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# KRILL OIL EXTRACTION METHOD: LIPID EXTRACTION METHOD

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## ABSTRACT

The marine crustacean krill (order Euphausiacea) has not been a traditional food in the human diet. Krill is a rich source of high-quality protein, with the advantage over other animal proteins of being low in fat and a rich source of omega-3 fatty acids. Antioxidant levels in krill are higher than in fish, suggesting benefits against oxidative damage (Tou et al. 2007). Krill oil extracted with different methods among that methods lipid extraction method is most widely used. The EH method yielded similar amount of lipids (up to 97. 72% of total lipids) with subcritical butane extraction method (97.60%). The recovery rate of ethanol and hexane was 83.6% and 86.86%, respectively (Sun et al. 2019).

## INTRODUCTION

Krill is by far the most dominant member of the Antarctic zooplankton community in terms of biomass, which is estimated to be between 125 and 750 million metric tons (Ulven et al. 2011). Krill play a major role in the transfer of energy in marine food webs, being important consumers of phytoplankton and other zooplankton, and prey of many higher tropic level predators that are often commercially important (Murphy, 2001). Antarctic krill (*Euphausia superba*) is considered as a new alternative, sustainable source to long chain n–3 PUFA. Krill oil also contains antioxidants such as astaxanthin and vitamins A and E (Ulven, 2016). Krill oil, a relatively new source of long-chain omega-3s, is extracted from Antarctic krill (*Euphasia superba*) and Pacific krill (*Euphasia pacifica*). The main characteristic of this oil is that the EPA and DHA in it are structurally attached to phospholipids molecules (Hernandez, 2016).

Krill oil has therapeutic potential in the treatment of chronic disorders, including inflammation, cardiovascular disease, hyperlipidemia, arthritis, neurological disorders, kidney disease and diabetes. Krill oil is an important source of n-3 and is particularly rich in long chain polyunsaturated fatty acids EPA and DHA. Krill contains 26.1% saturated fat, 24.2% monounsaturated fat, 48.5% polyunsaturated fat, 17.4% EPA, and 12.4% DHA (Tou et al. 2007). Krill oil has a unique biomolecular profile of phospholipids naturally rich in omega-3 fatty acids and diverse antioxidants significantly different from the usual profile of fish oils. The association between phospholipids and long-chain Krill oil has a unique biomolecular profile of phospholipids naturally rich in omega-3 fatty acids and

diverse antioxidants significantly different from the usual profile of fish oils. The association between phospholipids and long-chain (Bunea et al. 2004).

## MATERIAL & METHOD

### MATERIAL

Antarctic krill were obtained from China National Fisheries Co., Ltd. Then, the shrimp meal was obtained after the process of Microwave thawing, heating at 95°C for 5 min, and centrifugation, followed by freezing. Frozen Antarctic krill were then delivered to our laboratory and stored at −30°C until use. Prior to the experiments, the krill were crushed using a hammer crusher and then stored in a low temperature (−30°C) warehouse (Sun et al. 2019).

### KRILL OIL EXTRACTION TECHNOLOGIES

### CONVENTIONAL EXTRACTION TECHNIQUES

Lipid- extraction Method is most common solvent extraction method (Colletti et al. 2021). When ethanol and hexane were used as extraction solvents, the following experiments were conducted: 10 g of frozen Antarctic krill was weighed, and KO was extracted using EH (ethanol/hexane = 4:6). After extraction, the filtrate was stratified, and the upper hexane layer and the lower ethanol layer were separated. KO in the upper and lower layers was obtained via rotary evaporation and then collected in a glass vessel for analysis (Sun et al. 2019).

$$\text{Lipid extraction efficiency\%} = \frac{\textit{The KO extracte by EH}}{\textit{The KO extracted by Folch}} \times 100$$

A pilot-scale subcritical extraction unit purchased from Henan Subcritical Bio Technology Co., Ltd. was used to conduct the subcritical extraction. The experiment was performed at 30°C for 1 hr at a pressure range of 0.3–0.8 MPa and was repeated four times with butane and butane-dimethyl ether as solvent. For comparison, Antarctic krill oil was assessed via Folch method after the Antarctic krill cells were thoroughly homogenized (Sun et al. 2019).

