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Production of three types of krill oils from krill meal by a three-step solvent extraction procedure

Dan Xie[a,b], Hongyan Mu[c], Tianpei Tang[a], Xiaosan Wang[a], Wei Wei[a], Jun Jin[a], Xingguo Wang[a], Qingzhe Jin[a,*]

[a] Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province, National Engineering Research Center for Functional Food, School of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue, 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
[c] College of Food Science and Engineering, Qingdao Agricultural University, 700 Changcheng Road, Qingdao, Shandong 266109, PR China

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Hexane (PubChem CID: 8058)
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Sodium hydroxide (PubChem CID: 14798)
Retinol (PubChem CID: 445354)
5α-Cholestane (PubChem CID: 2723895)
1,1-Diphenyl-2-picylhydrazyl (PubChem CID: 74358)
Eicosapentaenoic acid (PubChem CID: 446284)
Docosahexaenoic acid (PubChem CID: 445580)

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ABSTRACT

In this study, a three-step extraction method (separately use acetone, hexane, and ethanol as extraction solvent in each step) was conducted to selectively extract three types of krill oils with different compositions. The lipid yields were 5.08% in step 1, 4.80% in step 2, and 9.11% in step 3, with a total of 18.99%. The krill oil extracted with acetone in step 1 (A-KO) contained the lowest contents of phospholipids (PL) (2.32%) and n-3 polyunsaturated fatty acids (PUFA) (16.63%), but the highest levels of minor components (505.00 mg/kg of astaxanthin, 29.39 mg/100 g of tocopherols, 34.32 mg/100 g of vitamin A and 27.95 mg/g of cholesterol). By contrast, despite having traces of minor components, the krill oil extracted using ethanol in step 3 (E-KO) was the most abundant in PL (59.52%) and n-3 PUFA (41.74%). The krill oil extracted using hexane in step 2 (H-KO) expressed medium contents of all the testing indices. The oils showed significant differences in the antioxidant capacity (E-KO > H-KO > A-KO) which exhibited positive correlation with the PL content. These results could be used for further development of a wide range of krill oil products with tailor-made functions.

1. Introduction

Antarctic krill (*Euphausia superba*), a zooplankton crustacean, is an abundant krill species found in the Southern Ocean. It is composed of 11.9–15.4% protein, 0.5–3.6% lipids, 3% ash, 2% chitin and carbohydrates, and 77.9–83.1% water (Grantham, 1977). Recently, nutraceutical aspects of krill have been well reported (Tou, Jaczynski, & Chen, 2007), contributing to the increasing interest in the research and development of krill products.

Krill oil is the lipid extracts obtained from whole Antarctic krill or krill meal. It comprises diverse types of lipid classes, such as phospholipids (PL), triacylglycerols (TAG), diacylglycerols (DAG), monoacylglycerols (MAG), free fatty acids (FFA), *etc* (Phleger, Nelson, Mooney, & Nichols, 2002). It has been recognized that krill oil contains substantial concentrations of n-3 polyunsaturated fatty acids (n-3 PUFA) in the form of PL, which is an alternative form of n-3 PUFA as TAG in fish oil (Gigliotti, Davenport, Beamer, Tou, & Jaczynski, 2011). Furthermore, minor compounds including astaxanthin, tocopherols, vitamin A, and cholesterol are also present in krill oil with varying amounts (Xie et al., 2017).

Recent studies have recommended use of krill oil to prevent chronic disorders like cardiovascular diseases, non-alcoholic fatty liver disease, hyperlipidemia, glycemia, hypercholesterolemia, obesity, premenstrual syndrome and inflammation (Deutsch, 2007; Sampalis et al., 2003; Sebastiano Banni, Murru, & Cordeddu, 2011; Tandy et al., 2009). Presently, considerable research interest exists in comparing the performance of krill oil and fish oil supplements. Some studies indicated that krill oil was superior to fish oil due to the higher

* Corresponding author.
E-mail addresses: jqzwx12@163.com, jqzwuxi@jiangnan.edu.cn (Q. Jin).

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bioavailability of eicosapentaenoic acid (EPA; C20:5) and docosahexaenoic acid (DHA; C22:6) in the form of PL (Rossmeisl et al., 2012; Ulven et al., 2011). However, this is controversial because several studies about krill oil and fish oil did not compare the health outcomes at identical doses of EPA and DHA (Laidlaw, Cockerline, & Rowe, 2014; Ramprasath, Eyal, Zchut, & Jones, 2013). Köhler, Sarkkinen, Tapola, Niskanen, and Bruheim (2015) found that krill meal had the lower bioavailability of EPA and DHA than krill oil, while the same with fish oil. This finding implied that the chemical form of EPA and DHA in PL appeared not to be mainly responsible for the better bioavailability of krill oil as the lipid in krill oil and krill meal was identical. Till now, no direct evidence supports the fact that the better properties of krill oil are dominated by EPA and DHA in the form of PL. In addition to EPA and DHA, astaxanthin, tocopherols, vitamin A are also important bioactive compounds, which are beneficial to human health at low doses. Cholesterol has been considered to potentially increase the risk of cardiovascular disease. The presence of these compounds in krill oil certainly have an extra impact to human health. However, which composition mainly determines the quality of the krill oil product and further dominates its functionalities or whether they do as a whole remain to be studied.

