KINETICS OF SUPERCRITICAL FLUID EXTRACTION OF OIL FROM MICROALGA *Nannochloropsis* sp

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Marine microalgae are recognised as an important source of bioactive lipids with a high proportion of polyunsatured fatty acids (PUFA), which have been shown to be effective in preventing or treating several diseases.

For the extraction of oil from microalgae, supercritical CO_2 (ScCO_2) is regarded with interest being safer than hexane and offering a negligible environmental impact, a shorter extraction time and a high quality final product, above all under a toxicological point of view.

Whilst some experimental papers are available on the supercritical fluid extraction (SFE) of oil from microalgae, only limited information exists on the kinetics of the process. In such a contest a mathematical model, able to describe the kinetics of a SFE process, was applied to the extraction with ScCO_2 of oil from *Nannochloropsis* sp, a marine microalga   commonly used in aquaculture and characterised by a lipid fraction with a high PUFA content. The kinetic model, which allows the optimisation of working parameters and the determination of process yields, is reported together with the experimental protocol followed and the results obtained.

INTRODUCTION

Bioactive lipids containing polyunsatured fatty acids (PUFA), especially *n*-3 PUFA such as α–linolenic acid (ALA, C18:3*n*-3), eicosapentaenoic acid (EPA, C20:5*n*-3), docosapentaenoic acid (DPA, C22:5*n*-3), and docosahexaenoic acid (DHA, C22:6*n*-3), have been shown to be effective in preventing or treating several diseases including cardiovascular disorders, cancer, type 2 diabetes, inflammatory bowel disorders, asthma, arthritis, kidney and skin disorders, depression and schizophrenia [1-5]. Marine fish lipid is the main conventional source of *n*-3 PUFA used in functional food, nutraceuticals and pharmaceuticals, but microalgae are recognised as an additional important source [5].

Although the oil extraction from lipid-bearing biomasses is normally carried out by using organic solvents, supercritical CO_2 (ScCO_2) is regarded with interest being safer than hexane and offering a negligible environmental impact, a shorter extraction time and a high quality final product, above all under a toxicological point of view.

Whilst some experimental papers are available on the supercritical fluid extraction (SFE) of oil from microalgal strains, only limited information exists on the kinetics of the process

[6-10]. In such a contest, a mathematical model able to describe the kinetics of a SFE process was applied to the extraction with $ScCO_2$ of oil from the *Nannochloropsis* sp, a marine microalga commonly used in aquaculture and characterised by an oil with a high proportion of *n*-3 PUFA [11-16].

MATERIALS AND METHODS

Wet biomass of the unicellular microalga *Nannocloropsis* sp was supplied by Reed Mariculture Inc. (San Jose, CA, USA). Moisture and oil content (dry basis), determined according to the standard AOCS methods, resulted 82.8 % and 25.3 % respectively. Cells were lyophilised, ground to a particle size of 0.37 mm and then stored at – 20°C until use.

Pure solvents and chemicals were used in the analyses, while commercial grade CO_2 was utilised for SFE. Extractions were performed using a commercial pilot plant apparatus (Sitec, Maur, Switzerland) described in a previous paper [17]. $ScCO_2$ extractions were carried out using 180 g of lyophilised biomass (mixed with 100 g of glass microspheres) per each run, with a working pressure (P) of 40, 55, 70 MPa and a temperature (T) of 40, 55 °C. The flow rate of $ScCO_2$ (Φ) was maintained constant and equal to 10 kg·h^{-1}. The extracted oil fractions were collected after a predetermined amount of CO_2, ranging from 0.8 to 60 kg, had passed through the bed of microalga. The extraction yields were determined gravimetrically.

Extraction by percolation with n-hexane for 6 hours was also performed using a Soxhlet apparatus.

RESULTS AND DISCUSSION

To optimise the extraction parameters, a kinetic approach developed during a previous research activity was adopted [18]. This approach, based on the Fick's law, uses the following exponential equation to describe the evolution of extracted oil over time (t):

$$Oe = H^* \cdot [Os] \cdot (1 - e^{-k \cdot t}) \tag{1}$$

where: Oe = amount (g) of oil extracted at a random time t per gram of microalgal biomass submitted to SFE (adimensional); H^* = adimensional constant, ranging from 0 to 1, related to the equilibrium constant H ($H^* = H/(H+1)$); [Os] = oil concentration in starting material (adimensional); k = kinetic constant (s^{-1}).

