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Impact of divalent salts and bovine gelatin on gel properties of phosphorylated gelatin from the skin of unicorn leatherjacket

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ABSTRACT

The impact of zinc chloride ($ZnCl_2$) and calcium chloride ($CaCl_2$) as well as bovine gelatin (BG) on the gel strength of phosphorylated fish gelatin (PFG) from the skin of unicorn leatherjacket was investigated. The gel strength of PFG increased with increasing concentrations of $ZnCl_2$ and $CaCl_2$ (2.5–40 μmol L^{-1}). A higher gel strength was observed with $CaCl_2$, compared with $ZnCl_2$. The gel strength of PFG with 20 μmol L^{-1} $CaCl_2$ increased by 15.7%, compared to the control gel. Nevertheless, at higher concentration (40 μmol L^{-1}) of both salts, gel strength of PFG decreased. Hardness of gels decreased with increasing PFG content ($P < 0.05$). Nevertheless, no differences in hardness were found amongst gels with BG/PFG ratios of 4:0 and 3:1 ($P \geq 0.05$). Thus, PFG could be used in combination with $CaCl_2$ to substitute for BG at a level of 25%.

1. Introduction

Gelatin is a protein derived from collagen by thermal denaturation or partial hydrolysis. It has the rheological properties of a thermo-reversible transformation between solution (sol) and gel form. Gelatin is composed of long chains of peptides (Rahman & Al-Mahrouqi, 2009). However, gelatin is almost completely lacking tryptophan and is low in methionine, cystine and tyrosine (Jamilah & Harvinder, 2002). The quality of gelatin depends on its physical, chemical and structural characteristics. The most important physical properties of gelatin are gel strength and viscosity (Karim & Bhat, 2009). The quality of gelatin is measured as the gel strength or Bloom value, which can be classified as low (<150), medium (150–220) and high Bloom (220–300) (Johnston-Banks, 1990).

Alternative gelatins have gained increasing attention in recent years as the demand for non-bovine and non-porcine gelatins has increased due to the BSE (bovine spongiform encephalopathy) crisis as well as religious and social reasons. Pig skin gelatin is not acceptable in Judaism and Islam and beef gelatin is acceptable only if it has been prepared according to religious requirements (Badii & Howell, 2006). As a consequence, fish gelatin has been of increasing interest as a potential alternative. Nevertheless, fish gelatins generally have poorer gelling property, compared with bovine and porcine gelatins. Therefore, the improvement of the gel properties of gelatin from marine sources is needed, so that it can be used for a wider range of applications as a food ingredient.

Phosphorylation of gelatin can be a means to improve gel properties, in which phosphate could provide negatively charged domain for protein aggregation (Guo, Li, Zhu, & Zhao, 2005; Kaewruang, Benjakul, & Prodpran, 2014a, 2014b). Network formation via salt-mediated interactions of the soluble proteins can take place at a low temperature, depending on the types of protein used and gelation time required (Hongsprabhas & Barbut, 1997). Kaewruang et al. (2014a, 2014b) reported that the phosphorylation of gelatin using STPP (0.25 g 100 g^{-1} gelatin) at pH 9 for 1 h gave a gelatin with improved gel strength. To augment the salt bridge between charged residues of phosphorylated gelatin, the introduction of divalent cations should be beneficial. Divalent cations have a much greater effect on the gel properties than monovalent cations (Tang, Tung, & Zeng, 1995). This is because divalent salt ions screen electrostatic interactions between the charged protein molecules (Yasuda, Nakamura, & Hayakawa, 1986). Additionally, the mixing of fish gelatin with mammalian gelatin at an appropriate

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ratio might bring about improved gelling properties of the mixture. Recently, Kaewudom, Benjakul, and Kijroongrojana (2012) reported that a mixture of fish gelatin and bovine gelatin at a ratio of 1:1 or 1:2 in conjunction with microbial transglutaminase (MTGase) could make surimi gels from threadfin bream with acceptability equivalent to the control surimi gel (without gelatin addition).

The objectives of this study were to evaluate the effect of type and concentration of divalent salts on gel property of phosphorylated fish gelatin (PFG) from unicorn leatherjacket skin and to study the impact of bovine gelatin (BG)/PFG ratios on the textural properties of the resulting mixed gelatins.

2. Materials and methods

2.1. Chemicals/gelatin

All chemicals were of analytical grade. Sodium tripolyphosphate (STPP) was purchased from Sigma (St Louis, MO, USA). Zinc chloride ($ZnCl_2$) and calcium chloride ($CaCl_2$) were obtained from Lab-Scan (Bangkok, Hat Yai, Thailand). Food grade bovine bone gelatin with a Bloom strength of 150–250 g (according to the manufacturer) 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, in Songkhla, Thailand. The fish (250–330 g fish^{-1}) were deskinned manually by the workers after off-loading, which was approximately 48 h after capture. The skin with attached scales was stored in ice with the approximately 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. Three different lots of skin (4–5 kg each) were obtained during January and March, 2013. Upon arrival, the skin with scales was washed with iced tap water (0–2 °C) and cut into small pieces (0.5 × 0.5 cm^2) with a scissor, placed in polyethylene bags and stored at −20 °C until use. The storage time was less than 2 months.

