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The Hansen theory to choose the best cosolvent for supercritical CO_2 extraction of β-carotene from *Dunaliella salina*

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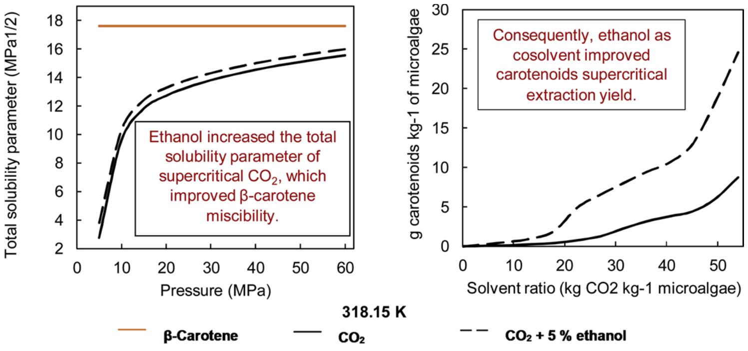
GRAPHICAL ABSTRACT



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ABSTRACT

The Hansen solubility theory was used to predict the best cosolvent for supercritical carbon dioxide (sc-CO_2) to achieve the selective extraction of β-carotene from *Dunaliella salina*. Among four organic cosolvents, ethanol was predicted to be the best based on minima R_a values. The predictions were validated through equilibrium data and extraction curves from the microalgae. The addition of ethanol reduced the bubble pressures, and therefore increased the solubility of the β-carotene. With 5% mass fraction, at 318.15 K and 20 MPa, the extraction yield was 25 g carotenoids kg microalgae^{-1}, much more than in pure sc-CO_2 (6 g).

1. Introduction

The search for natural sources of antioxidants keeps increasing over time. These products are used to reduce the oxidative deterioration of both, food and the human body [1,2]. Microalgae represent one of the sources that are being studied due to the high amounts of bioactive compounds within their structure. Some of these compounds include antioxidants, polyunsaturated fatty acids (PUFAs), sterols and others [1,3].

Carotenoids, for instance, are a class of organic pigments from the group of isoprenoids that have numerous biological functions. They are fat-soluble and therefore, they present high solubility in non-polar solvents. The β-carotene is one of the main types of carotenes, and it is mainly known for being a natural precursor of vitamin A. It is a high-molecular-weight compound that is formed by a hydrocarbon chain without oxygen ($C_{40}H_{56}$) [4]. It is easily degradable by light, heat and air, and its colour can vary from yellow to dark red, depending on its purity, source and location. Some of the main natural sources of β-carotene include plants, algae, microalgae [4] and fungi [5], but most part of its production comes from synthetic methods.

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Carotenoids have been commonly extracted from these raw materials using organic solvents. However, the increasing awareness towards the environmental and health issues that derive from the use of these substances has led to the implementation of stronger regulations. Thus, such technologies are being replaced by the development of sub- and supercritical carbon dioxide (sc-CO_2) extraction [6].

On the other hand, a cosolvent (modifier or entrainer) is added to a supercritical gas in order to increase the solubility of a specific compound, increase separation factor and/or reduce operating pressure and CO_2 consumption. In order to achieve so, the cosolvent changes some characteristics of the main supercritical solvent such as polarity or creates specific interactions with the solute or with the active sites of the solid matrix [7–9]. However, the selection of the best cosolvent requires long and expensive experimental effort. Additionally, after the extraction, the cosolvent must be recovered and reused. This procedure is a difficult task and could involve additional steps such as distillation [10]. Consequently, the benefits of cosolvent addition must be balanced against its disadvantages for a specific application [7,11].

The Hansen solubility theory (HST) can be used to assess the affinity among substances and in that way, to evaluate the miscibility among them. The suitability of the of the HST to predict the best cosolvents to solubilise fatty acids in sc-CO_2 was demonstrated in a previous work by our team [11]. The purpose of this study was to use the HST to predict the best cosolvent for sc-CO_2 to achieve the selective extraction of β-carotene from microalgae. Acetone, ethanol, n-hexane and methanol were used in the predictions that were validated with bubble pressures. Extraction curves were also obtained using ethanol and methanol as co-solvents.

2. Hansen solubility theory

Hildebrand and Scott [12] introduced the solubility parameter (δ) for first time in 1950 being a numerical estimation of the level of interaction between two substances. Thus, materials with similar δ are more likely to be miscible with each other. At that time, the authors defined the concept as the square root of the cohesive energy density (CED), which was usually found by dividing the latent heat of vaporization of a fluid (ΔE) by the molar volume (V) of the involved fluid, such as it is described in Eq. (1).

$$\delta = \sqrt{\left(\frac{\Delta E}{V}\right)} \tag{1}$$

The latent energy of vaporization could be usually calculated by Eq. (2), where ΔH is the latent heat of vaporization, R is the universal gas constant, and T is the absolute temperature. The SI units for HSP are $MPa^{1/2}$, which are 2.0455 times larger than the older Hildebrand unit (H).