The extraction rate (R) calculated as first derivative of the exponential equation (1):

$$R = dOe/dt = H^* \cdot [Os] \cdot k \cdot e^{-k \cdot t} \tag{2}$$

reaches its maximum value (R_{max}) at the beginning of extraction, when t is close to 0:

$$R_{max} = k \cdot H^* \cdot [Os] \tag{3}$$

According to Yu et al. [19], the value of R_{max} (s^{-1}) was assumed as an index to evaluate the efficiency of the SFE system *vs* the oil fraction of microalga. In particular, while the constant k gives information on the kinetics of the SFE, the product $H^* \cdot [Os]$, representing the asymptotic value of the extraction curve when t → ∞, measures the maximum amount of oil extractable in the working conditions adopted. In presence of a highly efficient SFE process, H^* tends to 1 and therefore the maximum amount of oil extractable per unit of biomass is equal to the concentration of oil in the starting material.

The identification of the best values to be assigned to the equation parameters k and H*·[Os] was carried out by a commercially available statistical program (BURENL[©]) described in a previous paper [20].

Table 1 reports the amount of oil extracted from *Nannochloropsis* as a function of run time and working conditions adopted, while Table 2 reports the values assumed by R_{max} and by the functional parameters k and H*·[Os].

Table 1: Amount (mg) of oil extracted per gram of *Nannochloropsis* submitted to SFE, as a function of run time and working conditions (temperature and pressure) adopted

Run time (min)	40 °C --------- 40 MPa	40 °C --------- 55 MPa	40 °C -------- 70 MPa	55 °C --------- 40 MPa	55 °C --------- 55 MPa	55 °C --------- 70 MPa	Soxhlet extraction with n-hexane
5	--	12.1	16.7	--	21.5	19.5	--
10	22.5	31.1	50.6	22.8	44.3	43.5	--
15	--	--	90.3	--	--	70.1	--
20	44.3	70.3	90.4	39.9	86.5	112.3	--
30	48.6	109.8	118.2	84.2	119.4	151.8	32.9
40	75.6	139.4	188.2	101.9	134.6	189.2	--
50	--	147.2	192.3	106.8	168.8	201.9	--
60	101.9	177.1	227.7	132.8	191.3	230.2	64.5
80	--	--	230.5	--	224.9	245.7	--
90	152.6	207.7	--	183.4	--	--	94.9
100	--	--	244.1	--	227.9	248.4	--
120	182.2	238.8	252.4	193.3	238.3	253.3	121.4
150	205.7	250.2	--	--	--	--	147.2
180	212.0	246.7	252.4	221.1	252.7	253.4	164.7
210	--	-	--	--	--	--	176.8
240	232.8	249.2	--	248.4	254.2	--	196.8
270	--	--	--	--	--	--	212.6
300	233.0	--	--	248.5	--	--	220.9
330	--	--	--	--	--	--	228.0
360	248.2	--	--	253.5	--	--	238.1

On the basis of the data tabled, the following remarks can be done:
a) ScCO2 is proven to be a good solvent for *Nannochloropsis* oil, as testified by the values assumed by the equation parameter H*·[Os]. This parameter, which represents the mgs of oil extracted from 1 g of microalga when the equilibrium is reached (extraction time = ∞), assumes in fact for all SFE runs values close to the concentration of oil in starting material and to that obtained when percolation with n-hexane is adopted;
b) P highly affects the kinetics of SFE, as confirmed by the values assumed by the constant k when working at the same T. In particular, for both T adopted, the value of k increases

markedly when P passes from 40 to 55 MPa, while the increases are lower when P passes from 55 to 70 MPa;

c) also the increase of T affects the kinetics of SFE, as testified by the value that k assumes when working at the same P, but such influence appears relatively low;

d) the binomial pressure-temperature is more crucial than T and P alone in determining the kinetics of the SFE process. In fact the value of k increases of three times passing from the mildest (40 °C and 40 MPa) to the hardest (55 °C and 70 MPa) working conditions;

e) in all conditions adopted, the extraction with $ScCO_2$ resulted faster than that carried out by percolation with n-hexane, as testified by the values assumed by k and/or R_{max}. For example, when working at the hardest SFE conditions (55 °C and 70 MPa), the rate at the beginning of extraction (R_{max}) is about seven times that measured using the Soxhlet apparatus.

