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Micro-emulsification/encapsulation of krill oil by complex coacervation with krill protein isolated using isoelectric solubilization/precipitation

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ABSTRACT

This study determined feasibility of krill protein isolated with isoelectric solubilization/precipitation (ISP) as wall material to microencapsulate krill oil by freeze-drying. Effects of krill oil/krill protein ratio on properties of microcapsules were investigated. With increased ratio, crude protein of microcapsules decreased, while total lipid increased. Although microcapsule oil loading capacity increased, loading and encapsulation efficiencies decreased. Thin layer chromatography (TLC) confirmed abundance of phospholipids, which are amphiphilic; and thus, resulted in stable emulsion (emulsion stability index). Microcapsules contained ω-3 polyunsaturated fatty acids (PUFAs) at 43–60, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at 28–41 and 9–11 g/100g of total FAs, respectively. SDS-PAGE electrophoresis revealed proteolysis of ISP krill protein, probably causing reduced loading and encapsulation efficiencies. SEM showed that krill oil/krill protein ratio affected surface microstructure. ISP krill protein showed potential as a wall material to microencapsulate krill oil; and thus, expand application of krill oil/protein for human consumption.

1. Introduction

In the recent years krill oil has become a popular dietary supplement due to multi-faceted nutraceutical benefits including management of dyslipidemia, chronic inflammatory conditions, cardiovascular health, cognitive disorder, and many more (Massrieh, 2008). Krill oil is considered a rich source of eicosapentaenoic acid (EPA, C20:5ω-3) and docosahexaenoic acid (DHA, C22:6ω-3) (Castro-Gómez, Holgado, Rodríguez-Alcalá, Montero, & Fontecha, 2015; Le Grandois et al., 2009; Winther, Hoem, Berge, & Reubsaet, 2011). A unique property of krill oil is that EPA and DHA are almost exclusively esterified to phospholipids (PLs), particularly phosphatidylcholine (PC). In contrast, EPA and DHA in fish oil are present in triacylglycerol (TAG). Esterification of EPA/DHA to PLs seems to increase bio-efficacy and bio-availability (Wijendran et al., 2002). Importantly, both EPA and DHA in various permutations have been found on one PL molecule (i.e., EPA/DHA dual-bonded PL) in krill oil. PLs are amphiphilic; and therefore, display greater polarity than TAGs. Based on dielectric constant, methanol is a more polar organic solvent than ethanol. Although methanol may have a greater affinity for PLs than ethanol, both alcohols target PLs more efficiently than TAGs. Using this principle, Castro-Gómez et al. (2015) purified PLs (i.e., 99.7% PC) with methanol from a commercial crude krill oil. Because PLs had higher concentration in the purified PC fraction, the detection limit of the ultra-sensitive mass spectrometer (UPLC/QToF-MS) increased. This technique allowed quantification of EPA/DHA dual-bonded PC (i.e., EPA/DHA in different permutations on one PC molecule). Castro-Gómez et al. (2015) found it remarkable that the methanol extract of crude krill oil contained 6.5% of the EPA/DHA dual-bonded PC. This translated into approximately 3% of this dual-bonded PL species in the commercial crude krill oil that was subjected to methanol extraction in their study. Organic solvents such as ethanol/acetone/hexane/supercritical-CO_2/etc. are most commonly used for commercial oil extraction including krill oil (Bruheim, Tilseth, & Mancinelli, 2016; Sampalis & Harland, 2016). However, krill oil can likely be extracted with inorganic solvents such as water or without solvents at all (Sclabos, Guerra, & Lay, 2015).

Another key difference between fish and krill oil is that krill oil contains astaxanthin, which imparts a deep purple color to the oil (Deutsch, 2007; Massrieh, 2008). Astaxanthin is a carotenoid-based antioxidant with greater antioxidant capacity than α-tocopherol

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(vitamin E) (Naguib, 2000). Astaxanthin does not protect ester bond on PLs from hydrolytic degradation. However, it does protect EPA/DHA from oxidative decomposition by being preferentially oxidized. When astaxanthin is oxidized, it loses its antioxidant capacity. Therefore, EPA/DHA may be spared from oxidation, but the overall benefits of krill oil may be reduced due to the loss of antioxidant capacity. This often is manifested by a gradual loss of the vivid red color of astaxanthin and a gradual shift towards dark red-brown oil (Choubert, Dentella, Atgie, & Baccaunaud, 2005). Astaxanthin has its own nutraceutical benefits that are independent from EPA/DHA. This is why krill oil has a great potential to exhibit multi-faceted health benefits with additive or synergistic effects in humans.

At present, krill oil is exclusively used as a dietary supplement in an encapsulated and gelled form such as gummies. However, it could be used as a nutraceutical ingredient in a variety of foods such as functional food products. Based on a chemical composition, krill oil could find numerous applications similar to lecithin (i.e., PL) such as that derived from soybean and egg yolk. Additional potential applications for krill oil could be functional foods that require EPA/DHA (i.e., ω-3 polyunsaturated fatty acids, ω-3 PUFAs) fortification such as foods with added fish, algae, flax, and the like oils. PLs are amphiphilic, which suggests excellent emulsification properties. However, depending on the actual food application, krill oil may show relatively limited solubility due to its high bulk viscosity. In addition, it may result in poor oxidative stability leading to potential rancidity. These issues may restrict incorporation of krill oil in food products. One potential way to provide a means to expand application of krill oil is microencapsulation by coacervation. Microencapsulation is a broad term that generally encompasses a process in which fine droplets or particles are surrounded by a coating or embedded in a matrix. It provides desired properties that were absent in the starting material. Coacervation is a more defined term that in principle is based on electrostatic interactions between oppositely charged molecules (i.e., cations and anions). Therefore, it results in molecular attraction enhancing microcapsule integrity and stability (Aziz, Gill, Dutilleul, Neufeld, & Kermasha, 2014; Kaushik, Dowling, Barrow, & Adhikari, 2015). Microencapsulation has been widely used to encapsulate bioactive food components, particularly lipids such as ω-3 PUFAs including krill oil and various antioxidants. Microencapsulation extends shelf-life as well as to improves miscibility and controlled release Aziz et al., 2014; Shahidi & Han, 1993).