$$\Delta E = \Delta H - RT \tag{2}$$

Dr Hansen proposed the division of δ into three components: dispersion, polar and hydrogen bonding forces, later on, known as Hansen solubility parameters (HSP) [13]. From then on, the HSP found many applications, from academic laboratories to industrial facilities to judge the miscibility of polymers, biopolymers, pharmaceuticals, pigments, colourants and some biological materials in different solvents [14]. In the three-parameter Hansen approach, ΔE can be obtained as the sum of three contributions (Eq. 3): the nonpolar atomic (dispersion) interactions, ΔE_d, permanent dipole-permanent dipole interactions, ΔE_p, and the hydrogen bonding interactions, ΔE_h [14].

$$\Delta E = \Delta E_d + \Delta E_p + \Delta E_h \tag{3}$$

Dividing Eq. (3) by V, gives the respective Hansen cohesion energy parameters (Eq. 4).

$$\frac{\Delta E}{V} = \left(\frac{\Delta E_d}{V}\right) + \left(\frac{\Delta E_p}{V}\right) + \left(\frac{\Delta E_h}{V}\right) \tag{4}$$

Taken into account that δ is defined by Eq. (1), the total Hansen solubility parameter (δ_T) will be the square root of a sum of each Hansen component squared, including the dispersion (δ_d), polar (δ_p) and hydrogen bonding (δ_h) contributions (Eq. 5).

$$\delta_T = \sqrt{\delta_d^2 + \delta_p^2 + \delta_h^2} \tag{5}$$

Temperature and pressure influence δ_T and individual HSP of solvents [15]. An increase of pressure at a constant temperature generally increases δ_T by increasing solvent density. For the same reason, an increase in temperature at constant pressure leads to a decrease in δ_T [16]. In any case, the influence of temperature and pressure on δ_T and HSP can be calculated by Eqs. (6), (7) and (8) as a function of molar volume [15]. In these equations, subscript “ref” makes reference to HSP and molar volumes of the fluid at room temperature (298.15 K).

$$\frac{\delta_{d\,ref}}{\delta_d} = \left(\frac{V_{ref}}{V}\right)^{-1.25} \tag{6}$$

$$\frac{\delta_{p\,ref}}{\delta_P} = \left(\frac{V_{ref}}{V}\right)^{-0.5} \tag{7}$$

$$\frac{\delta_{h\,ref}}{\delta_h} = exp\left[-1.32\,x\,10^{-3}(T_{ref} - T) - ln\left(\frac{V_{ref}}{V}\right)^{-0.5}\right] \tag{8}$$

In addition, solubilization properties can be performed by using the Hansen sphere technique. To do so, a group of solvents with known HSP is used to test their capability to solubilize a target compound. The internal part of the sphere is constituted by every solvent that dilutes the compounds of interest or target compound. The ones that are not able to dilute the target compound can be found in the external part of the sphere. The centre of the sphere corresponds to the three-dimensional HSP coordinates of the target compound. The radius is defined by the largest distance between the coordinates of the target compound and those of one solvent that dilutes the solute at the limit of the sphere. This radius is known as R_0 and it is just related to the target compound. On the other hand, the distance between the coordinates of the solvent (δ_{d1}, δ_{p1} and δ_{h1}) and those of the target compound or centre of mass of the Hansen sphere (δ_{d2}, δ_{p2} and δ_{h2}), is represented by R_a, and can be calculated as it is shown in Eq. (9). The smaller R_a, the more miscibility.

$$R_a = \sqrt{4(\delta_{d1} - \delta_{d2})^2 + (\delta_{p1} - \delta_{p2})^2 + (\delta_{h1} - \delta_{h2})^2} \tag{9}$$

More information about the Hansen solubility theory is provided in the Hansen’s handbook [14].

3. Materials and methods

3.1. Reagents

Table 1 shows the suppliers and purities of the solvents used in this work. All substances were used without any pre-treatment.

Table 1
Reagents used with suppliers and purities.

Material	Supplier	Purity ($kg\ kg^{-1}$)	CASRN
CO_2	Air Products	≥ 0.9998	124-38-9
Acetone	Sigma-Aldrich	≥0.999	67-64-1
Butylated hydroxytoluene	Sigma-Aldrich	≥ 0.99	128-37-0
β-carotene	Sigma-Aldrich	≥ 0.97	7235-40-7
Ethanol	Fisher Chemical	≥ 0.998	64-17-5
n-Hexane	Fisher Chemical	≥ 0.99	110-54-3
Methanol	Fisher Chemical	≥ 0.999	67-56-1

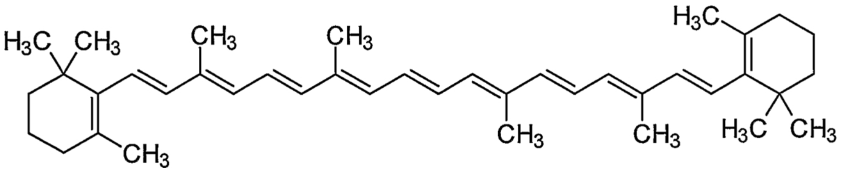


Fig. 1. The molecular structure of β-carotene.

