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Original Research Article

Recovery of Polyunsaturated Fatty Acids Rich Extracts from European Carp Viscera Using Supercritical Carbon Dioxide: Artificial Neural Network Modelling

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ABSTRACT

Applying green engineering principles to conventional fish processing bio-waste (viscera matrixes) suggests its reconsideration as a secondary raw material rich in highly valued polyunsaturated omega-3 and omega-6 fatty acids. Supercritical fluid carbon dioxide extraction was introduced as a separation technique with a minimal ecological footprint. The operating variables of the extraction process were set at 20, 30, 35, and 40 MPa; 313, 323, and 333 K; and 3.23, 4.62, and 5.9 g CO_2/min. The artificial neural network model was designed based on the investigated output − polyunsaturated fatty acids' solubility in supercritical carbon dioxide employing the backpropagation training algorithm with the proposed 3-10-1 architecture. The model provided high accuracy ($r = 0.9905$), and the resulting three-dimensional space depicted the omega-3 and omega-6 solubility. The maximum yield of the total extract, 0.52 g/g freeze-dried product, was achieved at 40 MPa and 333 K.

KEYWORDS

European carp viscera, Polyunsaturated fatty acids' solubility, Supercritical fluid carbon dioxide extraction, Feed-forward artificial neural network modelling.

INTRODUCTION

The modern lifestyle imposes significant requirements on the food and pharmaceutical industries in terms of accessing the increasing demands for high-quality functional products beneficial to the overall human health. Respectively, increased use of supplements requires the modern chemical, food, and pharmaceutical industries to develop new technologies, aligning with the general modern and ecological world trends that would produce final products following the rigorous environmental criteria. Using fatty acids has become a necessity, and among the most common are polyunsaturated omega-3 (ω-3) and omega-6 (ω-6) acids [1, 2].

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The problems of efficient use of raw materials, the creation of industrial and any other type of waste, and environmental pollution have been particularly topical in recent years. Conventional technological processes based on the fish processing industries generate a significant amount of by-products, which represent potentially cheap and renewable secondary resources and raw materials, rich in bioactive compounds that can be used as alternative sources for obtaining new products, biomaterials, fuel pellets, biogas etc.

European carp (*Cyprinus carpio* L.) entrails containing polyunsaturated fatty acids (PUFA) are generally discarded as a by-product in the fish industry. Therefore, in this research, European carp viscera was studied as a prospective secondary organic matter containing highly sought after ω-3 (Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA)) and ω-6 enriched fish oil [3, 4]. Double maceration, digestion, and percolation, as conventional techniques, are frequently used to separate biologically active compounds from matrixes of animal or plant origin [5]. Semi-industrial and conventional industrial processes based on extracting components of natural origin are mainly designed as continuous percolation-constant extract release systems. In contrast, laboratory-scale processes are conducted in batch mode [6].

Industrial practices concerning fish processing observed from the chemical engineering viewpoint have several deficiencies, such as moderate extraction efficiency or fish oil oxidation when cooking at high temperatures. One contemporary and high-precision separation technique that has an acceptable environmental impact is supercritical carbon dioxide extraction (SC-CO_2) from natural raw materials [7], described as supercritical fluid carbon dioxide extraction (SFE-CO_2) [8, 9]. The moderate working temperatures, solvent-free products generated by this process, and higher selectivity and experimental-process adaptability make this separation technique more appropriate for isolating PUFA-rich fish oil than traditional extraction processes [10]. Recent literature research favours applying the described separation system as adequate and selective towards non-polar fatty acid molecules [11].

Unlike classical extraction processes, SC-CO_2, performed under moderate operating temperatures and requiring no auxiliary step for solvent removal, has ecological and practical advantages. SC-CO_2 is a method used to extract various compounds from different materials using carbon dioxide in its supercritical state. This method is mainly favoured because it offers significant advantages in terms of process selectivity (targeting specific compounds, depending on the pressure (P) and temperature (T) conditions) and non-toxicity (CO_2 is a safer alternative to conventionally used solvents). It has green properties (CO_2 is abundant and non-polluting, making the process more environmentally friendly compared to some conventional extraction methods that use potentially harmful solvents), and the process conditions are adjustable (operating T and P can be varied, providing a high level of control over the extraction) [12]. SC-CO_2 extraction is widely used in various industries, including food, pharmaceuticals, and cannabis, to extract essential oils, flavours, fragrances, and other valuable compounds from natural sources [13]. Process characteristics ensure the favourable extraction of thermally unstable and highly sought-after components.

The engineering concept of this work, which is under the modern trends of process engineering and the postulates of the circular economy, has a confirmed economic and environmental impact. These aspects derive from generating commercial value through new products (PUFA-rich extracts) obtained from conventional processing industry waste materials, as well as due to eliminating the amounts of waste and their transformation into secondary raw material in a new technological process.

The modelling and mathematical interpretation are essential to the integral chemical engineering approach in any lab-scale experimental process [14]. The conventional approach to mathematical modelling description is usually a one-factor-at-a-time analysis of the impact, while other parameters are constant. Artificial Neural Network (ANN) [15], as an artificial intelligence system and modern data processing system, imitates the functions of the organic

nervous system and biological neurons and is increasingly being used in various fields because of their diversity and ability to adapt to a specific purpose [16, 17]. The application of ANNs in modelling different processes, in most cases, is to predict the values of dependent variables (outputs) based on known values of independent variables (inputs). ANNs offer several advantages that contribute to their widespread use in control engineering, as they offer adaptability to varying input conditions [18], parallel processing and robustness [19]. The Three-Dimensional Response Surface Methodology (3D-RSM) optimisation technique of response surface is commonly employed to mathematically depict the ANN model by showcasing the functional relationship between ANN outputs and input vectors and variable interactions [20].

The primary goal of this study is to utilise ANN modelling to forecast the solvability of detected ω-3 and ω-6 isolated from European carp viscera. SFE-CO_2 process performance was formulated as a solubility output plotted as a functional dependency from the experimental conditions: P, T , and Q_{CO_2}, and independent variables' interactions.

EXPERIMENTAL PROCEDURES

An experimental plan was designed to fulfil the required investigative part of this work. Performed analyses can be divided into several experimental sections depicting the generation of operating raw material matrices, conducting the selected extraction process and consequent 3D-RSM and ANN predictive modelling. Data from each of the stated procedures were further used to evaluate the overall performance of the designed separation system.

