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Physical, rheological and antioxidant properties of gelatin gel as affected by the incorporation of β-glucan

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ABSTRACT

Rheological properties and antioxidative activities of fish gelatin (FG) gel incorporated with β-glucan (BG) at different levels (0, 5, 10, 15 and 20% substitutions) were investigated. Gel strength of FG-BG mixed gel decreased as BG content increased ($P < .05$). Rheological measurements revealed that incorporation of BG slightly influenced elastic modulus (G') and loss modulus (G'') of FG as observed from time and temperature sweep. Gelling temperature of FG-BG mixed gel slightly decreased when BG was higher than 5% substitution ($P < .05$). Melting temperatures of all mixed gels were higher than that of FG ($P < .05$), but no differences in melting temperature were obtained among FG-BG mixed gel, regardless of levels ($P > .05$). FG-BG mixed gels exhibited the lower L^*, a^* and b^*-values, compared with FG gel. Based on gel microstructure, FG gel had a fine network with small voids. Conversely, looser gel network with larger voids were found in mixed FG-BG gels. When FG-BG mixed gels were subjected to a gastrointestinal tract model system (GIMs), the addition of BG increased antioxidative activity of resulting mixed gels in a dose dependent manner as indicated by the increases in ABTS radical scavenging activity, FRAP (ferric reducing antioxidant power) and reducing power. Therefore, the incorporation of β-glucan into fish gelatin gel slightly affected the rheological and textural properties, but yielded the gel product with enhanced health benefits.

1. Introduction

Gelatin is a fibrous protein obtained by thermal denaturation or partial hydrolysis of collagenous materials (Sinthusamran, Benjakul, & Kishimura, 2015). It has a wide range of applications in food, pharmaceutical, material, cosmetic and photographic industries (Benjakul, Oungbho, Visessanguan, Thiansilakul, & Roytrakul, 2009; Sinthusamran, Benjakul, & Kishimura, 2014). In recent year, fish gelatin has been considered as an alternative to bovine and porcine counterparts in order to avoid bovine spongiform encephalopathy and religious constraint (Abedinia, Ariffin, Huda, & Nafchi, 2017; Sinthusamran, Benjakul, & Hemar, 2016). Gelatins from various raw materials generally have different characteristic and gelling properties (Sinthusamran et al., 2014). Rheological and gelling properties are the major properties of gelatin, which are related to its structure and biochemical characteristics such as amino acid composition, molecular weight and the β/α ratio of chains, etc. (Karim & Bhat, 2009).

β-Glucan is a linear polysaccharide of β(1→3) and β(1→4) linked D-glucose molecules. It is a cell-wall polysaccharide present in barley and oats. It is found at small amount in some other cereals (Böhm & Kulicke, 1999). β-glucan is varied in the chain conformation and number of β(1→3) or β(1→4) linkage, which significantly affect its bioactivity (Ghavami, Goliaei, Taghizadeh, & Nikoofar, 2014). β-glucan exhibits health benefits such as reducing blood serum cholesterol, regulating blood glucose levels, using for the treatment of various cancers and possessing the immune modulatory activity as well as antioxidant activities (Kontogiorgos, Ritzoulis, Biliaderis, & Kasapis, 2006; Shah, Masoodi, Gani, & Ashwar, 2015). Some polysaccharide or dietary fiber can be used in foods as antioxidants agent, which could improve bioactivity in final food products (Alakhrash, Anyanwu, & Tahergorabi, 2016). Biological activities of polysaccharide depend on the molecular structure including molar mass, degree of branching and branch length (Shah et al., 2015). β-glucan is one of the polysaccharides possessing antioxidant activity (Johansson, Tuomainen, Ylinen, Ekholm, & Virkki, 2004). Surimi product with potential health benefits could be produced with addition of oat bran. Recently Alakhrash et al. (2016) reported that incorporation of oat bran up to

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6% (w/w) to surimi increased gel hardness of surimi and decreased cooking loss of surimi gel.

Incorporation of β-glucan into a gelling material, such as gelatin can be another promising approach to bring about the new product with health benefit, especially antioxidant properties. Nevertheless, the interaction between gelatin and β-glucan might affect the gelling and rheological properties of gelatin gel. No information regarding the rheological properties, gelling properties and antioxidative activity of gelatin mixed with β-glucan has been reported. Therefore, the aims of the study were to develop fish gelatin gel with increased antioxidant activity by incorporating β-glucan at various levels. Gastrointestinal tract model system was used to investigate antioxidative activity of the developed gels after ingestion.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade. Pepsin from porcine stomach mucosa (750 unit/mg dry matter), pancreatin from porcine pancreas (protease activity: 40 unit/mg, amylase activity: 5 unit/mg and lipase activity: 0.075 unit/mg), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,4,6-tripyridyl-triazine (TPTZ) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Amylase from *Bacillus subtilis* (20 unit/mg dry matter) was obtained from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Fish gelatin (FG) produced from tilapia skin (~240 bloom) was procured from Lapi Gelatine S.p.A (Empoli, Italy). β-Glucan (purity of 70%) was obtained from Hangzhou Asure Biotech Co, Ltd. (Hangzhou, China).

