Determination of biologically active substances in supercritical carbon dioxide sea buckthorn extract

N Lapteva¹, V Kazenina² and A Petrova¹

¹ Yaroslav-the-Wise Novgorod State University, 41, B. St. Petersburgskaya Str., Veliky Novgorod, 173003, Russia
² LLC “Grumant”, 13, Meretskova-Volosova st., Veliky Novgorod, 173003, Russia

E-mail: Natalya.Lapteva@novsu.ru

Abstract. Malnutrition and lack of essential nutrients require food enrichment with biologically active components. Polyunsaturated fatty acids play a great role in lipid metabolism. Results of research on fatty acid composition of oil obtained by the method of supercritical CO₂-extraction of sea buckthorn meal are considered in the article. The obtained extract differs from analogues in the higher content of carotenoids and polyunsaturated fatty acids. The results of evaluation of the extract quality show the possibility of its use in the production of enriched fat-containing foods, including confectionery.

1. Introduction
Extraction is a widely used technological process to extract biologically active components from vegetal raw materials. In this case, the processing of raw materials with any solvent is provided. The solvent for supercritical fluids – compressed gases such as CO₂ - is the most effective and safe as it does not pollute environment and ready products by solvent residues. A similar solvent can be removed simply by pressure relief.

The advantage of the supposed extraction method is its ability to provoke processes which allow one to extract raw materials components more fully, this being impossible when using common solvents.

Despite the fact that the studies of supercritical fluids have been conducted back in the 1970s, in recent decades the interest in application of carbon dioxide as an extractant of biological materials has increased. The process is being performed at temperature over 30°C and pressure over 74 bar. Herewith, liquefied dioxide demonstrates properties of a nonpolar solvent like hexane, and can solve triglycerides. Thanks to supercritical fluids it has become possible to obtain such products as caffeine-free coffee, fat-free meat products, shark liver squalene, and others [1].

Scientists of Kazan Technological University develop mathematic models of extracting oil from seeds of oil plants. Models of supercritical fluidal extraction of ground seeds of lupine [2] and amaranth [3] have been proposed. Such parameters as the degree of seed grinding, temperature and pressure have been taken into account. The published models allow calculating the process regime parameters and output of such components as squalene, and certain fatty acids: oleic, stearic, palmitic, linoleic ones. Investigation of solubility of certain components of the oil extracted allows the authors to suggest the possibility of fractioning of a ready product.
Sea buckthorn oil is widely spread in cosmetic industry and pharmacology. Sea buckthorn oil with a specific taste and flavor is produced from pulp of sea buckthorn fruits (sea buckthorn berries contain, on the average, from 3 to 10 % of a valuable vegetable fat). Orange reddish color of sea buckthorn oil is caused by the high concentration of carotenoids (precursors of Vitamin A) in this plant product.

Sea buckthorn oil is rich with Vitamin E which contributes to optimal hormonal balance and good work of the cardiovascular system. Group B Vitamins in its composition take an active part in protein, carbohydrate, water-salt, and lipid metabolisms, as well as participate in the synthesis of hormones produced by adrenal glands, thyroid, and pancreas [4].

Using the method of supercritical carbon dioxide extraction, it is possible to obtain phytosterols from sea buckthorn seeds. Czech scientists reached the maximum concentration of the extract β-sitosterol at pressure of 15 MPa, temperature 40 °C, and carbon dioxide consumption of 50 grams per 1 gram of seeds. Comparative extraction with hexane showed that the output of β-sitosterol and its concentration in the obtained extract were lower than those with carbon dioxide. As raw materials, they used seeds separated from the pulp after preliminary squeezing of the juice and drying [5].

In berries and green biomass of sea buckthorn collected in experimental fields of Federal Research Center Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences (Siberia, Russia) volatile compounds and essential oils were isolated, among which phenylpropanoids, alkanes, carboxylic acids and their esters predominated [6].

Fatty acid composition of sea buckthorn oil is quite various. It contains the most useful monounsaturated and polyunsaturated fatty acids, such as palmitoleic, oleic, linoleic, linolenic ones. The last two are essential fatty acids, and must be included into a healthy diet.

