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Physicochemical properties and film-forming ability of fish skin collagen extracted from different freshwater species

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ABSTRACT

Acid soluble collagens (ASCs) were extracted from tilapia, grass carp and silver carp skins, and the physicochemical properties and film-forming ability of ASCs were examined. Similar amino acid composition and electrophoretic patterns were observed in the obtained skin ASCs, but differences were found in the peptide maps of collagen digested by V8 protease, trypsin and lysyl endopeptidase. The reduced viscosity value of tilapia skin ASC was higher than that of grass carp and silver carp skin ASCs at 25 °C. Furthermore, the tensile strength of films based tilapia skin collagen was 51.24 MPa, and much higher than that of films based grass carp or silver carp skin collagen. On the other hand, the nucleation time of fibril formation of skin collagen from tilapia, grass carp and silver carp was about 2 min, 6 min and 3 min, respectively. The obvious coarser microstructure was found on the upper surface of films prepared from grass carp skin collagen by scanning electron microscopy. It is concluded that difference in the film-forming ability of collagen from freshwater fish skins could be correlated with the primary structures and conformation of collagen molecules.

1. Introduction

In recent years, biodegradable polymers based on proteins, polysaccharides and lipids as well as combinations of them have got increasing interest [1]. Among them, collagen-based films have been considered as very successful commercially natural polymer materials, which are mainly used for packaging material of meat product or sausage casing in the food industry, and drug delivery carriers or wound dressings in the medical fields [2,3].

With the outbreak of diseases such as bovine spongiform encephalopathy (BSE) and foot/mouth diseases, collagen from fish processing wastes has received increasing considerable attention as a safe and attractive alternate to mammal collagen [4]. In order to take advantage of fish collagen, extraction and functional characterization of acid- and/or pepsin-soluble collagen has been reported for different fish species recently, such as surf smelt skins, rohu and catla scales, deep-sea redfish skins, black carp skins, marine eel-fish skins and Amur sturgeon cartilage [5–10]. The physical and chemical properties of collagen from different fish species were found to mainly depend on their living environments [11]. It was reported that intrinsic viscosities of warm-water fish collagen were higher than that of cold-water fish collagen [12]. The denaturation temperature (Td) of collagens from rohu scale and black carp skins were very close to that of porcine skin collagen [6,8], indicating that freshwater fish collagen was potential alternative to replace mammalian collagen as biopolymer materials. In addition, slight difference was found in amino acid composition and amino acid sequence of the collagens from skins and bones of bigeye snapper [4]. Different imino acid content with similar thermal behavior was found between dusky spinefoot skin collagen and sea chub skin collagen although both of them are marine species [13]. However, comparative study of physicochemical properties and film-forming ability of collagen from different freshwater fish has rarely reported.

Tilapia (*Oreochromis niloticus*), grass carp (*Ctenopharyngodon idella*) and silver carp (*Hypophthalmichthys molitrix*) are common freshwater fish species in China, altogether accounting for 42.9%

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of the total freshwater fish production [14]. Great amounts of fish skins are generated in fish processing factories, causing pollutions to environment if they were not used effectively. Therefore, it is quite meaningful to clarify the physicochemical properties of collagen from different freshwater fish for using fish collagen to replace mammal collagen in some applications in the future.

Collagens extracted from various freshwater fish skins have received considerable attention recently. It was reported by Potaros et al. [15] that the biochemical properties and thermal stability of acid-solubilized collagen (ASC) and pepsin-solubilized collagen (PSC) from Nile tilapia skins were compared. The effect of pHs and salt concentrations on tilapia skin collagen solubility has been investigated, revealing that the ASC was soluble at acidic pH and lost the solubility when salt concentrations increased [16]. Wang et al. [17] investigated the effects of acetic acid concentration, temperature, time, solvent to material ratio on the extraction efficiency of collagen from grass carp skins and focused to obtain optimum extraction conditions. It was also reported by Zhang et al. [18] that the ASC from silver carp skins was isolated and some properties such as denaturation temperature and subunit composition were analyzed. However, strict comparisons among these physicochemical properties of fish skin collagens are difficult since methodologies may differ from one work to another. Therefore, it is necessary to investigate the physicochemical properties of skin collagen from tilapia, grass carp and silver carp in same manner.

The present study was firstly undertaken to extract collagen from the skins of three freshwater fish, tilapia, silver carp and grass carp. Secondly, effect of fish species on the physiochemical properties and film-forming ability of collagen was also investigated.

2. Materials and methods

2.1. Materials

Tilapia (0.50 ± 0.05 kg), grass carp (0.73 ± 0.06 kg) and silver carp (0.80 ± 0.10 kg) were purchased alive from local fish stall. Upon arrival, fish were washed using tap water and deskined. The scales and residual meats in the fish skins were removed manually. The obtained skins were stored at −18 °C until use. All other chemicals were analytical grade.

