Food Chemistry 233 (2017) 434–441

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Comparison of solvents for extraction of krill oil from krill meal: Lipid yield, phospholipids content, fatty acids composition and minor components

Dan Xie [a,b], Jun Jin [a], Jiang Sun [b], Li Liang [a], Xiaosan Wang [a], Wei Zhang [b], Xingguo Wang [a], Qingzhe Jin [a,*]

[a] State Key Laboratory of Food Science and Technology, Synergetic Innovation Center of Food Safety and Nutrition, School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi, Jiangsu 214122, PR China
[b] Zhonghai Ocean (Wuxi) Marine Equipment Engineering Co., Ltd., Jiangnan University National University Science Park, 100 Jinxi Road, Wuxi, Jiangsu 214125, PR China

ARTICLE INFO

Article history:
Received 22 December 2016
Received in revised form 9 April 2017
Accepted 23 April 2017
Available online 25 April 2017

Chemical compounds studied in this article:
Ethanol (PubChem CID: 702)
Isopropanol (PubChem CID: 3776)
Acetone (PubChem CID: 180)
Ethyl acetate (PubChem CID: 8857)
Isohexane (PubChem CID: 7892)
n-Hexane (PubChem CID: 8058)
Butane (PubChem CID: 7843)
Chloroform (PubChem CID: 6212)
Methanol (PubChem CID: 887)
Sodium hydroxide (PubChem CID: 14798)

Keywords:
Krill oil
Solvent extraction
Subcritical butane
Lipid yield
Phospholipids
Fatty acids
Minor components

ABSTRACT

The effects of seven different extraction solvents (ethanol, isopropanol, acetone, ethyl acetate, isohexane, n-hexane, and subcritical butane) on the lipid yield and quality of the oil extracted from krill meal were investigated in this study. Phospholipids (PL), fatty acids (FA) composition and minor components including sterols, astaxanthin, vitamin A and tocopherols in the extracted krill oil were analyzed. The results indicated that ethanol and isopropanol led to comparatively higher lipid yields (16.33 and 14.52%, respectively) and PL contents (39.2 and 38.7%, respectively) but lower contents of the minor components than the other solvents. The krill oil extracted with acetone had the lowest PL content (20.63%) but contained more astaxanthin (206.74 mg/kg), vitamin A (27.84 mg/100 g), and sterols (39.00 mg/g). Moreover, high levels of n-3 FA were present in the extracts with high PL contents. Further analysis revealed that 23.65–28.10% of eicosapentaenoic acid (EPA) and 16.71–21.03% of docosahexaenoic acid (DHA) were present in the PL, while only 2.83–3.48% of EPA and 1.40–1.74% of DHA were detected in the triacylglycerols (TAG). In addition, subcritical butane proved to be an alternative to n-hexane and isohexane; krill oil extracted with these three solvents had similar qualities.

1. Introduction

Antarctic krill (*Euphausia superba*) is the dominant krill species in the Antarctic Ocean with an estimated biomass of approximately 379 million metric tons and a gross post-larval production of 342–536 million metric tons per year (Atkinson, Siegel, Pakhomov, Jessopp, & Loeb, 2009). The main composition of Antarctic krill is protein (11.9–15.4%), lipid (0.5–3.6%), ash (3%), carbohydrates (2%), and water (77.9–83.1%) (Grantham, 1977).

Recently, krill oil has attracted great interest because of its unique composition and positive health effects. Polar lipid and triacylglycerols (TAG) are the major lipids in Antarctic krill, accounting for 56–81 and 12–38% of the total, respectively (Phleger, Nelson, Mooney, & Nichols, 2002). Antarctic krill oil is rich in phospholipids (PL) (≥40% of total lipid) (Fricke, Gercken, Schreiber, & Oehlenschläger, 1984); this is vastly different from common edible oils which consist chiefly of TAG (>95%). In addition, krill oil is especially abundant in n-3 polyunsaturated fatty acids (n-3 PUFA), particularly in eicosapentaenoic acid (EPA; C20:5) and docosahexaenoic acid (DHA; C22:6). Most of the EPA and DHA in krill are bound to PL (Gigliotti, Davenport, Beamer, Tou, & Jaczynski, 2011), while n-3 PUFA in fish are mainly associated with TAG.

* Corresponding author.
E-mail address: jqzwx12@163.com (Q. Jin).

http://dx.doi.org/10.1016/j.foodchem.2017.04.138
0308-8146/

Some studies have found that the special structure of EPA and DHA esterified as PL in krill oil led to a significantly higher incorporation of EPA and DHA into tissue and more profound cardioprotective effects than EPA and DHA as TAG (Liu et al., 2014; Rossmeisl et al., 2012). Moreover, some active substances such as astaxanthin, tocopherols, and vitamin A are also present in krill (Suzuki & Shibata, 1990; Tou, Jaczynski, & Chen, 2007); they may further improve the economic value of krill oil. Many studies have demonstrated that krill oil exerts numerous positive effects on human health, such as improving human brain function (Konagai et al., 2013), reducing cardiovascular risk (Berge, Musa-Veloso, Harwood, Hoem, & Burri, 2014), managing premenstrual syndrome (Sampalis, Bunea, Pelland, Kowalski, Duguet, & Dupuis, 2003), and reducing chronic inflammation (Deutsch, 2007). Therefore, krill oil can be considered a potential source of lipid with health-enhancing value. Especially, considering the global decline of wild-harvested fishstock, it may be a better alternative to fish oil for satisfying the growing demand from the n-3 PUFA market.