3.2. Hansen solubility parameters of β-carotene

There was not enough data in the literature regarding the physical properties and solubility parameters of the solute-solvent pairs of this study. Therefore, the HSP values of β-carotene from the dispersion, polar and hydrogen bonding forces were calculated using the group contribution method (GCM) that was proposed by Hansen and Beerbower [14]. δ_T was calculated using the GCM described by Fedors [17]. Some other authors used this same procedure [18,19]. Fig. 1 shows the β-carotene structure elaborated by the Aspen Plus® V 10.0 software [20], which was used for the implementation of the GCM.

Temperature dependence of the HSP of β-carotene was calculated on the basis of Jayasri and Yaseen [21] method described by Eq. (10), such as other authors did in the past [11,18]. There, T_r is the reduced temperature at temperatures 1 (298.15 K) and 2 (desired temperature). The critical properties that were required to calculate the variation of HSP of β-carotene as a function of temperature were predicted using the GCM proposed by Joback and Reid [22].

$$\frac{\delta_2}{\delta_1} = \left(\frac{1 - T_{r2}}{1 - T_{r1}}\right)^{0.34} \tag{10}$$

3.3. Hansen solubility parameters of solvents

The HSP of CO_2 and of the mixtures CO_2 plus a volume fraction of 5% (0.05 $m^3\,m^{-3}$) cosolvent were calculated as a function of temperature and pressure using Eqs. (6), (7) and (8). An additive relation between the HSP of each component was assumed as commonly done [11]. This means that the total HSP value of the mixture was equal to the sum of the product of the respective volume fractions of the components present in the mixture, and their corresponding HSP value.

The solubility parameters for CO_2 and the cosolvent at 298.15 K were obtained from the Hansen Handbook [14], while the molar volume at different temperatures and pressures studied were calculated using the Reference Fluid Properties (REFPROP) model from the National Institute of Standards and Technology (NIST), with help of the Aspen Plus V 10.0 software [20].

3.4. Miscibility predictions for the selection of the best cosolvent

The miscibility of β-carotene in CO_2 and its mixtures with four organic solvents (acetone, ethanol, hexane and methanol) frequently used in the manufacture of food and pharmaceutical products [23] was estimated using the R_a parameter. The smaller R_a value of the SCM, the more miscibility of ®-carotene. Consequently, and according to the theory, supercritical systems with lowest R_a would represent the most suitable one [11,18]. The calculations were performed at a constant cosolvent volume fraction equal to 5%. Pressure and temperature conditions to calculate the R_a values were selected to match the range of conditions used for the experimental validations.

A previous work of our group [11] demonstrated that the Hansen theory could not predict well the behaviour of cosolvents with ring structure; apparently, the ring provides stability to the molecule that the GCM does not predict accurately, avoiding entrainer/solute interactions that are necessary to achieve the cosolvent effect. For that reason, cosolvents with ring structure were not pre-selected for this work. Water was neither selected as a cosolvent because a mixture with a mass fraction of 5% (0.05 kg kg^{-1}) water in sc-CO_2 was not a homogeneous SCM within the explored interval of operating conditions. Besides, based on our experience, water forms emulsions with oily extracts hampering the extraction.

3.5. Bubble pressures of β-carotene in the supercritical fluids

The predictions were validated with experimental data obtained in a high-pressure variable volume view cell, following the static synthetic method described in a previous work [24]. Bubble pressures at 333.15 K using a molar fraction of 4 10^{-7} β-carotene pure sc-CO_2 and its mixtures with the four cosolvents were obtained. A cosolvent mass fraction of 5% ± 0.1% was used. Mass fraction and not volume fraction was used in the experimental determination because of the practical limitations of the variable volume view-cell. However, considering the low amount of cosolvent used, the variation with respect to the volume fraction was not significant in the range of conditions tested. The temperature controller was connected to a thermocouple (J type, accuracy of ± 1.0 K) that was in direct contact with the fluid mixture inside the equilibrium cell. The desired temperature was controlled to within 0.5 K. The phase transition was determined visually, as described by McHugh and Krukonis [25]. The assays were repeated at least twice, and the bubble pressure was determined a minimum of three times, $n = 2 \times 3$. The standard deviations in the bubble pressures were on average 0.2 MPa.

3.6. Supercritical fluid extraction of β-carotene from Dunaliella salina

Supercritical fluid extraction of β-carotene from *Dunaliella salina* was carried out in our laboratory at different conditions of pressure (20 MPa and 30 MPa) and temperature (308.15 K, 318.15 K and 328.15 K) using CO_2 and its mixture with a mass fraction of 5% cosolvent. It was not possible to perform extractions with n-hexane and acetone as cosolvents because of safety limitations. Methanol and ethanol have similar functionality, but the Hansen theory predicted very different behaviour. On the other hand, mass fraction and not volume fraction was used as the flow rate in the experimental set up was measured in mass.

