1 New developments in the modelling of carotenoids extraction from microalgae with supercritical CO₂

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9 Abstract

10 Literature data on the extraction of carotenoids from microalgae with supercritical carbon dioxide
11 (scCO₂) were used to describe the process as an extraction of a solution of carotenoids in oil. A
12 mathematical model for oil extraction from microalgae, including also the adsorption on microalgal
13 surfaces, was extended for this purpose. The working hypothesis was that the extraction process is
14 primarily controlled by phase equilibrium where the solubility of carotenoids in scCO₂, as minor
15 components, depends on the solubility of the major component - microalgal triglycerides. The above
16 assumption was supported and confirmed by the results of the modelling of the vapor-liquid phase
17 equilibria of the system carotenoid+oil+scCO₂, which was performed applying the predictive Soave-
18 Redlich-Kwong cubic equation of state. The well-known increase of extraction rate and yield with
19 extraction pressure and temperature was shown to be a result of both increasing carotenoid solubility
20 in scCO₂ and decreasing adsorption capacity of microalga.

21 Keywords: modeling, phase equilibrium, supercritical fluid extraction, carotenoids, oil, microalgae

22
23 1. Introduction

24 Microalgae are unicellular organisms which, through photosynthesis, produce proteins, carbohydrates,
25 lipids, pigments, antioxidants and other important nutrients which cannot be synthesized by humans or

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animals and must be acquired through diet, as well as a wide spectrum of other components of high value products [1].

In recent years the biorefinery concept has been identified as the most promising way for the creation of a biomass-based industry, which employs the full potential of any biomass via maximizing its conversion into high value products. Microalgae are considered to be a futuristic raw material, because they are easy to grow, and arid or low quality agricultural land is required for their cultivation.

Furthermore, they multiply quickly, have much higher biomass productivities in comparison to conventional land plants, quickly adapt to changing, etc. [2]. Those are just few of the advantages that made algae recognized as an alternative, so-called third generation feedstock.

The major pigments of microalgae are chlorophylls, phycobiliproteins, and carotenoids as β -carotene, astaxanthin, and other xanthophylls [3]. The role of carotenoids in algae and plants is to absorb light used in photosynthesis and protect chlorophylls from photodamage [4].

Carotenoids, as derivatives of tetraterpenes, contain 40 carbon atoms in molecule. They form two classes, carotenes like β -carotene that contain no oxygen and xanthophylls like astaxanthin, which contain oxygen. Their yellow, orange or red color is due to their long chain of conjugated double bonds that interacts with light. Carotenoids have many medicinal effects including protection against oxidative damage, and are popular as food supplements.

It has been shown that microalgae, in particular, are a mighty source of natural carotenoids for food industry and medicine [5]. Besides, astaxanthin from microalga *Haematococcus pluvialis* is used as a source of pigments in aquaculture. This compound as well as a mixture of carotenoids from *Chlorella vulgaris* are contained in poultry feed formulations where they serve also as precursors of vitamin A [6,7].

Carotenoids, as well as glycerides, free fatty acids, sterols, etc. belong to lipids that can be extracted from microalgae using non-polar solvents. In contrast to organic solvents leaving potentially toxic residues in extracts, supercritical CO₂ (scCO₂) is characterized by high selectivity, avoidance of thermal damage to labile bioactive compounds, non-toxicity, and pressure- and temperature-tunable properties [8]. An economic and environmental benefit of CO₂ is its easy recycling, however, the capital expenditure on the high-pressure extraction equipment is relatively high [9].

1 The area where scCO₂ has been widely applied to produce carotenoids from microalgae at industrial
2 scale is the extraction of astaxanthin from *H. pluvialis*. Companies specialized in astaxanthin
3 production (Algatechnologies, Fuji Chemical Industry Co., Nutrex Hawaii, Valensa International,
4 AstaMAZ NZ Ltd) conduct the scCO₂ extraction as a part of the whole process. In addition, as demand
5 for CO₂-extracted astaxanthin is steadily increasing, also other *H. pluvialis* producers submit dry algae
6 for scCO₂ extraction, though the high-pressure facility remains expensive compared to an equipment
7 for conventional separation methods [10].

