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Supercritical Extraction Technology of Obtaining Polyunsaturated Acids from Starfish (*Lysastrosoma anthosticta* Fisher, 1922)

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Abstract.
Introduction. Starfish (*Asteroidea*) are marine echinoderms with more than 160 species. Starfish are a valuable source of protein and fats. The present research featured the chemical composition of starfish, which can be used as a commercial source of lipids.
Study objects and methods. The study defined the optimal parameters for extracting the lipid fraction of *Lysastrosoma anthosticta* with supercritical carbon dioxide, as well as the qualitative composition of the obtained extracts.
Results and discussion. The yield of fatty acids obtained with supercritical carbon dioxide co-solvent was 1.8 times higher than that obtained with standard extraction according to the Folch method. The content of impurities was lower than in the samples with chloroform-methanol system. The polyunsaturated fatty acids isolated from *L. anthosticta* mainly belonged to ω-3 (18.0%), ω-6 (11.7%), ω-7 (21.2%), ω-9 (10.1%), and ω-11 (6.5%). The rest was saturated fatty acids, mainly palmitic (14%) and myristic (6%). The qualitative composition of the lipid fraction did not depend significantly from the isolation method. However, the supercritical extraction increased the product yield, extraction rate, and the quality of the extraction residue. Supercritical carbon dioxide left a dry residue, which had no typical smell and was brittle enough for grinding. Such residue can presumably be used to produce protein concentrate.
Conclusion. Supercritical extraction with chloroform can be recommended to isolate fatty acids from marine organisms at 60°C and 400 bar.

Keywords. Starfish, echinodermata, unsaturated fatty acids, lipids, sea

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Применение технологии сверхкритической экстракции для получения полиненасыщенных кислот из морской звезды (*Lysastrosoma anthosticta* Fisher, 1922)

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Аннотация.
Введение. Морские звезды (*Asteroidea*) насчитывают более 160 видов, что делает их ценным сырьем для производства белков и жиров. Настоящее исследование позволило определить химический состав морских звезд и доказало целесообразность использования этого ресурса в качестве коммерческого источника жиров.
Объекты и методы исследования. В ходе исследования были определены оптимальные параметры экстракции липидной фракции *Lysastrosoma anthosticta* сверхкритическим диоксидом углерода, а также описан качественный состав полученных экстрактов.
Результаты и их обсуждение. Выход жирных кислот, полученных со сверхкритическим сорастворителем диоксида углерода, был в 1,8 раза выше, чем при стандартной экстракции по методу Фолча. Содержание примесей оказалось ниже, чем в образцах, где использовалась система хлороформ-метанол. Полиненасыщенные жирные кислоты, выделенные из *L. anthosticta*, принадлежали к ω-3 (18,0 %), ω-6 (11,7 %), ω-7 (21,2 %), ω-9 (10,1 %) и ω-11 (6,5 %). Остальное составляли насыщенные жирные кислоты: пальмитиновая (до 14 %) и миристиновая (до 6 %). Качественный состав липидной фракции не отличался от метода выделения. Однако сверхкритическая экстракция увеличила выход продукта, скорость экстракции и качество экстракционного остатка. Сверхкритический диоксид углерода оставил твердый осадок, который не имел характерного запаха и был достаточно хрупким для дальнейшего измельчения. В будущем такой остаток можно использовать для получения белкового концентрата.
Выводы. Сверхкритическая экстракция хлороформом может быть рекомендована для выделения жирных кислот из морских организмов при 60°C и 400 бар.

Ключевые слова. Морская звезда, иглокожие, ненасыщенные жирные кислоты, липиды, море

Финансирование. Работа выполнена при финансовой поддержке гранта Президента Российской Федерации (SP-3156.2019.4).

Для цитирования: Применение технологии сверхкритической экстракции для получения полиненасыщенных кислот из морской звезды *Lysastrosoma anthosticta* Fisher, 1922 / А. М. Захаренко [и др.] // Техника и технология пищевых производств. 2021. Т. 51. № 4. С. 753–758. (На англ.). https://doi.org/10.21603/2074-9414-2021-4-753-758.

