixture was homogenized at 13,000 rpm for 1 min using Wiggen Hauser D-500 homogenizer. Then nano clay solution was added to FMP-FFS and homogenized at 13,000 rpm for 1 min. MTGase powder (1, 2 and 3% on solid protein) was dissolved in 5 mL of deionizaed water and was well mixed using a homogenizer until all powder was in solution. The MTGase solution was then added into nanocomposite solution. Glycerol was then added at 50% (w/w) of protein content. The mixture was stirred gently for 30 min at room temperature. The pH of the mixture was adjusted to 3 using 1N HCl, to solubilize the protein. The solution was filtered through a layer of nylon cloth to remove undissolved debris Limpan (Pires et al., 2011), and then it was degassed under vacuum for 5 min in order to remove all bubbles.

2.4. Film casting and drying

The film-forming solution (4 g) was cast onto a rimmed silicone resin plate (50 × 50 mm) and drying at 25 °C and 50% relative humidity (RH) for 24 h in an environmental chamber (WTB Binder, Tuttlingen, Germany). The resulting films were manually peeled off and used for analyses. Dried films were then peeled and stored in a desiccator containing saturated magnesium nitrate solution at 25 °C and 52.89% relative humidity until evaluation.

2.5. Determination of FMP film properties

2.5.1. Film thickness

A manual digital micrometer (0.001 mm, Mitutoyo, Mizonokuchi, Japan) was used to measure thickness of the FMP films. Average values of ten measurements in the different regions of each sample were calculated and used in water vapor permeability and tensile properties calculations.

2.5.2. Mechanical properties

Prior to testing the mechanical properties, films were conditioned for 48 h at 50 ± 5% relative humidity (RH) at 25 °C. Tensile strength (TS) and elongation at break (EAB%) of the film samples were determined according to ASTM standard method D882-02. The film samples were cut in rectangular specimens (2.5 × 10 cm). Initial grip separation was set at 50 mm, and cross-head speed was set at 50 mm/min. This test was repeated five times for each sample to confirm [h6] its repeatability.

2.5.3. Fourier transfer infrared spectra (FTIR)

Fourier transform infrared (FTIR) spectra were collected in transmission mode by using a Bruker (EQUI-NOX 55, Ettlingen, Germany) FTIR spectrophotometer with DTGS detector (16 scans) in the range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1}.

2.5.4. X-ray diffraction (XRD) characterization

The structure of nanoparticle, MTGase and its nanocomposites was evaluated by XRD measurement. XRD patterns were taken with a Philips X'Pert MPD Diffractometer (Eindhoven, Netherlands), with Co Karadiation at a wavelength of 1.544 nm, at 40 kV and 30 mA. nanocomposite films were scanned over the range of diffraction angle $2\theta = 1–12^{\circ}$, with a scan speed of 1°/min at room temperature.

2.5.5. Scanning electron microscopy (SEM)

The FMP and FMP/MTGase/MMT nanocomposite films were mounted on the specimen holder with aluminum tape and then sputtered with gold in a BAL-TEC SCD 005 sputter coater (BAL-TEC AG, Balzers, Liechtenstein). All the specimens were examined with a Philips XL 30 scanning electron microscope (Philips, Eindhoven, Netherlands) under high vacuum condition and at an accelerating voltage of 20.0 kV.

2.5.6. Surface color measurement

Color properties of films were measured using a color meter. Measurements are expressed as L^* (lightness), a^* (red/green), and b^* (yellow/blue). The parameters were determined by placing film samples on a standard plate ($L^* = 94.63$, $a^* = -0.88$, and $b^* = 0.65$). Color difference (ΔE) and whiteness index (WI) were calculated with respect to standard plate parameters by using following Eqs. (1) and (2), respectively.

$$E = \sqrt{\left[(a^*)^2 + (b^*)^2 + (L^*)^2\right]} \quad (1)$$