Raw material

Full-grown samples of freshwater carp (*Cyprinus carpio* L.) were procured from a local lake in the central part of North Macedonia. Obtained fish specimens were scaled and eviscerated subsequently. Extracted viscera material was ground using a commercial mincing machine with a 4 mm sieve to produce sufficiently homogenised natural matrixes. These have an increased mass-transfer contact surface area required to complete subsequent freeze-drying and separation processes successfully. Prepared viscera matrixes were measured in mass (approximately 20 g) and appropriately stored in refrigerated conditions without moisture and light.

Raw matrix freeze-drying

Each viscera batch was subjected to a freeze-drying (lyophilisation) process to eliminate the high moisture content in pre-defined matrixes. The high moisture content in eviscerated material was undesirable in the closed system SC-CO_2 – viscera matrixes, as it directly affects the overall polarity and the mechanisms of the extraction process. The freeze-drying process was designed and regulated to produce treated material portions with moisture content not higher than 5% (w/w) and enhance material stability.

Lab-scale freeze dryer − Freeze Zone Freeze Dryer LABCONCO was used to lyophilise the eviscerated material. The optimal freeze-drying process conditions were experimentally determined at 72 h and an operating P of 13.3 kPa. The process runs in three consecutive steps:

- Initial material freezing at an operating temperature of 233 K;
- Online regulated lyophilisation at 233 K and 13.3 kPa with a duration of 60 hours;
- Cascade-regulated freeze-drying within a temperature region of 233 K to 293 K for 10 hours.

Supercritical fluid carbon dioxide extraction of biologically active compounds

A laboratory-scale extraction in a supercritical fluid unit produced by NOVA-Swiss, HPEP 565.0156 (Nova Werke LTD, Effertikon) was used for the isolation of PUFA from the investigated raw material – carp viscera samples (**Figure 1**).

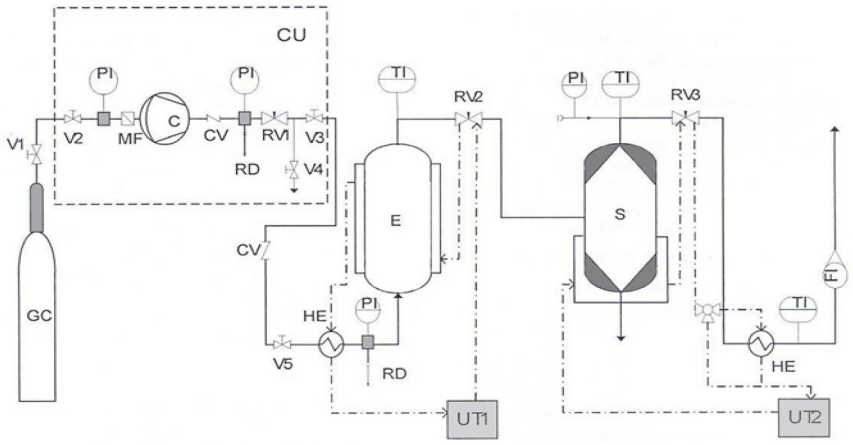

Figure 1. Lab-scale SFE-CO_2 unit HPEP 565.0156; GC − gas tank (CO_2), CU − compressor unit, C − diaphragm compressor, E − extractor, S − separator, UT1, UT2 − ultra thermostats, HE − heat exchanger, RD − ruptured disk, FI − flow measuring instrument, PI – pressure measuring instrument, TI – temperature measuring instrument

A pre-prepared matrix of lyophilised viscera material (20±1 g) was placed between two layers of 4 mm glass beads to eliminate the void volume in the extractor. The total capacity of the SFE extractor unit is 200 ml. A stabilisation period of 30 min was defined to provide uniform thermal distribution within the extractor and the necessary initial contact time in the system solvent − natural matrix. The SC-CO_2 extraction process was performed according to a pre-defined experimental plan regarding the operating values of the process parameters: *P* (20, 30, 35, and 40 MPa), *T* (313, 323, and 333 K), and Q_{CO_2} (3.23, 4.62, and 5.9 g/min). The SFE-CO_2 separator unit was operated at constant *P* (2 MPa) and *T* (298 K), as these values of operating parameters are in the range of optimal *P-T* conditions for the described closed system. The resulting total extracts (252 separate samples) were collected and stored in borosilicate dark glass vials at 253 K for further instrumental analysis.

The total extract yield (Y_{TOTAL}), as well as the yield of PUFA (Y_{PUFA}) and PUFA solubility (R_{PUFA}), were mathematically defined according to equations (1), (2) and (3):

$$Y_{TOTAL}[\%] = \frac{m_{extract}}{m_{sample}\ (dry\ basis)} \times 100 \tag{1}$$

$$Y_{PUFA}[\%] = \frac{m_{extract} \times x_{PUFA}}{m_{sample}\ (dry\ basis)} \times 100 \tag{2}$$

$$R_{PUFA}[\text{mg PUFA/dm}^3\ {}_{\text{N}}CO_2] = \frac{m_{extract} \times x_{PUFA}}{V_{CO_2}} \times 100 \tag{3}$$

Where: $m_{extract}$ – mass of extract [g]; $m_{sample}\ (dry\ basis)$ – mass of lyophilised raw material [g]; x_{PUFA} – mass fraction of PUFA in the extract; Y_{TOTAL} – yield of extract [g/100 g lyophilised raw material]; Y_{PUFA} – yield of PUFA [g/100 g lyophilised raw material]; R_{PUFA} – solubility of PUFA [mg PUFA/dm^3 $_{\text{N}}CO_2$], where $_{\text{N}}CO_2$ – CO_2 at NTP conditions, 101.325 kPa and 293 K; V_{CO_2} – volume of CO_2 [dm^3] used in the extraction process, calculated from the formula $V_{CO_2} = \left(Q_{CO_2}/\rho_{CO_2}^{NTP}\right) \times t$, where Q_{CO_2} – mass flow rate of CO_2 used in the extraction [g/min], $\rho_{CO_2}^{NTP}$ – density of CO_2 at NTP [g/dm^3], t – extraction time [min].