Introduction

The population of the earth is growing every year, which makes technologies for food obtaining and processing very important for humanity. Efficient processing technologies produce more useful products while doing less harm to the environment. The oceans are the least explored part of the earth. Every year, dozens of new compounds are isolated from marine aquatic organisms around the world. Many of them possess various beneficial biological properties that can be used, for instance, in pharmacology. Every year, new secondary metabolites of great practical and fundamental interest are extracted from echinoderms.

Starfish (*Asteroidea*) are widespread marine echinoderms of more than 160 species. Starfish are predators that damage shellfish plantations and coral reefs. In the XX–XXI centuries, world fisheries have been busy looking for new sources of nutrients, especially for marine carriers of biologically active substances that can be used to obtain highly effective medicines [1]. The present research featured *Lysastrosoma anthosticta*; the research objective was to select a promising method of supercritical extraction.

Supercritical fluid extraction and supercritical fluid chromatography have been used since the late 1970s in food analysis for determining lipids and toxicants. Supercritical fluid extraction is an effective means of natural product extraction. The supercritical extraction process has potential advantages over conventional extraction processes, such as shorter extraction time, reduced organic solvent volume, and more selective extraction [2].

Study objects and methods

Samples of *Lysastrosoma anthosticta* Fisher, 1922 were harvested in the Peter the Great Bay (Russia, Sea of Japan) in 2020. Starfish with a total weight of 42 000 g were gutted, cut with scissors into small pieces (about 1 cm long), and stored in a plastic bag at –70°C. Frozen samples were lyophilized and crushed. The dry weight was 3630 g. After that, 30 g of aliquots was used for extraction. All experiments were done in triplicates. Extraction with supercritical CO_2 was performed using THAR SFC 500 (USA). Figure 1 shows the technological scheme of the supercritical extraction unit. Extraction was carried out using supercritical CO_2: pressure – 200, 400, and 600 bar per square inch; t – 30, 40, 50, 60, and 70°C; flow rate – 20 g/min. At the second step, the experiment extraction was carried out using CO_2 and 5% solvent (chloroform): pressure – 200, 400, and 600 bar per square inch; t – 30, 40, 50, 60, and 70°C; flow rate – 20 g/min. The control extraction of 30 g of dried starfish was carried out using the Folch method with a mix of chloroform-methanol (2:1) at the rate of 20 parts of the extraction mixture per one part of the tissue at 30, 40, 50, 60, and 70°C [3].

The extracts were analyzed by high-performance liquid chromatography (HPLC) with tandem mass spectrometry

