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Mass balance for isoelectric solubilization/precipitation of carp, chicken, menhaden, and krill

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ABSTRACT

Mass balance analysis was conducted for isoelectric solubilization/precipitation (ISP) processing of carp, chicken, menhaden, and krill based on proximate composition of input materials and recovered fractions (i.e., protein, lipid, insoluble, and process water). Protein recovery yield and lipid reduction were also determined. Thin layer chromatography (TLC) and SDS-PAGE electrophoresis allowed determination of lipid classes and protein electrophoretic patterns. ISP concentrated crude protein (72–90 g/100 g, dry basis) and reduced total lipid (3–16 g/100 g, dry basis) in the protein fraction recovered with ISP when compared to the input materials (48–68 g of crude protein and 15–45 g of total lipid per 100 g, dry basis). Protein recovery yield and lipid reduction were 45–66 and 79–98 g/100 g, respectively. However, lipid fraction did not form when menhaden and krill were processed with ISP. Krill and menhaden lipids were distributed in the process water in addition to the protein and insoluble fractions. TLC showed that krill and menhaden lipids had high phospholipid (PL), but low triglyceride (TAG) content, contributing to emulsification and preventing formation of lipid fraction. SDS-PAGE confirmed presence of myosin and actin in protein fraction recovered from carp and chicken as well as proteolysis in menhaden and severe protein degradation in krill.

1. Introduction

Isoelectric solubilization/precipitation (ISP) is a protein recovery process that solubilizes and precipitates protein based on its isoelectric behavior when subjected to pH changes (Matak, Tahergorabi, & Jaczynski, 2015). Hultin and Kelleher (1999) first proposed to use protein isoelectric behavior in order to separate the protein from a muscle source. Their pioneering developments were supported with earlier works by Meinke, Rahman, and Mattil (1972) as well as Meinke and Mattil (1973).

Protein tends to aggregate when the pH is at the protein's isoelectric point (pI) due to prevailing protein-protein hydrophobic interactions. However, protein becomes water-soluble when the pH is far from the protein's pI due to prevailing protein-water electrostatic attraction. While protein is dissolved during ISP, it may be separated from lipid and insolubles such as bones, scales, exoskeleton, and skin. ISP has been shown to yield functional protein and lipid from low-value, under-utilized aquatic and terrestrial species as well as their processing by-products (Mireles DeWitt, Gomez & James, 2002; Yongswawatdigul & Park, 2004; Chen & Jaczynski, 2007a, 2007b; Pérez-Mateos & Lanier, 2007; Taskaya, Chen, & Jaczynski, 2010; Jin, Choi, Jeong, & Kim, 2011; Tahergorabi, Beamer, Matak, & Jaczynski, 2011; Tahergorabi, Beamer, Matak, & Jaczynski, 2012).

Silver carp (*Hypophthalmichthys molitrix*) and chicken are considered as abundant and inexpensive sources of animal protein for human consumption. A fresh-water species, silver carp provides the greatest biomass of animal protein from aquatic origin

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consumed by humans. It is especially popular in China where it is traditionally aquacultured on a large scale (FAO, 2013). However, commercial processing of silver carp presents challenges due to potential off-flavors as well as particularly bony and oily nature of the carp carcass. Carp is not commercially processed with ISP. However, ISP can be applied to recover functional protein and lipid from this challenging aquatic resource. On the other hand, consumption of chicken meat has been steadily increasing worldwide. The U.S. produces approximately 20% of chicken meat consumed worldwide (FAO, 2009). Chicken processing generates by-products that contain muscle protein that could be recovered for human consumption. In addition, dark chicken meat is typically considered of lower value, especially in the U.S. ISP can be applied to recover nutrients (i.e., protein and lipid) from chicken meat processing by-products as well as to convert dark chicken meat to white meat counterparts. Due to their wide acceptability and potential applicability of ISP, chicken and carp were selected for this research as representative sources from terrestrial and fresh-water aquatic environments.

The popularity of fish oil dietary supplements has been steadily growing due to their high content of omega-3 polyunsaturated fatty acids (ω-3 PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Atlantic menhaden (*Brevoortia tyrannus*) is not utilized for direct human consumption. Instead, this vast marine resource is mainly used by reduction fisheries to manufacture fish oil and to render fish meal and fertilizer. Menhaden is considered as a low-value fish due to its oily, bony, and dark flesh. Antarctic krill (*Euphausia superba*) has recently attracted significant attention at a commercial level due to its high EPA/DHA content in a unique phospholipid (PL) form (Gigliotti, Davenport, Beamer, Tou, & Jaczynski, 2011). Although krill have one of the largest biomass of any multicellular animal species in earth, they are significantly under-utilized for direct human consumption mainly due to processing challenges associated with rapid hydrolysis of krill muscle tissue by very potent krill endogenous enzymes in addition to fluoride level that is toxic to humans and a small but fragile krill body structure. Similar to menhaden, krill are mainly used to manufacture krill oil and to render krill meal. Menhaden and krill were selected for this research because of their abundance, but lack of direct human consumption.