All supercritical fluid extractions were done in a homemade semi-continuous flow apparatus that consisted of a CO_2 feeding line and a 1 10^{-4} m^3 capacity 316 stainless-steel vessel. A high purity CO_2 was used to reduce the oxygen concentration, which could degrade carotenoids during the extraction [26]. Moreover, the air from all pipes and valves and from the extractor was purged before experiments. A heating jacket was placed around the extractor to control the temperature inside. Two membrane pumps (Milroyal D; Dosapro Milton Roy, Spain) connected in parallel were used to pump the CO_2 and the cosolvent up to the extraction pressure. The pump for CO_2 had an attached chamber in which a cooling fluid flowed to avoid CO_2 cavitation during pumping. The CO_2 flow rate was measured in the mass-flow meter (M-10 SLPM-D, Alicat Scientific, USA) that was placed at the end of the line, while pressure was controlled by a back-pressure regulator (BPR, 26-1761-24-161, TESCOM Europe, Germany). Complete details of the equipment have been described in a previous report [27].

For each experiment, the extractor was filled with 0.01 kg of microalga *D. salina* (Algalimento S.L., Spain) with 6 kg moisture kg dry microalgae^{-1}, dominant size of 45 μm and an oil content of 0.21 kg oil kg dry microalgae^{-1}. The moisture was measured in an oven (Digiheat, J.P. Selecta, S.A., Spain) at 376.15 K until the weight did not vary more

Table 2
R_a values between β-carotene and supercritical fluids (CO_2 and its mixtures with a volume fraction of 5% cosolvent) at 20 MPa and different temperatures.

System	R_a		δ_d at 313.15 K ($MPa^{1/2}$)
	313.15 K	323.15 K	
sc-CO_2 + ethanol	12.14	13.57	10.77
sc-CO_2 + hexane	12.14	13.59	10.74
sc-CO_2 + acetone	12.21	13.64	10.76
sc-CO_2 + methanol	12.26	13.67	10.74
sc-CO_2	12.63	14.14	10.50

than 0.1%. The powdered microalgae was introduced with 0.016 kg of sea-sand (extra pure, particle size 1 mm - 0.315 mm ≥ 90%, Sigma-Aldrich, Spain). The use of sea-sand prevented the microalgae bed compaction avoiding the formation of preferential channels that reduced the extraction yield. For example, at 20 MPa and 318.15 K, by using sea-sand as packing material there was an increase of approximately 76% carotenoid extraction compared to the bed without sand. Furthermore, no adsorption of the extracts on the sea sand particles was observed. The procedures for the fill up of the raw material, start-up and stop of the equipment could be consulted in a previous work of this same research group [28].

Samples were taken each 15 min and the total extraction time was 180 min. When a cosolvent was used, this one was removed from the extract with a nitrogen stream after the extraction process was completed. The extract was a red oil whose total weight and its content in carotenoids were measured. The results shown are the average of at least two measurements. The experimental error was deduced from selected tests that were repeated six times. The maximum standard deviation was 4% and 10% for oil and carotenoids yield, respectively.

Finally, the extraction curves were constructed by plotting the extraction yield of carotenoids based on the total biomass loaded in the extractor (g carotenoids kg dry microalgae^{-1}), *versus* the solvent ratio that passed through the bed (kg CO_2 kg dry microalgae^{-1}) at constant intervals of time.

3.7. Content of total carotenoids of extracted samples

The extract was dissolved in methanol, stabilized with a volume fraction of 0.025% butylated hydroxytoluene (BHT) and immediately analysed to avoid degradation of the carotenoids. The total concentration of carotenoids was determined by measuring the absorbance of the samples using a UV–vis spectrophotometer (MRC, model UV-1800, Tel-Aviv, Israel) and the Eqs. (11), (12) and (13) proposed by Wellburn [29]. Based on the analysis of the microalgae provider, the β-carotene represented a mass fraction of 86% of the total carotenoids content. Therefore, the β-carotene was assumed as the most abundant compound present in the enriched-carotenoid extract.

$$C_{(x+c)}\left(\frac{\mu g}{mL}\right) = \frac{1000A_{470} - 1.63C_a - 104.96C_b}{221} \tag{11}$$

$$C_a\left(\frac{\mu g}{mL}\right) = 16.72A_{665.2} - 9.16A_{652.4} \tag{12}$$

$$C_b\left(\frac{\mu g}{mL}\right) = 34.09A_{652.4} - 15.28A_{665.2} \tag{13}$$

where $C_{(x+c)}$ is the total carotenoid content (xanthophylls and carotenes), C_a and C_b are chlorophyll *a* and chlorophyll b concentrations, respectively, and A_{470}, $A_{652.4}$ and $A_{665.2}$ are the absorbances at 470 nm, 652.4 nm and 665.2 nm, respectively.

