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Kinetics of the supercritical fluid extraction of carotenoids from microalgae with CO_2 and ethanol as cosolvent

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ABSTRACT

The kinetics of the supercritical fluid extraction of carotenoids from microalgae has been studied. The solvent systems used were supercritical carbon dioxide and CO_2 + 5% ethanol as a cosolvent. A penetration model developed by other authors was applied that, due to the spherical form of the cellular microalgae, was based on the application of the mass balance to a spherical particle. The fit of the model is satisfactory and it was able to predict in a reasonable way the extraction yield of the process. The comparison between experimental and calculated data provided a value for the internal diffusion coefficient and this allowed an analysis of the influence of the different variables in the extraction process.

1. Introduction

At present, microalgae offer great possibilities for the isolation of natural substances of significant commercial interest in industries such as pharmaceuticals and alimentary or cosmetics products. This situation means that microalgae are raw materials with a great deal of added value.

Nowadays, society demands products made with additives that are natural in origin and, wherever possible, are beneficial to human health. In this sense, marine microalgae offer great potential as sources of these substances and have attracted close attention from the aforementioned industries due to the economic and social repercussions that the use of this type of additive has in the production of their products. Many of these products are designed for direct human consumption and the extraction technique is extremely important in terms of the appropriate technology to apply.

There are numerous reports in the literature related to the analysis of diverse types of microalgae as potential sources of carotenoids. We report here a study into the extraction and application of three microalgae that represent three of the five groups in which are usually classified algae. Each one has a cellular structure different: *Nannochloropsis gaditana* [1–6], *Synechococcus* sp. [7–10] and *Dunaliella salina* [11,12].

Studies into the effects of pressure, temperature and the addition of a cosolvent to supercritical carbon dioxide in the extraction of carotenoids from the aforementioned three microalgae have been reported previously [13–17].

The results obtained with supercritical carbon dioxide indicate that the optimum extraction conditions, in the cases of *N. gaditana* and *D. salina*, are 400 bar and 60 °C, while the best results in the case of *Synechococcus* sp. were obtained at a pressure of 300 bar and a temperature of 50 °C.

The addition to the supercritical carbon dioxide of ethanol (5%) as a cosolvent led to increases in the extraction yields of carotenoids. In these cases, the optimum conditions of pressure and temperature were 500 bar and 60 °C when the raw material was *N. gaditana*. In the cases of *Synechococcus* sp. and *D. salina* the best conditions were 400 bar and 60 °C.

Conventional extraction techniques for carotenoids from natural matrices involve the use of organic solvents, and this is a practice that is currently being phased out for environmental, health and safety reasons. Supercritical extraction with carbon dioxide is an advanced technology that has a low environmental impact due to the undisputed advantages of carbon dioxide as a solvent, *i.e.* low toxicity, low cost and easy separation from extracts [18]. In addition, the use of carbon dioxide gives an extra advantage in terms of quality, as extracts do not suffer the excessive heating that would destroy thermally unstable compounds.

The design and scale up of an industrial process for supercritical extraction requires the development of a model that allows the behaviour of the process to be predicted. The most representative

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Nomenclature

N	density of the molar flux of the solute (mol/(m^2 s))
D_m	internal diffusion coefficient (m^2/s)
C	concentration of the solute in the particle (mol/m^3)
R	equivalent particle radius (m)
k_e	external individual mass transfer coefficient
t	extraction time (s)
r	spherical coordinate
x_r, x_θ, x_ϕ	adimensional concentrations
Sh	Sherwood number
Re	Reynolds number
Sc	Schmidt number
Bi	Biot number
v	linear velocity (m/s)
d	equivalent particle diameter (m)
D_e	external diffusion coefficient (m^2/s)
L	characteristic lenght (m)

Greek symbols

β	solutions of Eq. (4)
θ, ϕ	spherical coordinates
ρ	density of the solvent (kg/m^3)
μ	viscosity of the solvent (kg/(m s))

Subscripts

k	vector index of the solution of Eq. (4)
0	initial
f	in the fluid

models are based on the film model or the penetration model. The film model is preferred due to its conceptual and computational simplicity. On the other hand, a penetration model results in a better description of the process and allows values for the internal diffusion coefficient to be obtained.

In the work described here, the penetration model was applied to the supercritical extraction of carotenoids from the three microalgae discussed above. In this study the raw material was considered to consist of spherical particles. The application of this model allowed a value to be obtained for the diffusion coefficient of the solute within the solid matrix.

2. Materials and methods

2.1. Raw materials and chemicals

The raw materials employed in the experiments were obtained from the marine Microalgae Culture Collection of the Instituto de Ciencias Marinas de Andalucía (CSIC, Spain)—ICMAN.

The biomass was freeze-dried after being cultivated in seawater enriched with f/2 medium [19] at a temperature in the range 20–35 °C and aerated with atmospheric air. Once the sample had been obtained, it was stored under vacuum in darkness until the extraction procedure was carried out. *D. salina* was freeze-dried in glycerol in order to protect the cellular structure of the microalga.

The chemicals and their characteristics, purity levels and commercial sources are shown in Table 1.

The choice of ethanol was based on literature data [20–22] that show ethanol to be a very effective cosolvent for the supercritical extraction of hydroxycarotenoids from different matrices. The presence of ethanol (in traces) in the final extracts does not compromise the use of the products in nutraceutical or pharmaceutical applications [23].

In one study it was found that the use of 15% of ethanol led to the best extraction yields [20], but previous experiments with *Synechococcus* sp. showed that the appropriate percentage of cosolvent was 5% [24]. The same percentage of cosolvent was used for the other microalgae studied in order to make the yields directly comparable.

2.2. Extraction equipment

The experimental development was carried out in a micro-scale supercritical extraction apparatus obtained from *Isco* (Nebraska) (model SFX 220) (Fig. 1). The equipment consisted of an extractor, a SFX 200 controller, a restrictor and two syringe pumps (models 260D and 100DX) for the introduction of carbon dioxide and ethanol, respectively [13].

