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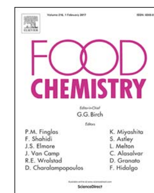

Sequential extraction of gel-forming proteins, collagen and collagen hydrolysate from gutted silver carp (*Hypophthalmichthys molitrix*), a biorefinery approach

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ABSTRACT

Collagen and collagen hydrolysate (CH) was recovered from the bone and skin containing sediment residue emerging during pH-shift-based protein isolation from silver carp. Hydrolysis resulted in higher yield (15.1–15.4%) compared to collagen isolation by acid or pepsin (3.1–5.9%) ($p < 0.05$). Isolated collagens were characterized as type I and maintained their triple-helical structure, confirmed by SDS-PAGE and FTIR. Pepsin-hydrolysis and sequential hydrolysis by pepsin and trypsin hydrolyzed all heavy molecular weight chains of collagen but sequential hydrolysis yielded higher degree of hydrolysis. When CH was added to a silver carp protein isolate prior to gelation, the gel behavior was dependent on molecular weight of the added CH. More hydrolyzed collagen emerging from sequential hydrolysis improved water holding capacity of the gel while reducing its breaking force. Thus, residue from pH-shift processing of fish can be used for isolation of high quality collagen/CH and provides a promising basis for a multiple-product fish biorefinery.

1. Introduction

Increasing demand for seafood products in parallel with global population growth and ongoing wild fish supply decline has caused great interest in using aquaculture species and seafood processing by-products as protein sources for human consumption (Gehring, Gigliotti, Moritz, Tou, & Jaczynski, 2011). Silver carp provides one of the most abundant biomasses of farmed aquatic resources in the world. However, processing of silver carp with conventional methods like mechanical meat-bone separation and washing of minced fillets results in high amount of by-products (>80% w/w) (Paker, Beamer, Jaczynski, & Matak, 2013). Further, even with meat-bone separator it is difficult to remove impurities (e.g. bones) due to the bony nature of the carp body (Shi et al., 2017).

In this regard, acid and/or alkaline solubilization followed by isoelectric precipitation, also called the pH-shift processing (Hultin & Kelleher, 1999; Abdollahi, Marmon, Chaijan, & Undeland, 2016; Hultin et al., 2001), has been successfully recognized as a promising technique for direct protein recovery from unconventional complex aquatic raw materials, including gutted fish (Abdollahi, Rezaei, Jafarpour, & Undeland, 2017) and seafood processing by-products (Chen & Jaczynski, 2007). The process involves selectively extracting proteins from homogenized raw material in water using a high (>10.5) or a low (<3.5) pH to solubilize the muscle proteins followed by centrifugation to separate the solubilized proteins from high and low density undissolved material. The solubilized protein is then recovered using isoelectric precipitation (usually pH 5.5) and dewatered by centrifugation or filtration. Since pH-shift processing does not require a pre-filleting step, and since it recovers both myofibrillar and sarcoplasmic proteins, proteins yields generally become higher with this method compared to methods based on mincing and washing (Nolsøe & Undeland, 2009). However, other fractions produced, as the fat layer and bone and skin containing sediment residue emerging during the first centrifugation also have great potential for recovering higher value products for food application. For example, it was shown that that sediments produced after the first centrifugation during the pH-shift processing of gutted silver carp (Taskaya, Chen, Beamer, Tou, & Jaczynski, 2009) and trout by-products (Chen, Tou, & Jaczynski, 2007) still contain 42–67% of protein. When using gutted fish or fish by-products, the sediment mainly contains collagenous residues including bone, skin and connective tissue that seems to be a great source for isolation of fish collagen or collagen hydrolysate.

Collagen is made of a three polypeptide chain and is the major structural protein in vertebrate body constituting about 30% of their

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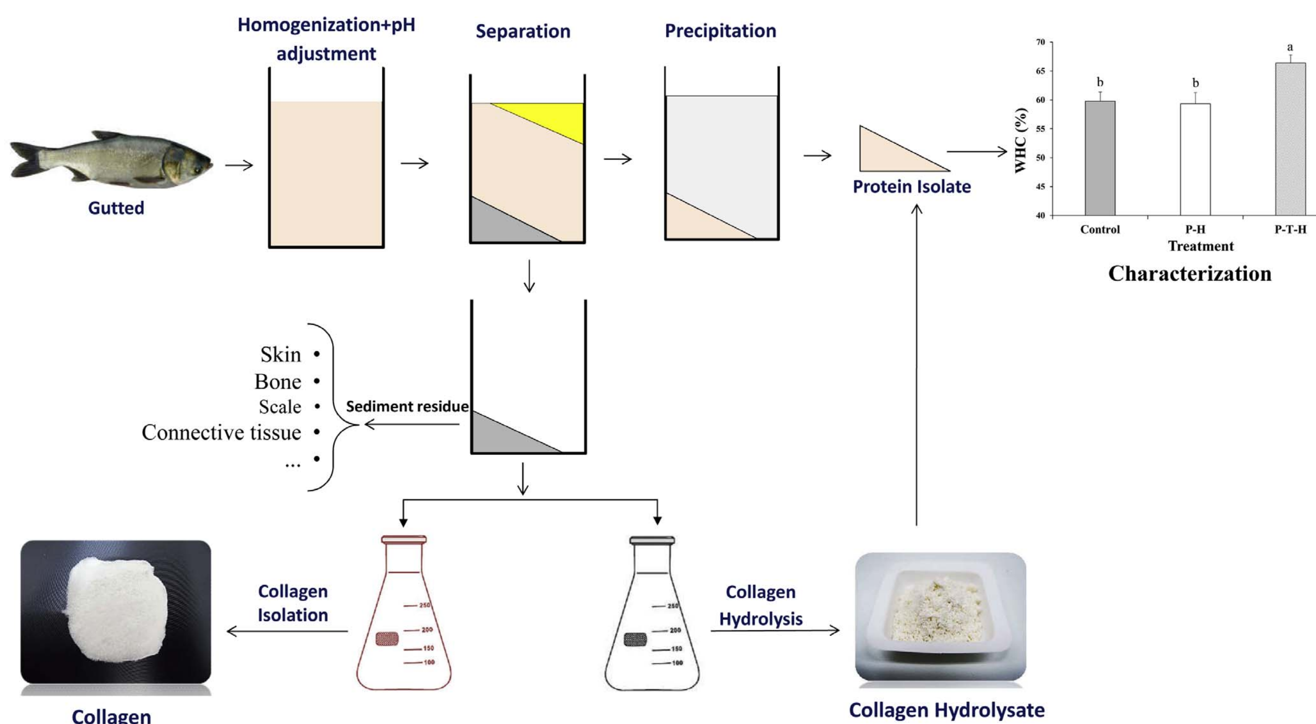

