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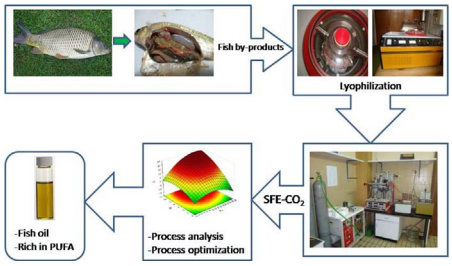
Supercritical fluid extraction of fish oil from common carp (*Cyprinus carpio* L.) tissues

Stefan Kuvendziev[a,*], Kiril Lisichkov[a], Zoran Zeković[b], Mirko Marinkovski[a], Zehra H. Musliu[c]

[a] Faculty of Technology and metallurgy, University Ss. Cyril and Methodius, Skopje, Macedonia
[b] Faculty of Technology in Novi Sad, University of Novi Sad, Serbia
[c] Faculty of Veterinary Medicine, University Ss. Cyril and Methodius, Skopje, Macedonia

GRAPHICAL ABSTRACT



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ABSTRACT

The goal of this work is isolation and characterization of biological active compounds in fish caviar, viscera and fillet obtained from fresh water carp (*Cyprinus carpio* L.) from Tikveš Lake, Republic of Macedonia. A solid-liquid extraction by Soxhlet method was applied in order to obtain total extracts from analyzed samples of animal origin. During the experimental investigation, the influence of some process parameters over the total yield of extract was examined. The SFE-CO_2 was introduced as a desirable alternative in the defined system solvent-lyophilized matrixes. Further, the influence of the operating parameters: operating temperature, operating pressure and extraction time on the yield of total extract, were determined through maximization of the functional dependency. Obtained total extracts were characterized by GC-FID method for qualitative and quantitative determination of the presence of fatty acids. From resulting chromatograms it was concluded that these total extracts contain monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids.

1. Introduction

Fish and fish products fit in the modern trends of food processing industry and human nutrition. Namely, recent studies support the inclusion of these products as essential for health improvement and proper functioning of the human organism. The reason behind these recommendations is the actual presence of bioactive compounds in the fish tissue [1].

Especially important bioactive compounds, that are present in the fish tissue, are omega – polyunsaturated fatty acids. Omega-3 (ω-3) and omega-6 (ω-6) polyunsaturated fatty acids cannot be synthesized by the human metabolism, but still are very important to human health through prevention of numerous medical conditions, which is supported by significant number of studies. Namely, these essential fatty acids represent a solid prevention from cardiovascular diseases. The most suitable way to introduce these bioactive compounds in human metabolism is through consummation and therefore they are labeled as essential fatty acids [2–5].

* Corresponding author.
E-mail address: stefan@tmf.ukim.edu.mk (S. Kuvendziev).

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Fish and fish products have been considered a necessary and crucial part of human nutrition for a long time. It is the presence of bioactive components, such as ω-3 and ω-6 polyunsaturated fatty acids, in fish tissues, that makes them extremely valuable to the food-processing and pharmaceutical industries. The fish processing industry is a large production sector that incorporates various production processes, such as filleting, scaling, curing, smoking, canning etc. More than 70% of harvested fish represents a raw material to the fish processing industry, and only 50% (mass) is produced as an edible portion, where by-products are discarded as waste. Inspired by the basic concepts of Green process engineering that promotes implementation of cyclic production technologies where no by-product is considered as waste, common carp viscera was investigated in the frames of this work, as a possible raw material for isolation of ω-3 and ω-6 enriched fish oil [2–5].

Some of the recent studies suggest that the global fish consumption as well as the consumption of other marine and fresh water products, has significantly increased over the past decade. The common carp (fresh water carp) *Cyprinus carpio* L. is probably one of the most consummated fish species in the republic of Macedonia. It is present at most of the lakes countrywide, including the Tikveš Lake in the southern part of Macedonia. Common carp, regarding its chemical composition, is considered to be an "oily" fish compared to other fresh water species, which is supported by numerous comparative studies in relation to fish tissues [3–8].

As a result of increased production of fish products (fish fillets and fish caviar), the food processing industry has increased the quantities of byproducts (fish viscera) that are considered in most cases as waste. The zero emission concept, that is an aim within all of the production process technologies, can also be achieved within the fish processing industry, through defining a potential use of its waste (viscera). Physic – chemical characterization of fish viscera presented in several studies, suggests that this byproduct is relatively rich in polyunsaturated fatty acids and therefore can be considered as a potential source of these bioactive compounds [9–13].