The operating methodology involved loading the extraction cartridge with approximately 0.20 g of *N. gaditana*, or 0.10 g of *Synechococcus* sp. or 0.105 g of *D. salina* (in this case 0.025 g in dry weight). These raw materials had previously been homogenised to maintain a constant apparent density in all experiments. The cartridge was then introduced into the extractor and left for 15 min to reach the operating temperature.

The flow was regulated with a micrometric valve, which was thermostated at 50 °C, until a constant flow rate of 4.5 mmol/min was achieved. An extraction time of 3 h was chosen because the majority of the carotenoids and chlorophyll extracted with supercritical carbon dioxide had been obtained after this period.

The extracts were collected in glass tubes containing ethanol. After the extraction process was complete, the solvent was removed by passing a nitrogen stream over the extract obtained at a temperature of 40 °C. Then, this extracted product was dissolved in methanol (5 mL) and stored at 4 °C with the exclusion of light until subsequent analysis. Finally, the extractor and the pipes were cleaned with acetone.

The total concentrations of carotenoids were determined by measuring the absorbance of the different samples using a spectrophotometer (Japan, Hitachi U-2010).

The equations proposed by Wellburn [25] were used in the analysis of carotenoids and these equations are given in a previous publication [13].

3. Extraction model

Different approaches from the literature can be adopted to model supercritical fluid extraction. In the successful approach adopted to model the supercritical fluid extraction from seeds, the overall fixed bed behaviour was represented by the differential mass balance for the solute in the supercritical phase and in the solid phase [26,27]. These balances were combined with an equilibrium relationship to evaluate the mass transfer coefficient of the extraction process. This approach can be adopted when solubility equilibria or external diffusion are the limiting factors.

When intraparticle or internal diffusion controls the mass transfer process, it is opportune to study mass transfer between a single particle and the supercritical solvent and then extend the results to the whole bed. Although typical vegetable particles do not conform to these assumptions, Reverchon et al. [28] developed a simplified model based on the hypothesis of a bed formed by spherical particles of the same shape and size. Bartle et al. [29] employed a similar approach and based their solution on the hypothesis that mass transfer resistance on the fluid side is equal to zero.

In the study described here, the model developed by Reverchon et al. [28] was applied to the extraction of pigments from microalgae. Due to the spherical form of the raw material, it was decided to apply the mass transfer model to a spherical particle.

Table 1
Characteristics of the chemicals used in the present work.

Chemical	Purity	Company	Characteristic
Carbon dioxide	99.995%	Carburos metálicos	Supercritical extraction solvent
Methanol	HPLC grade	Panreac	Storage solvent for the extracts
Ethanol	Instrumental analysis grade	Panreac	Collection solvent for the extracts. Used as a cosolvent
Acetone	Industrial	PQS	Cleaning of supercritical extraction equipment
Nitrogen	99.99%	Carburos metálicos	Evaporation of solvents

The material balance across an internal particle surface of radius r, according to Fick's first law, for a constant density and diffusivity in the solid and spherical coordinates (r, θ, ϕ) is

$$N = -C_0 D_m \left(\frac{1}{r^2} \frac{\partial (r^2 x_r)}{\partial r} + \frac{1}{r \operatorname{sen} \theta} \frac{\partial (x_\theta \operatorname{sen} \theta)}{\partial \theta} + \frac{1}{r \operatorname{sen} \theta} \frac{\partial x_\phi}{\partial \phi} \right) \quad (1)$$

where $N = N(t)$ is the molar flux density per unit of area, C_0 is the initial concentration in the solid matrix, x_r, x_θ, and x_ϕ are the dimensionless concentrations (functions of the extraction time) along coordinates r, θ, and ϕ, respectively, and D_m is the diffusivity in the solid matrix.

If the concentration of pigments is independent of coordinates θ and ϕ, the material flux within the particle has the following expression:

$$N = -D_m \frac{1}{r^2} \frac{\partial r^2 C}{\partial r} \quad (2)$$

The same material flux must fit the transport equation on the external surface:

$$N = k_e (C - C_f) \quad (3)$$

where C is the concentration of pigment at the particle surface, C_f is the concentration of pigment in the supercritical phase and k_e is the particle-to-supercritical phase mass transfer coefficient.

From the heat–mass transfer analogy [30] it is possible to obtain a solution for the concentration based on the extraction time and the intraparticle position according to the expression:

$$X(t, r) = \frac{C - C_f}{C_0 - C_f} = \sum_k^\infty 2 \frac{\operatorname{sen} \beta_k - \beta_k \cos \beta_k}{\beta_k - \operatorname{sen} \beta_k \cos \beta_k} \frac{\operatorname{sen}(\beta_k (r/R))}{\beta_k (r/R)} \exp \left[- \left(\frac{\beta_k}{R} \right)^2 D_m t \right] \quad (4)$$

where β_k are the k solutions for Eq. (5):

$$\beta_k \cot \beta_k = 1 - k_e \frac{R}{D_m} \quad (5)$$

The equation for the molar flux density is obtained when the concentration is obtained of expression (4) and is substituted into