Fatty acids quantification by gas-chromatography with flame-ionisation-detector

The SFE-CO_2 extracts from the viscera matrixes were investigated to characterise their fatty acid profile and quantify the presence of ω-3 (including EPA and DHA) and ω-6 PUFA. The analysis followed the AOAC Official Method 996.06 Gas Chromatography with Flame Ionisation (GC/FID) [2] using an Agilent 7890A unit equipped with a J&W HP-88 GC column: 60 m (length), 0.25 mm (internal diameter), 0.20 μm (particle size), 7-inch cage. The exact volume of 1 μl total extract was used for each chromatographic analysis, performed according to the previously stated AOAC Official Method: initial temperature of 393 K (1 min), sequenced by 10 K/min gradient rise to 448 K (10 min), then 5 K/min rise to 483 K (5 min) and a final 5 K/min rise to 503 K (12 min). The working temperatures for the injector and detector were 523 K and 573 K, respectively. The carrier gas was helium, with a 0.8 ml/min flow rate.

RESULTS AND DISCUSSION

The proposed research and development concept within this work, in addition to the established economic benefits, has a significant environmental impact because it incorporates the utilisation of bio-waste and thereby contributes to reducing the amount of waste.

Considering the properties of the designed experimental system consisting of the separation complex – pre-treated viscera matter versus supercritical fluid (carbon dioxide), the employed supercritical fluid CO_2 extraction process represents a highly prospective and efficient separation method: the non-polar bioactive compounds were isolated using the non-polar solvent (CO_2). The valorisation of the analysed fish processing waste as a possible resource of important biologically active compounds, through separation of ω-3 (including EPA and DHA) and ω-6 PUFA by the SFE-CO_2, represents the basis of this work. The presentation of the green engineering principles substantiated by the experimental results for the closed system SC-CO_2-viscera matrixes and the designed multi-layered ANN-generated innovative aspects deliver an engineering justification for the study. The determined impact of the working parameters on the yield of the observed compounds and the SC-CO_2 process modelling were considered focal points and an essential part of this study.

Experimentally developed separation process based on the supercritical carbon dioxide – freeze-dried secondary organic matter, established as supercritical fluid CO_2 extraction of valuable bioactive compounds, provided significant yields of PUFA-containing extract from lyophilised matrixes. The biologically active compounds were validated through the previously defined instrumental analysis (gas chromatography with flame ionising detector). These results represent the central aspect of experimental planning.

Supercritical fluid carbon dioxide extraction on lyophilised matrixes

The experimental analysis correlated to the isolation yield of multicomponent extract through the SFE-CO_2 on analysed freeze-dried viscera samples produced satisfactory results for this output. The value of the whole extract yield was maximal at 40 MPa and 333 K, Y_{TOTAL} = 51.5 g/100 g lyophilised material. All experimental results were calculated as the average of three experiments. Discussed experimental data points indicate that each obtained result for the observed response was confined to statistically insignificant processing error (less than 2%), thus providing sufficient validity of the results and adequacy of the employed extraction system design. This repeatability is valid for all values of the entire data set. The results from the established experimental plan produced the required data set for the ANN model. The initial experimental work provided the needed data depicting the experimental conditions and obtained extraction yield outputs, eq. (1). Each produced extract was subjected to GC/FID analysis to determine the fatty acid profile. **Figure 2** presents one of the chromatograms, generated at optimal operating conditions regarding the presence and resulting R_{PUFA} as a function of the operating process variables.

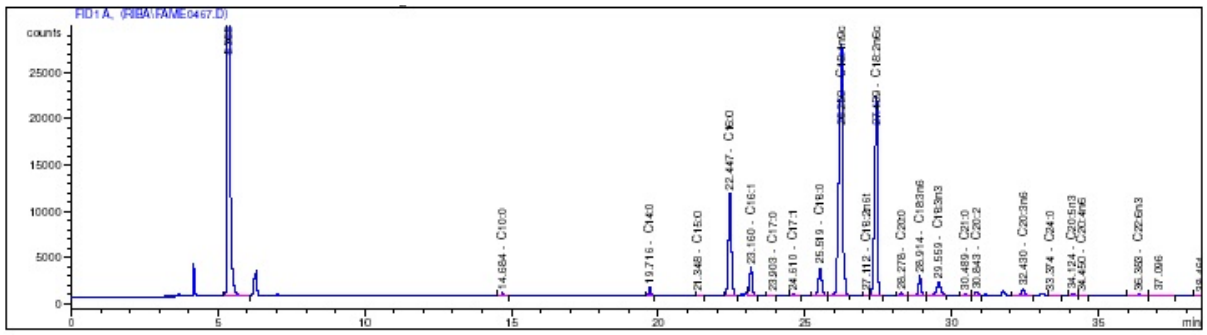

Figure 2. Chromatogram of SFE-CO_2 extract (P = 35 MPa, T = 313 K, Q_{CO_2} = 3.23 g/min, t = 180 min)

The interpretation of the results obtained from the presented chromatogram regarding the resulting fatty acid profile of the fish oil is given in **Table 1**. Considering all aspects regarding the operating variable values and the comparative analysis of the resulting Y_{PUFA} and R_{PUFA} values serve as a basis for process modelling and optimisation. The optimisation includes the economic aspect of the investigated process, and therefore, **Table 1** and **Figure 2** present the fatty acid profile and the chromatogram obtained at P = 35 MPa, T = 313 K, Q_{CO_2} = 3.23 g/min. These operating conditions require low operating costs − lowest values for operating temperature and CO_2 flow rate, whereas the operating P enables high value for Y_{PUFA} extracted per Q_{CO_2} (4.77±0.043 mg/g CO_2).

