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Characteristics and gelling property of phosphorylated gelatin from the skin of unicorn leatherjacket

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ABSTRACT

The characteristics and gelling property of gelatin from the skin of unicorn leatherjacket, phosphorylated with sodium tripolyphosphate (STPP) at various concentrations (0.25%, 0.50%, 0.75% and 1.00% w/w), for different times (1 and 3 h) at 65 °C, were studied. With the increase of STPP concentration and time, no increase in bound phosphate was observed. The highest gel strength was obtained for gelatin phosphorylated using 0.25% STPP for 1 h ($P < 0.05$). When the effect of pH (5, 7, 9 and 11) on phosphorylation and gel property of gelatin was investigated, gelatin phosphorylated at pH 9 had the highest gel strength (204.3 g) ($P < 0.05$) and exhibited a finer and more compact network structure with smaller pores. Gelatin became negatively charged (−3.89 mV) and might undergo an ionic interaction to a higher extent, thereby strengthening the gel network. Thus, the phosphorylation, under the appropriate condition, could improve the gelling property of gelatin from the skin of unicorn leatherjacket.

1. Introduction

Gelatin, the denatured form of collagen, has been widely used in the food and pharmaceutical industries, as well as for other technical applications (Kaewruang, Benjakul, & Prodpran, 2013b). Generally, gelatin is produced from the skin and skeleton of bovine and porcine (Kittiphattanabawon, Benjakul, Visessanguan, Nagai, & Tanaka, 2005). However, the occurrence of bovine spongiform encephalopathy (BSE) and foot-and-mouth disease (FMD) are of major concern to human health. Furthermore, porcine and bovine gelatins are prohibited in some religions (Sadowska, Kolodziejska, & Niecikowska, 2003). As a consequence, fish gelatin has gained increasing interest as the potential alternative for land animal counterpart. Fish skin, fin, scale and bones are the abundant by-products from the fish processing industry. They have been used as promising raw material for gelatin production (Ahmad & Benjakul, 2011). However, fish gelatins have lower gel strength, compared with mammalian counterpart, more likely due to the lower imino acid content (Foegeding, Lanier, & Hultin, 1996).

Phosphorylation has been used to improve the functional properties of food proteins. Water solubility, emulsifying activity and gel-forming properties of food proteins are improved by phosphorylation (Li, Ibrahim, Sugimoto, Hatta, & Aoki, 2004). Zhang, Li, and Ren (2007) reported that phosphorylation of the soy protein isolate with sodium tripolyphosphate (STPP) could improve its functional properties. Recently, Kaewruang, Benjakul, and Prodpran (2013a) reported that phosphorylation of gelatin by incorporation of STPP during extraction at an appropriate level was able to increase the gel strength of the resulting gelatin. Nevertheless, phosphorylation of gelatin might be governed by several factors, e.g. amount of phosphate and pH. Additionally, the level of phosphates bound to gelatin might affect gelation of the gelatin. Therefore, the objective of this investigation was to study the effect of the phosphate level, time and pH on phosphorylation and gel properties of gelatin from the skin of unicorn leatherjacket.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade. Sodium tripolyphosphate (STPP), ammonium molybdate and glutaraldehyde were purchased from Sigma (St Louis, MO, USA). Food grade bovine bone gelatin with the bloom strength of 150–250 g was obtained from Halagel (Thailand) Co., Ltd. (Bangkok, Thailand).

2.2. Collection and preparation of fish skin

The skin of unicorn leatherjacket (*Aluterus monoceros*) was obtained from a dock, Songkhla, Thailand, stored in ice with a skin/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 1 h. Upon arrival, the skin was washed with iced tap water (0–2 °C) and cut

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into small pieces (0.5 × 0.5 cm^2), placed in polyethylene bags and stored at −20 °C until use. The storage time was less than 2 months.

2.3. Pretreatment of skin

Removal of non-collagenous proteins and swelling of prepared skin were carried out according to the method of Ahmad and Benjakul (2011). Fish skin (0.5 × 0.5 cm^2) was soaked in 0.05 M NaOH with a skin/solution ratio of 1:10 (w/v) to remove non-collagenous proteins. The mixture was stirred continuously at room temperature using an overhead stirrer equipped with a propeller (RW 20.n, IKA Labortechnik, Germany) at a speed of 150 rpm. The alkaline solution was changed every 2 h for a total of 4 h. The alkaline-treated skin was washed with tap water until a neutral or faintly basic pH of wash water was obtained. For the swelling process, the alkaline-treated skin was soaked in 0.05 M phosphoric acid with a skin/solution ratio of 1:10 (w/v) with gentle stirring at room temperature. The acidic solution was changed every 3 h for a total of 6 h. The swollen skin was washed thoroughly with tap water until the wash water became neutral or faintly acidic.

2.4. Extraction of gelatin

The swollen skin was mixed with distilled water (65 °C) at a ratio of 1:5 (w/v). The mixture was incubated at 65 °C for 12 h in a temperature controlled water bath (Memmert, Schwabach, Germany) and stirred continuously at a speed of 150 rpm using the overhead stirrer equipped with a propeller. The mixture was centrifuged at 5000*g* for 10 min at 25 °C using a Beckman model Avanti J-E centrifuge (Beckman Coulter, Inc., Palo Alto, CA, USA) to remove insoluble material. The solution was freeze-dried using a Scanvac Model Coolsafe 55 freeze dryer (Coolsafe, Lynge, Denmark). The resulting gelatin was referred to as "control gelatin".

