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Chemical properties of ω-3 fortified gels made of protein isolate recovered with isoelectric solubilisation/precipitation from whole fish

Reza Tahergorabi [a], Sarah K. Beamer [b], Kristen E. Matak [b], Jacek Jaczynski [b,*]

[a] Oregon State University, Seafood Research and Education Center, 2001 Marine Dr., Astoria, OR 97103, USA
[b] West Virginia University, Animal and Nutritional Sciences, P.O. Box 6108, Morgantown, WV 26506, USA

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ABSTRACT

Protein isolate was recovered from whole gutted fish using isoelectric solubilisation/precipitation (ISP). The objective was to determine chemical properties of heat-set gels made of the ISP protein isolate fortified with ω-3 polyunsaturated fatty acids (PUFAs)-rich oils (flaxseed, fish, algae, krill, and blend). The extent of the PUFAs increase, ω-6/ω-3 FAs and unsaturated/saturated FAs ratios, and the indices of thrombogenicity and atherogenicity depended on specific ω-3 PUFAs-rich oil used to fortify protein isolate gels. Lipid oxidation in ω-3 PUFAs fortified gels was minimal, although greater ($P < 0.05$) than control gels (without ω-3 PUFAs fortification). However, all gels were in the slightly rancid, but acceptable range. The commonly used thiobarbituric-acid-reactive-substances (TBARS) assay to determine lipid oxidation in seafood may be inaccurate for samples containing krill oil due to its red pigment, astaxanthin. Protein degradation (total-volatile-basic-nitrogen) was greater ($P < 0.05$) in ω-3 PUFAs fortified gels than control gels. However, all gels were considerably below the acceptability threshold for protein degradation. The shear stress of ω-3 PUFAs fortified gels was generally greater than the control gels and the shear strain was generally unchanged. This study demonstrates that ω-3 PUFAs fortification of protein isolates recovered with ISP from fish processing by-products or whole fish has potential application in the development of functional foods.

1. Introduction

Globally, fish provide 15% of the total dietary intake of animal protein for 4.3 billion people. In 2010, the global consumption of fish was 18.6 kg per capita (FAO, 2012). A global decline in marine fish stocks has become a widely disputed and publicized issue. Freeman et al. (2006) estimated that the amount of large marine fish is 10% of their original stocks prior to global industrialisation. It has also been stated that by mid-21st century most commercial fisheries may collapse (Mooney, Nichols, & Elliott, 2002). These declines, when coupled with increasing human population necessitate a development of processing strategies to maximise the recovery of functional and nutritious fish muscle proteins from low-value/underutilized species and fish processing by-products. The recovered proteins would be subsequently used in value-added seafood products destined for human consumption.

Isoelectric solubilisation/precipitation (ISP) allows selective, pH-induced water solubility of muscle proteins with concurrent separation of lipids and removal of materials not intended for human consumption such as bones, skin and scales. Muscle protein isolates from fish have thus far been recovered using ISP in a batch mode at the laboratory-scale (Choi & Park, 2002; Kim, Park, & Choi, 2003; Kristinsson & Hultin, 2003; Undeland, Kelleher, & Hultin, 2002) and pilot-scale (Mireles DeWitt, Nabors, & Kleinholz, 2007). ISP processing has been applied to beef and fish processing by-products (Chen & Jaczynski, 2007a; Chen and Jaczynski, 2007b; Mireles DeWitt, Gomez, & James, 2002). Most recently, ISP has been used to recover a muscle protein isolate from chicken meat by-products (Tahergorabi, Beamer, Matak, & Jaczynski, 2011; Tahergorabi, Sivanandan, & Jaczynski, 2011). ISP processing allows high protein recovery yields, while significantly reducing fat content (Chen & Jaczynski, 2007b; Taskaya, Chen, Beamer, & Jaczynski, 2009). Recovered protein isolates retain functional properties and nutritional value (Chen, Tou, & Jaczynski, 2007, 2009; Gigliotti, Jaczynski, & Tou, 2008; Nolsoe & Undeland, 2009; Taskaya, Chen, & Jaczynski, 2009; Taskaya, Chen, Beamer, Tou, & Jaczynski, 2009). Due to extreme pH during ISP, this technology results in a non-thermal microbial reduction (Lansdowne, Beamer, Jaczynski, & Matak, 2009a, 2009b). Therefore, ISP offers several advantages over mechanical filleting and may be a useful technology to recover functional and nutritious protein isolates from whole gutted fish (i.e., without prior filleting) or fish processing by-products for subsequent application in value-added food products.