Table 1. FA profile of SFE-CO_2 viscera extract (P = 35 MPa, T = 313 K, Q_{CO_2} = 3.23 g/min)

Detected FA		Yield [mg/0.1 g extract]
Decylic acid	C10:0	0.36
Dodecanoic acid	C12:0	0.22
Tetradecanoic acid	C14:0	0.94
Pentadecanoic acid	C15:0	0.12
Hexadecanoic acid	C16:0	14.17
Heptadecanoic acid	C17:0	0.11
Octadecanoic acid	C18:0	4.18
Icosanoic acid	C20:0	0.42
Heneicosanoic acid	C21:0	0.20
Docosanoic acid	C22:0	0.17
Tetracosanoic acid	C24:0	Not detected
Saturated Fatty Acids (SFAs)		20.89
9-tetradecenoic acid (n5)	C14:1	Not detected
cis-6-Hexadecenoic acid	C16:1	3.79
Heptadecenylsalicylic acid	C17:1	0.22
(9Z)-Octadec-9-enoic acid	C18:1 n9c	41.19
(13Z)-Docos-13-enoic acid	C22:1 n9	Not detected
Mono Unsaturated Fatty Acids (MUFAs)		45.20
(9Z,12Z)-Octadeca-9,12-dienoic acid	C18:2 n6t	0.08
cis, cis-9,12-Octadecadienoic acid	C18:2 n6c	26.47
(6Z,9Z,12Z)-Octadeca-6,9,12-trienoic acid (GLA)	C18:3 n6	2.27
(9Z,12Z,15Z)-Octadeca-9,12,15-trienoic acid (ALA)	C18:3 n3	2.37
(11E,14E)-icosa-11,14-dienoic acid	C20:2 n6	0.53
(8Z,11Z,14Z)-Icosa-8,11,14-trienoic acid (DGLA)	C20:3 n6	0.62
(5Z,8Z,11Z,14Z)-Icosa-5,8,11,14-tetraenoic acid (AA)	C20:4 n6	0.97
docosa-13,16-dienoic acid	C22:2 n6	0.14
(5Z,8Z,11Z,14Z,17Z)-Icosa-5,8,11,14,17-pentaenoic acid (EPA)	C20:5 n3	0.26
(4Z,7Z,10Z,13Z,16Z,19Z)-Docosa-4,7,10,13,16,19-hexaenoic acid (DHA)	C22:6 n3	0.20
PUFAs		33.91

Analysis of the experimental results suggests the satisfactory presence of highly sought-after ω-3 (including EPA and DHA) and ω-6 PUFA, thus confirming the established SFE-CO_2 process as an adequate separation system for the analysed raw material. Experimentally derived results concerning the total extraction yield coupled with the generated chromatograms of implemented instrumental analysis were the basis for the calculation of the Y_{PUFA}, eq. (2). Considering the physical properties of gaseous CO2, as the solvent mass flow rate was measured at the plant exit (at NTP operating conditions), PUFA yield results were used to calculate the solubility of these bioactive components in SC-CO2, eq. (3). An excerpt of these results is presented in **Table 2**. According to conducted experimental work, the data regarding Y_{PUFA} obtained at various operating conditions and total extraction time of 180 min represents the calculation basis for R_{PUFA}, according to previously defined equations (1) to (3). Initial calculations present the mass of polyunsaturated fatty acids obtained per unit mass of CO_2 (m_{PUFA}/m_{CO_2} [mg PUFA/g CO_2]) and these values are then used to express the studied response, R [mg PUFA/dm^3 $_N$$CO_2$].

Table 2. Solubility of PUFA at different operating conditions and extraction time of 180 min

T [K]	P [MPa]	Q_{CO_2} [g/min]	m_{PUFA}/m_{CO_2} [mg PUFA/g CO_2]	R [mg PUFA/dm^3 $_N$$CO_2$]
333	30	3.23	3.02±0.042	5.56
333	30	5.90	1.54±0.029	2.83
323	20	4.62	0.58±0.012	1.07
333	20	5.9	0.39±0.007	0.71
313	40	4.62	2.38±0.038	4.37
323	40	4.62	1.96±0.045	3.61
313	20	3.23	1.33±0.012	2.44
323	20	3.23	1.08±0.011	1.99
323	40	3.23	3.44±0.038	6.32
323	30	5.90	1.27±0.022	2.33
313	30	4.62	2.25±0.038	4.14
323	35	3.23	3.27±0.039	6.01
333	20	3.23	0.76±0.006	1.39
333	35	3.23	3.71±0.019	6.82
313	35	5.90	2.59±0.039	4.76
313	20	5.90	0.67±0.009	1.24
313	35	3.23	4.77±0.043	8.78
333	30	4.62	1.63±0.02	2.99
333	40	4.62	2.88±0.046	5.29
333	35	4.62	2.54±0.043	4.67
313	40	3.23	4.24±0.038	7.80
323	20	5.90	0.55±0.01	1.01
333	20	4.62	0.41±0.005	0.75
313	40	5.90	2.15±0.032	3.96
323	40	5.90	1.85±0.033	3.41
313	30	5.90	2.13±0.049	3.91
333	40	3.23	5.34±0.091	9.83
333	35	5.90	1.88±0.039	3.46
313	20	4.62	0.72±0.013	1.32
323	35	5.90	1.65±0.026	3.04
323	30	4.62	1.34±0.027	2.47
323	30	3.23	2.49±0.03	4.59
313	35	4.62	2.67±0.027	4.92
323	35	4.62	1.76±0.033	3.23
313	30	3.23	4.18±0.071	7.69
333	40	5.90	2.71±0.043	4.99

Artificial neural network predictive model for PUFA solubility

The process of ANN creation consisted of three steps: training by the introduction of experimentally obtained data, validation, and determining the deviation of the ANN-generated output from the experimental data. The task of the ANN model is to predict the solubility of PUFA in supercritical CO_2 during SFE of biologically active components from lyophilised tissue samples. For this purpose, MATLAB − Neural Network Toolbox software kit was employed. The created ANN consists of three neural layers: the input layer, the hidden (operative) layer, and the output layer. The input layer is defined by the experimental variables (P, T, Q_{CO_2}) matrix, whereas the output layer represents the resultant PUFA solubility (R_{PUFA}). From an engineering point of view, the main goal was to design a hidden (operative) layer and to define the transfer functions for the artificial neurons to generate an optimal ANN architecture for the investigated system. The structural architecture of the operational (hidden) layer of the designed ANN in terms of the number of neurons resulted from the minimisation of the mean squared error (MSE) function of output values versus experimental data (**Figure 3**).

