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Effect of transglutaminase induced crosslinking on the properties of starch/gelatin films

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ABSTRACT

This paper was to measure the effect of fish gelatin and Transglutaminase enzyme (TGs) on properties of sago starch and fish gelatin films. The concentration of glycerol was 30% of polymers (db) and the sago starch to fish gelatin ratios were (1:0 and 3:1) with TGs concentrations (1, 5 and 10 mg/g gelatin). The results were discussed in terms of 'gelatin and TGs effect'. In a general manner, fish gelatin and TGs have an effect on both physicochemical and functional properties of the produced films. Addition of fish gelatin to sago starch films significantly reduced tensile strength (TS), water vapor permeability (WVP) but increased the percentage of elongation at break (%EAB). Positive effects of TGs addition on mechanical properties were observed. FTIR-ATR showed an evident of interaction between polysaccharides and protein. Furthermore, the transmittance percentage of amide I and amide II bands in treated films reduced with increasing enzyme concentration as an evident of enzyme crosslinking.

1. Introduction

Quality and shelf life of foods can be improved by using edible films (starch and gelatin) that provide barriers to mass transfer. Edible films functional properties depend on the material characteristics and the method of their preparation (Flores, Famá, Rojas, Goyanes & Gerschenson, 2007). The main film-forming materials used and investigated to enhance the food qualities and shelf-life are polysaccharides, proteins and lipids; their derivatives and their mixtures. Polysaccharides materials including starch, carrageenan and alginate have been used as a result of their ability of form films. Hydrocolloid films are considered to be good oxygen and carbon dioxide barriers but less effective as moisture barriers (De Carvalho & Grosso, 2004). Polysaccharides-protein mixed systems have been extensively studies where understanding the interactions between these two biopolymers are of great importance in developing edible films to enhance film properties as well as for food processes and products. Several studies have examined different film properties including physical, mechanical and thermal such as mixed starch and gelatin films (Arvanitoyannis, Nakayama & Aiba, 1998), gelatin and chitosan films (Kolodziejska & Piotrowska, 2007), cassava and gelatin films (Veiga-Santos, Oliveira, Cereda & Scamparini, 2007) and soy protein and gelatin films (Cao, Fu & He, 2007). Modification of gelatin and gelatin based films could be achieved through several methods including electrostatic forces and establishment of salt bridges as conducted by Kaewruang, Benjakul, Prodpran, Encarnacion, and Nalinanon (2014) and (Sow & Yang, 2015) or through protein bonds cross-linking (De Jong & Koppelman, 2002). Transglutaminase enzyme (TGs) is a protein polymerizing agent that results in forming isopeptide bonds between proteins based foods which enhance their properties. It improves food products properties such as firmness, viscosity, elasticity and water binding capacity (Kieliszek & Misiewicz, 2014). Examining the affects of transglutaminase enzyme on the properties of films (mechanical, water vapor permeability (WVP) and solubility) have been conducted in some studies as in gelatin-casein films (Chambi & Grosso, 2006), gelatin films (De Carvalho & Grosso, 2004; Lim, Mine & Tung, 1999) and in ground beef to enhance its texture (Bilic et al., 2005). Addition of TGs to casein-protein blends resulted in formation of protein cross-linking and high molecular mass polymers, thus resulting in stronger gels between 22 and 37 °C (Mylla et al., 2007).

In the this study, the objectives were to evaluate the effects of adding fish gelatin to sago starch based film and incorporating TGs enzyme on the rheological properties of the film forming solutions and the physical, thermal and mechanical properties of the developed sago starch/fish gelatin films.

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2. Materials and methods

Biopolymers as sago starch (*Metroxylon sagu*) (Nitsei Industrial Sdn. Bhd, Malaysia) and gelatin was extracted from surimi fish wastes (Norziah et al., 2009). Transglutaminase enzyme was from Ajinomoto (Activa TG-S, Japan) with activity as 100 U/g powder. All reagents were of analytical grade.

2.1. Starch/gelatin film forming solutions

Biopolymers with glycerol were used to prepare the film forming solutions (FFS) following the procedures described by (Al-Hassan & Norziah, 2012). Sago starch/fish gelatin solutions were prepared with the ratios (1:0 and 3:1) giving a total weight of (5.2 g) in 200 mL including 30% (w/w) glycerol. Sago starch was dissolved and heated in distilled water (at 85 ° C/30 min) (solution A) followed by addition of plasticizer at 60 °C. Likewise, sago starch/fish gelatin film forming solutions (3:1) were prepared by adding fish gelatin to (solution A) at 60 °C and continue stirring (30 min), then adding glycerol (solution B). The mixture (3:1) was incubated with transglutaminase enzyme (TGs) at 50 °C/15 min as reported by (Kolodziejska & Piotrowska, 2007), then enzyme was deactivated (De Carvalho & Grosso, 2004). The concentrations of added TGs to starch:-gelatin(3:1) were 1.0, 5.0 and 10.0 mg/g gelatin.

2.2. Starch/gelatin edible film casting

Film forming solution was poured onto casting plates (16 × 16 cm & 3 mm height) and dried at 35 ° C/24 h using a ventilated oven.