4. Results and discussion

4.1. Solubility parameters estimation and selection of the best cosolvent

The predicted HSP for dispersion, polar, hydrogen bonding and total contributions for β-carotene at 298.15 K were 16.74 $MPa^{1/2}$, 2.32 $MPa^{1/2}$, 5.39 $MPa^{1/2}$ and 17.60 $MPa^{1/2}$, respectively. The δ_T of β-carotene was highly influenced by the dispersion contribution due to the presence of a long straight hydrocarbon chain in its structure. Furthermore, as can be seen in Fig. 1, β-carotene contains eleven unsaturations (olefin groups), which enhances the dipole-dipole contribution.

The mixtures sc-CO_2 + cosolvent should be in the supercritical state (completely miscible) at the operating conditions to reach the desired cosolvent effect [7]. The existence of a single homogeneous phase for all the mixtures at the explored interval of conditions was corroborated by visual inspection in the high-pressure variable volume view cell. Thus, the selection of the best cosolvent was done at conditions over the critical point of all the mixtures.

The R_a values of the mixtures CO_2 + cosolvent indicated the cosolvent order. According to Table 2, the most adequate cosolvents for β-carotene extraction were in decreasing order: ethanol, hexane, acetone and methanol. Similar results were obtained with cosolvent volume fractions of 10% (data not shown as the experimental extractions were only done with a mass fractions of 5% cosolvent). This ranking did not correspond to the solubility of β-carotene in the pure solvents at room conditions [30] shown in Table 3. For example, at 298.15 K and atmospheric pressure, the best solvent to solubilise β-carotene was hexane, while ethanol was the best cosolvent at supercritical conditions. Furthermore, all SCM had lower R_a and consequently higher solvent power than sc-CO_2. The addition of cosolvents increased the dispersion, polar and hydrogen bonding contributions to the CO_2, which reduced the energy difference between the target compound and the SCM.

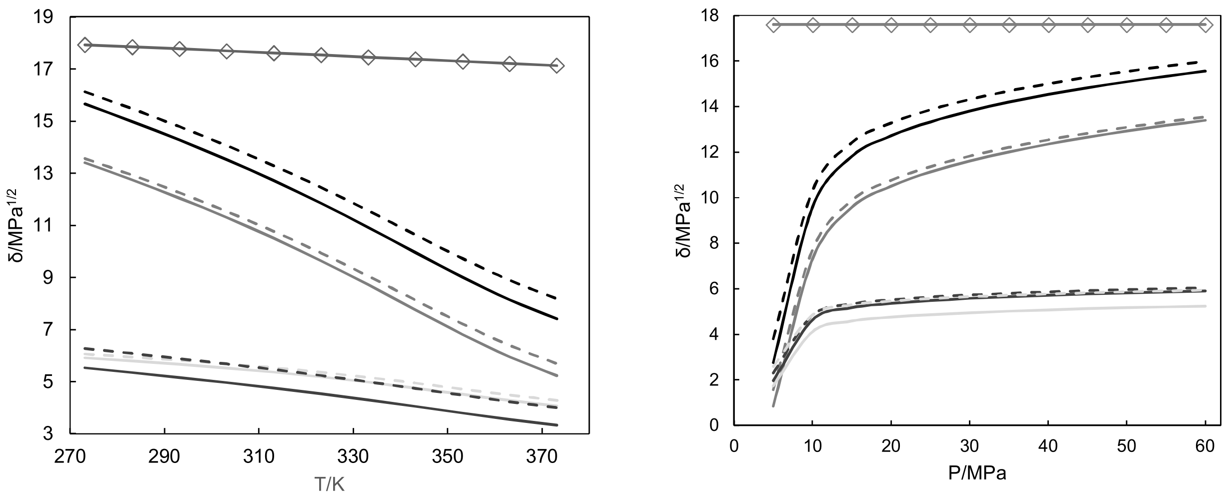
The entrainer effect is obtained when there are specific intermolecular interactions between the cosolvent and the solute, such as dipole-dipole, dipole-induced dipole, or induced dipole-induced dipole (dispersion) interactions and specific interactions such as H-bonding [7,31]. The β-carotene mainly interacts through induced dispersion and dipole-dipole forces, but mostly by the first one, due to the non-polar nature of β-carotene. Table 2 shows dispersion forces at 20 MPa and 313.15 K of CO_2 and its mixtures with all the cosolvents. The mixture of CO_2 with ethanol has the greatest and the closest dispersion contribution to that of the β-carotene. Therefore, the miscibility enhancement in the presence of ethanol could be due to the highest induced dispersion interactions between this cosolvent and β-carotene.

On the other hand, Fig. 2 shows the variation with temperature (Fig. 2a) and with pressure (Fig. 2b) of dispersion, polar, hydrogen bond and total solubility parameters of CO_2 and its mixture with the best cosolvent (ethanol) at a volume fraction of 5%.

Both temperature and pressure strongly influenced δ_T and HSP of the supercritical mixture. An increase in temperature at constant pressure decreased the total solubility parameter. Furthermore, the dispersion contribution (δ_d) decreased at a higher velocity than the polar (δ_p) and hydrogen bonding (δ_h) forces. On account of that, the

Table 3
The solubility of β-carotene in pure organic solvents at 298.15 K and 0.1 MPa.