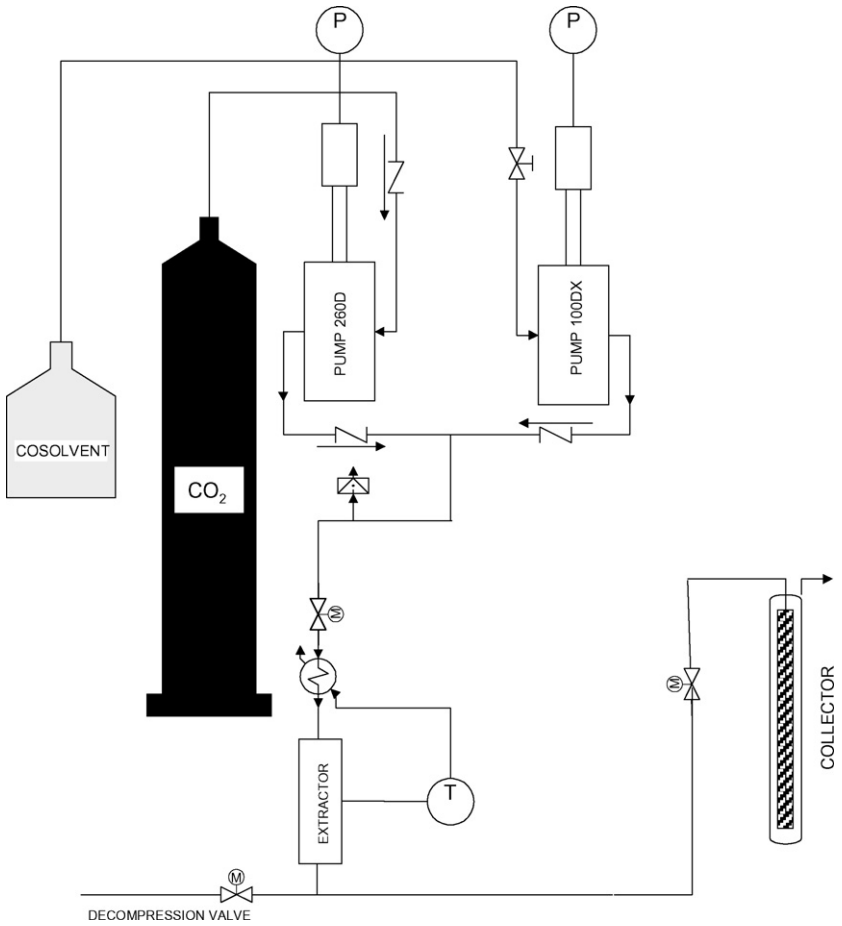

Fig. 1. Schematic diagram of the experimental apparatus.

Eq. (2) (for $r = R$). The resulting equation is as follows:

$$N = -\frac{2D_m C_f}{R} - 4\frac{D_m}{R}(C_0 - C_f) \times \sum_{k}^{\infty} \frac{2\ \text{sen}\,\beta_k - \beta_k^2 \cos^2\beta_k - \beta_k \,\text{sen}\,\beta_k \cos\beta_k}{\beta_h^2 - \beta_k\ \text{sen}\,\beta_k \cos\beta_k} \exp \times \left[-\left(\frac{\beta_k}{R}\right)^2 D_m t\right] \quad (6)$$

This expression is related to the yield of the process. Comparison of the experimental results with those obtained using the model allows the value of the internal diffusivity coefficient, D_m, to be estimated.

According to the literature, the spherical particle model has been applied with success to herbaceous matrices such as rosemary, basil and marjoram leaves [28,31,32], red grape pomace [33], olive husk [34] and vegetables seeds [35].

4. Results and discussion

Plots of the extraction yields (points) vs. time are shown in Figs. 2–4 for the three microalgae studied under the different operating conditions selected. The extraction yields are expressed as percentages with respect to the initial weight of raw material in the extractor.

4.1. Mass transfer model results

The proposed model is based on the following assumptions:

1. The value of the external mass transfer coefficient can be calculated using semiempirical equations that relate this coefficient with dimensionless numbers. The equation used for the solid–fluid transfer case in a supercritical extractor is that proposed by Tan et al. [36]:

$$Sh = 0.38 Re^{0.83} Sc^{1/3} \quad (7)$$

where *Re*, *Sh*, and *Sc* are the dimensionless numbers of Reynolds, Sherwood, and Schmidt, respectively:

$$Re = \frac{\rho v d}{\mu}, \quad Sh = \frac{2Lk_e}{D_e}, \quad Sc = \frac{\mu}{\rho D_e}$$

where ρ and μ are the density and viscosity of the solvent system, respectively, v is the linear velocity, d is the equivalent diameter of the particle, and D_e is the external diffusion coefficient.

The application of this expression requires knowledge of the values of the external diffusion coefficients of beta-carotene, one