Fig. 1. Schematic overview of production of fish protein isolate and the solid sediment residue using the pH-shift process with subsequent collagen and collagen hydrolysate production from the residue.

total protein. Collagen, gelatin and their hydrolysates are widely utilized in various fields and industries including food, pharmaceutical, cosmetic, biomedical and leather. Nowadays, animal by-products like skin and bone from pig, bovine and chicken skin and bone are the main sources of commercial collagen. However, some health concerns related to outbreak of bovine sponge encephalopathy, foot-and-mouth disease and avian influenza together with religious limitations for application of pig and bovine collagen in kosher and halal foods, have caused great interest in finding alternative collagen resources (Liu et al., 2015).

Among the alternatives, fisheries by-products have shown great potential due to high availability an less religious limitations and relatively proper collagen yield (Li et al., 2013). Previous studies have reported when using different fisheries by-products including skin (Muyonga, Cole, & Duodu, 2004a), bone (Duan, Zhang, Du, Yao, & Konno, 2009; Li et al., 2013), scale (Chuaychan, Benjakul, & Kishimura, 2015; Liu et al., 2015;), and swim bladder (Liu et al., 2015), depending on by-product type, biochemical and functional properties of the recovered collagen have however differed. In some studies (Guo et al., 2015; Huang, Wu, Yang, Li, & Kuo, 2015), recovered collagen has also been subjected to enzymatic hydrolysis to produce bioactive peptides.

Appling the sediment residue from pH-shift processing of gutted fish or fish by-products may provide a more purified source for collagen since a large part of non-collagenous proteins are then removed as main product. This concept was successfully tested with turkey processing by-products (Du, Keplova, Khiari, & Betti, 2014), but to the best of our knowledge, it has never been evaluated using the pH-shift processing residue of fisheries resources. Succeeding here would allow for resource efficient biorefining of bone containing fish raw materials into two different products; functional proteins and collagen or collagen hydrolysate. Thus, the present study was aimed to: a) recover and characterize acid-solubilized and pepsin-solubilized collagen from the pH-shift processing residue of gutted silver carp, b) isolate and characterize collagen hydrolysate using pepsin or pepsin and trypsin sequential hydrolysis from the residue, and c) evaluate the effect of the collagen hydrolysate with different molecular weight on the properties of gels made from silver carp protein isolate.

2. Material and methods

2.1. Chemicals

Pepsin, trypsin, acetic acid, EDTA, tris(hydroxymethyl)aminomethane, sodium dodecyl sulphate (SDS), β-mercaptoethanol (β-ME), glycerol, egg white albumin, and glutaraldehyde were purchased from Sigma-Aldrich Corp. (USA). Sodium hydroxide, hydrochloric acid, trichloroacetic acid (TCA), and sodium chloride were provided by Scharlo (Scharlo Co., Spain).

2.2. Fish sample preparation

Live silver carp (*Hypophthalmichthys molitrix*) with an average weight of 1000 ± 100 g were purchased from a local aquaculture farm. Within 1 h, they were transported to the laboratory in sealed foamed polystyrene boxes containing flaked ice (1:1 ratio). Then, the fish were gutted and washed with tap water, manually. Then, the gutted fish were grinded using a table top meat mincer (C/E22 N, Minerva Omega group, Italy) equipped with a plate with 3 mm holes. Finally, the mince was frozen at -80 °C in plastic zip-lock bags for further use.

2.3. Protein recovery by the pH-shift process

The mince was thawed under running tap water and subjected to alkaline pH-shift processing following the main principle described by Undeland, Kelleher, and Hultin (2002) but with some modifications. Hence, 340 g fish mince was homogenized with 6 volumes of cold distilled water for 1 min at speed 4 using a Polytron Homogenizer (IKA, Brazil). Then, the homogenate was adjusted to pH 11.5 using 2 M NaOH with a titrator (907 Titrando, Metrohm AG, Zurich, Switzerland) automatically in set pH mode with a maximum titration rate. The pH was monitored with a calibrated Hamilton double pore electrode (Bonaduz, Switzerland) coupled to the titrator. The pH-adjusted slurry was allowed to stand in ice for 10 min and then centrifuged at 8500*g* in a precooled (4 °C) Beckman centrifuge (Beckman Coulter Avanti® J-20XP

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