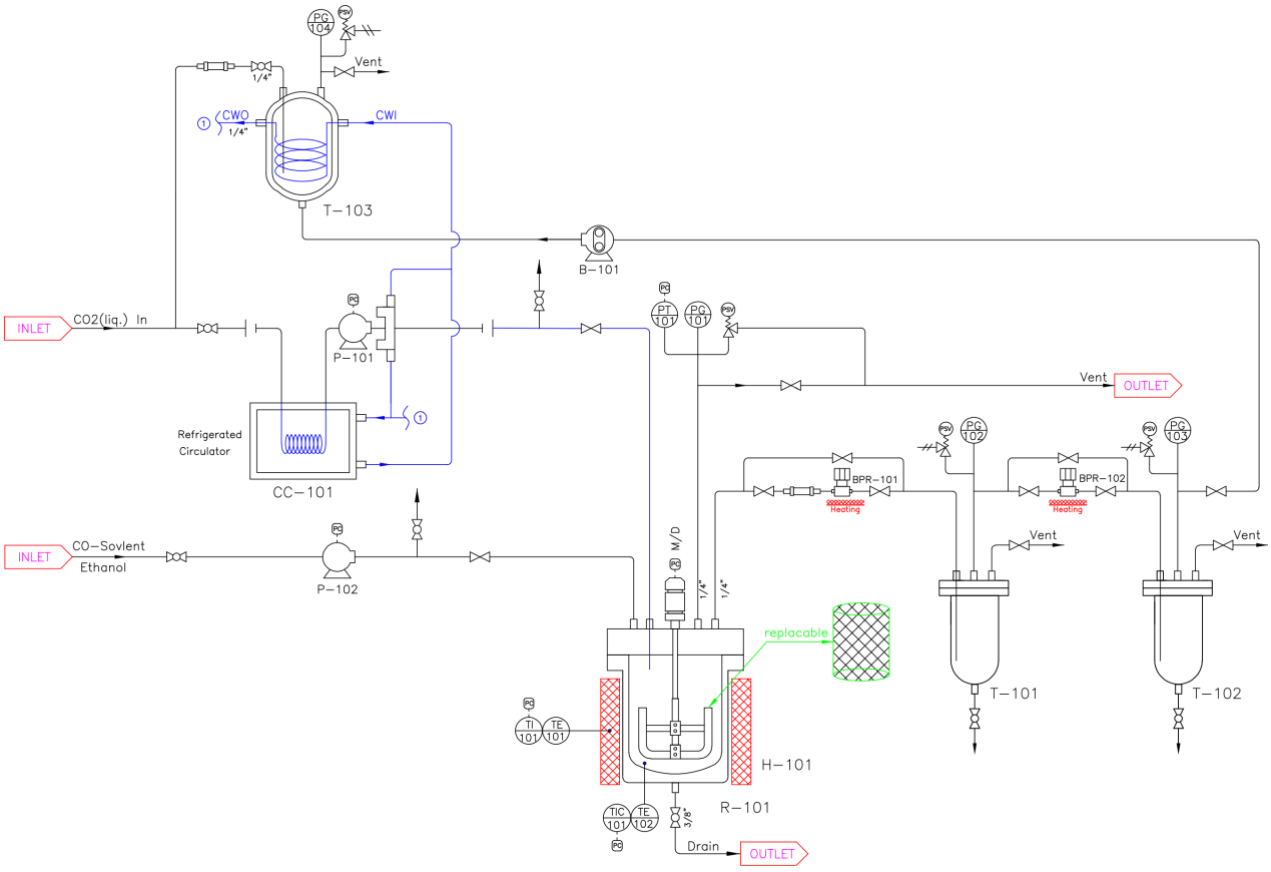

Figure 1. Technological scheme of the supercritical extraction unit

Figure 2. HPLC and ion trap joint system with tandem mass spectrometry

(LC-MS/MS). Reverse-phase HPLC was performed using a Shimadzu LC-20 liquid chromatograph (Shimadzu, Japan) equipped with a CTO-20A thermostat (Shimadzu, Japan) and a UV-VIS SPD-20A detector (Shimadzu, Japan). ZORBAX Eclipse XDB C_{18} (150×4.6 mm, particle size: 5 microns) was used as an analytical column at a temperature of 30°C and a total flow rate of 0.22 mL/min. Gradient elution with two mobile phases

(A – deionized water; B – acetonitrile with formic acid 0.1% v/v) was programed as follows: 0 min 0% V, 25 min 100% V, 60 min 100% V. The chromatograph and the mass spectrometric detector were linked by the Compass software, which made it possible to integrate the entire system into a single complex (Fig. 2).

The mass spectra of electrospray ionization mass spectrometry (ESI-MS) and electrospray ionization tandem mass spectrometry (ESI-MSN) were performed using an Amazon SL ion trap (Bruker, Germany) equipped with an electrospray ionization source. The ESI MS ionization parameters were optimized as follows: capillary voltage of 4500 V, nitrogen spraying at 29 psi, dry gas consumption of 10 l/min at 160°C. Mass spectra were recorded in the mass range m/z 50–2000 in the mode of negative and positive ions. The mass spectra of the ions were recorded in the auto MS/MS mode.