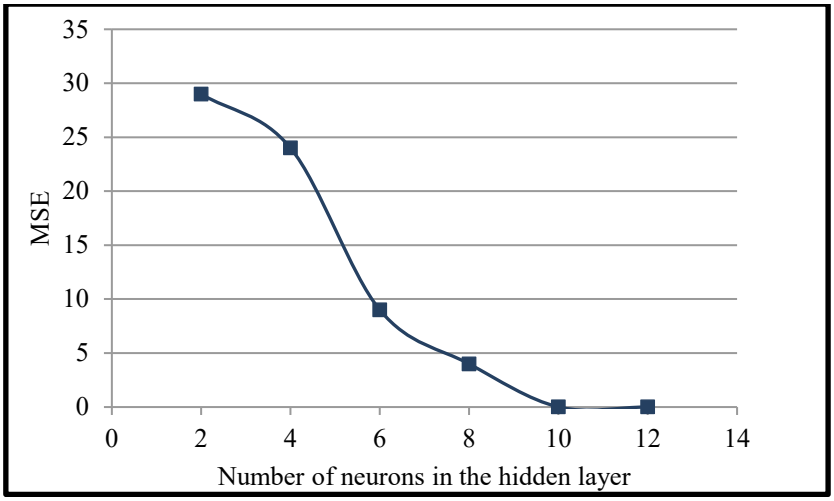

Figure 3. MSE as a function of the number of neurons in the hidden layer

Considering the network performance related to the number of neurons in the operative (hidden) neural layer presented in **Figure 3**, one can conclude that the optimal number of neurons in this ANN layer is 10. Thus, the minimum value of the MSE is achieved using the 3-10-1 architecture of the ANN. **Table 3** shows the designed ANN's architectural properties, including the designated transfer functions for the hidden and output neural layers. The input layer consists of 3 neurons (operating P, T, and Q_{CO_2}) and the data matrix includes the operating values of these parameters: 20, 30, 35, and 40 MPa (P), 313, 323 and 333 K (T), and 3.23, 4.62 and 5.9 g/min (Q_{CO_2}). The output layer consists of one neuron (R_{PUFA}).

Table 3. Artificial neural network properties for R_{PUFA} = f (P, T, Q_{CO_2})

Algorithm	Feed-forward backpropagation
Minimised error function	MSE
Training	Levenberg-Marquardt
Training type	Supervised
Input layer	No transfer function
Hidden layer	Hyperbolic tangent transfer function
Output layer	Hyperbolic tangent transfer function
Number of neurons in the input layer	3
Number of neurons in the hidden layer	10
Number of neurons in the output layer	1

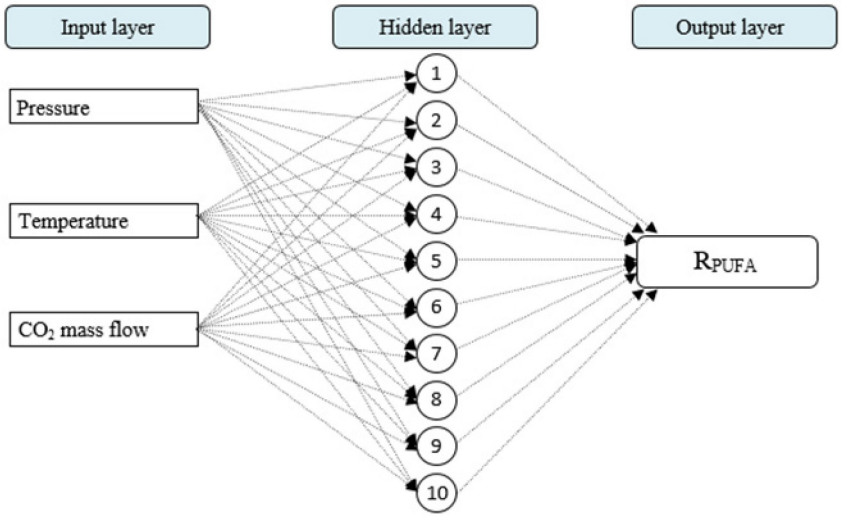

Figure 4. ANN model for the prediction of PUFA solubility in SC-CO_2, R_{PUFA} = f (P, T, Q_{CO_2})

Figure 4 presents a scheme of the complete architecture of the generated ANN. The results generated after completing the training procedure are illustrated in **Figure 5** and **Figure 6**. Figure 5 depicts the successful completion of the training procedure using the selected Levenberg-Marquardt backpropagation training algorithm. **Figure 6** demonstrates the ANN training and validation performance; the presented graph suggests that the designed ANN provides the minimal MSE value of 0.17912 for the validation data set. This result confirms that the created ANN and completed training give a highly accurate predictive model to produce a good fit of experimentally observed values R_{PUFA} in the range of experimental operating conditions and extrapolated data points. The ANN model generated R_{PUFA} output values that fit the experimental data adequately. The results of regression analysis of the ANN model are presented in **Figure 7** and **Figure 8**. From these results for the employed simulations of the ANN model for the prediction of R_{PUFA}, it can be concluded that the created ANN predicts the experimentally obtained data with high precision with a correlation coefficient of $r = 0.9905$ for the entire data set.

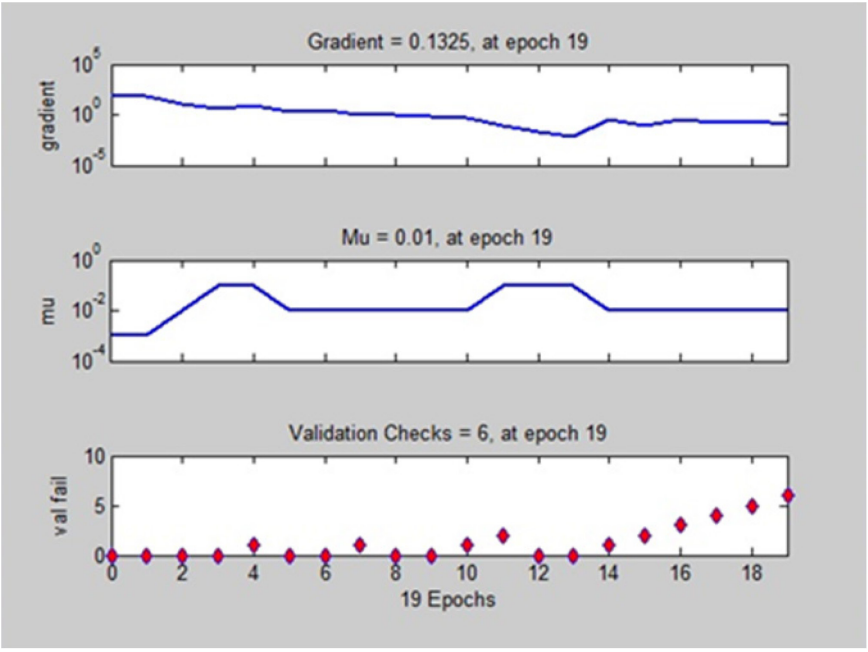

Figure 5. Training procedure for the ANN model R_{PUFA} = f (P, T, Q_{CO_2})

