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Effects of salt and sugar addition on the physicochemical properties and nanostructure of fish gelatin

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ABSTRACT

Application of fish gelatin as a food component in replace of mammalian sources has attracted attentions recently. However, physicochemical properties of fish gelatin might be affected by other food components thus affecting its application. To determine whether and how sugar and salt components in food affect the physicochemical properties of fish gelatin, nanostructure of tilapia fish gelatin was studied by atomic force microscopy (AFM) with the secondary structure investigated by Fourier transform infrared (FTIR) spectroscopy. The results indicated that 1.5% NaCl addition led to a loss in molecular order in secondary structure which was accompanied with reducted gel strength; however, addition of 1.5% sucrose did not affect physicochemical and structural properties of fish gelatin. Fish gelatin possessed heterogeneous nanostructure including spherical aggregates, ring like structure, short and long rods as well as continuous fibre network. Incorporation of NaCl with fish gelatin increased diameter of spherical aggregates to more than two folds of control. These data suggest that addition of NaCl reduced gel strength through inducing large nano-aggregates, which could be at improper alignment that prevented the formation of a rigid gel. Interestingly, the fish skin gelatin studied here showed good storage stability over 30 days of storage at 4 °C. Sodium chloride affects fish gelatin's nanostructure and physicochemical properties more than sucrose at the same concentration.

1. Introduction

Currently, about 98.5% of world gelatin production is extracted from cattle hides, beef bones and pork skin (Karim & Bhat, 2009). Applications of gelatin from pork and beef byproducts have religious restrictions as well as food safety concern because these gelatin may be contaminated by pathogenic vectors such as prions from the diseased animal (Karim & Bhat, 2009). Due to these concerns alternative sources of gelatin such as gelatin extracted from fish would be valuable and has attracted many interests in recent years.

To expand the application of fish gelatin in food, the understanding of how properties of fish gelatin are impacted by common food components such as salt and sugar is important. In food application, both salt and sugar are common solutes present in most of the formulations. The level of sugar present in food could range from high in dessert and confectionary (more than 10%) to a low concentration as what exists in pasta sauces, soups, meat and ham (less than 5%) (USDA, 2013). While the addition of salt is often self-limited due to the intense salty taste, the concentration of salt in restaurant food, meals and fast food could range from less than 0.1% to as high as 2.15% (USDA, 2013).

The effect of solute addition on gelatin has been studied in terms of physicochemical properties. The solutes that have been studied include electrolytes such as NaCl (Haug, Draget, & Smidsrød, 2004), $MgCl_2$ and $MgSO_4$ (Sarabia, Gomez-Guillen, & Montero, 2000), $CaCl_2$ and phosphate salt (Kaewruang, Benjakul, Prodpran, Encarnacion, & Nalinanon, 2014), and non-electrolytes such as sucrose (Choi, Lim, & Yoo, 2004; Choi & Regenstein, 2000; Koli, Basu, Nayak, Kannuchamy, & Gudipati, 2011). It was proposed that electrolytes such as salts could affect gelatin via modification of the electrostatic forces and formation of salt bridges (Kaewruang et al., 2014), while non-electrolyte such as sugars could affect gelatin gel properties due to the hydration effect or stabilising of hydrogen bond (Choi et al., 2004). However the observed changes in physical properties of fish gelatin due to solute addition have not been fully understood especially the underlying mechanism.

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As a thermo reversible gel, gelation of gelatin is induced by transiting coil to helix below gelling temperature, the individual α-chain could be partially renatured to triple helix like that occurred in collagen, which serves as junction zones cross-linked by flexible peptide chains (Haug et al., 2004). The structure of gelatin is affected by distribution of polypeptide fragments when collagen is partially hydrolysed into gelatin, the subsequent aggregation of the peptide fractions as well as the inherent amino acid composition (Yang, Wang, Regenstein, & Rouse, 2007). Structural studies of gelatin have been conducted using different methodologies. The secondary structure of gelatin such as the relative proportion of helix and coil could be determined using Fourier transform infrared spectroscopy (FTIR) (Ahmad & Benjakul, 2011; Muyonga, Cole, & Duodu, 2004) and circular dichroism (Giménez, Turnay, Lizarbe, Montero, & Gómez-Guillén, 2005). The X-ray diffraction analysis has been used to determine the fibril distribution (Zhang, Xu, & Wang, 2011). However, these methods provide a sample-wide average information of gelatin structure (Feng, Lai, & Yang, 2014; Yang & Wang, 2009).

Atomic force microscope (AFM) has been successfully applied to investigate detailed nanostructure of fish gelatin that had not been sophisticatedly prepared (Yang & Wang, 2009; Yang, Wang, Regenstein, et al., 2007). Nanostructure of colloid is closely correlated to its physical properties including stability, diffusivity and permeability (Díaz-Calderón, Caballero, Melo, & Enrione, 2014).

The objectives of this study were to investigate whether and how the addition of salt and sugar at low concentration (1.5%, w/w) affect the physicochemical properties of fish gelatin. The physicochemical properties including texture and viscosity were analysed together with gelatin's secondary structure and nanostructure in order to elucidate the underlying mechanism of physicochemical property changes. These results could be extended to understand the effects of other components on the properties of fish gelatin.