Results and discussion

An analysis of yields of lipids fraction from *Lysastrosoma anthosticta* under various conditions showed that the extraction with supercritical carbon dioxide with no co-solvent was less effective than the standard extraction according to the Folch method (Fig. 3). However, when extra 5% chloroform was added to the extraction system as a co-solvent, it significantly increased the yields of the total fatty acid fraction. The choice of the solvent and the extraction range parameters was based on the results of previous works where starfish material was treated with a plant matrix [4, 5]. Despite the fact that the main target components are soluble in liquid CO_2 only above 200 bar, it is possible to separate significant amounts of the lipid fraction [6, 7]. The lipid fraction obtained by supercritical extraction with chloroform was 1.8 times higher than in the standard Folch method. The extraction was found to be quite effective only when a co-solvent was used. The optimal parameters for extraction with a co-solvent included a temperature of 60°C and a pressure of 400 bar. The data obtained correlated with the most frequently selected parameters. Most often, when using this technology, the authors chose a pressure of 300–350 bar as the optimal one [8–11]. With these parameters, a good yield of lipids can be obtained as quickly as possible; a further increase in the temperature and extraction pressure did not lead to a significant increase in the yield.

Obtaining chemical profiles is an extremely important result for any biological analysis system. In this work, we used the HPLC-ESI-MS/MS method with additional ionization and analysis of fragmented ions. High accuracy mass spectrometric data were recorded on an ion trap amaZon SL BRUKER DALTONIKS equipped with an ESI source in the mode of negative and positive ions. The experiment used a four-stage ion separation mode (MS/MS mode). A qualitative analysis showed that the ratio of polyunsaturated to saturated fatty acids did not depend on the extraction method (Table 1). An analysis of polyunsaturated fatty acids isolated from *L. anthosticta* showed that they mainly belonged to ω-3 (18.0%), ω-6 (11.7%), ω-7 (21.2%), ω-9 (10.1%), and ω-11 (6.5%). The rest was saturated fatty acids, mainly palmitic (up to 14%) and myristic (up to 6%).

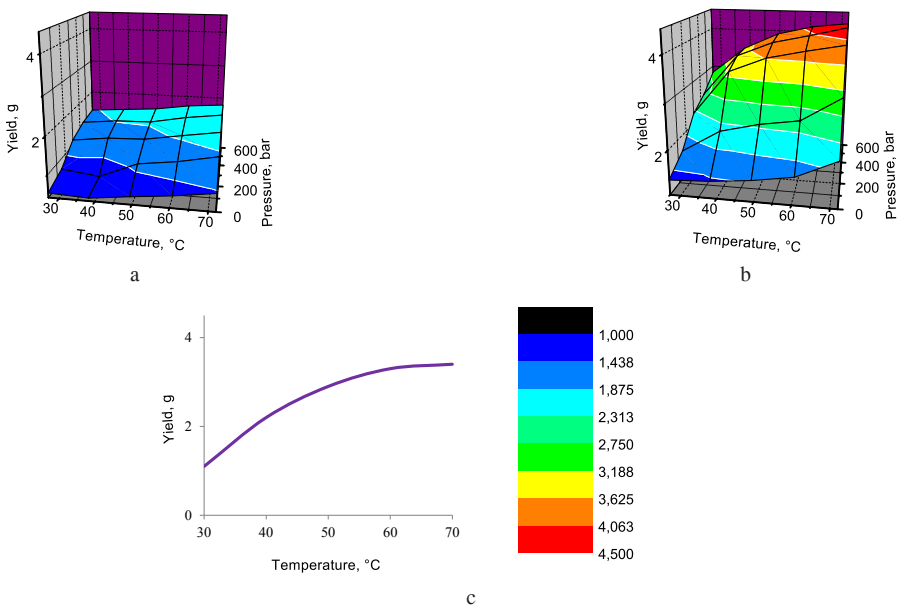

Figure 3. Effect of extraction conditions on the yield of the fatty acid: a – extraction with supercritical CO_2; b – extraction with supercritical CO_2 with a solvent (chloroform); c – extraction according to the Folch method at various temperatures