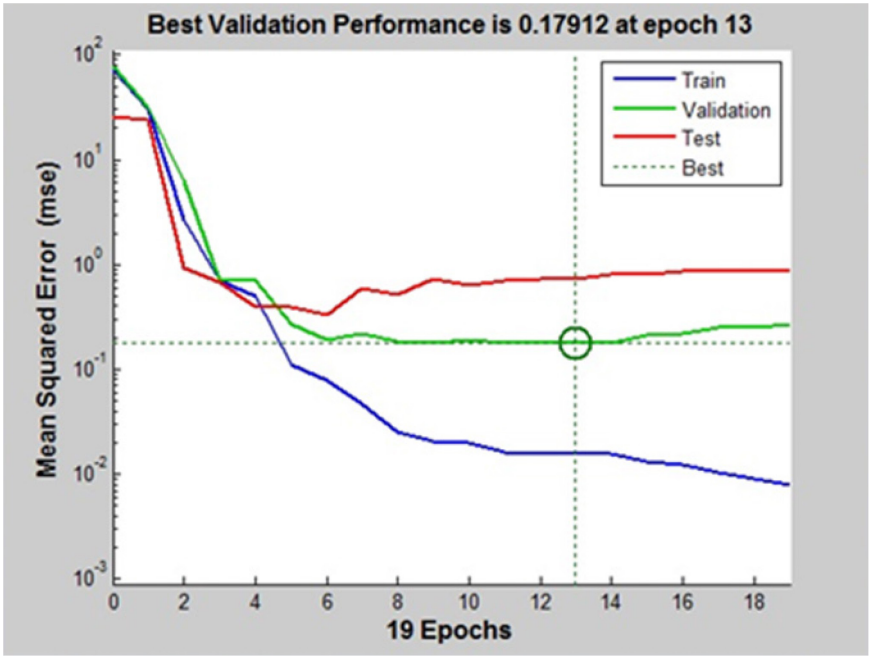

Figure 6. ANN training performance - deviation of model output values from the experimental data for PUFA solubility for each data matrix set

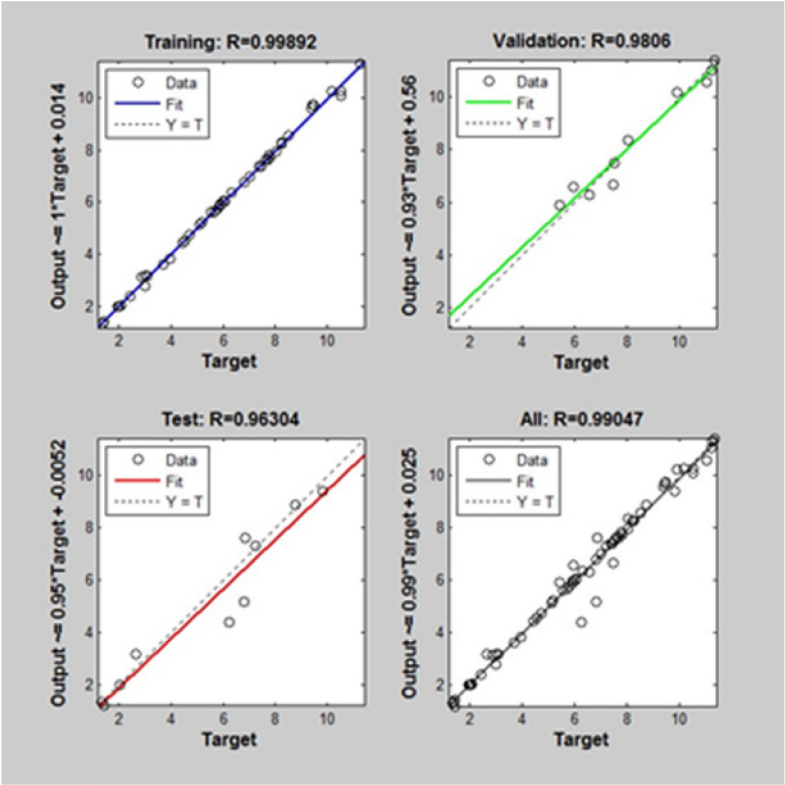

Figure 7. Correlation of model and experimental data values − validation of the ANN model for the prediction of R_{PUFA} = f (P, T, Q_{CO_2})

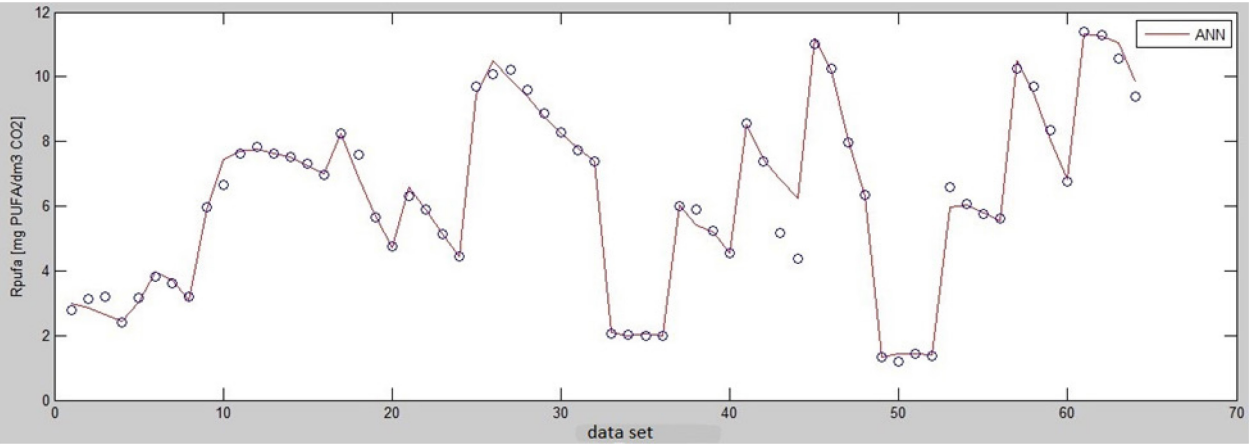

Figure 8. Comparative plot − model and experimental data values for the entire data set

The mathematical interpretation of the created ANN is:

$$R = a_0 + a_1 \times T + a_2 \times P + a_3 \times Q + a_4 \times T^2 + a_5 \times T \times P + a_6 \times T \times Q + a_7 \times P^2 + a_8 \times P \times Q + a_9 \times Q^2 \quad (4)$$

Where the dimensions of variables are: P [MPa], T [K] and Q [g/min]. **Table 4** shows the values of the model coefficients that were determined.