* Corresponding author. Tel.: +1 304 293 1893.
E-mail address: Jacek.Jaczynski@mail.wvu.edu (J. Jaczynski).

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The western diet is characterised by the increased dietary intake of saturated fat, ω-6 fatty acids (ω-6 FAs), and trans-FAs, as well as decreased intake of ω-3 FAs. As a result, the ratio of ω-6 FAs to ω-3 FAs is at 15–20 to 1, instead of the suggested 1 to 1 (Eaton & Konner, 1985; Eaton, Konner, & Shostak, 1988; Simopoulos, 1991; Simopoulos, 1999a; Simopoulos, 1999b; Simopoulos, 1999c). Alpha-linolenic (ALA, 18:3ω-3), eicosapentaenoic (EPA, 20:5ω-3) and docosahexaenoic acids (DHA, 22:6ω-3) are the main ω-3 polyunsaturated FAs (ω-3 PUFAs), while linoleic (LA, 18:2ω-6) and arachidonic acids (AA, 20:4ω-6) are the main ω-6 PUFAs in aquatic animals (Chen, Nguyen, Semmens, Beamer, & Jaczynski, 2006). There is increasing interest in the fortification of food products with ω-3 PUFAs because of their health benefits, especially the reduction of cardiovascular disease (CVD) (Nair, Leitch, Falconder, & Garg, 1997). According to the American Heart Association, CVD has had an unquestioned status of the number one cause of death in the U.S. since 1921 (American Heart Association, 2009). In 2004, the Food and Drug Administration (FDA) approved a health claim for reduced risk of CVD for foods containing ω-3 PUFAs, mainly EPA and DHA (FDA, 2004). This provided a marketing leverage for functional foods fortified with ω-3 PUFAs and initiated a development of food products addressing the diet-driven CVD. Anderson and Ma (2009) provided an up-to-date and comprehensive review of health benefits specific for ALA, EPA, and DHA. Since the seafood products developed from the ISP-recovered fish protein isolate would be formulated products associated with aquatic sources, they are a logical vehicle for increasing the consumption of ω-3 PUFAs; and therefore, addressing the diet-driven CVD without the need for dietary supplements in a pill or capsule form.

The overall objective of this study was to recover a fish protein isolate by ISP from whole gutted rainbow trout (bone-in, skin- and scale-on) as a model for fish processing by-products. The ISP-recovered isolate was subsequently used for the development of heat-gelled functional seafood product fortified with ω-3 PUFAs. Specific objectives were to determine (1) FA composition including indices of thrombogenicity and atherogenicity, (2) lipid oxidation, (3) protein degradation, and (3) fundamental texture properties of fish protein isolate gels fortified with ω-3 PUFAs-rich oils (flaxseed, fish, algae, krill, and blend (flaxseed:algae:fish, 8:1:1)).

2. Materials and methods

2.1. Sample preparation and recovery of fish protein isolate with isoelectric solubilisation/precipitation

Whole gutted rainbow trout (bone-in, skin- and scale-on) were purchased from a local aquaculture farm. Whole gutted trout were used as a model for fish processing by-products. The fish were subjected to isoelectric solubilisation/precipitation (ISP) to recover muscle protein isolate. A processing flowchart for the recovery of fish protein isolate and subsequent development of heat-set gels is shown in Fig. 1.

The fish were ground (meat grinder model 812 with 2.3 mm grinding plates, Biro, Marblehead, OH) followed by homogenisation with distilled and de-ionised water (dd H_2O) at 1:6 ratio (ground fish:water, w:v) using a laboratory homogenizer (PowerGen 700, Fisher Scientific, Fairlawn, NJ) set at speed five for five minutes. During the entire ISP processing, temperature was carefully controlled at 4 °C. The processing time did not exceed 60 min. The homogenisation/mixing was continued with the PowerGen homogenizer set at speed three during subsequent pH adjustment steps.