Table 1. Fatty acids analysis of extracts

Fatty acids	Yield, %		
	Control	Supercritical fluid extraction with CO_2	Supercritical fluid extraction with CO_2 and co-solvent
12:0	0.2	0.1	0.2
14:0	6.0	5.9	6.1
15:0	0.2	0.2	0.2
16:0	14.5	14.2	14
17:0	0.7	0.8	0.8
18:0	0.3	0.3	0.3
19:0	0.6	0.7	0.6
20:0	0.1	0.1	0.2
22:0	1.0	0.9	0.9
ω-3	17.8	18.0	18.0
ω-4	2.2	2.2	2.1
ω-5	0.3	0.2	0.3
ω-6	11.8	11.6	11.7
ω-7	21.0	20.9	21.2
ω-9	10.2	10.4	10.1
ω-11	6.1	6.3	6.5
ω-13	4.8	4.7	4.5
Other	2.2	2.5	2.3

The predominance of polyunsaturated acids is typical of starfish [12, 13].

A review of scientific publications showed that starfish can be considered as a valuable source of feed additives for agricultural animals and birds. For instance, Danish compound feed producers are considering the possibility of industrial use of starfish to produce additives for compound feeds. According to recent studies, such compound feed can reduce the excretion of nitrogen in pigs. Scientists from the Center for Aquatic Animals in Denmark proved that starfish can be an effective alternative to traditional sources of feed protein, e.g. soybeans. Starfish meal could replace the most commonly used protein sources and increase the weight gain in piglets. In fact, starfish-based animal feed may be more economically rational than traditional protein sources. In addition, starfish are being rigorously tested as a possible protein source for bird nutrition. If the results are confirmed, starfish will become a rich source of protein, which will create high demand from egg producers, who are constantly searching for new sources of protein [14].

Conclusion

Starfish are a valuable raw material for protein and lipid production. The chemical composition of starfish makes it possible to use it in food and feed industry. The content of proteins was 9.5–14.0%, lipids – 0.5–3.5%, minerals – 1.5–32.0%. In comparison with the integumentary tissue, the internal organs of starfish have a higher content of potassium and iron [15, 16].

The yields of fatty acids obtained under conditions of supercritical carbon dioxide co-solvent were 1.8 times higher than those obtained with the standard Folch method, while the content of impurities was lower than when the extraction was performed using a chloroform-methanol system. The analysis of polyunsaturated fatty acids isolated from *Lysastrosoma anthosticta* showed that they mainly belonged to ω-3 (18.0%), ω-6 (11.7%), ω-7 (21.2%), ω-9 (10.1%), and ω-11 (6.5%). The rest was saturated fatty acids: palmitic (14%) and myristic (6%). Thus, isolating fatty acids from marine organisms using supercritical extraction with chloroform can be recommended as an effective commercial method.

In addition, supercritical carbon dioxide with a solvent left a dry residue, brittle enough for further grinding and without typical smell. Such a residue can presumably be used to produce protein concentrate.

Contribution

All the authors contributed equally to the study and bear equal responsibility for information published in this article.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

References

1. Yavnov SV. Atlas morskikh zvezd dal′nevostochnykh morey Rossii [Atlas of starfish of the Far Eastern seas of Russia]. Vladivostok: Russian Island; 2010. 240 p. (In Russ.).

2. Taylor SL, King JW, Montanari L, Fantozzi P, Blanco MA. Enrichment and fractionation of phospholipid concentrates by supercritical fluid extraction and chromatography. Italian Journal of Food Science. 2000;12(1):65–76.

3. Pan M, Qin C, Han X. Quantitative analysis of polyphosphoinositide, bis(monoacylglycero)phosphate, and phosphatidylglycerol species by shotgun lipidomics after methylation. Methods in Molecular Biology. 2021;2306:77–91. https://doi.org/10.1007/978-1-0716-1410-5_6.

4. Zakharenko A, Romanchenko D, Thinh PD, Pikula K, Thuy Hang CT, Yuan W, et al. Features and advantages of supercritical CO_2 extraction of sea cucumber *Cucumaria frondosa japonica* Semper, 1868. Molecules. 2020;25(18). https://doi.org/10.3390/molecules25184088.

