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Artificial neural network modelling of supercritical fluid CO_2 extraction of polyunsaturated fatty acids from common carp (*Cyprinus carpio* L.) viscera

Stefan Kuvendziev[a,*], Kiril Lisichkov[a], Zoran Zeković[b], Mirko Marinkovski[a]

[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*

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ABSTRACT

Common carp viscera, obtained from Tikveš Lake in Macedonia, was investigated as a possible source of polyunsaturated (PUFA) fatty acids. Supercritical fluid CO_2 extraction (SFE-CO_2) was employed for extraction of investigated bioactive components. The GC-FID analysis on the total extract obtained by supercritical fluid CO_2 extraction confirmed the assumption of presence of these bioactive components. A three layer artificial neural network was created for prediction and modelling of the extraction yield of polyunsaturated fatty acids from lyophilized viscera matrixes. Operating values of pressure, temperature, mass flow of CO_2 and extraction time were defined as input vectors to the ANN where PUFA extraction yield was considered as an output vector. Created ANN model provided adequate fitting of experimental data, with a correlation coefficient of 0.9968 for the entire data set. RSM-3D method was employed for mathematical modelling of the ANN output values as a function of operating variables and their interactions.

1. Introduction

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 [1–5]. The fish processing industry is a large production sector that incorporates various production processes, such as filleting, scaling, curing, smoking, canning [4]. 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 [4,6]. 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.

Fish oil production is a constitutional part of the fish-processing industry, where the conventional fish oil production process includes several production sequences: cooking at high temperatures, dewatering (pressing), centrifuging, separation and purification of crude fish oil [4,5]. There are several disadvantages to the conventional fish oil production, such as high cooking temperatures that may lead to fish oil oxidation, moderate extraction efficiency etc. Considering the importance of ω-3 and ω-6 enriched fish oil, there are plenty of studies investigating alternative separation techniques [7,8]. Supercritical fluid CO_2 extraction (SFE-CO_2) is a contemporary precise process eco-separation technique that produces a minimal impact on the environment [9]. Furthermore, this extraction procedure is conducted at relatively low operating temperatures and produces solvent-free extracts and proved to be more selective towards polyunsaturated fatty acids compared to conventional separation techniques. Several studies have confirmed the supercritical carbon dioxide as an adequate solvent for the polyunsaturated fatty acids [9–14].

Artificial neural network (ANN) represents a modern data processing system, whose design, structure and functioning principles are based on the principles of biological neural system. The fundamental processing element of ANN is an artificial neuron that receives inputs such as values of operating parameters and outputs the desired result, such as extraction yield. Designed neural architecture includes multiple interconnected process elements (neurons) that simultaneously process data. Basic characteristics

* Corresponding author. Tel.: +389 23088238.
E-mail addresses: stefan@tmf.ukim.edu.mk, stefan.kuve@gmail.com (S. Kuvendziev).

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of a neural network are adaptive learning (training), data self-organization capability and parallel processing ability [15,16]. These advantages exclude the need of conventional mathematical modelling. Response surface-3D optimization method was employed in order to mathematically describe the ANN model through the functional dependence of ANN outputs from the input vectors and their interactions [17–28].

The aim of this work is the application of artificial neural network modelling in prediction of the extraction yield of SFE-CO_2 of polyunsaturated fatty acids (ω-3 and ω-6) from lyophilized common carp viscera in correlation to the operating conditions: pressure, temperature, CO_2 mass flow and extraction time, as well as their interactions.

2. Materials and methods

2.1. Common carp viscera

Adult samples of common carp (*Cyprinus carpio* L.) were captured from the Tikveš Lake in the central part of Republic of Macedonia. Captured samples were measured in length and weight and subsequently scaled and eviscerated. Obtained viscera was measured in weight and treated with manually operated mincing machine through 4 mm openings in order to adequately homogenize the raw material and increase the contact surface. Minced viscera was divided into 20 g portions and stored at −20 °C for further use [1,29].

2.2. Sample preparation

Considering the large amount of moisture (up to 66 mass%) within the viscera samples, that interferes with the SFE-CO_2 process through increase of solvent polarity, lyophilization was defined as a mandatory procedure for sample preparation. Lyophilization or freeze-drying provides satisfactory moisture elimination while preserving the chemical properties of the viscera tissues [7]. Freeze-drying was conducted in a laboratory scale lyophilizer 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. Residual moisture for treated samples was below 1% (mass). Freeze-dried viscera matrixes were stored −20 °C for further use [1].

2.3. Supercritical fluid extraction with CO_2

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. The stabilization period can be reduced to 20 min and thus reduce the total process time. Extraction process was conducted at various values of the operating conditions: pressure (200, 300, 350 and 400 bar), temperature (40, 50 and 60 °C), and CO_2 mass flow (0.194, 0.277 and 0.354 kg/h). 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.

2.4. Determination of fatty acid (FA) profile

Qualitative and quantitative analysis of obtained total extracts in order to define their fatty acid (FA) profile, was performed by GC-FID analysis, according to the official BF_3 method for determination of fats and oils in food samples – AOAC 996.06 [7,31]. Obtained SFE-CO_2 extracts, containing the triglycerides of interest (PUFA) were transferred to methyl esters and subsequently subjected to GC-FID analysis [31]. GC-FID analysis was performed using Agilent 7890A unit with a HP-88 (J&W 112-8867) column, with a length of 60 m, internal diameter of 0.25 mm and stationary phase thickness of 0.2 μm. 1 μl of each extract was used in these analyses. The operating temperature of the chromatographic separation included initial temperature of 120 °C, which was maintained for 1 min, further increase to 175 °C with a gradient of 10 °C/min (maintained for 10 min), additional increase to 210 °C with a gradient of 5 °C/min (maintained for 5 min) and a final increase to 230 °C with a gradient of 5 °C/min (maintained for 12 min). Injection and detector temperatures were set at 250 and 300 °C, respectively. Helium was used as a carrier gas with a flow of 0.8 ml/min.

2.5. Definition of PUFA extraction yield

The yield of total extract was mathematically defined as a mass ratio of obtained extract in the used sample:

$$\text{Yield of total extract}\,(\%) = \frac{m_{\text{extract}}}{m_{\text{sample}}} \times 100$$

The extraction yield of the PUFA group was determined considering the FA profile of each extract that enables mathematical calculation of the mass of PUFA within the obtained extract, x_{PUFA}:

$$\text{PUFA yield}\,(\%) = \frac{m_{\text{extract}} \times x_{\text{PUFA}}}{m_{\text{sample}}} \times 100$$

2.6. Artificial neural network (ANN) design

Artificial neural network was created for prediction of the extraction yield of PUFA as a function of the operating parameters and their interactions, using MATLAB/Neural Network Toolbox. The selected data are passed into the input layer and then propagated from the input layer to the hidden layer, before they finally reach the output layer of the network. Every node in the hidden or output layer will firstly act as a summing junction which combines and modifies the inputs from the previous layer, using the following equation [17–27]:

$$y_i = \sum_{j=1}^{p} x_i \times w_{ij} + b_j$$

where y_i is the net input to node j in hidden or output layer, i is the number of nodes, x_i is the inputs to node j (or the outputs of the previous layer), w_{ij} is the weights representing the strength of the connection between the ith node and jth node and b_j is the bias associated with node j.

The hidden layer neurons were assigned a sigmoidal (*tansig*) transfer function which is most commonly used for non-linear relationships and represents an activation function for the respective