There are published reports on recovery of protein and lipid from carp (Paker, Beamer, Jaczynski, & Matak, 2013a; Paker, Beamer, Jaczynski, & Matak, 2013b; Taskaya, Chen, Beamer, & Jaczynski, 2009; Taskaya, Chen, Beamer, Tou, & Jaczynski, 2009), menhaden (Pérez-Mateos & Lanier, 2007), chicken (Jin et al., 2011; Tahergorabi, Sivanandan, Beamer, Matak, & Jaczynski, 2012; Tahergorabi et al., 2011), and krill (Chen & Jaczynski, 2007a, 2007b; Gigliotti, Jaczynski, & Tou, 2008; Sun et al., 2014; Wang, Xue, Wang, & Yang, 2011). These studies focus on characterization of functional and nutritional properties of protein and lipid recovered with ISP. However, there is no systematic information regarding mass balance for ISP processing. As a principle, the weight of the input material (e.g.; carp, chicken, menhaden, or krill) for ISP processing should theoretically equal the sum of weights of output fractions (i.e., recovered protein and lipids ad well as remaining insolubles and process water). Mass balance shows distribution of nutrient fractions separated during ISP in relation to the whole material that was used as input for ISP processing. Mass balance is useful because it allows determination of nutrient (i.e., protein and lipid) recovery yields. In this study, mass balance for ISP processing was based on proximate composition (dry basis for crude protein, total fat, and ash content) and biochemical characterization (SDS-PAGE electrophoresis and thin-layer-chromatography) of the input material (i.e., carp, chicken, menhaden, or krill) in relation to their respective fractions (i.e., protein, lipid, insolubles, and process water) recovered with ISP.

The primary objectives of this study was to determine mass balance for ISP processing of representative sources such as carp, chicken, menhaden, and krill. The secondary objective was to use the mass balance to calculate protein recovery yield and fat reduction rate as well as to investigate protein fractions and lipid classes in the ISP input and output.

2. Materials and methods

2.1. Sample preparation and ISP processing

Fresh whole gutted silver carp (bone-in, skin-on, and scale-on) were purchased from RCB Fish Company (Ledgetter, KY, USA). Fresh Atlantic menhaden were purchased from LD Amory Company (Hampton, VA, USA). Fish were only manually eviscerated (i.e., bone-in, skin-on, and scale-on). Menhaden were harvested from the Hampton portion of the Chesapeake Bay. Fresh whole chicken (gutted, bone-in, and skin-on) was purchased from a local grocery store. Frozen whole Antarctic krill were purchased from Rod's Food (Dekalb, IL, USA). Immediately upon arrival, all materials except krill were separately pre-cut and ground twice with a meat grinder using 2.3 mm grinding plates (812, Biro, Marblehead, OH, USA) resulting in a homogenous carp, chicken, or menhaden paste. The paste samples of approximately 1 kg were separately vucuum-packed and stored at −80 °C until experiments. Storage time did not exceed 1 month. Frozen krill was partially thawed and ground in a blender (51BL31, Waring Commercial, Torrington, CT, USA) until homogenous paste was obtained. Krill paste was not stored. Instead, krill paste was immediately subjected to isoelectric solubilization/precipitation (ISP).

ISP was carried out according to Tahergorabi, Beamer, Matak, and Jaczynski (2013) with minor modification. A flowchart for ISP processing of carp, chicken, menhaden, and krill is shown in Fig. 1. Temparature during ISP processing was carefully controlled below 4 °C. The processing time was approximately 60 min. Brifely, frozen paste was thawed at 4 °C overnight except for krill (see previous paragraph). A sample of 857 g of carp, chicken, menhaden, or krill paste was separately blended with 5.143 L of distilled deionized ice water. The mixture was homogenized (PowerGen 700, Fisher Scientific, Fairlawn, NJ, USA) at high speed for approximately 1 min followed by pH adjustment to 11.5 using 1 mol/L NaOH in order to induce isoelectric solubilization. After the pH was adjusted to 11.5, the solution was mixed for additional 10 min. The solution was centrifuged at 10,000 × g and 4 °C for 10 min (SLC-6000, Sorvall, Asheville, NC, USA). Centrifugation resulted in three distinct layers that were seperately collected. Lipid layer was collected from the top; while protein and insoluble layers were collected from the middle and bottom, respectively. The lipid and insoluble layers were retained for freeze-drying and subsequent analysis.

The pH of the collected protein solution was adjusted to 5.5 using concentrated HCl (8 mol/L) in order to induce isoelectric precipitation. After the pH was adjusted to 5.5, the solution was mixed for additional 10 min. Centrifugation, as above, was applied to de-water precipitated protein. Centrifugation resulted in two distinct layers that were separately collected. Protein layer was collected from the bottom and process water was collected from the top. All of the collected fractions (i.e., lipid, insolubles, protein, and process water) were freeze-dried (VirTis Genesis 35XL, SP Industries, Warminster, PA, USA), vacuum-packed, and kept at −20 °C until analysis. The storage time did not exceed 1 month.

The following exceptions to ISP processing were made. ISP processing for krill was conducted at pH 11.0 and 5.0 instead of 11.5 and 5.5 for isoelectric solubilization and precipitation, respectively (Sun et al., 2014). Lipid layer did not form for krill or menhaden;