2.3. Preparation of phosphorylated fish gelatin (PFG)

2.3.1. Pretreatment of skin

Removal of non-collagenous proteins and swelling of prepared skin were carried out according to the method of Kaewruang et al. (2014a, 2014b). Fish skin (0.5 × 0.5 cm^2) was soaked in 0.05 mol L^{-1} NaOH with a skin/solution ratio of 1:10 (*w*/*v*) to remove non-collagenous proteins. The mixture was stirred continuously at room temperature (28–32 °C) using an overhead stirrer equipped with a propeller (RW 20.n, IKA Labortechnik, Staufen, Germany) at a speed of 150 rpm. The alkaline solution was changed every 2 h for a total of 4 h. Alkaline-treated skin was washed with tap water until a neutral or faintly basic pH wash water was obtained (pH 7.2–7.8) using a pH metre (Schott, Mainz, Germany). For the swelling process, the alkaline-treated skin was soaked in 0.05 mol L^{-1} phosphoric acid with a skin/solution ratio of 1:10 (*w*/*v*) with a gentle stirring at a speed of 150 rpm using the overhead stirrer at room temperature. The acidic solution was changed every 3 h for a total of 6 h. Swollen skin was washed thoroughly with tap water until the wash water had neutral or faintly acidic pH (pH 6–7) as measured by the pH metre.

2.3.2. 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. 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 materials. Soluble gelatin was then phosphorylated.

2.3.3. Phosphorylation of gelatin

The phosphorylation of gelatin was carried out according to the method of Kaewruang et al. (2014a, 2014b). STPP was added to the gelatin solution to obtain a final concentration of 0.25 g 100 g^{-1} gelatin. The solution was adjusted to pH 9 using 1 mol L^{-1} NaOH using the pH metre. The solution was continuously stirred at 65 °C for 1 h. The solution was cooled, frozen and freeze-dried using a Scanvac Model Coolsafe 55 freeze dryer (Coolsafe, Lynge, Denmark).

2.4. Study on the effect of $ZnCl_2$ and $CaCl_2$ at different concentrations on the gel properties of PFG

The freeze-dried PFG was dissolved when needed (less than 2 months) in distilled water at 60 °C to obtain a final concentration of 6.67 g 100 mL^{-1}. The gelatin solution was stirred until the gelatin was solubilised completely. $ZnCl_2$ or $CaCl_2$ was added to the gelatin solutions to obtain the different final concentrations (2.5, 5, 10, 20 and 40 μmol L^{-1}). PFG without the addition of divalent salt was used as the control. The solution was continuously stirred using a magnetic stirrer at a low speed at room temperature for 15 min and cooled in a refrigerator at 10 °C for 16 h for gel maturation. All gel samples were then subjected to the analyses.

2.5. Study on the effect of bovine gelatin/PFG ratios on the gel properties of mixed gelatin

Bovine gelatin (BG) was mixed with PFG at different ratios (BG/PFG = 4:0, 3:1, 2:2, 1:3 and 0:4). Solutions of mixed gelatins at various BG/PFG ratios (6.67 g 100 mL^{-1}) were prepared as previously described. Thereafter, $CaCl_2$ was added at a final concentration of 20 μmol L^{-1}. The gel samples were incubated at 10 °C for 16 h for gel maturation. The resulting gelatin gels were subjected to the analyses.

2.6. Analyses

2.6.1. Determination of gel strength

The gelatin gel at a concentration of 6.67 g 100 mL^{-1} was prepared as per the method of Kaewruang et al. (2014a, 2014b) as described above. The gelatin solution was transferred to a cylindrical polyvinylchloride mould (diameter of 3 cm and height of 2.5 cm). After setting or maturation, 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 g) was recorded when the penetration distance reached 4 mm. The speed of the plunger was 0.5 mm s^{-1}.

2.6.2. Texture profile analysis

TPA was measured using the Texture Analyser. Gelatin gel samples that were 3 cm in diameter and 2.5 cm in height were used after careful removal from the moulds. The gels were compressed by an aluminum plate (100 mm diameter) until the deformation reached 30% at a speed of 1.0 mm s^{-1}. The pause between the first and second compressions was 3 s. The testing was done immediately after the samples were removed from the refrigerator (~4 °C). Hardness, brittleness, springiness, cohesiveness, adhesiveness, gumminess and chewiness of samples were recorded (Yang, Wang, Zhou, & Regenstein, 2008).