Table 4. Values of coefficients of the ANN model − R_{PUFA} = f (P, T, Q_{CO_2})

Coefficient	Value
a_0	36.0475
a_1	−1.2805
a_2	0.0683405
a_3	−86.1971
a_4	0.0110531
a_5	$3.92458 \cdot 10^{-4}$
a_6	0.0997967
a_7	$-6.87433 \cdot 10^{-5}$
a_8	−0.0931169
a_9	169.391

The 3D-RSM was designed for a detailed analysis of the influence of the experimental variables and resulting variable interactions on the predicted R_{PUFA} in SC-CO_2 extraction of ω- 3 and ω-6 from freeze-dried viscera tissues, and to initiate process optimisation. The analysis of the defined mathematical model involves interpreting the values of resulting regression coefficients (**Table 4**). The study suggests that the operating pressure positively impacts the output − R_{PUFA}. On the other hand, the operating temperature has a more complex impact on the extraction process and mass transfer phenomena occurring in the extractor. Initially, the operating temperature has a negative effect due to the decreased viscosity of the supercritical fluid. Further temperature increase positively impacts R_{PUFA} due to increased vapour pressure of extracted compounds. The complex impact of model interactions can be observed and elaborated through the analysis of the 3D surfaces generated by the software package Statgraphics Centurion, shown in **Figure 9** to **11**.