8 In order to efficiently extract carotenoids and other sensitive substances also from other microalgae
9 species, NATECO2 GmbH has built an ultra-high pressure extraction unit operating at pressures up to
10 100 MPa. Within an EU-funded research project The Microalgae Biorefinery [11], a CO₂ extract
11 containing around 30 % carotenoids was produced from *D. salina* by this company. Nevertheless, as
12 stated in the final report of the project, supercritical CO₂ operations are exceptionally expensive to
13 develop and need to be large scale for cost efficiencies.

14 Many researchers focus on optimizing microalgae pretreatment and on determining the best operating
15 conditions of carotenoid extraction with scCO₂. Thus, on the one hand, it has been found that the yield
16 of astaxanthin and its content in the extract increased with increasing temperature and pressure
17 [12,13], which holds also for supercritical fluid extraction (SFE) of carotenoids from other microalgae
18 [14-19]. On the other hand, degradation of carotenoids, which occurs at high temperatures, was
19 occasionally reported already at extraction temperature 60 °C [20].

20 The efficiency of SFE of carotenoids from microalgae was increased when a polar liquid was
21 dissolved in scCO₂, namely ethanol [8,12,21-28] or ethanol-water mixture [29]. Interesting results on
22 SFE of carotenoids from *Dunaliella salina* were reported by Mouahid et al. [30], who observed that
23 water present in microalgae at 23 wt. % enhances the extraction of carotenoids (mainly β -carotene)
24 while excessive drying of algae might provoke their degradation. However, polar modifiers decrease
25 the selectivity of extraction, and thus polar compounds like chlorophylls are co-extracted [6, 31-34].
26 Besides, if separation of a liquid modifier from the extract is required, heat must be applied.

27 Other modifiers examined in the extraction of carotenoids are edible oils. The main advantage of an oil
28 modifier is that because it is present already in the microalgae and is a major component of all scCO₂

extracts, there is no need to separate it subsequently from the product. For example, it has been demonstrated that the effect of soybean oil used as a scCO₂ modifier was either comparable to that of ethanol [35] or slightly lower [36], in dependence on the type of carotenoid extracted.

In view of the above, the purpose of this paper is to develop a mathematical model based on the knowledge accumulated in the literature on scCO₂ extraction of carotenoids from microalgae, and apply it subsequently to model the published data. It is the authors' belief that the novel tool will reveal and explain the dependence of extraction rate and yield on process conditions. It can also help finding the optimum between fixed and variable costs of the process because on the one hand the fixed cost of high pressure equipment increases when it is designed for higher specific pressures, but on the other hand, as the extraction rate and yield also increase with increasing extraction pressure, the necessary batch time is shorter and the variable cost per unit decreases.

2. Preliminary considerations

When the solubility of an extracted substance is known, the analysis of kinetic extraction data begins by comparison of its concentration in scCO₂ with the solubility. The correlation of carotenoid solubility with pressure and temperature required can be obtained from the literature, and where experimental data is missing, a close relation between molecular structure of carotenoids (Fig. 1) and their solubility in scCO₂ can be expected.

Published data on the solubility of carotenes (β -carotene and lycopene) and five xanthophylls in scCO₂ were reviewed by de la Fuente et al. [4]. As the solubility of β -carotene was measured repeatedly, the authors could compare the results for one carotenoid from more than ten different sources, which exhibit large differences. These discrepancies can be attributed not only to the limitations of the different experimental techniques applied but also to differences in the purity of β -carotene, in its state – crystalline or amorphous, and, moreover, to its limited stability. Thermal degradation, oxidizing, and isomerization of β -carotene can substantially affect the results of measurement [4,37]. These factors affect also the solubility data of other carotenoids. Yet, de la Fuente et al. [4], on the basis of analyses of literature data and their own measurements, were able to conclude that though solubilities of

carotenes in scCO₂ are slightly higher than those of xanthophylls, still the differences are very small due to their structural similarity.

