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Extraction and characterisation of lipids from Antarctic krill (*Euphausia superba*)

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ABSTRACT

There is significant commercial interest in oil extraction from krill because it is rich in omega-3 polyunsaturated fatty acids (*n*-3 PUFA) such as eicosapentaenoic (EPA, 20:5*n*3) and docosahexaenoic (DHA, 22:6*n*3) acids. The objectives were to determine oil extraction efficiency using different solvent systems and the composition of extracted oil and spent krill following extraction. Extraction efficiency was the highest ($P < 0.05$) for one-step extraction using freeze-dried krill with 1:12 or 1:30 krill:solvent ratio (w:v) compared to Folch, Soxhlet, and conventional two-step extraction. Extracted oils contained predominantly phospholipids (20–33%), polar non-phospholipids (64–77%), and minor triglycerides (1–3%). Triglycerides contained much less ($P < 0.05$) total *n*-3 (4.0%), DHA (1.1%), and EPA (2.3%), but more ($P < 0.05$) saturated FA (38.7%) than phospholipids (total *n*-3-47.4%, DHA-18.0%, EPA-28.2%, saturated FA-23.5%). Antioxidant capacity of krill oil extracted by one-step extraction (9.4–14.2 μmol Trolox Equivalents/ml oil) was generally similar to antioxidant capacity of krill oil extracted by ethanol (22.9), but greater ($P < 0.05$) than antioxidant capacity of krill oil extracted by acetone (1.2) and Folch method (1.5). The spent krill following oil extraction contained protein (72.9–75.8%, dry basis). Based on the extraction efficiency and composition of the extracted oil, the one-step extraction using 1:12 krill:solvent ratio is recommended.

1. Introduction

Antarctic krill (*Euphausia superba*) are small, shrimp-like crustaceans. Commercial capture is simple because krill form high-density surface swarms. Despite their small size, krill likely has the largest biomass of any multi-cellular animal species on earth (Nicol, James, & Pitcher, 1987). Although it is difficult to accurately determine the sustainable biomass for krill harvest, this significant resource may be comparable to the biomass of all other aquatic species currently harvested. The total annual capture from all fisheries has been approximately 130 million tons (MT) since 2000 (FAO, 2007). By comparison, krill biomass has been estimated at 400–1550 MT with a sustainable harvest at 70–200 MT (Suzuki & Shibata, 1990). However, newer estimates suggest that the krill biomass may be lower (Priddle, Boyd, Whitehouse, Murphy, & Croxall, 1998; Smetacek & Nicol, 2005). Nicol and Foster (2003) estimated the annual krill capture to be 0.1 MT, making krill an underutilized species. However, due to the role that krill play in marine ecology, an internationally monitored and governed ecosystem approach is a necessity for a long-term sustainability of this fishery (Everson, 2000; Hureau, 1985; Laws, 1985).

Grantham (1977) reported that krill contains 77.9–83.1% moisture, 0.4–3.6% lipids, 11.9–15.4% protein, and ~2% chitin and glucides. Saether, Ellingsen, and Mohr (1986) determined that due to seasonality lipid content ranges widely from 12–50% (dry basis). Lipid content and its composition in krill also depend on species, age, and the time between capture and freezing (Kolakowska, 1991). Kolakowska, Kolakowski, and Szczygielski (1994) reported that the *n*-3 PUFA, EPA and DHA are particularly abundant, which was attributed to krill consuming single-cell marine micro-algae. However, shellfish are often perceived as high in cholesterol; and therefore, reduce its acceptance as food by consumers. Cholesterol level in krill is higher than fish, but lower than shrimp (Tou, Jaczynski, & Chen, 2007). Also, it is important to emphasise that two-thirds of the sterols in shellfish are non-cholesterol sterols, which interfere with absorption of dietary cholesterol (Feeley, Criner, & Watt, 1972; Vahouny, Connor, Roy, Lin, & Gallo, 1981).