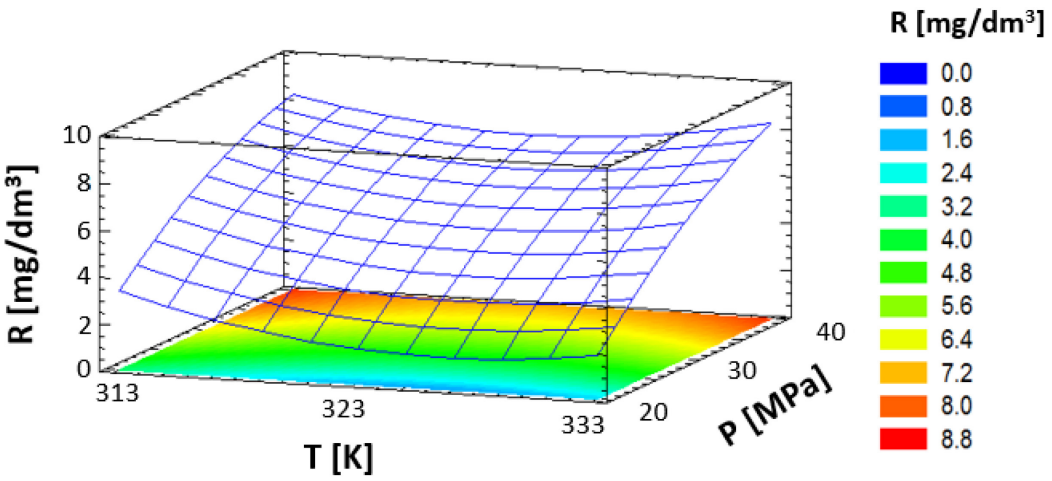

Figure 9. 3D response surface R_{PUFA} = f (P,T); Q_{CO_2}=3.23 g/min

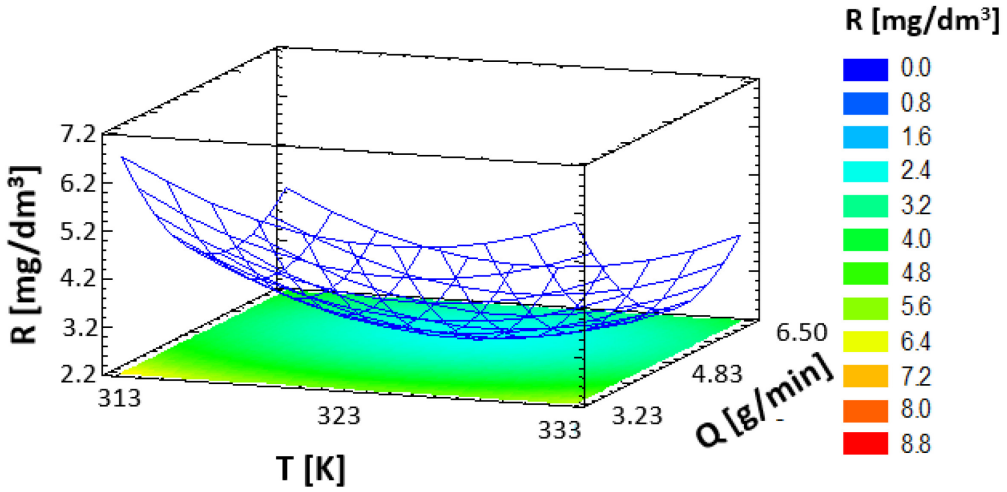

Figure 10. 3D response surface R_{PUFA} = f (T, Q_{CO_2}); P=30 MPa

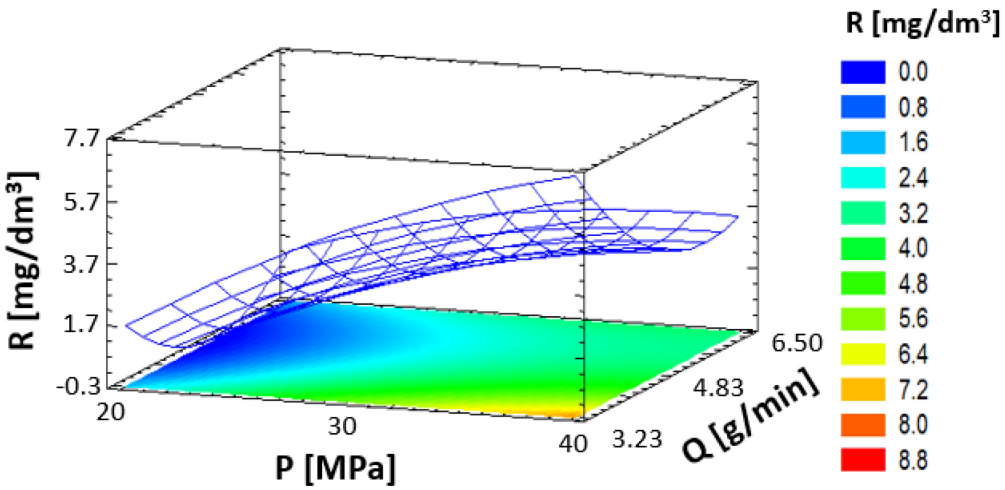

Figure 11. 3D response surface R_{PUFA} = f (P, Q_{CO_2}); T=323 K

The presented response surface in **Figure 9** suggests the highest values for R_{PUFA} in the operating areas of the most elevated P and T values of 313 and 333 K at constant values of Q_{CO_2}=3.23 g/min. The interpretation of these outcomes originates from the complex interaction between the operating P and T, influencing the physical properties of the extracted components and the supercritical fluid. The function maximum, located in these regions, is the optimal operating region for this system.

Operating values of the experimental variables (P and T) exhibit a significant impact on the observed physical characteristics of the designed extraction setup supercritical carbon dioxide – freeze-dried viscera and, consecutively, on the solvability of isolated ω-3 and ω-6 in SC-CO_2. These process variables affect the vapour pressure of the isolated material and the physical behaviour of the introduced supercritical fluid.

The temperature had a complex influence on the solubility of PUFA in supercritical CO_2 at constant pressure (P=const). In fact, at lower constant P, the increase in the operating temperature decreases the solubility of PUFA in supercritical CO_2. At the higher constant operation P (from 35 to 40 MPa), the result of the increase in the operating T is an increase in the solubility.

The 3D response surface in **Figure 10** indicates a function maximum or highest R_{PUFA} value in the operating region of high values for temperature and CO_2 flow rate, as well as in the area of low values for T and CO_2 flow rate, at a constant pressure of 30 MPa. The analysis of the 3D response surface presented in **Figure 11** shows the highest values for PUFA solubility were obtained, as expected, in the operating region of the highest values of the working P and Q_{CO_2} at a constant operating T of 323 K.

CONCLUSIONS

The lyophilised viscera tissues from the conventional fish processing industry were collected from adult European carp (*Cyprinus carpio* L.) specimens. This material of natural origin was proven to contain highly valuable polyunsaturated fatty acids ω-3 and ω-6. From the obtained results, one can conclude that European carp viscera is a satisfactory secondary resource of aquatic origin (as a usually discarded by-product) rich in the abovementioned biologically active components.

This result asserts the designed separation system and derived extensive approach model based on the supercritical fluid extraction of freeze-dried organic by-products as an adequate and representative concept within the frames of green process engineering. Furthermore, the applied ANN architecture and performed process optimisation concluded the broad engineering approach for the proposed solution that brands the viscera tissues as a secondary raw material instead of conventional discarding as bio-waste. Satisfactory results regarding the effectiveness of separating biologically active compounds from the lyophilised fish tissues have been obtained by implementing the green extraction process – SFE-CO_2, using non-toxic solvents that are eco-friendly process technologies. The experimental work resulted in determining optimal values for applied operating parameters P, T, and Q_{CO_2} to obtain maximum solubility of PUFA (R_{PUFA}).

Experimental analysis and obtained results showed that the designed separation system based on the green SFE-CO_2 process, coupled with a functional ANN as an integral product of process engineering, is an adequate process of isolation of important biologically active compounds from low-cost, bio-waste raw material - European carp viscera. Choosing from a variety of approachable models, a feed-forward backpropagation ANN containing an optimal structure of 3-10-1 along with the Levenberg-Marquardt training algorithm was designed to obtain the optimal predictive model for this process, allowing the prediction of R_{PUFA} in SC-CO_2 as a function of P, T and Q_{CO_2}. The utilised ANN model generated a matrix of output data, and RSM was used to mathematically describe the ANN-predicted values as a function of those working parameters.

This work presents a solid engineering basis for introducing contemporary green transformations and generating highly valuable products (PUFA-rich extracts) through the optimised process of biowaste valorisation according to the principles of green process engineering and circular economy. The investigated secondary raw material – fish processing bio-waste (viscera matrixes) represents a solid source of valuable bioactive components (ω-3 and ω-6) through the application of modern and precise eco-separation procedures (SFE-CO_2). The presented extraction system has a confirmed economic and environmental impact, expected by the generation of commercial value through new products obtained from waste materials of the conventional processing industry, as well as due to the elimination of the amounts of waste and their transformation into secondary raw material in a new process. Furthermore, the experimental results obtained and the created ANN model provide a basis for a consecutive process scale-up. Designed ANN provided a highly accurate fit of experimentally obtained results ($r = 0.9905$), thus representing a predictive model for the observed R_{PUFA}.

NOMENCLATURE

$m_{extract}$	mass of extract	[g]
m_{sample}	mass of sample	[g]
P	pressure	[MPa]
Q_{CO_2}	mass flow of CO_2	[g/min]
r	correlation coefficient	
R_{PUFA}	solubility of PUFA	$\left[\frac{\text{mg PUFA}}{\text{dm}^3\ {}_N CO_2}\right]$
T	temperature	[K]
t	extraction time	[min]
V_{CO_2}	volume of CO_2	[dm^3]
x_{PUFA}	mass fraction of PUFA in the extract	
Y_{PUFA}	yield of PUFA	[%]
Y_{TOTAL}	yield of total extract	[%]

Greek letters

ω-3	omega-3 fatty acid	
ω-6	omega-6fatty acid	
$\rho_{CO_2}^{NTP}$	density of CO_2 at NTP	[g/dm^3]

Abbreviations

3D	Three Dimensional
ANN	Artificial Neural Network
DHA	Docosahexaenoic Acid
EPA	Eicosapentaenoic Acid
GC-FID	Gas Chromatography with Flame Ionisation Detection
MSE	Mean Squared Error
MUFA	Mono Unsaturated Fatty Acids
NTP	Normal Temperature-Pressure conditions, 101.325 kPa and 293 K
PUFA	Poly Unsaturated Fatty Acids
RSM	Response Surface Methodology
SFA	Saturated Fatty Acids
SC-CO_2	Supercritical Carbon Dioxide
SFE-CO_2	Supercritical Fluid Carbon Dioxide Extraction

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