Table 2: SFE of oil from *Nannochloropsis* sp. Maximum extraction rate (R_{max}) and values of the equation parameters k and $H^* \cdot [Os]$ as a function of temperature (T) and pressure (P) adopted. c.i. = confidence interval (p = 0.05); r = correlation coefficient

T (°C)	P (MPa)	$(R_{max} \pm c.i.) \cdot 10^6$ (s^{-1})	$(k \pm c.i.) \cdot 10^4$ (s^{-1})	$(H^* \cdot [O_S] \pm c.i.) \cdot 10^3$ (adimensional)	r^2
40	40	39.38 ± 0.65	1.57 ± 0.02	250.84 ± 0.99	0.99
40	55	74.98 ± 0.99	2.97 ± 0.03	252.45 ± 0.81	0.99
40	70	107.76 ± 1.37	4.20 ± 0.04	256.58 ± 0.85	0.98
55	40	49.58 ± 0.67	1.99 ± 0.02	249.13 ± 0.77	0.99
55	55	86.94 ± 1.03	3.45 ± 0.03	252.00 ± 0.80	0.99
55	70	118.04 ± 1.39	4.58 ± 0.04	257.75 ± 0.80	0.98
Soxhlet extraction		17.44 ± 0.47	0.70 ± 0.01	249.13 ± 2.49	0.99

To relate R_{max} to density of $ScCO_2$, the value of which is influenced by both the pressure and temperature adopted, the following equation introduced by Chrastil [21] was adopted:

$$R^*_{max} = \rho^a \cdot e^{(b/T + c)} \qquad (4)$$

where: R^*_{max} is R_{max} expressed in grams of extracted oil per litre of $ScCO_2$ flowed through the bed of microalga; ρ = density of ScCO2 ($g \cdot l^{-1}$); a, b, c = equation parameters; T = temperature (°K). The identification of the best values to be assigned to the equation parameters a, b and c was carried out by the statistical program BURENL$^{©}$ introduced above.

Table 3 reports the value of the parameters a, b and c calculated using the experimental values of R_{max} reported in Table 2. Each value was previously expressed in $g \cdot l^{-1}$ by using the following equation:

$$R^*_{max} (g \cdot l^{-1}) = R_{max} (s^{-1}) \cdot m \cdot \rho \cdot \Phi^{-1} \qquad (5)$$

where: m = amount of lyophilised microalga submitted to each SFE run (g); ρ = density of $ScCO_2$ at T and P adopted ($g \cdot l^{-1}$); Φ = flow rate of $ScCO_2$ ($g \cdot s^{-1}$);.

Table 3: Values of parameters involved in the Chrastil equation 4 adopted to relate the solvent power of $ScCO_2$ (evaluated by R^*_{max}) to its density (ρ). c.i. = confidence interval (p = 0.05); r = correlation coefficient

Equation	a ± c.i.	- (b±c.i.)	- (c ± c.i.)	r^2
$R^*_{max} = \rho^a \cdot e^{(b/T + c)}$	10.92 ± 2.57	3506.57 ± 1225.65	62.66 ± 16.18	0.97

Data in Tables 2 and 3 are of high practical interest, allowing the calculation of the extraction yield for any value of P, T and t selected. All these data are well related to those previously calculated for the SFE of oil from some oilseeds (sunflower, soybean, rapeseed) and already reported [18, 22, 23]. This consideration, together with the high values assumed by the square of the correlation coefficient, testifies the suitability of the hypotheses introduced and give a measure of the validity of the mathematical model proposed.

The fatty acid composition of extracted oils, together with the economical aspects of such process is under evaluation within a research project supported by the Italian Ministry of Research (MIUR) .

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