5. Razgonova MP, Zakharenko AM, Ercisli S, Grudev V, Golokhvast K. Comparative analysis of far east sikhotinsky rhododendron (*Rh. sichotense*) and East Siberian Rhododendron (*Rh. adamsii*) using supercritical CO_2-extraction and HPLC-ESI-MS/MS spectrometry. Molecules. 2020;25(17). https://doi.org/10.3390/molecules25173774.

6. Kas′yanov GI, Nematullaev I, Shaftan EhA, Shaposhnikov VG. Rastvorimost′ natural′nykh veshchestv v zhidkom diokside ugleroda [Solubility of natural substances in liquid carbon dioxide]. News of Institutes of Higher Education. Food Technology. 1997;237–238(2–3):54–57. (In Russ.).

7. Buranachokpaisan K, Muangrat R, Chalermchat Y. Supercritical CO_2 extraction of residual oil from pressed sesame seed cake: Optimization and its physicochemical properties. Journal of Food Processing and Preservation. 2021;45(9). https://doi.org/10.1111/jfpp.15722.

8. Zhou J, Gullón B, Wang M, Gullón P, Lorenzo JM, Barba FJ. The application of supercritical fluids technology to recover healthy valuable compounds from marine and agricultural food processing by-products: A review. Processes. 2021;9(2). https://doi.org/10.3390/pr9020357.

9. Gong T, Liu S, Wang H, Zhang M. Supercritical CO_2 fluid extraction, physicochemical properties, antioxidant activities and hypoglycemic activity of polysaccharides derived from fallen *Ginkgo* leaves. Food Bioscience. 2021;42. https://doi.org/10.1016/j.fbio.2021.101153.

10. Guzmán-Albores JM, Bojórquez-Velázquez E, De León-Rodríguez A, Calva-Cruz ODJ, Barba de la Rosa AP, Ruíz-Valdiviezo VM. Comparison of *Moringa oleifera* oils extracted with supercritical fluids and hexane and characterization of seed storage proteins in defatted flour. Food Bioscience. 2021;40. https://doi.org/10.1016/j.fbio.2020.100830.

11. Muangrat R, Jirarattanarangsri W. Physicochemical properties and antioxidant activity of oil extracted from Assam tea seeds (*Camellia sinensis* var. *assamica*) by supercritical CO_2 extraction. Journal of Food Processing and Preservation. 2020;44(3). https://doi.org/10.1111/jfpp.14364.

12. Svetashev VI, Kharlamenko VI. Fatty acids of abyssal Echinodermata, the sea star *Eremicaster vicinus* and the sea urchin *Kamptosoma abyssale*: A new polyunsaturated fatty acid detected, 22:6(n-2). Lipids. 2020;55(3):291–296. https://doi.org/10.1002/lipd.12227.

13. Beppu F, Li H, Yoshinaga K, Nagai T, Yoshinda A, Kubo A, et al. Dietary starfish oil prevents hepatic steatosis and hyperlipidemia in C57BL/6N mice fed high-fat diet. Journal of Oleo Science. 2017;66(7):761–769. https://doi.org/10.5650/jos.ess17038.

14. Bogdanov VD, Maximova SN, Tungusov NG, Shadrina EV. Techno-chemical description of starfish as an object of industrial processing. Izvestiya TINRO. 2015;181:241–251. (In Russ.).

15. Stonik VA. Morskie polyarnye steroid [Marine polar steroids]. Russian Chemical Reviews. 2001;70(8):763–808. (In Russ.).

16. Malyarenko TV. Izuchenie struktury i biologicheskoy aktivnosti asterosaponinov i drugikh polyarnykh steroidnykh soedineniy morskikh zvezd [Study of the structure and biological activity of asterosaponins and other polar steroid compounds of starfish]. Cand. sci. chem. diss. Vladivostok: G.B. Elyakov Pacific Institute of Bioorganic Chemistry; 2012. 134 p.