Sea buckthorn oil can be used in pharmacology to correct the disturbances of lipid metabolism in humans. It is related to the optimally recommended ratio of polyunsaturated fatty acids ω-6 to ω-3. However, the amount of these acids varies depending on the ways of getting the oil, raw materials preparation, growing areas, etc. According to data of many authors, the amount of linoleic fatty acid is, on the average, from 6 to 15%, and that of α-linolenic acid is from 2 to 6% of the total amount. Palmitic fatty acid (to 37%) and palmitoleic fatty acid (to 35 %) predominate in the composition. The sea buckthorn being analyzed was obtained by the liquefied gases extraction. The determination of fatty acid composition was performed by the methods of gas fluid distributive chromatography and infrared spectroscopy [7, 8].

Thus, the oil of sea buckthorn obtained by the carbon dioxide extraction method is a valuable source of biologically active substances, and can be used not only in the pharmaceutical industry, but also in the industry of biologically active food supplements, as well as for enrichment of products of special purposes. Increased proportion of such valuable components as polyunsaturated fatty acids and carotenoids can be made possible due to improved preparation of raw materials and corrected technological regimes of extraction.

2. Methods and Equipment
For the research the sea buckthorn (Hippóphaë rhamnóides) meal was used after the extraction of juice and oil from it by the diffusion method. Meal samples from various suppliers of Russia were selected. Preparation of samples and production of the extract was performed by Limited Liability Company “Grumant” (Veliky Novgorod, Russia) using equipment Thar Technologies Inc. (the USA).

Drying was performed with an apparatus “Musson-2-modul”, grinding into fractions of 3 mm was made by a chopper UIM-2. Next, the sea buckthorn meal was subjected to the fluid extraction. Carbon dioxide was used as an extractant.

Samples were extracted according to the general technological scheme (Figure 1).

In the obtained extract, organoleptic indicators, solubility in different solvents, mass fraction of moisture, carotenoids and fatty acids were determined.

To determine solubility, 10 grams of the extract were weighed in a cup with an error of no more than ±0.1 gram, distilled water or vegetable oil in the amount of 30 grams, continuously shaken for 10 minutes at temperature 20 ± 2 °C, was added. Sea buckthorn extract is allowed to be heated in a water
bath to 30 °C. Observations were carried out after cooling the solution to 20 ± 2 °C. The settled diluted extract was viewed in a transmitting light. Absence of clouding and clots indicates the full solubility of the extract.

![Technological scheme of production of experimental samples.](image)

3. Results

As the research result, the obtained CO₂-extract of sea buckthorn represented the paste-like mass, with color from orange to dark-red and flavor characteristic of sea buckthorn. The extract is soluble in 65% solution of ethyl alcohol, in sunflower oil, and slightly soluble in water.

Two variants of the samples from the raw materials of harvest of 2017 and 2018 were obtained (sample 1 and sample 2, correspondingly). As a control sample, the analogue was used: a sea buckthorn extract produced in Germany, Flavex company.

The results of determination of the mass fraction of moisture and the carotenoids sum are presented in Table 1.

The moisture content in all samples was within the normalized indicators (not more than 5%). Increased dispersion of the prepared meal in comparison with the standard technology (2.5–3 mm)
allowed obtaining the extract with the higher content of carotenoids. Herewith, both samples exceeded the control by the given indicator (by 3.5 and 6.9%, correspondingly).

**Table 1.** Mass fraction of moisture and carotenoids sum in the sea buckthorn extract.

|                | W, %  | \( \omega \) of carotenoids, mg % |
|----------------|-------|------------------------------------|
| Control        | 3.22  | 290                                |
| Sample 1       | 3.38  | 300                                |
| Sample 2       | 3.06  | 310                                |

The mass fractions of fatty acids revealed in the samples of sea buckthorn are presented in Table 2.