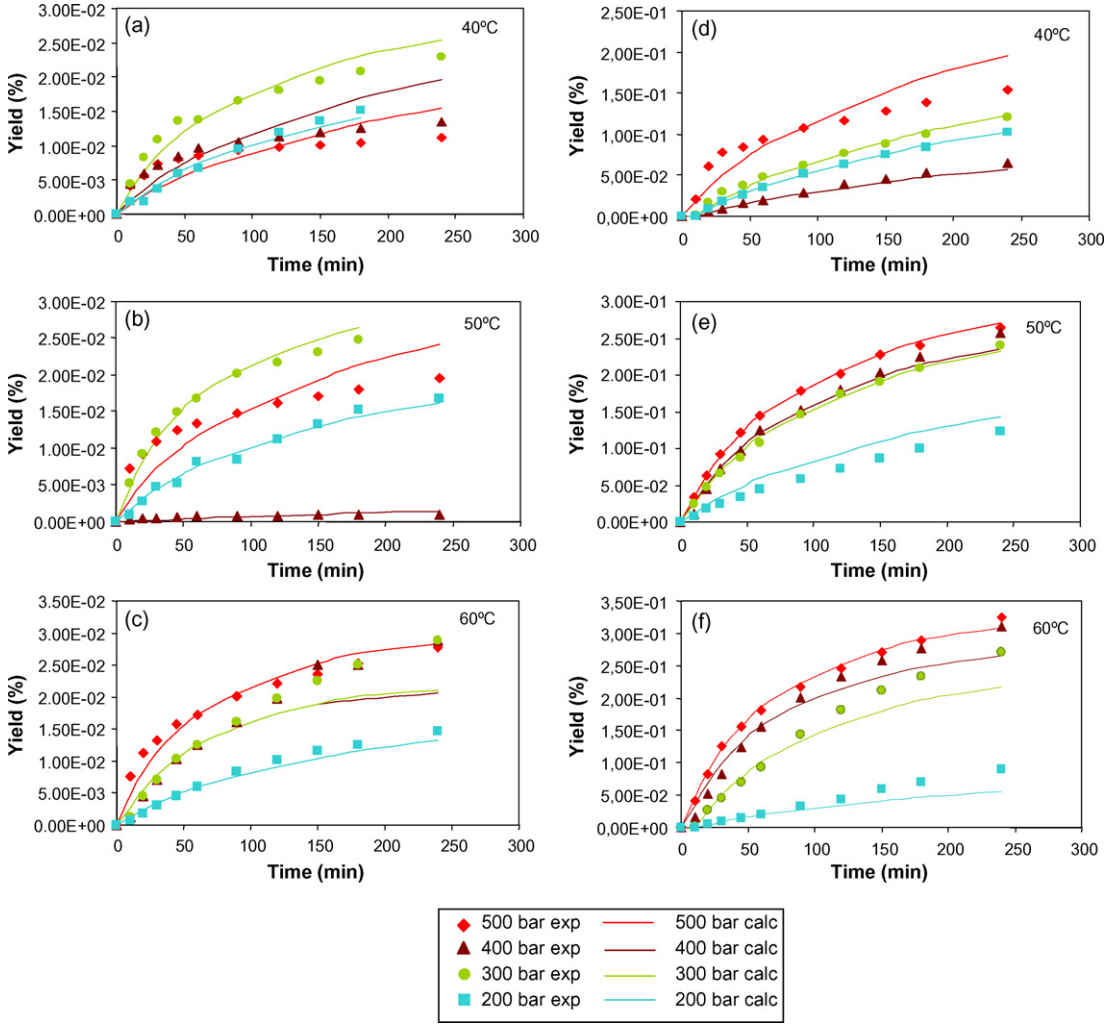

Fig. 2. Comparison between experimental and calculated values of the extraction yields for *Nannochloropsis gaditana* when using SC CO_2 (a–c) or SC CO_2 + 5% of ethanol (d–f).

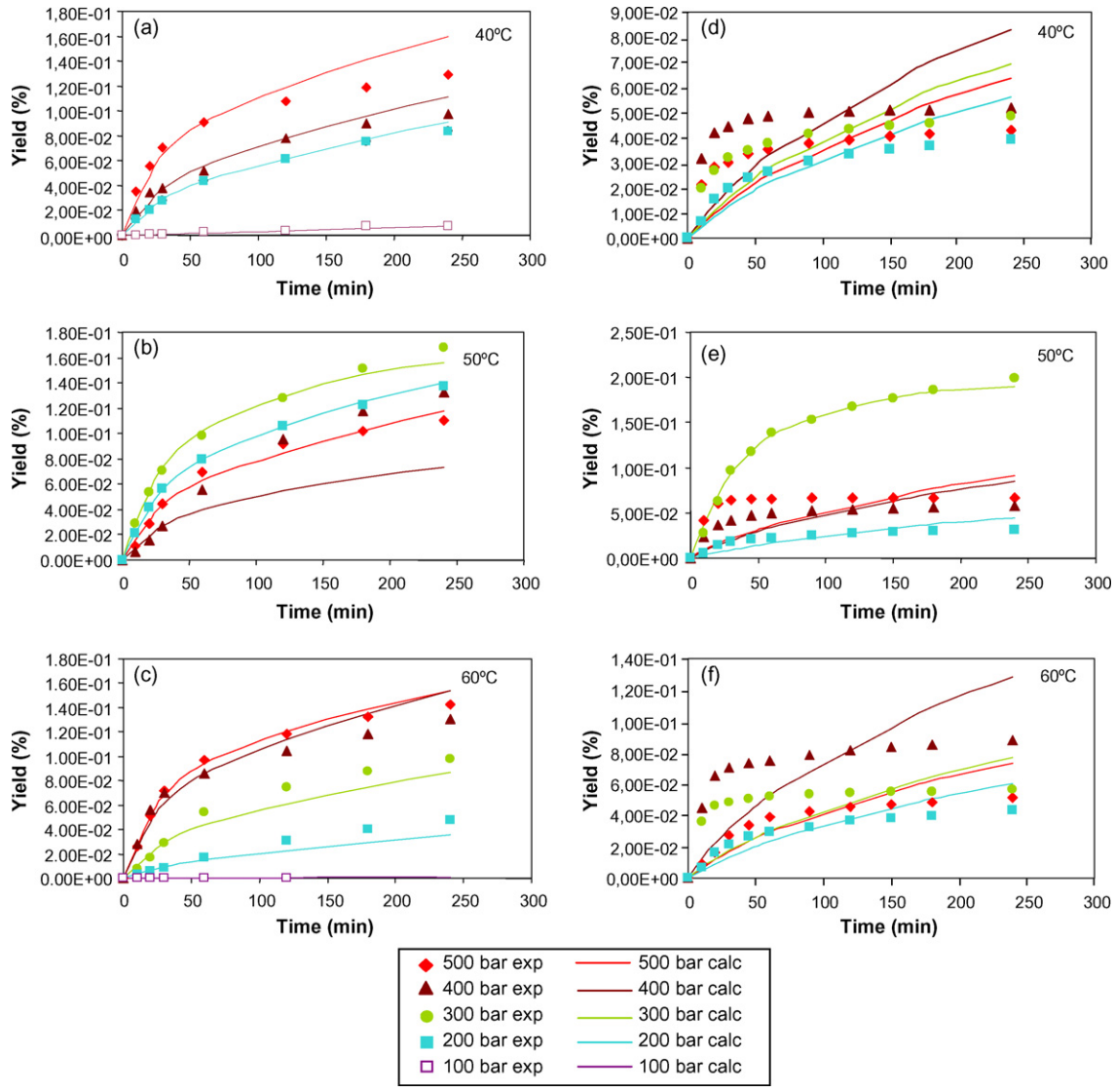

Fig. 3. Comparison between experimental and calculated values of the extraction yields for *Synechococcus* sp. when using SC CO_2 (a–c) or SC CO_2 + 5% of ethanol (d–f).