2.2. Extraction of acid-solubilized collagen

The collagen was extracted from fish skins according to the method described by Kittiphattanabawon et al. [4] with some modifications. All procedures were carried out at 4 °C. To remove non-collagenous proteins, fish skins were soaked in 0.1 M NaOH with a skin/solution ratio of 1:10 (w/v) for 36 h, and alkali solution was changed every 12 h. The treated skins were then washed with cold tap water to neutral pH. The deproteinized skins were then defatted using 10% butyl alcohol with a solid/solvent ratio of 1:10 (w/v) for 36 h and the solvent was changed every 12 h. Defatted skins were washed with cold tap water and subsequentially extracted with 0.5 M acetic acid with a skin/acetic acid ratio of 1:30 (w/v) for 2 days. The insoluble components were separated with double layers of gauze and the filtrate was centrifuged at 4 °C, 10,000 × *g* for 30 min. The obtained supernatant was precipitated by adding NaCl to a final concentration of 2.6 M. The resulting sediment was collected by centrifuging at 4 °C, 10,000 × *g* for 30 min. The pellet was dissolved in minimum volume of 0.5 M acetic acid, and dialyzed against 0.1 M acetic acid, followed by distilled water. The dialysate was freeze dried, and the dry collagen was stored at −18 °C until use.

2.3. Characterization of acid-solubilized collagen

2.3.1. Amino acid composition

Raw fish skins and collagen samples were hydrolyzed in 6 M HCl at 110 °C for 22 h in presence of 0.1% phenol. After hydrolysis, HCl was removed using rotary evaporator (model RE-52AA, Shanghai YaRong Biochemistry Instrument Factory, Shanghai, China) at 60 °C. When the solution was evaporated, the residue was dissolved in 0.02 M HCl and filtered through 0.22 μm membrane filter. The amino acid composition of an aliquot was analyzed by a HITACHI model 835-50 high speed amino acid analyzer (HITACHI Co., Tokyo, Japan). The content of hydroxyproline in the same sample was determined by a hydroxyproline assay kit (Nanjing JianCheng Bioengineering Institute, Nanjing, China). All determinations were carried out at least in triplicate.

2.3.2. Peptide mapping

Peptide mapping of collagen samples was performed according to the method reported by Kittiphattanabawon et al. [4] with a slight modification. V8 protease (EC 3.4.21.19, Sigma Chemical Co., St. Louis, MO, USA), lysyl endopeptidase (EC 3.4.21.50, Wako Pure Chemical Industries, Ltd., Tokyo, Japan) and trypsin (EC 3.4.21.4, 1:250, Amresco LLC, USA) were utilized for digestion in this study. The collagen samples were dissolved in 0.1 M sodium phosphate (pH 7.2), containing 0.5% SDS (w/v) to a final concentration of 5 mg/ml. The V8 protease digestion and lysyl endopeptidase digestion were carried out at 37 °C for 30 min at enzyme/substrate ratio of 1/100 (w/w) and 1/1000 (w/w), respectively. In the case of digestion by trypsin, the collagen was dissolved in 20 mM Tris–HCl (pH 7.0), containing 0.5% (w/v) SDS and digested at 37 °C for 30 min with an enzyme/sample ratio of 1/20 (w/w). The reaction mixtures were then subjected to boiling water for 5 min to terminate the digestion. Peptides were analyzed by SDS–PAGE using 10% separating gel and 4% stacking gel.

2.3.3. Determination of denaturation temperature

The denaturation temperature (Td) was measured from viscosity changes according to the method described by Nagai et al. [5] with an Ubbelohde viscometer (Shanghai Glass Instrument Institute, Shanghai, China). The viscometer was filled with 15 ml 0.1% (w/v) collagen solution in 0.1 M acetic acid. After incubation in a water bath at each designed temperature (15–45 °C) for 30 min, efflux time (t) of collagen solution was measured at those temperatures. The efflux time (t_0) of 0.1 M acetic acid was also determined under the same conditions. All determinations were carried out at least in quintuplicate. The specific viscosity (η_{sp}) and reduced viscosity (η_{re}) were calculated by the following equations:

$$\eta_{sp} = \frac{t - t_0}{t_0}$$

$$\eta_{re} = \frac{\eta_{sp}}{c} (\mathrm{dL/g})$$

where c is the concentration of collagen. The Td is the temperature at which the change of reduced viscosity reached by 50%.

On the other hand, the thermal transition of collagen was measured by differential scanning calorimetry (DSC-1, Mettler Toledo instruments Co., Zurich, Switzerland). The lyophilized collagen samples were dissolved in a 0.05 M acetic acid solution with a sample/solution ratio of 1:40 (w/v). About ten milligram rehydrated samples were accurately weighed into aluminum pans and scanned over a range of 15–45 °C at a heating rate of 5 °C/min. An empty pan was used as the reference.