A 6 L aliquot of the homogenate was transferred to a beaker and the pH was adjusted to 11.50 ± 0.05 with 5 and 0.5 M NaOH (Chen & Jaczynski, 2007a, 2007b; Tahergorabi, Beamer, et al., 2011; Taskaya, Chen, Beamer, & Jaczynski, 2009). The 5 and 0.5 M reagents were used for crude and fine pH adjustments, respectively, during both protein solubilisation and subsequent precipitation (pH = 5.5) (see below). Once the desired pH was obtained, the solubilisation reaction was allowed to take place for 10 min, followed by centrifugation at 10,000*g* and 4 °C for 10 min using a laboratory batch centrifuge (Sorvall Evolution RC Refrigerated Superspeed centrifuge equipped with Sorvall Fiber Lite rotor SLC-6000, Sorvall Centrifuges, Asheville, NC). The centrifugation resulted in three layers: top – fish lipids, middle – fish muscle protein solution, and bottom – insolubles (bones, skin, insoluble proteins, membrane lipids, etc.).

The fish muscle protein solution was collected and its pH was adjusted to 5.50 ± 0.05 by 5 and 0.5 M HCl to precipitate the proteins. Once the desired pH was obtained, the precipitation reaction was allowed to take place for 10 min. The solution with precipitated proteins was de-watered by centrifugation as above. The centrifugation resulted in two layers: top – process water, and bottom – precipitated and de-watered fish proteins isolate. The precipitated and de-watered fish protein isolate was collected. The final moisture of the isolate was adjusted to 82 g/100 g by manual squeezing of the isolate wrapped in a cheese cloth. The isolate was used in the preparation of fish protein isolate paste.

2.2. Preparation of fish protein isolate paste

Fish protein isolate pastes were made using the procedure described by Jaczynski and Park (2004). The ISP-recovered protein isolates were chopped in a universal food processor (Model UMC5, Stephan Machinery Corp., Columbus, OH) at low speed for 1 min. A fish protein paste was obtained by extracting myofibrillar proteins in the fish protein isolate with 0.34 M of KCl-based salt substitute (AlsoSalt® sodium-free salt substitute, AlsoSalt, Maple Valley, WA) and chopping at low speed for 0.5 min in the universal food processor. This level of salt substitute was found to be optimum and similar to salt (NaCl) in terms of protein gelation and endothermal transitions as well as texture and colour in heat-set fish protein gels (Tahergorabi, Beamer, Matak, & Jaczynski, 2012; Tahergorabi & Jaczynski, 2012). The concentration of 0.34 M of the salt substitute was equivalent to 2 g of NaCl per 100 g of the fish protein isolate. The salt substitute contained 68 g of KCl per 100 g of the salt substitute and L-lysine mono-hydrochloride. According to the manufacturer, the patented L-lysine derivative masks the metallic-bitter aftertaste of KCl.

The final moisture content of the fish protein isolate paste was adjusted to 68 g/100 g by adding functional additives at the following final concentrations: 10 g/100 g of a ω-3 PUFAs-rich oil (see below), 3.7 g/100 g crab flavor (F-11019, Activ International, Mitry-Mory Cedex, France), 2 g/100 g of potato starch (PS) (Penbind 1000 modified potato starch, Penford Food Ingredients Corp., Centennial, CO), 0.5 g/100 g of microbial transglutaminase (MTGase) (Activa RM, Ajinomoto USA Inc., Teaneck, NJ), and 0.3 g/100 g of polyphosphate (PP) (Kena FP-28, Innophos, Cranbury, NJ). The above levels of functional additives were found in previous studies as optimal for gelation of ISP-recovered fish protein isolates and consequently physicochemical properties of heat-set gels as well as closely resembling commercial surimi-based seafood products (Chen & Jaczynski, 2007b; Perez-Mateos, Boyd, & Lanier, 2004; Taskaya, Chen, Beamer, & Jaczynski, 2009; Taskaya, Chen, & Jaczynski, 2009; Taskaya, Chen, & Jaczynski, 2010). A 0.5 g/100 g of titanium dioxide (TiO_2) [Titanium (IV) oxide, Sigma–Aldrich, Inc., St. Louis, MO] was also added to the paste (Tahergorabi, Beamer, et al., 2011; Taskaya et al., 2010). TiO_2 is commonly added up to 1 g/100 g as a whitening agent in food products. The PS, MTGase, TiO_2, and PP were in a dry powder form. The crab flavor was a water-soluble liquid. The ω-3 PUFAs-rich oils (see below) were