Despite its potential as a high quality lipid and protein source (Bridges, Gigliotti, Altman, Jaczynski, & Tou, 2010; Chen, Tou, & Jaczynski, 2009; Gigliotti, Jaczynski, & Tou, 2008; Gigliotti, Smith, Jaczynski, & Tou, 2010; Tou et al., 2007), the use of krill as human food has been limited (Suzuki & Shibata, 1990). Krill is mainly used by reduction fisheries for manufacture of fish feeds due to its high astaxanthin content. In addition, encapsulated krill oil is used as a dietary supplement with various potential health benefits including protection against cardiovascular disease (CVD) (Bunea, El

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Farrah, & Deutsch, 2004; Sampalis, 2007; Sampalis et al., 2003). Bunea et al. (2004) attributed some of these benefits to the *n*-3 PUFA in krill being mainly associated with phospholipids (PL); unlike in fish where the *n*-3 PUFA are associated with triglycerides (TG). The oxidative stability of krill oil has been attributed to its antioxidants content, in particular astaxanthin (Suzuki & Shibata, 1990). Krill oil may be valuable in the development of nutraceutical food products (Kassis, Beamer, Matak, Tou, and Jaczynski, 2010; Kassis, Drake, Beamer, Matak, & Jaczynski, 2010; Kassis, Gigliotti, Beamer, Tou, and Jaczynski, submitted for publication).

A major hindrance to commercial processing of krill and development of new krill-based food products may be due to high activity of krill lipases and proteases (Anheller, Hellgren, Karlstam, & Vincent, 1989). These enzymes are released immediately upon the demise of krill, resulting in autolysis, which leads to a rapid spoilage. The enzymes combined with its small size makes krill processing for human food a significant challenge. Another concern is high fluoride content in the exoskeleton. However, centrifugation removes fluoride (Christians & Leinemann, 1983; Karl et al., 1986).

Krill oil is currently extracted by two-step solvent extraction using acetone and ethanol in the first and second step, respectively (Beaudoin & Martin, 2004; Sampalis, 2007). However, this extraction requires two separate extraction steps and takes a relatively long time. In addition, the two-step extraction does not mention water removal from krill prior to oil extraction. Water interferes with solvent extraction and water removal prior to oil extraction results in greatly improved extraction efficiency and less water in the extracted oil (Dunford, Temelli, & LeBlanc, 1997; Nilsson, 1996). Another process to extract krill oil takes advantage of superctitical-CO_2 entrained with up to 20% ethanol (Bruheim et al., 2008). However, this process requires thermal inactivation of lipases at over 50 °C prior to oil extraction. Although heat likely inactivates lipases resulting in reduced hydrolysis of ester bonds and consequently fewer free FA, it simultaneously denatures heat-labile krill muscle proteins (Carvajal, Lanier, & Macdonald, 2005).

Due to structural changes, the recovery of denatured proteins would be difficult and even if krill proteins were recovered, the proteins would exhibit reduced functionalities (i.e., gel-forming ability, extractability, water-holding-capacity, etc.). Bruheim et al.'s (2008) process is similar to the two-step solvent extraction (Beaudoin & Martin, 2004; Sampalis, 2007), but does not require water removal prior to processing. However, freeze-drying of krill prior to oil extraction with superctitical-CO_2 has been shown to increase extraction efficiency approximately three times (Yamaguchi et al., 1986). The protein remaining in the residual spent krill following oil extraction can be recovered with techniques such as isoelectric solubilisation/precipitation and if protein functionalities are retained, this protein could be used in human food products contributing to the fuller use of this tremendous resource (Chen & Jaczynski, 2007a,b; Chen et al., 2009; Gehring, Gigliotti, Moritz, Tou, & Jaczynski, 2010; Jaczynski, 2010; Torres, Chen, Rodrigo-Garcia, & Jaczynski, 2007).

It is hypothesised that one-step extraction with acetone:ethanol mixture for 2 h from whole krill will result in high extraction efficiency. The objectives were to determine oil extraction efficiency from whole krill using different solvent systems and characterise the composition of extracted lipids and residual spent krill following oil extraction.

2. Materials and methods

2.1. Sample preparation and oil extraction

Whole frozen Antarctic krill (*Euphausia superba*) was obtained from Krill Canada (Langley, BC, Canada). The krill blocks were transported overnight to the West Virginia University food science laboratory in heavily insulated industrial strength boxes filled with dry ice. Upon arrival the boxes were immediately stored at −80 °C until use. Whole frozen krill was freeze-dried without thawing (VirTis Genesis 35SQ Super XL freeze-dryer, Virtis, Gardiner, NY, USA), vacuum-packed and stored at −80 °C until processed. A flow diagram of oil extraction from krill is shown is Fig. 1.