The common methods for obtaining krill oil are by extraction using organic solvents and supercritical carbon dioxide ($SC-CO_2$). The most used two-step extraction process using acetone and ethanol with whole fresh krill led to high oil yield (Beaudoin & Martin, 2004). However, the two separate extraction steps are laborious and time-consuming. Gigliotti et al. (2011) improved the process to extract lipid from freeze-dried krill by using a one-step strategy with isochoric acetone and ethanol; a high efficiency of lipid extraction could also be achieved by this method, but the extraction procedures are simpler than the previous two-step extraction. Furthermore, the extrusion of krill meal prior to oil extraction could promote lipid yield when using n-hexane as the extraction solvent (Yin et al., 2015). $SC-CO_2$ was first applied for krill oil extraction in 1986 (Yamaguchi et al., 1986) but it failed to extract PL. Thus, ethanol was added as a co-solvent in the supercritical fluid extraction to enhance the solute solubility for polar lipid. Notably, most of the previous studies on krill oil extraction have focused on the lipid yield and PL content of the extracts. However, available data regarding the minor components of extracted krill oil are scarce, especially with regard to tocopherols, vitamin A and sterols. As fat-soluble substances, these compounds could be co-extracted during krill oil production; and their presence in krill oil not only determine the substantial nutritional value but also influence the oxidative stability of the products. Therefore, it is essential to conduct a comprehensive assessment of krill oil composition when different extraction solvents were used.

Additionally, although supercritical fluid technique has gained wide acceptance as an alternative to organic solvent extraction for oil production given some advantages (i.e., solvent-free extract, mild supercritical conditions, environmental friendliness, etc.), the large-scale application of this technique is still limited due to high operating costs, high equipment investment and low processing capacity. Subcritical extraction has the same advantages as supercritical extraction but is easier to industrialize to apply in large-scale production and has a higher productivity (Xu, Han, Zhou, Wu, & Ding, 2015). Although some studies have focused on the use of subcritical extraction technology for obtaining vegetable oil (Gnayfeed, Daood, Illés, & Biacs, 2001; Santos et al., 2015), the literature lacks information on krill oil extraction using subcritical technology.

The purpose of this study was to investigate the effects of seven widely used solvents (ethanol, isopropanol, acetone, ethyl acetate, isohexane, n-hexane, and subcritical butane) on the lipid yield and quality of krill oil extracted from krill meal. PL, fatty acids (FA), and minor components including astaxanthin, sterols, tocopherols and vitamin A, in the extracted krill oil were analyzed. Soxhlet extraction and the Folch method were conducted as references. The comparative results will be beneficial in providing powerful insights into the influences of the different extraction solvents on the quality of krill oil.

2. Materials and methods

2.1. Materials

Krill meal was purchased from Antarctic Farm Biotechnology Co., Ltd. (Jinan, Shandong, China) and kept frozen at −40 °C until processed.

The standards of 37 fatty acid methyl esters (FAME), α-, β-, γ-, and δ-tocopherols (purity > 95%), retinol, and 5α-cholestane were purchased from Sigma-Aldrich Chemical Co., Ltd. (Shanghai, China). The astaxanthin standard was purchased from Aladdin Industrial Co., Ltd. (Shanghai, China). Other reagents and solvents were provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Lipid extraction

When ethanol, isopropanol, acetone, ethyl acetate, isohexane, and n-hexane were used as extraction solvents, the experiments were conducted as follows: 100 g of krill meal was weighed in a continuous shaker, and the lipid was extracted using 1200 mL of solvent at 30 °C for 2 h. The solvent was then removed at 50 °C in a rotary evaporator. Similar to the procedure of Gigliotti et al. (2011), the krill meal to solvent ratio was set at 1:12 (*w:v*).

A pilot-scale subcritical extraction unit purchased from Henan Yalinjie Bio Technology Co., Ltd. (Anyang, Henan, China) was used to conduct the subcritical butane extraction. The experiment was performed at 30 °C for 1 h at a pressure range of 0.3–0.8 MPa. The krill meal to butane ratio was 1:2 (*w:v*), as reported by Xu et al. (2015) for wheat germ oil extraction using subcritical butane.

For comparison, krill oil was extracted from krill meal using the Folch method (Folch, Lees, & Sloane, 1957) and Soxhlet extraction. In the Folch method, 10 g of krill meal was weighed, and the lipid was extracted using 200 mL of a 2:1 chloroform:methanol (*v:v*) mixture, as described by Gigliotti et al. (2011). The Soxhlet method used in this study was that of the International Organization for Standardization (ISO), which involves the gravimetric determination of the oil from the petroleum ether extract (ISO 659, 1988). The extraction process lasted 8 h.

2.3. Determination of lipid yield

For each lipid extraction, the weight of the initial krill meal and the weight of the extracted lipid were recorded to calculate the lipid yield. That yield was determined gravimetrically based on the following formula:

$$\text{Lipid yield } \% = \frac{y}{a} \times 100$$

where y is the mass of the extracted lipid (g) and a is the initial krill meal weight (g).

2.4. Determination of PL in extracted krill oil

PL content and composition were determined according to the method described by Avalli and Contarini (2005) with some modifications. A certain amount of krill oil (0.1 g) was dissolved in 1 mL chloroform:methanol (2:1, *v:v*) and transferred into a capped test tube. Five microliters of the solution was injected into a high-performance liquid chromatographic system (HPLC) (1260 Infinity, Agilent, Santa Clara, CA, USA) with an evaporative light scattering detector (ELSD) for separation of PL. A silica column (5 μm, 250 × 4.6 mm; Grace, Columbia, Maryland, USA) was used. Gradi-