This is evidenced also by the consistent set of experimental data on the solubility of astaxanthin (> 98 %), lycopene (95.4 %) and β -carotene (≥ 95 %) in scCO₂ at temperatures 40, 50, and 60 °C shown in Fig. 2. The data was measured using a static method combined with HPLC analysis of saturated solution [4, 38]. In these studies, solubility was correlated with temperature T (K), pressure P (MPa), and CO₂ density ρ (kg m⁻³) using the model of Méndez-Santiago and Teja [39]

$$y = \frac{10^{-6}}{P} \exp\left(\frac{A+B\rho}{T} + C\right) \quad (1)$$

where y is the carotenoid mole fraction in the solution and model parameters A , B , C are listed in Table 1. (The typing error in the original equation for β -carotene solubility has been corrected.)

The effect of oil or its representative triolein on the solubility of β -carotene in scCO₂ was measured using either the dynamic method, where CO₂ was saturated with oil before it flowed to the column containing carotenoid [40], or an equilibrium cell loaded with both carotenoid and triolein [37,38]. When oil was added to CO₂ as co-solvent, the solubility of β -carotene increased only 1.3-1.5 times, as evident from the results of Sovová et al. [40].

The positive effect of oil on the solubility of β -carotene was even higher in the second case, when β -carotene and liquid triolein were equilibrated with the scCO₂ phase. Furthermore, the enhancement of β -carotene solubility in the presence of triolein was increasing with increasing CO₂ density. This observation is confirmed by the change in the exponent of the density in the Chrastil equation used to correlate the solubility with temperature and density, which was determined as $k = 9.7$ for the solubility of pure β -carotene [40], and as $k = 15.73$ for the mixture of β -carotene and triolein [37]. The difference between the vapor phase concentrations of triolein, which was two orders of magnitude higher than the saturated concentration of β -carotene, and the solubility values of pure triolein in scCO₂ was within experimental error [37, 38].

The β -carotene solubility data measured by Araus et al. [38] in an equilibrium cell loaded with 0.6 g of β -carotene and 1 cm³ of triolein correlate well either with the isotherms of triolein or with the oil solubility in scCO₂, as shown in Fig. 3, where the oil solubility w_{oil} was estimated using the equation

1 derived by del Valle et al. [41] for vegetable oils solubility. Evidently, when oil is present in the
2 condensed phase together with a carotenoid, dissolution of both substances in scCO₂ should be
3 considered. While the solubility of the oil is practically unaffected by the presence of a carotenoid, the
4 solubility of the carotenoid, the minor component, depends on the solubility of the oil. Besides, the
5 solubility of a carotenoid mixed with oil increases faster with increasing temperature than the
6 solubility of oil, which holds also for the solubility of these pure substances in scCO₂.

7 Bustamante et al. [8], who performed extraction of astaxanthin from *H. pluvialis*, observed that the
8 solubility of the carotenoid evaluated from the initial slope of the extraction curve was one or two
9 orders of magnitude higher than that extrapolated from the data of de la Fuente et. al. [4] on solubility
10 of free astaxanthin in scCO₂. They assumed that the high solubility values experimentally observed
11 could be explained by the effect of co-extracted microalgae components, mainly triglycerides [8].

12 The relationship between the solubility of β -carotene and that of the oil, displayed in Fig. 3, implicates
13 that carotenoids and oil behave similarly during SFE, which lead us spontaneously to the conclusion
14 that carotenoids are extracted from their solution in the oil. Therefore, the equilibrium between the oil-
15 rich phase and the CO₂ phase should be taken into account and studied. According to this hypothesis,
16 the vapor phase is in contact with a solution of carotenoids and CO₂ in the oil, contained in disrupted
17 microalgal cells. Thus, phenomenological models for the extraction of carotenoids must include also
18 the extraction of oil, or, more precisely, the extraction of triglycerides as major oil components. To test
19 the hypothesis, the liquid-vapor equilibrium in the system consisting of CO₂ and representatives of oil
20 and carotenoids, under conditions of modelled extraction runs, was simulated as outlined concisely in
21 the sections to follow.