Krill oil can also be extracted using non-solvent techniques including mechanical pressing, which is commonly used for oilseed extraction, such as sesame oil and sun flower oil. Non-solvent sequential procedures, such as cooking, decanting, pressing and centrifuging, are able to successfully separate krill oil from the mixture (Colletti et al. 2021).

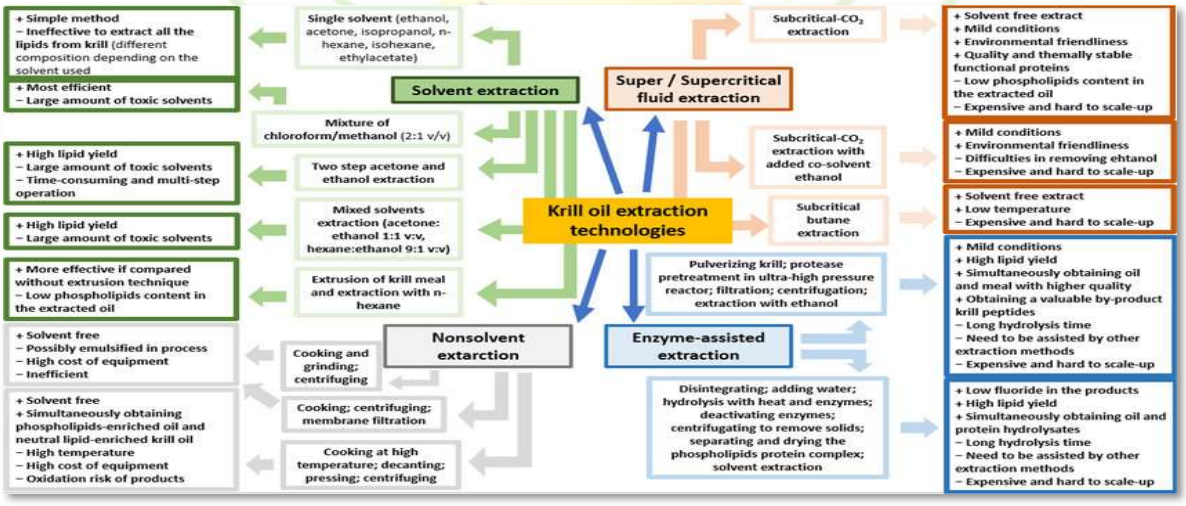


Figure 1: Krill oil extraction technologies (Colletti et al. 2021).

## UNCONVENTIONAL EXTRACTION TECHNIQUES

The supercritical fluid extraction method is an unconventional extraction technique that can be used for krill oil extraction and is known to be solvent-free. It is considered environmentally friendly as it uses supercritical carbon dioxide, which is safe, non-toxic and chemically inert (Colletti et al. 2021).

## RESULTS & DISCUSSION

As shown in Figure 2a, when the volume of ethanol was higher than hexane, the filtrate did not stratify. As the proportion of ethanol in the extraction solvent decreased, lipid extraction efficiency increased. When the volumetric ratio of ethanol/hexane was 6:4, lipid extraction efficiency increased up to 74.49%. When the volume of hexane was higher than ethanol, after standing for 30 min, the filtrate stratified into two layers. The total lipid extraction efficiency can approach 95.23% (54.77% for ethanol layer and 40.46% for hexane layer) at a volumetric ratio of ethanol/hexane 4:6 (Sun et al. 2019).

The effect of reaction time on lipid extraction efficiency of ethanol and hexane layers was evaluated and presented in Figure 2b. The lipid extraction efficiency of both layers increased gradually with time. The highest lipid extraction efficiency of 52.53% (ethanol layer) and 44.80% (hexane layer) was achieved at 4 hr, with no significant increase thereafter (Sun et al. 2019).