Several conventional and modern separation techniques can be applied for isolation of fish oil extract from fish tissues – fillets, caviar and viscera. Obtained fish oil consists of saturated, monounsaturated and polyunsaturated fatty acids, the latest of which is the focal point of this work. Soxhlet extraction or hot continuous percolation is a conventional separation technique that provides high yields of fish oil from abovementioned raw materials. Various solvents, with different polarities, were used in Soxhlet extraction on fish fillets, caviar and viscera which resulted in different yields of oil extracts. After rotary-evaporation of the solvent, gas chromatography was applied to define the presence and quantities of various saturated, mono- and polyunsaturated fatty acids in the obtained fish oil [1,14–20].

Several authors have reported on fish oil extraction through application of SC-CO_2, while emphasizing the good solubility of the fish oil in the supercritical CO_2 and avoiding possible degradation of the present polyunsaturated fatty acids which occurs during conventional fish oil production. The fish industry by-products such as fish skins, heads and viscera were identified as potential sources of polyunsaturated fatty acids. This work's research is focused on valorization of fish by-products that are discarded as waste in Republic of Macedonia, as a potential raw material rich in bioactive compounds – unsaturated fatty acids, while designing a contemporary system based on the SFE-CO_2 process. This way, the bio-waste issues are addressed in a manner of waste utilization as a low-cost raw material and introduction of a precise process eco-separation technique for isolation of components of interest to the food and pharmaceutical industries. This approach conforms to the main postulates of zero emission and introduction of so called "cyclic" process design, where no by-product is considered as waste.

The supercritical fluid extraction (SFE) has several practical and ecological advantages over the classical separation processes. This non-conventional extraction technique is performed under moderate operating conditions, regarding the operating temperature, and requires no additional energy consumption for solvent removal [1–5]. Supercritical carbon dioxide has been widely used within the supercritical fluid extraction processes as it represents a non-toxic, relatively cheap and inflammable "green" solvent with relatively mild critical values for the operating parameters – critical temperature of 304.15 K and critical pressure of 7.38 MPa. These properties ensure successful extraction of thermally unstable bioactive components such as ω-3 and ω-6 polyunsaturated fatty acids (PUFA) [1–5,7–10].

Gas chromatography with flame ionization detector – GC-FID is an appropriate an adequate instrumental method for determination of the fatty acid composition of extracted fish oil [3–5].

2. Materials and methods

2.1. Raw material – fish tissues and sample preparation

The common carp (*Cyprinus carpio* L.) that was analyzed within the frames of this work, was obtained from Tikveš Lake in the southern part of Macedonia. Only adult samples of this species were used in our research. The samples were measured in length and weight and scales and skin were removed. Each fish sample was divided in portions of flesh (fillets), viscera and caviar, separately. Each portion was homogenized, in order to obtain reproductive results, and stored at −20 °C until further use in laboratory analysis.

All of the portions were lyophilized in order to eliminate most of the water present in these fish tissues, as the presence of water would change the polarity of the solvents used for the Soxhlet extraction and interfere with the extraction process. The lyophilization was conducted in a laboratory scale lyophilizator Freeze Zone Freeze Dryer, at a Safe temperature set at −50 °C, Collector temperature at −80 °C, and pressure of 0.133 mbar, for a period of 72 h. Dry matter content was defined in each of the portions, according to the mass loss method [1,3,4].

2.2. SFE-CO_2 on lyophilized fish tissues

Supercritical fluid CO_2 extraction was performed in a laboratory scale unit NOVA-Swiss, High Pressure Extraction Plant 565.0156 by Nova Werke LTD, Effertikon. The extractor unit, with a capacity of 200 ml, was filled with 20 ± 1 g of freeze-dried matrixes and void volume was completed with glass beads (4 mm diameter) at the bottom and on the top. A stabilization period of 30 min was allowed for providing adequate contact between the operating matrix and the solvent and to ensure proper thermal distribution in the extractor. Extraction process was conducted at various values of the operating conditions: pressure (200, 300, 350 and 400 bar) and temperature (40, 50 and 60 °C). In order to study the dynamics of the separation process, extraction time sequences were set at 15, 30, 60, 90, 120, 150 and 180 min, as the extraction yield reached a plateau after 3 h for each of the extraction procedures. Separator operating conditions for each extraction procedure were kept at constant pressure-temperature values, at 20 bar and 25 °C, respectively. SFE-CO_2 experiments, considering the number of operating parameters and their values, generated 252 extract samples that were collected in the glass collector in the extractor, then transferred in adequate glass vials using Pasteur pipette and stored at −20 °C for further use in GC-FID analysis [1,3,4].

2.3. Influence of operating parameters on SFE-CO_2

The influence of three experimental factors – operating pressure, temperature and extraction time on the efficiency of SFE-CO_2 was studied. Operating pressure was varied at four levels, 200, 300, 350 and 400 bar, operating temperature was varied at three levels, 40, 50 and 60 °C and extraction time was varied at four levels, 30, 60, 120 and 180 min.