23 3. Mathematical models

24 3.1. Model compounds and phase equilibria of the system

25 As discussed briefly previously, the algal oil is a mixture of many compounds among which
26 triglycerides prevail. Hence, in order to simulate the liquid-vapor phase equilibria between the oil-rich
27 and the CO₂ phases, an acceptable approach will be to reduce the size of the modelling task by
28 representing the targeted groups, in our case carotenoids and triglycerides, by model compounds. As a

representative of the carotenoids we have chosen astaxanthin, and triolein – of the triglycerides. The reason behind that is that astaxanthin is a typical carotenoid of microalgae, and triolein - a typical trimer of glycerol and C18 fatty acids, which predominate in algal oil as well as in most vegetable oils. In other words, triolein will exemplify the extracted oil *minus* carotenoids, thus including not only the different triglycerides but also sterols, partial glycerides, and other minor components in the oil.

The general relation describing liquid-vapor equilibrium is:

$$f_i^L = f_i^V \quad (2)$$

where f_i^L and f_i^V are the fugacities of component i in the liquid and vapor phases, respectively.

Fugacities are related as shown:

$$\begin{aligned} f_i^L &= x_i \phi_i^L P \\ f_i^V &= y_i \phi_i^V P \end{aligned} \quad (3)$$

where P is the pressure, and x_i and y_i are mole fractions, and ϕ_i^L and ϕ_i^V are fugacity coefficients of the i -th component in the liquid and vapor phase, respectively.

To calculate the fugacity coefficients, thermodynamic models of the equation of state (EoS) type, flexible enough with regard to composition, are the usual choice. However, EoS models require data on pure components' critical properties, which, for high molar mass triglycerides and/or components like astaxanthin, cannot be determined experimentally. Hence, the critical properties of such compounds should be considered hypothetical and it is necessary to estimate them.

In our case, there are sets of estimated thermophysical parameters of triolein published and those were used by us to demonstrate the influence of the uncertainties in its properties on the quality of the VLE predictions for the system triolein + scCO₂ [40]. For astaxanthin, however, to the best of our knowledge, there are no estimations of its critical properties values, though its solubility in scCO₂ was correlated previously [4]. The reason behind that is that a density-based model which does not require such data was used rather than a rigorous thermodynamic model.

To estimate the critical temperature and pressure of astaxanthin required, we apply the methods suggested in [42, 43], and the values obtained are $T_c = 1200.7$ K; $P_c = 5.5$ bar, respectively. Nevertheless, taking into consideration that there are no other estimations published in the literature to

compare with, it is highly desirable to assess the reliability of our estimation. We do that by applying the generalized semi-theoretical expression advocated by Žbogar et al. [44], which correlates T_c/P_c with the van der Waals surface area. The result shows that the values estimated by us give an acceptable approximation to the theoretical T_c/P_c ratio, namely: $T_c/P_c = 222.73$ (theoretical) vs $T_c/P_c = 218.31$ (ours).

3.2. Model for extraction kinetics

Our model, like other phenomenological models for SFE, consists of a mass balance in particles and in fluid phase, an equation for mass transfer rate, and an equation describing the extraction yield increase. Similarly to the model applied to SFE of microalgal carotenoids by Bustamante et al. [8], an initial faster extraction is described, followed by a slower extraction with monotonously decreasing rate. However, the new developments in our model, in contrast to the previously published one, postulate that: *i)* The rate of carotenoid extraction should be determined *by the extraction of oil*; *ii)* The decrease in the extraction rate is not caused by an exhaustion of extract in the open cells **but** by the exhaustion of free extract that was not adsorbed on the microalgal matrix.