of the major pigments in the algal biomass studied, in the solvents under investigation. In order to estimate this parameter, expression (8) was applied as it relates the external diffusion coefficient with solvent density:

$$\mathrm{Ln}\, D_e = a + b\rho \tag{8}$$

where D_e is the diffusion coefficient (cm^2/s), ρ the density of the solvent system (g/cm^3), and a and b are adjusted parameters.

The adjusted parameters and the absolute deviations are indicated in Table 2.

The density values for supercritical carbon dioxide were obtained from the literature [37].

In order to estimate the density for the carbon dioxide + cosolvent system it was assumed that ethanol behaves as an incompressible liquid. Therefore, the density at 40, 50 and 60 °C for the different pressures is the same as that at atmospheric pressure. Correlation (9) [38] was used to obtain the density of the ethanol at the operating temperatures:

$$\rho = \frac{C_1}{C_2^{[1+(1-T/C_3)^{C_4}]}} \tag{9}$$

where ρ is density ($kmol/m^3$), T is temperature (K), and C_1, C_2, C_3 and C_4 are constants whose values depend on the component in question. In the case of ethanol, C_1 is 1.648, C_2 is 0.276, C_3 is 513.92 and C_4 is 0.233.

Bearing in mind that in the mixture the molar fractions of carbon dioxide and ethanol are 0.95 and 0.05, respectively, the density of the mixture was determined using Eq. (10):

$$\rho_m = 0.95\rho_{CO_2} + 0.05\rho_{EtOH} \tag{10}$$

The estimated diffusivity values are given in Table 4.

2. The parameters necessary to develop the model are shown in Tables 3 and 4.

The viscosity values of the solvent systems studied were estimated by the Chung method [39].

On the basis of the considerations outlined above, and using the spherical particle model, the values of the internal diffusion coefficients of the pigments, D_m, were evaluated. These values are shown in Table 5, for the different operating conditions studied, along with the absolute deviations for the experimental values.

The results do not show a clear trend for the operating temperatures and pressures used. However, it was observed that the

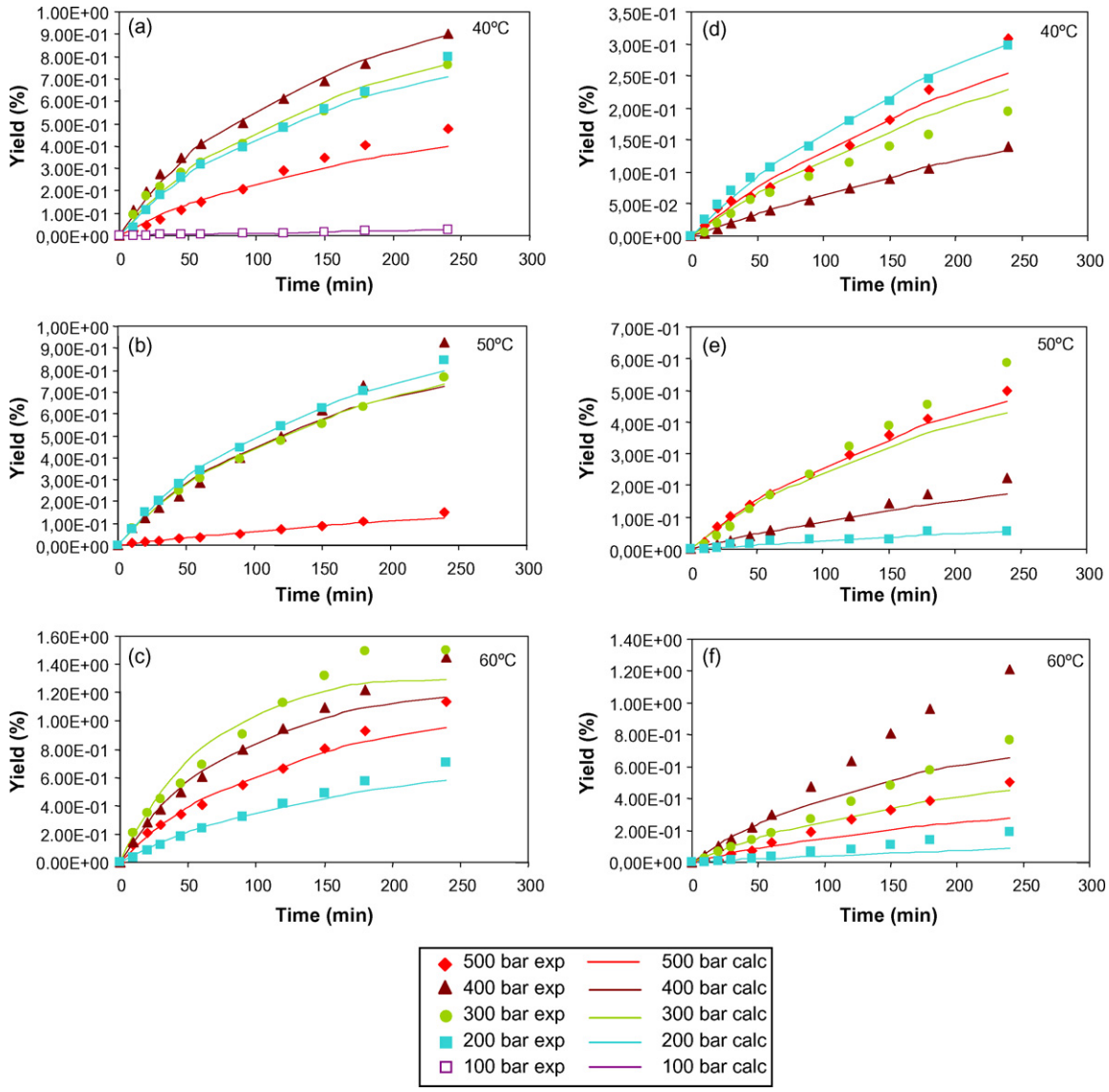

Fig. 4. Comparison between experimental and calculated values of the extraction yields for *Dunaliella salina* when using SC CO_2 (a–c) or SC CO_2 + 5% of ethanol (d–f).