In the one-step extraction, oil was extracted from freeze-dried krill using 1:1 acetone:ethanol (v:v) solvent mixture (ACS grade acetone, Fisher Scientific, Fairlawn, NJ, USA; ACS grade 95% ethanol, Pharmco, Brookfield, CT, USA). The following krill:solvent ratios were tested 1:6, 1:9, 1:12, and 1:30 (w:v). The weight of the initial freeze-dried krill was recorded in order to determine extraction efficiency (see below). The 1:30 ratio was used in the present study to mimic the 1:6 ratio of whole fresh krill as described by Beaudoin and Martin (2004) as well as Sampalis (2007). Fresh whole krill is currently used in commercial oil extraction (Beaudoin & Martin, 2004; Bruheim et al., 2008; Sampalis, 2007). However, in the present study freeze-dried krill was used (Dunford et al., 1997; Yamaguchi et al., 1986). Therefore, 1:30 ratio (freeze-dried krill) used in the present study approximately corresponded to 1:6 ratio (fresh whole krill) during commercial krill oil extraction based on the lipid content in relation to the solvent volume. Freeze-dried krill was dispersed in the solvent mixture (acetone:ethanol) by homogenisation for 30 s using a laboratory blender (model 51BL31, Waring Commercial, Torrington, CT, USA). Oil extraction was conducted for 2 h at 4 °C using a continuous shaker (model Excella E25R, New Brunswick Scientific, Edison, NJ, USA) followed by centrifugation at 10,000*g* and 4 °C for 20 min (model Sorvall RC-5B Refrigerated Superspeed, Kendro Laboratory Products, Newtown, CT, USA). The supernatant (i.e., extracted krill oil) was decanted and air dried at atmospheric pressure. The sediment (residual spent krill including protein, shell, etc.) was also dried under air at atmospheric pressure and analysed for crude protein (Kjeldahl), total fat (Soxhlet), and ash content (see below).

In the two-step extraction oil was extracted from freeze-dried krill using two separate extractions. The weight of the initial freeze-dried krill was recorded in order to determine extraction efficiency (see below). The freeze-dried krill was first mixed with acetone at a 1:6 ratio (krill:acetone, w:v), centrifuged, and then the sediment was mixed with ethanol at a 1:6 ratio (krill:ethanol, w:v), followed by final centrifugation. Therefore, two separate extracts were obtained. Acetone extract was obtained in step 1 and ethanol extract in step 2. In step 1, freeze-dried krill was dispersed in acetone by homogenisation for 30 s using the laboratory blender. Oil extraction in step 1 was conducted for 2 h at 4 °C using the continuous shaker followed by centrifugation at 10,000*g* and 4 °C for 20 min. The supernatant (i.e. acetone extract) was decanted and air dried at atmospheric pressure; while the sediment was subjected to step 2 of the extraction. Step 2 was conducted in the same manner as step 1 except ethanol was used instead of acetone. Therefore, total extraction time (i.e., step 1 and 2) was 4 h. Following step 2, final centrifugation at 10,000*g* and 4 °C for 20 min was applied. The supernatant (i.e., ethanol extract) was decanted and air dried at atmospheric pressure. The sediment (residual spent krill including protein, shell, etc.) was analysed for crude protein (Kjeldahl), total fat (Soxhlet), and ash content (see below).

Dry krill oils (i.e., krill oil from one-step extraction, acetone extract from two-step extraction, and ethanol extract from two-step extraction) were clarified in 2:1 chloroform:methanol (v:v) mixture (ACS grade chloroform, Fisher Scientific, Fairlawn, NJ, USA; HPLC grade methanol, Fisher Scientific, Fairlawn, NJ, USA) with 20 ml of 10% NaCl in water added to a separation funnel (Folch, Lees, & Sloane, 1957). This clarification removed any residual water in the krill oil samples. Sufficient volume of the chloroform:methanol mixture was added until there was no visible separate layers. Following clar-