2.5. Phosphorylation of gelatin

2.5.1. Effect of STPP levels and times

To the gelatin solution, obtained after extraction, STPP was added to obtain final concentrations of 0.25%, 0.50%, 0.75% and 1.00% (w/w gelatin). All solutions were adjusted to pH 7 using 1 M HCl. The solution was continuously stirred at 65 °C for different times (1 and 3 h). The solution was cooled and freeze-dried. The gelatin samples were subjected to analyses of the phosphate content and gel strength.

2.5.2. Effect of pH values

To study the effect of pH on phosphorylation, gelatin solution containing 0.25% STPP (w/w gelatin) was adjusted to various pH values (5, 7, 9 and 11) using 1 M NaOH or 1 M HCl. The mixture was continuously stirred at 65 °C for 1 h. The solution was cooled and freeze-dried. Gelatins phosphorylated at different pHs were subjected to analyses of the phosphate content, zeta potential and gel strength. Gelatins phosphorylated at the selected pHs were determined for FTIR spectra and microstructure.

2.6. Analyses

2.6.1. Determination of inorganic phosphate content

To determine the bound phosphate, gelatin samples were dissolved in distilled water to obtain the concentration of 0.5% (w/v). Then, the gelatin solution was dialysed against 20 volumes of distilled water overnight. The dialysis water was changed every 3 h, for four times. The resulting dialysate was then freeze-dried. Both non-dialysed and dialysed samples were determined for phosphate content.

Inorganic phosphate content was determined according to the method of Fiske and Subbarow (1925) with a slight modification. Gelatin sample (~1 g) was ashed at 550 °C to remove the organic compounds using a muffle furnace (Fisher Scientific Model 550-58, Napean, Ontario, Canada), for 9 h. Thereafter, the inorganic residue was dissolved in 85% nitric acid. The solution was adjusted to 10 ml with distilled water. An aliquot of the solution was subjected to phosphate determination using a spectrophotometric method, in which ammonium molybdate was used and the coloured reaction mixture was monitored at the absorbance of 640 nm (Fiske & Subbarow, 1925). Potassium dihydrogen phosphate (KH_2PO_4) solutions with the concentration range of 0–1 mM were used for the standard curve preparation. Phosphate content was expressed as μmol/g gelatin.

2.6.2. Determination of gel strength

Gelatin gel was prepared as per the method of Kaewruang et al. (2013b). Gelatin sample was dissolved in distilled water (60 °C) to obtain the final concentration of 6.67% (w/v). The gelatin solution was stirred using a magnetic stirrer (IKA-Werke, GMBH & COKG, Staufen, Germany), at a medium speed, at room temperature until the gelatin was solubilised completely. The gelatin solution was cooled in a refrigerator at 10 °C for 16–18 h for gel maturation. The dimension of gel sample was 3 cm in diameter and 2.5 cm in height. The gel strength was determined using a Model TA-XT2 Texture Analyser (Stable Micro System, Surrey, UK) with a load cell of 5 kN and equipped with a 1.27 cm diameter flat faced cylindrical Teflon® plunger. The maximum force (in gram) was recorded when the penetration distance reached 4 mm. The speed of the plunger was 0.5 mm/s.

2.6.3. Measurement of the zeta potential

Gelatin samples were dissolved in distilled water at a concentration of 0.5 mg/ml. Prior to analysis, the samples were adjusted to pH 7. The zeta potential was measured using a zeta potential analyser (ZetaPALS, Brookhaven Instruments Co., Holtsville, NY, USA) at room temperature.

2.6.4. Fourier transform infrared (FTIR) spectroscopy

FTIR analysis was performed using a Bruker Model EQUINOX 55 FTIR spectrometer (Bruker, Ettlingen, Germany) equipped with a deuterated L-alanine triglycine sulphate (DLATGS) detector. The horizontal attenuated total reflectance (HATR) accessory was mounted in the sample compartment. The internal reflection crystal (Pike Technologies, Madison, WI, USA), made of zinc selenide, had a 45° angle of incidence from the IR beam. For spectra analysis, the samples were placed onto the crystal cell and the cell was clamped into the mount of the FTIR spectrometer. The spectra in the range of 650–4000 cm^{-1} with an automatic signal gain were collected and averaged for 32 scans, at a resolution of 4 cm^{-1}, and were ratioed against a background spectrum recorded from the clean empty cell at 25 °C. Analysis of spectral data was done using the OPUS 3.0 data collection software programme (Bruker, Ettlingen, Germany).

2.6.5. Microstructure analysis

The microstructures of gelatin gels were visualised using a scanning electron microscopy (SEM). Gelatin gels having a thickness of 2–3 mm were fixed with 2.5% (v/v) glutaraldehyde for 12 h. The samples were then rinsed with distilled water for 1 h and dehydrated in ethanol solution 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 observed using a scanning electron microscope (Quanta 400, FEI, Eindhoven, Netherlands) at an acceleration voltage of 10 kV.