The present model is an extension of a model for SFE of microalgal oil [45], which was based on the following assumptions:

- Intact cell walls are completely impermeable and the available extract is *only* the one from crushed cells. The assumption is justified by the fact that the necessary condition for the extraction of intracellular components as lipids and carotenoids is breakage of the thick cell walls of microalgae [10];
- There is at least partial adsorption of oil on microalgae matrix, and the interface was situated at open cells inside particles formed by agglomerated open and intact cells.

What concerns the mass balance, the partial differential equations, most frequently used in SFE models for extraction column, were replaced by a set of ordinary differential equations written for n ideally mixed stages representing the column, where the number of stages increases with decreasing axial dispersion.

1 The new developments and extensions of the existing model, advocated in the present work, include *i*)
 2 rewriting the mass balance equations to describe the SFE of individual extract components; and *ii*)
 3 introducing a new relationship for carotenoid solubility.

4 The equations for the *i*-th component are:

$$\frac{dY_{i,j}}{dt} + \frac{N}{M} \frac{dX_{i,j}}{dt} + n \frac{Q'}{M} (Y_{i,j} - Y_{i,j-1}) = 0 \text{ for } j=1,2,\dots,n \quad (4)$$

$$\frac{dX_{i,j}}{dt} = -\frac{M}{N} \frac{Y_{i,j}^+ - Y_{i,j}}{t_{MT}} \quad \text{for } j=1,2,\dots,n \quad (5)$$

$$\frac{de_i}{dt} = \frac{Q'}{N} Y_{i,n} \quad (6)$$

5 where $Y_{i,j}$ (g/kg) is the fluid phase concentration, $Y_{i,j}^+$ (g·kg⁻¹) is the equilibrium fluid phase
 6 concentration in contact with oil in open cells, $X_{i,j}$ (g·kg⁻¹) is the concentration in microalgae, e_i (g·kg⁻¹)
 7 is the extraction yield, *j* is the ordinal number of the stage, *N* (kg) is the mass of particles, *M* (kg) is the
 8 mass of CO₂ in the space between particles, Q' (kg/s) is the mass flow rate, and t_{MT} (s) is the
 9 characteristic time of mass transfer resistance consisting of the resistance in particle pores filled with
 10 extract solution in scCO₂ and external mass transfer resistance, both of them decreasing with particle
 11 size. The volume of the pores is assumed to be small enough to neglect the amount of extracted
 12 substances in the pores in comparison to their amount in open cells.

13 The initial and boundary conditions are

$$X_{u,i} = X_{i0} + \frac{M}{N} Y_{i0} ; X_{i,j} = X_{i0} , Y_{i,j} = Y_{i0} \text{ for } j=1,2,\dots,n; e_i = 0 \text{ for } t = 0 \quad (7)$$

$$Y_{i,j-1} = 0 \quad \text{for } j = 0 \quad (8)$$

14 where $X_{u,i}$ (g·kg⁻¹) is the concentration of component *i* in open cells of microalga prepared for the
 15 extraction.

16 In the model for carotenoid extraction, only two pseudocomponents were considered: carotenoids
 17 (CAR) and total extract without carotenoids denoted TG according to its major components
 18 triglycerides. The equilibrium fluid phase concentration of TG is assumed to be independent of the
 19 minor extract component, carotenoids. The equilibrium relationship according to Perrut et al. [46] is
 20 introduced for TG:

$$Y_{TG}^+ = Y_s \text{ for } X_{TG} > X_{AC}, Y_{TG}^+ = KX_{TG} \text{ for } X_{TG} \leq X_{AC} \quad (9)$$

As long as the amount of TG in microalgae is greater than its adsorption capacity X_{AC} , the solubility of TG in scCO₂ is constant and equal to Y_s . Then, the decreasing equilibrium fluid phase concentration of TG desorbed from the microalgae matrix is characterized by the partition coefficient K .

The equilibrium fluid phase concentration of carotenoids depends on the solubility of TG and on the oil composition in the open cells, but since the type of this dependence was not known *a priori*, it had to be determined by comparison of experimental extraction kinetics data with the kinetics modelling results, as outlined below.