Solvent	Solubility of β-carotene [30] (kg m^{-3})
Ethanol	30
Hexane	600
Acetone	200
Methanol	10



a) 20 MPa

b) 313.15 K

Fig. 2. Dispersion (δ_d), polar (δ_p), hydrogen bonding (δ_h) and total (δ_T) contributions at a) constant pressure (20 MPa) and b) constant temperature (313.15 K). δ_T for β-carotene (—◇—); δ_d (——), δ_p (——), δ_h (——) and δ_T (——) for pure CO_2 and δ_d (- - -), δ_p (- - -), δ_h (- - -) and δ_T (- - -) for the mixture CO_2 + 0.05 $m^3\,m^{-3}$ ethanol.

total solubility parameter of CO_2 decreased more rapidly as well, because the larger contribution to δ_T came from δ_d. Contrarily, a rise in pressure at a constant temperature increased δ_T by increasing the solvent density, as can be seen in Fig. 2b. However, around 15 MPa, the increasing velocity was reduced, because above this condition, density maintained in more constant values. In addition, according to Fig. 2b, the addition of ethanol increased the dispersion, polar and hydrogen bond interactions of the solvent, thus reducing the distance between δ_T of the solvent mixture and δ_T of β-carotene.

4.2. Experimental validations

4.2.1. Bubble pressures

Bubble pressures were measured with acetone, ethanol, n-hexane and methanol as cosolvents. Table 4 shows the results at 333.15 K using a molar fraction of 4 10^{-7} of β-carotene. The bubble pressure of β-carotene in pure sc-CO_2 was similar to that reported by other authors at the same temperature [32–35]. In Table 4, lower bubble pressure values indicate higher miscibility. Therefore, the best cosolvent for the solubilization of β-carotene in sc-CO_2 was ethanol followed by n-hexane, acetone and methanol, which was consistent with the predictions made through the HST.

There are already published data on the use of methanol and ethanol as cosolvents to enhance the solubility of β-carotene in sc-CO_2. Cygnarowicz et al. [36] in their study of equilibrium solubilities of β-carotene in sc-CO_2 and its mixtures with 1% of methanol and 1% of ethanol found that ethanol was better cosolvent than methanol to solubilize β-carotene, as it was predicted in this work.

Table 4
Bubble pressures (MPa) obtained by the static synthetic method at 333.15 K for a molar fraction of 4 10^{-7} β-carotene in different supercritical mixtures formed by CO_2 and a 5% mass fraction of cosolvent.

Supercritical mixture	Bubble pressure (MPa)
CO_2 + ethanol	14.6
CO_2 + n-hexane	16.1
CO_2 + acetone	17.7
CO_2 + methanol	18.2
CO_2	26.0

4.2.2. Supercritical fluid extraction of β-carotene from microalgae

The predictions and previous results from the static synthetic method were tested versus extraction data from our laboratory and with reported data from the literature. The supercritical extractions from the microalgae by using pure sc-CO_2 and the SCM CO_2 + methanol and CO_2 + ethanol rendered a red oil. It was previously demonstrated that sc-CO_2 extraction is selective for the recovery of neutral lipids from microalgae [37]; thus, the extract was most likely composed by this lipid fraction enriched in carotenoids.

Table 5 shows the total oil and carotenoids extraction yield from *D. salina* at 180 min and different operating conditions when using sc-CO_2 and its mixtures with methanol and ethanol at a mass fraction of 5%. Moreover, Fig. 3 compares the carotenoids extraction curves at 20 MPa and temperatures of 318.15 K (Fig. 3a) and 328.15 K (Fig. 3b), respectively. The use of cosolvents improved the total extraction yield and the content of carotenoids in the extract, with the largest increase occurring in the SCM of CO_2 + ethanol, which agreed with the predictions based on the R_a values. It also increased the selectivity for the carotenoids extraction as shown by the carotenoids to oil ratio (Table 5). Thus, the extract obtained with pure sc-CO_2 at 20 MPa and 318.15 K contained approximately 16% of carotenoids while the extract obtained with the SCM sc-CO_2-methanol and sc-CO_2-ethanol at the same conditions contained approximately 21% and 46%, respectively.

The assumption that the cosolvent causes a local increase in the density of sc-CO_2 and therefore higher solubility was long time assumed in the scientific community, however, as can be seen in Table 5, this assumption would not be valid in this work, since the SCM with the cosolvent (ethanol or methanol), at the same operating conditions, do not exceed the sc-CO_2 density and therefore this could not be the reason for its higher extraction selectivity. Even though how entrainers work, is still under debate, this work supports the hypothesis that entrainers enhance solubility in sc-CO_2 through preferential entrainer – solute interactions, as it has been recently discussed [7,9,11].