Figure 2c showed that the lipid extraction efficiency of both layers was further enhanced with an increase in temperature from 20 to 40°C, which resulted in a prominent increase in lipid extraction efficiency from 76.47% to 97.37% (52.52% of ethanol layer and 44.85% of hexane layer). However, a slight reduction was observed beyond 40°C (Sun et al. 2019).

As shown in Figure 2d, lipid extraction efficiency of both layers gradually increased with addition of solvent until equilibrium was attained. A solvent ratio of 1:25 served as the optimum value to obtain a lipid extraction efficiency of 97.38% (52.53% of ethanol layer and 44.85% of hexane layer), beyond

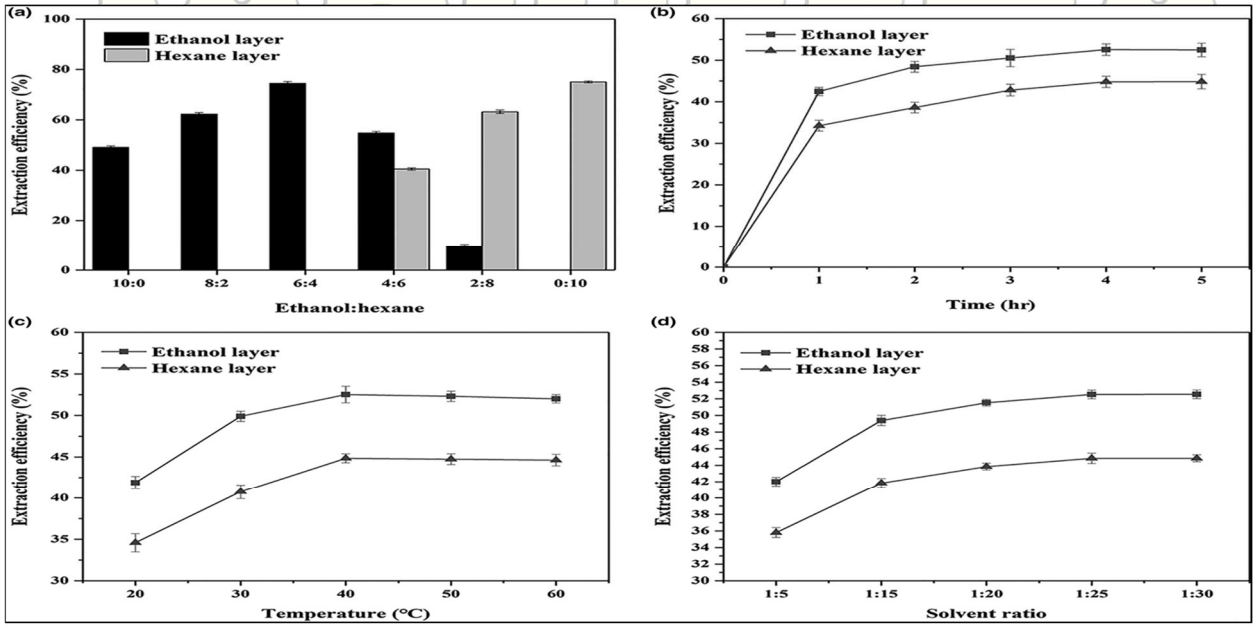


Figure 2: a) Effect of different ethanol–hexane ratios on lipid extraction efficiency of ethanol and hexane layers; (b) effect of different extraction times on lipid extraction efficiency of ethanol and hexane layers; (c) effect of different temperatures on lipid extraction efficiency of ethanol and hexane layers; (d) effect of different solvent ratios on lipid extraction efficiency of ethanol and hexane layers. The lipid extraction efficiency is the sum of both in ethanol and hexane layers

which there was no significant change in efficiency (Sun et al. 2019).

Meanwhile, the recovery rate of ethanol was 83.6%, and that of hexane was 86.86% (Sun et al. 2019).

## CONCLUSION

KO was extracted via the EH method and subcritical method. The results indicated that the EH method can be used to extract two kinds of KO with high nutritional value, simultaneously (Sun et al. 2019).

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