Therefore, as a starting point, the relative solubility defined as

$$S = \frac{Y_{CAR}^+ X_{TG}}{Y_{TG}^+ X_{CAR}} \quad (10)$$

was used in the model to calculate the solubility Y_{CAR}^+ , directly proportional to the triolein solubility Y_{TG}^+ and carotenoid-to-triolein ratio in the liquid phase, equal to X_{CAR}/X_{TG} . These quantities vary in the course of extraction run, while S remains constant in this simplified description.

For each stage, the model equations (4)-(6) for the CAR and TG were integrated using the Euler method, with Y_{TG}^+ and Y_{CAR}^+ substituted from eqs. (9), (10) in eq. (5). To avoid numerical oscillations at $X_{TG} = X_{AC}$ where the equilibrium fluid phase concentration decreases stepwise, the mass transfer rate was set equal to zero for $Y_{TG} > Y_{TG}^+$. The yield of extracted oil was calculated as:

$$e_{OIL} = e_{TG} + e_{CAR} \quad (11)$$

Then, the oil and carotenoids extraction curves, calculated from the model, were adjusted to the data from the literature, minimizing the sum of absolute values of differences between the experimental (*exp*) and calculated (*calc*) yields related to maximum experimental yields:

$$\Delta = \left\{ \frac{1}{e_{OIL,exp,m}} \sum_{k=1}^m |e_{OIL,exp,k} - e_{OIL,calc,k}| + \frac{1}{e_{CAR,exp,m}} \sum_{k=1}^m |e_{CAR,exp,k} - e_{CAR,calc,k}| \right\} / 2 \quad (12)$$

where m is the number of experimental points.

As the kinetic modelling offers estimates of S values (eq. 10), which are just numbers valid at very low carotenoid concentrations in oil, a real insight into the phase equilibrium of the TG+CAR+CO₂ system was obtained through modelling its liquid-vapor equilibria, the results of which confirmed that the

SFE of carotenoids from microalgae should be really regarded as extraction of a solution of carotenoids dissolved in oil and *not* as the extraction of solid carotenoids, which, to the best of our knowledge, is the governing assumption made in the literature till present.

4. Results and discussion

4.1. SFE kinetics

The purpose of modelling the kinetics data on the SFE of carotenoids from microalgae was to check whether the new model describes adequately the experimental results available in the literature. The kinetic data measured simultaneously for total extract (oil) and carotenoids were found in the papers published by Mendes et al. [14,47], Machmudah et al. [12], Nobre et al. [27], and Millao and Uquiche [19]. For the purpose of calculations, the graphs, where the extraction yields are plotted against either extraction time or scCO₂ consumption, had to be converted to digital data.

The next sections discuss in some details the dependence of model parameters on operating conditions and microalgae pretreatment. Also, in section 4.1.1. is explained how the contents of extractable solutes in microalgae, representing the initial conditions for the integration of model equations, were adjusted.

4.1.1. Characteristics of microalgae

Mendes et al. [14] extracted 5 g of freeze-dried *Chlorella vulgaris*, either crushed or whole. The content of carotenoids in microalga, 1.33 mg·g⁻¹, was determined after practically complete breaking the cell walls. Canthaxanthin followed by astaxanthin were the major carotenoid components. The content of lipids in microalga determined by extraction with hexane was 185 mg·g⁻¹. C18:1 (41%), C16:0 (22%), and C18:3 (9%) acids prevailed in the fatty acid profile. In our calculations, the contents of extractable pseudocomponents were adjusted to fit the extraction curves. The values $X_{u,CAR} = 0.90$ mg·g⁻¹ and $X_{u,TG} = 124.1$ mg·g⁻¹ for the extraction from crushed alga suggest that one third of cells remained intact. In the modelling of extraction from whole alga, $X_{u,CAR}$ varied between 0.05 and 0.25

1 $\text{mg}\cdot\text{g}^{-1}$ and $X_{u,TG}$ was $51 \text{ mg}\cdot\text{g}^{-1}$. Thus, according to the modelling, the process of lyophilization itself
2 opened about 28 % cells for oil extraction but only 4-19 % of carotenoids was extractable.