Further works show similar results from other raw materials. Casas et al. [38] for example, described the extraction of β-carotene from *Synechococcus* sp. These authors reported that β-carotene was only partially removed with pure CO_2 so its content in the extract did not exceed 0.48 g kg dry microalgae^{-1}. Nevertheless, the use of CO_2 with a volume fraction of 5% ethanol enhanced the extraction of β-carotene,

Table 5
Total oil and carotenoids extraction yield from *Dunaliella salina* at 180 min.

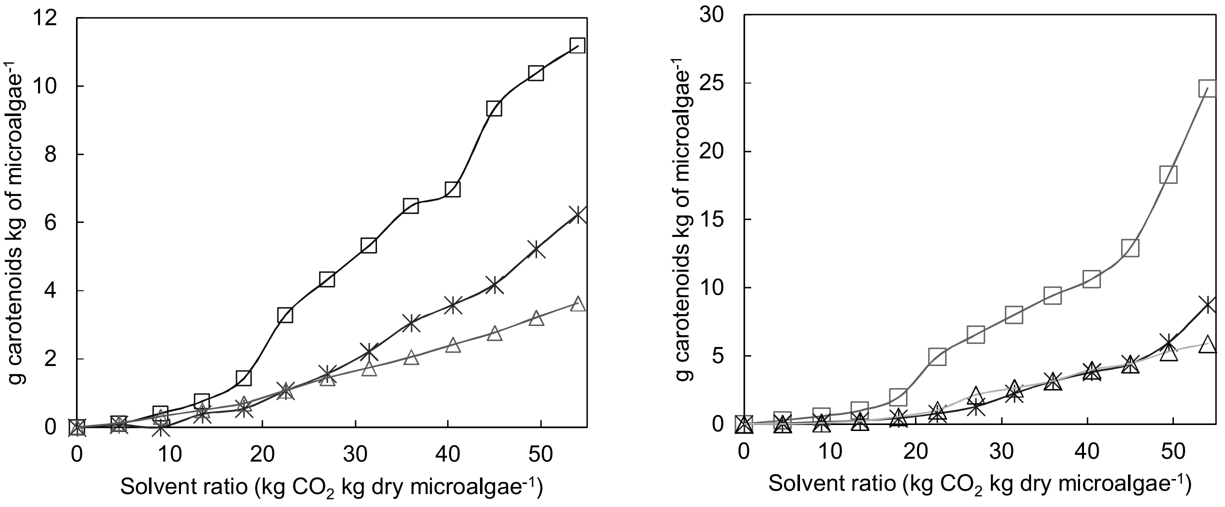
Pressure (MPa)	Temperature (K)	Density [20] (kg m^{-3})	Oil extraction yield (g oil extract kg microalgae^{-1})	Carotenoids extraction yield (g carotenoids kg microalgae^{-1})	Ratio Carotenoids/Oil
sc-CO$_2$					
	308.15	866	33	4	0.12
20	318.15	813	38	6	0.16
	328.15	755	45	12	0.27
	308.15	929	56	15	0.27
30	318.15	890	58	18	0.31
	328.15	850	64	23	0.36
sc-CO$_2$ + 0.05 kg methanol kg solvent^{-1}					
20	308.15	744	40	6	0.15
	318.15	602	42	9	0.21
sc-CO$_2$ + 0.05 kg ethanol kg solvent^{-1}					
20	308.15	739	49	11	0.22
	318.15	593	54	25	0.46

with concentrations higher than 0.70 g kg dry microalgae^{-1} at 40 MPa and 313.15 K. In the same way, Guedes et al. [39] compared the extraction yield of carotenoids from *Scenedesmus obliquus* using pure CO$_2$ and its mixture with 0.077 m^3 m^{-3} ethanol at pressures between 15 MPa and 25 MPa and temperatures from 313.15 K to 333.15 K. The highest content of carotenoids (0.30 g kg dry microalgae^{-1}) in the extract was obtained at the maxima pressure and temperature using ethanol as cosolvent.

The extraction yields notably increased with pressure due to the consequent increase of the solvent density and capacity. However, the impact of temperature was divergent to what was foreseen. R$_a$ values were lower at lower temperatures, suggesting a higher extraction yield in both types of extractions at these conditions with pure CO$_2$ and with its mixture with the alcohols. On the contrary, the carotenoids content in the extract was higher at the highest temperature in all cases. As an example, carotenoids content in the extract obtained using sc-CO$_2$ + 0.05 kg ethanol kg solvent^{-1} at 20 MPa and 318.15 K was 25 g carotenoids kg dry microalgae^{-1}, while at 308.15 K it was 11 g carotenoids kg microalgae^{-1}. This positive impact of temperature on carotenoids extraction yield was initially unexpected since a rise in temperature reduces the density of the supercritical solvent, which reduces its solvent power as reflected in the R$_a$ values. However, temperature positively affects the mass transfer. For instance, temperature increases the solute´s effective diffusivity within the solid and external transportation into the bulk of the supercritical phase and promotes the cell wall to rupture, so the sc-CO$_2$ could then extract more lipids [28] and so, more carotenoids.