Various protein- and carbohydrate-based wall materials are used for microencapsulation. Selecting proper wall material is critical. Not only can oil be extracted from krill, but protein can also be isolated with isoelectric solubilization/precipitation (ISP) from this tremendous resource. In terms of composition, protein is the main nutrient available from krill for human consumption (Tou, Jaczynski, & Chen, 2007). Therefore, attention should be focused to isolate krill protein in order to increase utilization of this tremendous resource for direct human consumption. ISP has already been shown to efficiently isolate muscle protein (myosin and actin) from krill (Chen & Jaczynski, 2007; Chen, Tou, & Jaczynski, 2009; Sun et al., 2013, 2014; Wang et al., 2015). ISP protein isolates have also been shown to retain their functional properties, including gelation (Tahergorabi, Beamer, Matak, & Jaczynski, 2012; Taskaya, Chen, Beamer, & Jaczynski, 2009; Taskaya, Chen, & Jaczynski, 2009). This specific protein functionality may be particularly applicable to form a wall material for encapsulation of krill oil. The ISP krill protein isolate might be a potential, promising protein-based wall material for krill oil microcapsule. In addition, both the wall material as well as the encapsulated oil would be of the same origin, krill. ISP is a protein isolation process that solubilizes and precipitates protein based on its isoelectric behavior when subjected to pH changes (Gehring, Gigliotti, Moritz, Tou, & Jaczynski, 2011; Matak, Tahergorabi, & Jaczynski, 2015). Krill protein isolates have high nutritional quality and appear safe for human consumption (Bridges, Gigliotti, Altman, Jaczynski, & Tou, 2010; Burri & Johnsen, 2015; Gigliotti, Jaczynski, & Tou, 2008). Encapsulation of krill oil has recently been reported by Haider, Majeed, Williams, Safdar, and Zhong (2017) and Aziz et al. (2014). They encapsulated krill oil with chitosan-tripolyphosphate and gelatin-gum Arabic, respectively. These wall materials are fundamentally different than the ISP krill protein isolate. In addition, they are not derived from krill unlike the ISP krill protein isolate. To our knowledge, ISP protein isolates including ISP krill protein isolate have not been investigated yet for microencapsulation of krill oil.

The primary objective of this study was to evaluate the feasibility of ISP krill protein isolate as the wall material for krill oil microcapsules. The secondary objective was to investigate the effects of different ratios of krill oil/krill protein isolate on properties of krill oil microcapsules, including proximate composition, oil loading capacity, loading efficiency, encapsulation efficiency, fatty acid profile, lipid classes, protein electrophoretic fractions, and microcapsule microstructure.

2. Materials and methods

2.1. Preparation of krill protein isolate by isoelectric solubilization/precipitation

Frozen whole Antarctic krill (*Euphausia superba*) blocks were purchased from Rod's Reef (Dekalb, IL, USA). Krill blocks were shipped overnight to the laboratory in heavily insulated industrial strength boxes filled with dry ice. Upon arrival, krill blocks were immediately cut into approximately 1 kg pieces, vacuum packed, and stored at −80 °C. Storage time did not exceed 1 month.

Protein was isolated from whole krill using isoelectric solubilization/precipitation (ISP) according to Chen and Jaczynski (2007) and Shi et al. (2017). Temperature during ISP processing was carefully controlled below 4 °C. The processing time was approximately 60 min. Frozen krill was partially thawed and ground in a blender (51BL31, Waring Commercial, Torrington, CT, USA) until homogenous paste was obtained. A sample of 857 g of ground krill paste was blended with 5143 ml 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 11.0 using NaOH (10 mol/L) in order to induce isoelectric solubilization of krill protein. After the pH was adjusted to 11.0, 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 two distinct layers. The bottom sediment layer containing insoluble fraction (exoskeleton, appendages, lipid, insoluble protein, etc.) was not collected; while the top supernatant layer mainly containing dissolved protein was collected. The pH of the collected supernatant was adjusted to 5.0 using concentrated HCl (8 mol/L) in order to induce isoelectric precipitation of krill protein. After the pH was adjusted to 5.0, the solution was mixed for additional 10 min. Centrifugation, as above, was applied to de-water precipitated krill protein. Centrifugation resulted in two distinct layers, supernatant layer containing process water on the top and sediment layer containing precipitated and de-watered krill protein isolate on the bottom. The krill protein isolate was collected and its pH was adjusted to 7.2. The moisture content of krill protein isolate was 85 g/100 g. Krill protein isolate was used to prepare krill oil/krill protein isolate emulsion.

2.2. Preparation of krill oil/krill protein isolate microemulsion

Krill oil was purchased from Jedwards International, Inc. (Braintree, MA, USA). Upon arrival and throughout the study, krill oil was stored under refrigeration in a N_2-flushed, dark container. Storage time did not exceed 1 month.

Krill oil was mixed with krill protein isolate at the following ratios, 0:100, 20:80, 40:60, 60:40, 80:20 (ml of krill oil:g of krill protein isolate) to prepare microemulsions. These ratios will be hereafter referred to as 0:100, 20:80, 40:60, 60:40, 80:20, respectively. Krill protein