Table 2
Adjusted parameters from Eq. (8) for the solvent system.

Solvent system	*b*	*a*	*AD*
CO_2 supercritical	−3.71	−8.05	4.03%
CO_2 + 5% ethanol	−4.87	−7.48	10.67%

values of the internal diffusion coefficient were low when CO_2 + 5% ethanol was used. This indicates the possibility that mass transfer has a major influence on the extraction process.

The choice of the most appropriate mass transfer model can be made by evaluating the values of the Biot number obtained from the experimental data for the internal diffusion coefficient D_m, external mass coefficient k_e and particle diameter d:

$$Bi = \frac{k_e d}{D_m}$$

If the Biot number is greater than 10, the internal diffusion is the controlling factor in the extraction process. In this case, the

Table 3
Parameters and characteristics of the pigment extraction process.

Characteristics of the raw material	Algal density	1.31 g/mL
	Equivalent radius *Nannochloropsis gaditana*	1.75×10^{-6} m
	Equivalent radius *Synechococcus* sp.	1.00×10^{-6} m
	Equivalent radius *Dunaliella salina*	6.00×10^{-6} m
Characteristics of the extractor	Length	1.30×10^{-2} m
	Diameter	7.00×10^{-3} m
	Volume	0.50 mL

Table 4
Parameters for the determination of the mass transfer coefficient, k_e, using the correlation proposed by Tan et al. [36] at the different operating conditions obtained with the penetration model applied to a spherical particle.

Solvent	P (bar)	T (°C)	ρ (g/mL)	μ (cP)	D_e ($\times 10^{-9}$ m^2/s)	v ($\times 10^{-4}$ m/s)	*Re*			*Sc*	k_e ($\times 10^{-4}$ m/s)		
							N.g.	*S.* sp.	*D.s.*		*N.g.*	*S.* sp.	*D.s.*
CO_2 SC	100	40	0.623	0.049	3.167	1.380	–	3.540	21.220	0.025	–	4.990	3.680
		60	0.292	0.024	0.108	2.940	–	7.180	–	0.756	–	0.960	–
	200	40	0.840	0.080	23.800	1.020	3.770	2.150	12.930	0.004	1.420	13.540	9.980
		50	0.784	0.070	1.753	1.090	4.300	2.460	14.750	0.051	1.460	2.600	1.920
		60	0.723	0.061	2.180	1.190	4.950	2.830	16.980	0.038	1.510	3.320	2.450
	300	40	0.911	0.093	1.092	0.940	3.220	1.840	11.030	0.094	1.420	1.560	1.150
		50	0.871	0.086	1.264	0.990	3.480	1.990	11.940	0.078	1.650	1.820	1.340
		60	0.830	0.079	1.472	1.030	3.820	2.190	13.110	0.064	1.950	2.140	1.580
	400	40	0.957	0.108	0.919	0.900	2.780	1.590	9.530	0.123	1.160	1.280	0.940
		50	0.924	0.099	1.039	0.930	3.040	1.740	10.420	0.103	1.330	1.460	1.080
		60	0.891	0.091	1.176	0.960	3.300	1.880	11.300	0.087	1.520	1.670	1.230
	500	40	0.992	0.119	0.805	0.870	2.520	1.440	8.660	0.149	1.000	1.100	0.810
		50	0.963	0.110	0.896	0.890	2.740	1.570	9.390	0.127	1.130	1.240	0.920
		60	0.934	0.102	0.998	0.920	2.950	1.690	10.110	0.109	1.270	1.400	1.030
CO_2 + 5% ethanol	200	40	0.837	0.084	1.416	1.020	3.540	2.020	12.150	0.071	1.820	2.000	1.480
		50	0.783	0.074	1.746	1.090	4.070	2.320	13.950	0.054	2.290	2.520	1.860
		60	0.725	0.064	2.169	1.180	4.680	2.670	16.040	0.041	2.910	3.200	2.360
	300	40	0.904	0.101	0.697	0.940	2.950	1.690	10.120	0.161	1.010	1.110	0.820
		50	0.866	0.091	0.839	0.990	3.290	1.880	11.270	0.125	1.220	1.350	0.990
		60	0.826	0.082	1.017	1.030	3.650	2.090	12.510	0.097	1.490	1.640	1.210
	400	40	0.948	0.115	0.563	0.900	2.610	1.490	8.920	0.215	0.810	0.890	0.660
		50	0.916	0.104	0.657	0.930	2.860	1.630	9.800	0.174	0.950	1.050	0.770
		60	0.884	0.084	0.768	0.970	3.550	2.030	12.170	0.124	1.190	1.310	0.970
	500	40	0.981	0.126	0.838	0.870	2.370	1.350	8.110	0.154	1.000	1.090	0.810
		50	0.953	0.116	0.929	0.900	2.570	1.470	8.820	0.131	1.120	1.230	0.910
		60	0.925	0.107	1.037	0.920	2.790	1.590	9.570	0.112	1.270	1.390	1.030

Note: *N.g.*: *Nannochloropsis gaditana*; *S.* sp.: *Synechococcus* sp.; *D.s.*: *Dunaliella salina*.

application of a penetration model is most appropriate. On the other hand, if the Biot number is less than 10, the controlling factor is the mass transfer in the interstitial fluid. The most appropriate model in such a case is the film model [40].

