Extraction of Omega-3 Fatty Acids from Atlantic Sea Cucumber (*Cucumaria frondosa*) Viscera Using Supercritical Carbon Dioxide

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1. Introduction

Marine invertebrates, sea cucumbers, are ideal functional food with high nutritional value and have been exploited and used for hundreds of years as a food delicacy and medicines for a wide variety of disease[1,2]. Sea cucumbers are comprised of three parts, including body walls, tentacles, and viscera[3]. The general practice of preparing sea cucumbers for food market almost relies on cutting, trimming, and removing tentacles and internal organs during processing; as such, considerable amount of sea cucumber by-products containing valuable matter ends up in the landfill. The growing market for nutraceutical supplements, especially for those are high in Omega-3 fatty acids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), provides an opportunity to enhance the profit margins through generating values from by-products that are typically discarded as wastes[4]. EPA and DHA promotes anti-inflammatory activities and supports cardiovascular health, brain function, metabolism and immune function, and anti-cancer[7]. Also, adverse environmental impact of waste drive research into extraction of value-added compounds, such as lipids and astaxanthin from marine waste[4–6].

To the best of our knowledge, current studies on sea cucumber lipid extraction mainly use conventional methods involving organic solvents like chloroform and n-hexane[8–12]. However, use of chlorinated solvents like chloroform is associated with the environmental and health concerns; whereas other solvents like n-hexane and n-heptane demonstrate low extraction yield[4]. The cost of large amount of solvents required, their health and environmental concerns, and strict requirements for products purity, challenge the use of organic solvents for the extraction of natural products at the industrial levels. Thus, to eliminate/reduce the use of organic solvents, researchers are motivated to seek for greener alternatives to conventional extraction methods; among which, the supercritical carbon dioxide ($scCO_2$) extraction method has attracted increasing interests.

2. Materials and Methods

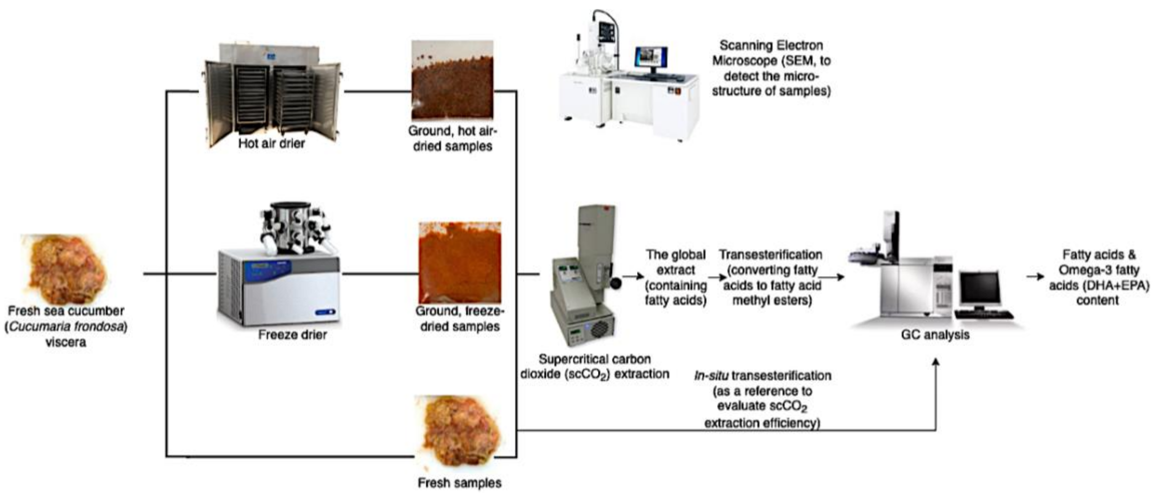

Figure 1. The flow chart of extraction of Omega-3 fatty acids from *Cucumaria frondosa* viscera using $scCO_2$.

Cucumaria frondosa viscera were prepared in the form of fresh, hot air-dried, and freeze-dried samples to evaluate the effects of different drying methods on $scCO_2$ extraction. Both conventional and $scCO_2$ extraction method were conducted and the extracts were transesterified and analyzed by GC-FID. The process variables were primarily screened using single-factor experiments by fixing all parameters except one variable, and a response surface method, central composite inscribed (CCI) design with selected parameters was created. The fatty acid yields and selected Omega-3 fatty acids (DHA+EPA) were evaluated as the response variables for optimization.

3. Results and discussion

The main findings include:

1. Experimental extraction condition including temperature of 55 ˚C, pressure of 35 MPa, dynamic time of 50 min, and co-solvent to biomass ratio of 2:1 yielded a maximum amount of fatty acids with recovery efficiency of 97.67 %, while the highest selected Omega-3 fatty acids yields with a recovery efficiency of 82.404% was obtained when the processing variables were set as temperature of 75 ˚C, pressure of 35 MPa, dynamic extraction time of 50 min, and mass ratio of co-solvent to biomass of 1:1.
2. In the optimization, temperature of 75 ˚C, pressure of 45 MPa, dynamic extraction time of 30 min, and co-solvent to biomass mass ratio of 2:1 was suggested by a statistical software, with experimental fatty acids and selected Omega-3 yields at 14.400 g/100 g of the hot air-dried samples and 2.939 g/100 g of the hot air-dried samples, respectively.
3. The Bligh & Dyer method extracted significantly more crude oils due to the co-extraction of some untargeted compounds, but scCO_2 extraction showed higher selectivity to fatty acids. These two methods yielded comparable selected Omega PUFAs and fatty acids composition.
4. Freeze drying was examined to be the most effective pre-treatment method with higher fatty acids yields and lower moisture content, but different pre-treatments did not alter yields of selected Omega-3 fatty acids significantly and only had marginal impacts on fatty acids profiles. Comparable Omega-6/Omega-3 ratios were also obtained from samples with different pre-treatments.

4. Conclusions

This is the first attempt to use scCO_2 extraction to recover fatty acids from sea cucumber by-products. The effects of process variables and various pretreatment processes on product yields was investigated. The study extended to optimization of process conditions to increase the extraction yield. The developed process is a greener alternative to conventional solvent extraction methods, which helped in eliminating the use of toxic organic solvents and addressed the concern over the presence of residual organic solvents in nutraceutical products.

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