2.2. Preparation of fish gelatin/β-glucan mixed gels

Fish gelatin (FG) and β-glucan (BG) were separately solubilized in distilled water at 60 °C and 95 °C, respectively. The mixtures were stirred until the samples were fully solubilized. BG solution was mixed with FG solution to obtain 5, 10, 15 and 20% gelatin substitution. The resulting solutions were referred to as "FG-5BG", "FG-10BG", "FG-15BG" and "FG-20BG", respectively. FG solution without BG was also prepared and named as "FG". Total solid content of all mixtures was 6.67% (w/v). All solutions were transferred to a cylindrical polyvinyl chloride (PVC) mold with 30 mm diameter and 25 mm height. Those solutions were incubated at the refrigerated temperature (4 °C) for 18 h for gel maturation. All gel samples were subjected to analyses.

2.3. Analyses of gels

2.3.1. Measurement of gel strength

Gel strength was determined at 8–10 °C using a texture analyzer (Stable Micro System, Surrey, UK) with a load cell of 5 kg, cross-head speed of 1 mm/s, equipped with a 12.7 mm diameter flat-faced cylindrical Teflon® plunger. The maximum force (gram) was recorded, when the plunger had penetrated 4 mm into the gel samples.

2.3.2. Determination of gel color

The color of gel samples was measured by a Hunter lab colorimeter (Color Flex, Hunter Lab Inc., Reston, VA, USA). L^*, a^* and b^* values indicating lightness/brightness, redness/greenness and yellowness/blueness, respectively, were recorded. The colorimeter was warmed up for 10 min and calibrated with a white standard. Total difference in color (ΔE^*) was calculated using the following equation (Gennadios, Weller, Hanna, & Froning, 1996):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

where ΔL^*, Δa^* and Δb^* are the differences between the corresponding color parameter of the sample and that of FG gel ($L^* = 72.23$, $a^* = -1.54$ and $b^* = 10.17$).

2.3.3. Measurement of rheological properties

Rheological properties of FG solution and FG-BG mixed solutions were determined using a controlled stress rheometer (RheoStress RS 1, HAAKE, Karlsruhe, Germany). The measuring geometry used was a 60 mm parallel plate and the gap was set at 1.0 mm. Different solutions (2.9 ml) were loaded on the peltier plate. The excess sample was removed and its periphery was coated with paraffin oil to minimize the evaporation. The sample was initially maintained at 60 °C for 15 min for equilibration. Then the sample was cooled from 60 to 5 °C. The measurements were performed at a constant frequency of 1 Hz, a constant applied stress of 3 Pa and a constant temperature of 5 °C for 180 min. The elastic modulus G′ and the loss (viscous) modulus G″ were recorded.

Gelling and melting temperatures of all samples were measured following the method of Sinthusamran et al. (2014). The measurements were conducted at a constant frequency of 1 Hz, and a constant stress of 3 Pa. The samples were heated from 5 to 60 °C and subsequently cooled to 5 °C at a constant rate of 1.0 °C/min. The gelling and melting temperatures were calculated, where tan δ became 1 or δ was 45°.

2.3.4. Microstructure analysis

Microstructure of FG gel and FG-BG mixed gels (6.67%, w/v) was visualized using a scanning electron microscopy (SEM). Gelatin gels having a thickness of 2–3 mm were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 12 h. The samples were then rinsed with distilled water for 1 h and dehydrated in ethanol with a serial concentration of 50, 70, 80, 90 and 100% (v/v). Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA, USA). The specimens were visualized with a scanning electron microscope (JEOL JSM-5800 LV, Tokyo, Japan) at an acceleration voltage of 20 kV.

2.3.5. Study on antioxidative activities of gels

2.3.5.1. Preparation of gastrointestinal tract model system. Gastrointestinal tract model system was prepared according to the method of Lo, Farnworth, and Li-Chan (2006) with a slight modification. All gel samples were added with distilled water to obtain a concentration of 25 mg solid/ml. The mixture was homogenized at 8000 rpm for 3 min using a homogenizer (IKA Labortechnik homogenizer, Selangor, Malaysia). To mimic the human mouth conditions, the solution was adjusted to pH 7.2 using 1 M NaOH and the α-amylase dissolved in 0.1 M NaCl, 10 mM Na_2HPO_4 and 10 mM KH_2PO_4, pH 7.2 was added to obtain the final concentration of 20 mg/g sample. The mixture was incubated at 37 °C for 5 min with a continuous shaking (Memmert Model SV 1422, Schwabach, Germany). Then the solution was adjusted to pH 2.0 with 1 M HCl and pepsin dissolved in 0.1 M HCl was added to obtain the final concentration of 40 mg pepsin/g sample. The mixture was incubated at 37 °C for 1 h with a continuous shaking. Thereafter, the pH of the reaction mixture was raised to 5.3 with 1 M NaOH before the addition of 20 mg pancreatin/g sample. Subsequently, the pH of mixture was adjusted to 7.5 with 1 M NaOH. Then the mixture was incubated at 37 °C for 3 h with a continuous shaking. After being