The calculated Biot number (excluding the experiments at 100 bar) is generally in the range between 10^7 and 10^{10} for the solvent systems studied. These values are greater than 10. The internal diffusion therefore controls the extraction process and the calculated values of the diffusion coefficient can be used in the design of the extraction of the extraction process.

The full Biot number data are shown in Table 6 for each biomass used. It can be seen that the values obtained with the adimensional number are very similar for biomasses with similar equivalent radius, e.g. *N. gaditana* and *Synechococcus* sp.

Table 5
Values of the internal diffusion coefficients, D_m, obtained with the penetration model applied to a spherical particle for the three microalgae studied.

Solvent	Pressure (bar)	Temperature (°C)	$D_m \times 10^{-19}$ (m^2/s)			E.A. (%)		
			N.g.	*S.* sp.	*D.s.*	*N.g.*	*S.* sp.	*D.s.*
CO_2 SC	100	40	–	0.020	0.220	–	38	6.8
		60	–	0.000	–	–	52	–
	200	40	12.400	0.600	83.400	13	16	9.9
		50	12.400	23.140	113.000	11	1.7	2.1
		60	7.300	0.500	50.000	14	18	13
	300	40	64.400	5.000	100.000	12	6.0	9.3
		50	120.000	45.600	90.000	6.6	2.7	6.4
		60	42.200	5.000	830.000	23	16	11
	400	40	20.000	9.300	160.000	27	11	7.1
		50	0.080	4.100	90.000	37	31	10
		60	42.200	31.500	470.000	24	12	6.6
	500	40	11.000	36.700	20.000	32	14	17
		50	50.000	12.120	1.850	23	9.7	6.4
		60	140.000	38.000	194.000	10	3.8	8.7
CO_2 + 5% ethanol	200	40	2.800	0.700	3.300	2.5	26	7.5
		50	5.000	0.420	0.320	30	29	32
		60	0.750	0.800	1.220	17	23	15
	300	40	4.380	1.240	2.000	9.3	34	29
		50	24.500	64.000	7.000	2.7	5.5	28
		60	26.300	2.000	12.600	11	40	14
	400	40	0.750	2.000	0.900	9.7	41	10
		50	27.000	2.140	1.300	19	36	26
		60	50.000	7.060	20.000	22	35	22
	500	40	13.000	1.000	2.430	19	37	12
		50	44.300	3.000	8.100	1.2	41	7.2
		60	90.000	1.300	3.000	3.4	24	42

Note: *N.g.*: *Nannochloropsis gaditana*; *S.* sp: *Synechococcus* sp.; *D.s.*: *Dunaliella salina*.

Table 6
Range of Biot number values for the raw materials used.

Solvent	Biomass	Biot number
CO_2 supercritical	*Nannochloropsis gaditana*	3.17×10^7–5.82×10^{10}
	Synechococcus sp.	5.99×10^7–4.51×10^{10}
	Dunaliella salina	2.28×10^7–5.97×10^9
CO_2 + 5% ethanol	*Nannochloropsis gaditana*	4.93×10^7–1.36×10^{10}
	Synechococcus sp.	4.21×10^7–1.20×10^{10}
	Dunaliella salina	5.82×10^8–6.98×10^{10}

In the case of *D. salina*, which has a radius three times larger than the other samples, the Biot number values are slightly lower than the other values when the solvent system is supercritical carbon dioxide. However, the values are slightly higher on addition of 5% ethanol as cosolvent.

The highest values for the internal diffusion coefficient were obtained when *D. salina* was used as the raw material. This microalga has the highest particle radius. In contrast, the lowest values were obtained with *Synechococcus* sp., which has the lowest particle radius of the biomass samples studied.

On the order hand, the absolute deviations for the experimental values are, in half of the cases, below 25%, 30% and 15% for the experiments with *N. gaditana*, *Synechococcus* sp. and *D. salina*, respectively. These results guarantee the applicability of the model for the adjustment of the experimental data.

Published diffusion coefficient data for the solid matrix are lacking and so the estimated values obtained from the proposed model cannot be compared. However, it is worth pointing out the results published by Reverchon et al. [28,32] concerning a vegetable matrix for the extraction of essential oils and cuticular waxes. The values for the internal diffusion coefficient in the extraction of essential oils were in order of 10^{-13} m^2/s. These values are higher than those obtained in the extraction of carotenoids from microalgae, which gave values in the order of 10^{-19} m^2/s, but are similar to those obtained by Reverchon et al. [28,32] in the extraction of cuticular waxes (approximately 10^{-17} m^2/s). However, it is also necessary to bear in mind that these waxes are found in a thick film that covers the vegetable matrix. In the microalgae studied here, carotenoids are found in an organelle named the chloroplast except in the case of *Synechococcus* sp., where these pigments are close to the cellular membrane due to the absence of this organelle.

In the application of this extraction technique to red grape pomace, the internal diffusion coefficients were in the order of 10^{-15} m^2/s. This value is similar to that obtained by Reverchon et al. [28,32] in the extraction of essential oils from vegetable matrixes, as mentioned previously. These matrixes have a similar structure to red grape pomace.

Experimental and calculated data for the extraction yields are represented graphically in Figs. 2–4 in terms of the mass transfer model selected vs. time. These graphs have been grouped according to the solvent system and the biomass used in order to allow an easy visual comparison to be made. The extraction yields are expressed as percentages rather than micrograms of carotenoids per miligram of dry weight of microalga.