**Table 2.** Fatty acid composition of extracts.

| Name of fatty acid | Mass fractions of fatty acids, % | Control | Sample 1 | Sample 2 |
|--------------------|----------------------------------|---------|----------|----------|
| Myristine          |                                  | 0.36    | 0.30     | 0.40     |
| Palmitic           |                                  | 35.86   | 28.10    | 25.70    |
| Palmitoleic        |                                  | 34.98   | 26.80    | 24.30    |
| Stearic            |                                  | 0.73    | 1.50     | 1.80     |
| Oleic              |                                  | 14.42   | 15.10    | 13.80    |
| Linoleic           |                                  | 5.99    | 17.50    | 21.10    |
| \( \alpha \)-linolenic |                              | 1.23    | 10.20    | 12.70    |
| Arachinic          |                                  | –       | 0.30     | 0.30     |
| Gondoic            |                                  | 0.12    | 0.1      | 0.10     |

Correction of parameters of meal preparation for extraction, selection of raw materials with the highest content of the specified components, as well as selection of extraction technological regimes allowed obtaining the product with the increased content of such essential polyunsaturated fatty acids as linoleic and \( \alpha \)-linolenic ones at the enterprise of LLC “Grumant”. Herewith, the fractions of palmitic, palmitoleic, and, in a less degree, stearic fatty acids decreased.

4. **Conclusion**

Physiologic need for carotene and polyunsaturated fatty acids for different population groups is presented in Methodical recommendations MP 2.3.1.2432-08 “Norms of physiological requirements for energy and nutrients for various groups of the population of the Russian Federation”, approved by Rospotrebnadzor on December 18, 2008.

The use of mono- and polyunsaturated fatty acids to increase a diet nutritious value is a relevant investigation trend due to the underdeveloped market of domestic food supplements in Russia.

As the research finding, the technology of sea buckthorn oil was developed, this oil is recommended as a functional ingredient to decrease the lack of polyunsaturated fatty acids in the human diet, since these micronutrients (some of them are essential) provide normal development and biobalance in the body.

The authors proved that the use of suggested technological extraction regimes, provided that the set quality indicators of raw materials and parameters of meal preparation for extraction are followed, had allowed obtaining the product with the higher content of such essential polyunsaturated fatty acids as linoleic and \( \alpha \)-linolenic ones at the enterprise of LLC “Grumant”.
In this regard, the technology of supercritical carbon dioxide sea buckthorn extract which was developed during the investigation can be implemented in the production of LLC “Grumant”. The extract obtained can be recommended for use as a biologically active food supplement in order to prevent vitamin deficiency.

References

[1] Sapkale G N, Patil S M, Surwase U S and Bhatbhage P K 2010 A review supercritical fluid extraction Int. J Chem Sci 8 (2) 729–743
[2] Egorov A G, Mazo A B and Maksudov R N 2010 Extraction from a polydisperse granular layer of milled oilseeds with supercritical carbon dioxide Theoretical Foundations Of Chemical Technology 44 (5) 498–506
[3] Maksudov R N, Tremasov E N, Egorov A G and Mazo A B 2010 Supercritical extraction of seeds of oil cultures Bulletin of Kazan Technological University 2 257-260
[4] Zinchenko A A, Krichkovskaya L V and Buzova V N 2005 Standardization of carotene containing drugs “Maslo oblepihovoe” and “Aekol” Belgorod State University Scientific Bulletin. Series: Medicine. Pharmacy, available at: http://dspace.bsu.edu.ru/bitstream/123456789/10636/1/Sinchenko_Standartisaziya.pdf/
[5] Marie Sajfrtová, Ivana Licková, Martina Wimmerová, Helena Sovová and Zdenek Wimmer 2010 Int. J. Mol. Sci. 11 (4) 1842–50
[6] Slynko N, Kuibida L, Tatarova L, Galitsyn G, Goryachkovskaya T and Peltek S 2019 Essential Oils from Different Parts of the Sea Buckthorn Hippophae rhamnoides L. Advances in Bioscience and Biotechnology 10 233–243
[7] Trineeva O V and Slivkin A I 2016 Study on fatty acid composition of vegetable oils and oil extracts for pharmaceutical purposes by methods of gas liquid chromatography and infrared spectroscopy Sorption and chromatographic processes 2 212–219
[8] Goremykina N V, Vereshchagin A L, Koshelev Yu A and Pershin N S 2014 Composition of glycerides of sea buckthorn oil of Altai Krai, obtained by different ways Polzunovskij Vestnik 3 190–194