Moreover, a rise in temperature increases the solute vapour pressure as expressed in the Antoine equation. At low pressures, the density effect is predominant and therefore the extraction is more effective at low temperature, whereas if the pressure is high, the increase of the vapour pressure with the temperature prevails. Therefore, the isotherms in the pressure-solubility diagrams intersect at a point called the crossover point, which represents the pressure at which the operating temperature increase, benefits the extraction. Sovová et al. [32] found that the crossover point of β-carotene solubility was around 16 MPa. So, at higher pressures, the solubility of this compound both in pure and ethanol-modified CO$_2$ increased when raising temperature from 313.15 K to 333.15 K. The Hansen theory considers neither the increase of the solute volatility with temperature, nor the enhancement of the kinetic which could turn into an inconvenient for the HSP approach when predicting the best conditions for an extraction. Therefore, a contribution of the impact of temperature on solute vapour pressure should be added to the Hansen model for this specific application due to the use of high pressure, e.g. a term with the Antoine equation.

Table 6 shows the reported data on the total extraction yield of oil and carotenoids obtained with sc-CO − CO$_2$ of *D. salina* in operating conditions close to those used in this work. The highest carotenoids



a) 308.15 K

b) 318.15 K

Fig. 3. Supercritical fluid extraction of carotenoids from *Dunaliella salina* at 20 MPa and a) 308.15 K and b) 388.15 K. Pure sc-CO$_2$ (– △–), sc-CO$_2$ + 0.05 kg methanol kg solvent^{-1} (▬ ✳ ▬), sc-CO$_2$ + 0.05 kg ethanol kg solvent^{-1} (▬ ⊡ ▬).

Table 6
Total oil and carotenoids extraction yield by supercritical carbon dioxide extraction from *Dunaliella salina* from several sources.

Pressure (MPa)	Temperature (K)	Solvent ratio (kg CO_2 kg microalgae^{-1})	Oil extraction yield (g oil extract kg microalgae^{-1})	Carotenoids extraction yield (g carotenoids kg microalgae^{-1})	Ratio Carotenoids/Oil	Reference
20	313.15	360	NR	6.43		[40]
	323.15			7.03		
30	313.15			6.30		
	323.15			6.31		
	333.15			14.92		
31	318.35	120	43.8	2.04	0.05	[41]
44	300.65		65.8	4.74	0.07	
30	303.15	97	NR	0.10		[44]
40	328.15			0.12		

NR = not reported.

content found by Macías-Sánchez et al. [40] was almost 15 g carotenoids kg dry microalgae^{-1} at 30 MPa, 333.15 K using 360 kg CO_2 kg dry microalgae^{-1}. Jaime et al. [41] found a maximum carotenoid extraction of 4.74 g carotenoids kg dry microalgae^{-1} at 43.7 MPa, 298.85 K (subcritical conditions) and 120 kg CO_2 kg dry microalgae^{-1}. However, in this work, using a much lower solvent ratio of 54 kg CO_2 kg dry microalgae^{-1}, 23 g carotenoids kg dry microalgae^{-1} were extracted at 30 MPa and 328.15 K. These discrepancies could be related to the initial carotenoid content of the microalgae due to their growth method and to the optimized methodology used in this work. Note that even better extraction yield (25 g of carotenoids kg of dry microalgae^{-1}) was obtained using ethanol as a cosolvent under milder conditions of both pressure (20 MPa) and temperature (318.15 K).

5. Conclusions

The Hansen theory can be used to predict the best cosolvent for supercritical CO_2 in the solubilization of carotenoids within a specific interval of operating conditions. However, its use is limited regarding the prediction of the best conditions to perform the extraction. The effect of pressure is well predicted due to the direct relationship between pressure and density. However, the influence of temperature in the extraction from a solid matrix is complex involving several mechanisms that cannot be predicted with a single approach.

According to the Hansen solubility theory, the best co-solvent for the extraction of carotenoids is ethanol, although these are best solubilized under room conditions in n-hexane. This is due to specific interactions that occur under supercritical conditions. Another interesting prediction is that methanol would be a much worse co-solvent than ethanol despite both being short-chain alcohols.

Experimental data corroborated these predictions. On the one hand, the bubble pressure of β-carotene in the CO_2-ethanol mixture was lower than those obtained in the other supercritical mixtures. On the other hand, the addition of ethanol not only improved the extraction yield but also the selectivity to carotenoids in the supercritical extraction from the microalgae *D. salina*. Thus, with ethanol as cosolvent, at 318.15 K and 20 MPa, the carotenoids extraction yield was more than double (25 g carotenoids kg^{-1} microalgae) than in the mixture sc-CO_2 + methanol (9 g carotenoids kg^{-1} microalgae) and slightly more than four times than in pure sc-CO_2 (6 g carotenoids kg^{-1} microalgae). Moreover, the extract obtained with the CO_2 – 5% ethanol blend, was an oil with a content of 46% carotenoids, which is the highest of those reported in the literature [40–42] for the same microalgae and also those that can be found commercially in a wide variety of products [43].

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