It can be seen from Fig. 3 that extraction yields calculated by the model on using CO_2 + 5% ethanol with *Synechococcus* sp. do not follow the same trend as the experimental yields. This is due to the fact that the model considers a maximum extraction value and, as a result, the curves tend to be asintotic. In this case, the maximum value considered was the value from the extraction yield at 300 bar and 50 °C. This value differs significantly from the other values. For this reason the values calculated by the model tend to this maximum value and do not follow the trend shown by the experimental values—except for the curve obtained at the maximum of extraction.

The kinetic model applied in this study has been used for experiments at laboratory scale. It would be interesting to compare these results with experiments at higher scale.

4.2. Kinetics of the extraction

In order to compare the kinetics results obtained in the extraction of carotenoids using both solvent systems (supercritical carbon dioxide and 5% ethanol in CO_2) with the three microalgae studied, the yields are represented in a normalized way vs. time in Fig. 5. In order to obtain the standardized yields the value of each one was divided by the yield corresponding at 250 min (the maximum operating temperature).

Analysis of Fig. 5 shows that curves obtained for the experimental extraction yields of carotenoids using CO_2 with *Synechococcus* sp. and *N. gaditana*, and using CO_2 + 5% ethanol with *N. gaditana*, show two types of behaviour. In the first region the data follow a linear trend with time, meaning that this period is characterized by extraction at a constant rate. During this phase a large proportion of the total carotenoids extracted are obtained. The straight line is expected to arise due to the solubility equilibrium or a constant resistance to the mass transfer.

In the second region, the extraction rate diminishes with time. Carotenoids at this point have been stripped from the solid–supercritical fluid interface. It is therefore necessary for the solvent to diffuse into the solid matrix to achieve further extraction, thus lowering the extraction rate. This behaviour indicates that the process would be controlled by the mass transfer.

In the case of *Synechococcus* sp. with CO_2 + 5% ethanol as cosolvent, it can be seen that the extraction rate decreases steadily with time. This behaviour indicates that this period is controlled by mass transfer and is typical for processes in which diffusional problems exist [41].

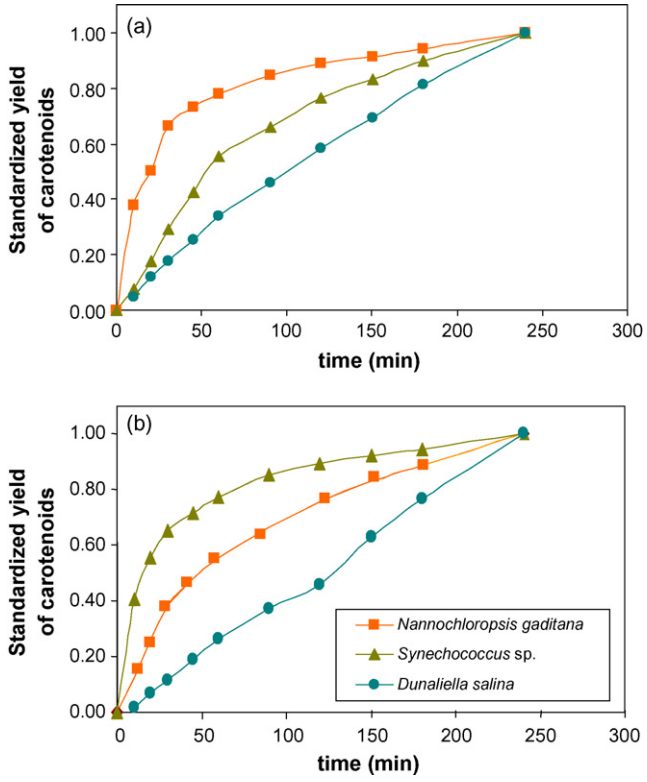

Fig. 5. Kinetic extraction curves normalized: (a) using supercritical carbon dioxide and (b) using CO_2 + 5% ethanol.

In the case of *D. salina* the same trend was observed in both solvent systems. The curves show one region that is reminiscent of the behaviour during the extraction process outlined above. The extraction rate remains constant over time, indicating that solubility could be the controlling factor for the process.

It has been reported in the literature that the use of a cosolvent can lead to the appearance of an inflection point in the extraction curve. This point indicates that the cosolvent starts to leave the extractor with the solute that it has extracted [33]. Such an inflection point was not observed in the carotenoid extraction from microalgae described here.

The results of the kinetic extraction indicate that in order to estimate the optimum extraction time, it is necessary to run the extraction process for more than 250 min. This length of time is necessary because the experimental curves do not show a clear trend to a maximum extraction yield.

5. Conclusions

A mass transfer model for a spherical particle was selected to study the extraction of carotenoids. The value of the internal diffusion coefficient was obtained as an adjustment parameter. The absolute deviations found on applying the model were below 25%, 30% and 15% in more than half of the cases studied for the microalgae *N. gaditana*, *Synechococcus* sp. and *D. salina*, respectively.

Analysis of the Biot number obtained enables the penetration model to be selected as the most appropriate mass transfer model, since the values obtained for this adimensional number are greater than 10.

From the kinetic extraction study it was deduced that it is not possible to determine an optimum extraction time for the two solvent systems investigated. This is because the carotenoid extraction yields do not tend to a maximum of extraction asymptotically in the operating time studied.

The representative curves obtained in the kinetic extraction study for each biomass used show three types of curves and these suggest the behaviour patterns described below. The yields are expressed in a normalized way vs. time:

The first type of curve is obtained for *N. gaditana* and *Synechococcus* sp. on using supercritical carbon dioxide and for *N. gaditana* on using CO_2 + 5% of ethanol. In this graph an initial region is observed in which the extraction rate is constant. This is followed by a second region in which the extraction rate diminishes with time.

The second type of curve shows a continuous decrease in the extraction rate with time. This is the case for *Synechococcus* sp. extracted with CO_2 + 5% of ethanol.

A third type of curve shows an extraction rate that is constant with time. This behaviour is shown by *D. salina* with the two solvent systems studied.

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