2. Materials and methods

2.1. Sample preparation

Commercial tilapia fish gelatin (180 Bloom) was purchased from Jiangxi Cosen Biology Co., Ltd (Yingtan, Jiangxi, China). The gelatin contained 83.14% protein, 0.68% ash, 9.12% moisture and 7.06% of other substances. Three groups of samples were prepared, i.e. control sample containing fish gelatin only (FG), fish gelatin with NaCl (FGN), fish gelatin with sucrose (FGS). Fish gelatin solution (6.67%, w/w) was prepared following the method modified from Yang and Wang (2009). Gelatin was soaked in distilled water until completely swollen, heated and stirred in a 65 °C water bath for 10 min. Sodium nitrite was added into the gelatin solution as antimicrobial agents at level of 0.1% to prevent microbial spoilage. To examine the effect of added solutes on gelatin gels, sucrose and NaCl were dry blended with fish gelatin powder before hydration, the final concentration of the solutes in gelatin solution was at 1.5% w/w. After heating, the solutions were immediately filled into a small cylindrical-shaped flat bottom plastic container (31 mm diameter × 25 mm height). The solution was then stored at 10 ± 2 °C for 17 ± 1 h to ensure the gels had matured evenly, and considered as sample at day 0. Subsequent storage of the gelatin gels were carried out at 4 ± 1 °C for 30 days.

2.2. Gel strength & texture profile analysis (TPA)

Day 0 results were obtained immediately after gel maturation at 10 °C for 17 ± 1 h. For subsequent storage time, gelatin gels were removed from the refrigerators and equilibrated to 10 °C for at least 0.5 h in cold water bath prior to measurement. Gel strength was determined by a TA.XT2-i Texture Analyser (Stable Micro System, Goldaming, Surrey, UK). For gel strength analysis, a 0.5″ radius cylinder probe (P/0.5R) was used to penetrate 4 mm into the gelatin gel at a speed of 0.5 mm/s. Gel strength of gelatin was defined as the maximum force required to penetrate 4 mm of gel, and recorded in unit of g (Yang & Wang, 2009). While for TPA, the gel sample was subject to two cycle compression to 40% of its original height with a flat cylindrical probe (47 mm). The detailed test settings were pre-test speed: 1.0 mm/s; Test speed: 0.5 mm/s; Target mode: Distance; Distance of compression: 12.4 mm (40% of original gel height); Time: 10.0 s; Trigger type: Auto (Force); Trigger Force: 0.05 N; Tare mode: Auto; and Advanced Options: On. Hardness, cohesiveness, springiness and chewiness were calculated from TPA curve according to the definition as described in Yang, Wang, Jiang, et al. (2007).

2.3. Viscosity

Fish gelatin gel was melt in a 60 °C water bath for less than 1 h. The viscosity of gelatin solution was measured at 60 °C using a Brookfield DV II + viscometer (Brookfield Engineering, Middleboro, MA, USA) equipped with No. 1 spindle at 100 rpm rotation. The gelatin solution was filled in a sample holding tube that was connected with circulated water bath which was set at 60 °C to maintain the temperature during measurement.

2.4. Fourier transform infrared (FTIR) spectroscopy

The gelatin gels were taken out after respective storage time and freeze dried for FTIR analysis. The freeze dried gelatin was milled into powder and grinded with KBr powder (Merck KGaA, Damstadt, Germany) at a ratio of 3 mg of gelatin to 100 mg of KBr. The KBr powder was stored and dried at 120 °C to eliminate moisture absorpted. The pellet was examined using a Spectrum One FTIR spectrometer (PerkinElmer, Waltham, MA, USA). The scan was conducted between 4000 and 450 cm^{-1} with resolution of 4 cm^{-1}. The background spectrum was collected before each scan. For each sample, at least triplicate of spectra were obtained. The spectra of a same sample with an average of 32 scans were smoothened, baseline corrected, normalised and averaged for qualitative interpretation of spectra.

For peak height and location of peak, amide A, amide I, amide II and amide III were selected for quantitative measurement of maximum peak height and location (wavenumber) using Spectrum software (version 5.0.1, PerkinElmer). Baseline of each band was defined by software, and the corrected peak height was taken as the absorbance difference from the peak to the baseline.

Deconvolution of amide I was also studied for further quantitative analysis. The spectra region between 1720 and 1590 cm^{-1} was selected as amide I band, of which each unprocessed spectra were cut and baseline corrected. Fourier self deconvolution was performed using the Spectrum software (version 5.0.1, PerkinElmer) with line narrowing factor, gamma, set at 1.0 and the smoothing length width set at 50%–60% by using Bessel type smoothing function. The deconvoluted amide I band was then fitted using Origin Pro 9 (OriginLab, Northampton, MA, USA). Gaussian curve fitting function was employed, using procedure described in Byler and Susi (1986) with slightly adjustment. The iteration was performed until the fit converged. The final fitting quality of the curve had corrected R^2 value greater than 0.99. The component peaks identified after deconvolution and curve fitting were compared to literature; the percent contribution of specific component was calculated by the area of the component peak divided by the total area of amide I band before deconvolution (Byler & Susi, 1986).