Recently, solvent extraction is the most commonly method to produce krill oil from krill meal or whole krill. Ethanol or the combination of ethanol and acetone are normally preferred to extract krill oil due to the high extraction efficiency of lipid (Beaudoin & Martin, 2004; Gigliotti et al., 2011). However, little attention has been paid to the chemical composition of krill oil during the krill oil extraction, especially the minor components. In fact, the content and composition of lipid in krill have shown to fluctuate with interannual changes, seasonal variations, sexual maturity, and capture stations of krill samples (Clarke, 1980; Phleger et al., 2002). Besides, our previous study has demonstrated that various extraction solvents have significant effects on the composition of krill oil, such as PL, fatty acids, and minor components (Xie et al., 2017). Thus, the complexity of krill lipid and the diversity of extraction method lead to the irregularity of the quality of krill oil product; and in turn, make it difficult to study the relationship between composition and function of krill oil.

In the present study, a novel three-step extraction was performed in an attempt to achieve three types of krill oils with different compositions. Acetone, hexane and ethanol were selected as the extraction solvent successively used in each step for their large differences in the krill lipid extraction (Xie et al., 2017). The lipid yield was determined and the krill oil extracted in each step was collected separately to characterize their chemical compositions. The antioxidant capacities of the three types of krill oils were also compared to preliminary explore the relationship between chemical composition and functional property of krill oil. This study will provide a new approach for extraction of krill oil and could be helpful to exploit tailored krill oils having targeted functionalities.

2. Materials and methods

2.1. Materials

Krill meal was provided by Antarctic Farm Biotechnology Co., Ltd. (Jinan, Shandong, China) and then stored at −40 °C until processed. Prior to the extraction procedure, the krill meal was dried to constant weight in the vacuum at 60 °C.

The 37-component fatty acid methyl esters (FAME), 5α-cholestane, retinol, tocopherols standards, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), and 1,1-diphenyl-2-picylhydrazyl (DPPH) were purchased from Sigma-Aldrich Chemical Co., Ltd. (Shanghai, China). The astaxanthin standard was bought from Aladdin Industrial Co., Ltd. (Shanghai, China). The distearoyl phosphatidylcholine (DSPC) and distearoyl phosphatidylethanolamine (DSPE) standards were purchased from A.V.T. Pharmaceutical Co., Ltd (Shanghai, China). All other reagents and solvents used were purchased from Sinopharm Chemical Regent (Shanghai, China).

2.2. Lipid extraction

Fig. 1 illustrates the flow diagram of oil extraction in the three-step method. The krill lipid was firstly extracted with acetone, followed by hexane, and finally ethanol step by step. The extracted oil in each step was separately collected: the krill oil extracted using acetone in step 1 (A-KO), the krill oil extracted using hexane in step 2 (H-KO) and the krill oil extracted using ethanol in step 3 (E-KO), respectively.

Specifically, in step 1, 100 g of krill meal was mixed with 200 mL of precooling acetone and the lipid was extracted at 4 °C for 15 min. The mixture was then filtered using a Buchner funnel. The A-KO was recovered from the filtrate by removing acetone at 30 °C using a vacuum rotary evaporator. The residual krill meal was then dried and weighed as the extraction material for the next step (A-KM). In step 2, the lipid extracted from the A-KM using hexane at 30 °C for 15 min. The ratio of A-KM to hexane was 1:2. H-KO was obtained in similar manner with step 1 and the residual krill meal obtained in step 2 was also dried and weighed as the extraction material for the next step (H-KM). Step 3 extracted E-KO from H-KM using ethanol at 30 °C for 20 min at a 1:3 ratio (H-KM/ethanol, *w:v*).

2.3. Determination of lipid yield

The amount of the lipid extracted in each step (A-KO, H-KO, and E-KO) and the amount of the initial krill meal (dry basis) were recorded. The lipid yield was calculated by the following equation:

$$\text{Lipid yield } (\%) = \frac{\text{m}}{\text{x}} \times 100$$

where m is the weight of extracted lipid (g) in each step, x is the mass of initial krill meal (g).

2.4. Determination of PL content

The determination of PL content was performed on a high-performance liquid chromatographic system (HPLC) (1260 Infinity, Agilent, Santa Clara, CA, USA) equipped with an evaporative light scattering detector (ELSD). One hundred milligrams of krill oil was dissolved in 1 mL chloroform/methanol (2:1, *v:v*) and 5 μL of the product was injected for analysis. A silica gel column (5 μm, 250 × 4.6 mm; Grace, Columbia, Maryland, USA) was used to separate PL. Nitrogen was used as the nebulizing gas with a flow rate of 1.5 L/min. The evaporating temperature was set at 60 °C. The elution program was operated at 35 °C. The mobile phase were: solvent A (10 mM ammonium acetate/isopropanol (1:2, *v:v*)); solvent B (*n*-hexane); solvent C (isopropanol). Gradient conditions for PL analysis were set as Table 1. Standard curves for phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were calculated from the area values obtained by injecting 5 μL of chloroform/methanol (2:1, *v:v*) serially diluted solutions of DMPC (5–25 μg), DMPE (5–25 μg).

The standard curve for PC was:

$$Y = 17435X - 2537.6; R^2 = 0.9953$$

where Y is the area value of PC, X is the mass (μg) of PC injected.

The standard curve for PE was:

$$y = 16814x - 3173.9; R^2 = 0.9953$$

where y is the area value of PE, x is the mass (μg) of PE injected.

The PC and PE contents were calculated according to the corresponding standard curve. The total PL content was the sum of PC and PE and reported in g/100 g oil.