2.3. Dynamic rheological measurements

Starch/fish gelatin film forming solutions (FFS) were cooled to room temperature (~28 °C) before dynamic rheological measurements (viscoelastic flow). An oscillatory shear test was applied to measure the rheological properties of the starch/gelatin mixtures using a controlled stress AR 1000 rheometer (TA Instruments, USA).

2.4. Measurement of crosslinking degree

The crosslinking degree of transglutaminase treated films was preformed according to the method of Bubnis and Ofner (1992) as described by Prasertsung, Mongkolnavin, Kanokpanont, and Damrongsakkul (2010). Formation of a yellow soluble complex was used to determine un-crosslinking groups due to gelatin free amino groups react with 2,4,6-trinitrobenzene sulfonic acid (TNBS).

2.5. Solubility of films

Films solubility was determined as described by (García, Pinotti, Martino, & Zaritzky, 2009). Sample films (2 × 3 cm) were placed in a desiccator containing P_2O_5 (0% RH/7days). Then samples were subject to constant agitation for (1 h/25 ± 2 °C) in test beakers with 80 mL deionized water and filtered (Whatman 4) and dried in an oven (60 °C) to a constant weight. Total soluble was expressed as percentage (% solubility) and calculated according to the follows equation: Percentage of Solubility = [(Initial dry weight-Final dry weight)/Initial dry weight] × 100.

2.6. FTIR spectroscopy

Films spectra were recorded with Fourier transform infrared spectrometry-Attenuated total reflectance (Thermo Scientific Nicolet iS10 FT-IR Spectrometer, Massachusetts, USA) from 4000 to 400 cm^{-1}.

2.7. Measurements of mechanical properties

Developed films were tested following the ASTM methods D882-00 (ASTM, 2000a) to measure the tensile strength and percentage of elongation at break (%EAB) plus Young's modulus. The film specimen strips (14 × 2 cm) were conditions with saturated sodium bromide solution (56% RH, 30 ° C/48 h) prior to testing.

2.8. Measurements of water vapor permeability

Developed films were tested for water vapor permeability (WVP) following ASTM E96-00 method (ASTM, 2000b). Glass permeation cells were used that contained silica gels (0% RH) and mounted films on top of the cell. Samples were placed in a desiccator (100% RH, 30 °C) and measurements were taken at 24 h intervals over a 7-day period.

2.9. Statistical analyses

The results of this study were analyzed using SPSS 18.0. ANOVA was applied using Duncan's test with confidence level as $p < 0.05$.

3. Results and discussion

3.1. Flow analysis

The flow behaviour and viscosity profile of film forming solutions (FFS) with 30% glycerol are shown in Table 1. Film-forming solutions showed non-Newtonian behaviour with thixotropy shear thinning (pseudoplastic behaviour) i.e increase in shear stress decreased the viscosity indicative of structural breakdown due to shearing. All samples were fitted to Cross-model. Starch suspension was reported as non-Newtonian presented pseudoplastic or shear-thinning behaviour (Bertuzzi, Armada & Gottifredi, 2007). The highest viscosity was observed for sago starch samples only (1:0). Glycerol as the plasticizer, reduced the intra and inter-molecuar forces in sago starch due to formation of hydrogen bonds of starch molecule. Kurata and Tsunashima (1998) reported that bigger molecules resulted in higher solution viscosity. Addition of fish gelatin to sago starch FFS reduced the viscosity as well as the consistency due to a reduction in the molecular weight compared to sago starch solution. Sago starch/fish gelatin solution (3:1) presented a high shear stress-shear rate relationship compared to other film forming solutions with transglutaminase. Addition of transglutaminase enzyme to (3:1) film forming solutions reduced the shear stress-shear rate relationship for all different concentrations (1, 5 and 10 mg) compared to untreated samples (TGs 0 mg). The observation may be due to reduction in hydrogen-bonding formed between starch and gelatin due to the formation of the isopeptide in the gelatin, which maybe resulted in excessive crosslinking. Therefore, presence of gelatin in the sample may reduce inter and intra molecular interactions of the sago starch.

Table 1
values of consistency, flow behaviour index and r^2 obtained for film forming solution of sago starch (1:0), sago starch/fish gelatin (3:1) and (3:1) with transglutaminase enzyme (TGs 1 mg, TGs 5 mg and TGs 10 mg).

Sample	η_0 [a]	η_∞ [b]	C (s) [c]	m [d]	r^2 [e]
1:0 (G)	0.432	0.0060	1.627	0.516	0.9896
3:1 (G)	0.033	0.0079	0.113	0.536	0.9990
TGs 1 mg	0.014	0.0071	0.016	0.936	0.9970
TGs 5 mg	0.015	0.0073	0.018	0.806	0.9918
TGs 10 mg	0.012	0.0058	0.009	0.976	0.9942

[a] infinite-rate viscosity.
[b] zero-rate viscosity.
[c] consistency.
[d] flow behaviour index.
[e] determination coefficient for the Cross model fit.