3 The kinetics of extraction of freeze-dried *Chlorella vulgaris* was published also in the paper of
4 Mendes et al. [47] for different degrees of crushing. Again, about 2/3 of carotenoids were represented
5 by astaxanthin and canthaxanthin. The contents of oil and carotenoids in microalga were not specified
6 in the paper. According to the SFE model, the $X_{u,TG}$ values were 56, 124, and $131 \text{ mg}\cdot\text{g}^{-1}$ for whole,
7 slightly crushed, and well crushed cells, respectively. The $X_{u,CAR}$ values were under these conditions
8 0.28 , 0.89 and $0.94 \text{ mg}\cdot\text{g}^{-1}$, respectively.

9 Nobre et al. [27] extracted *Nannochloropsis* sp. with both pure CO_2 and CO_2 modified with ethanol.
10 The content of oil in milled microalga, determined by Soxhlet extraction with hexane, was $407 \text{ mg}\cdot\text{g}^{-1}$.
11 Major fatty acids in the extract were C16:1 (37%), C16:0 (35%), and C18:1 (18%). The content of
12 carotenoids determined using three different methods was $0.97 \pm 0.06 \text{ mg}\cdot\text{g}^{-1}$, $1.34 \pm 0.02 \text{ mg}\cdot\text{g}^{-1}$, and
13 $1.47 \pm 0.10 \text{ mg}\cdot\text{g}^{-1}$, the lowest value being unreliable due to the application of high temperature, which
14 might have led to the degradation of carotenoids. The values used in the SFE model, optimized to
15 match kinetic data, were $X_{u,TG} = 343 \text{ mg}\cdot\text{g}^{-1}$ and $X_{u,CAR} = 1.0 \text{ mg}\cdot\text{g}^{-1}$, which suggests that about 16 % of
16 oil and 25-32 % of carotenoids were unavailable for SFE with pure CO_2 . The value of $1 \text{ mg}\cdot\text{g}^{-1}$ also
17 corresponds to the maximal experimental yield of carotenoids obtained by Nobre et al. [27] using the
18 more efficient SFE with modified CO_2 . In the present work, the SFE model is applied only to the
19 extraction with pure CO_2 .

20 Machmudah et al. [12] extracted astaxanthin from powdered *Haematococcus pluvialis*. Again, both
21 pure CO_2 and ethanol-modified CO_2 were tested as solvents, and only the kinetic data obtained with
22 pure CO_2 were modelled in the present work. The content of astaxanthin in microalga was determined
23 by Soxhlet extraction with dichloromethane as $34.3 \text{ mg}\cdot\text{g}^{-1}$, the content of oil was not measured.
24 According to the model, the contents of pseudo-components available for SFE were $X_{u,TG} = 200 \text{ mg}\cdot\text{g}^{-1}$
25 and $X_{u,CAR} = 32 \text{ mg}\cdot\text{g}^{-1}$. The high content of carotenoids in *H. pluvialis*, enabling their fast extraction, is
26 noticeable. Fatty acids C18:1, C18:2, C18:3 and C16:0 prevail in the fatty acid profile of *H. pluvialis*
27 oil [48].

1 Millao and Uquiche [19] extracted oil and carotenoids from the pellets of extruded *Nannochloropsis*
2 *gaditana*. They did not determine separately the contents of extracted substances in alga. According to
3 the SFE model, the extractable amounts were $X_{u,TG} = 165 \text{ mg}\cdot\text{g}^{-1}$ and $X_{u,CAR} = 1.4 \text{ mg}\cdot\text{g}^{-1}$. However, the
4 estimate of $X_{u,CAR}$ is questionable because, when extraction runs were finished, the extraction rate did
5 not yet decrease and indicate thus approaching the asymptotic yield of carotenoids.

6 7 4.1.2. Pressure and temperature effects on extraction yields

8 The effects of operating conditions on the extraction of oil and moreover on the extraction of
9 carotenoids are pronounced, as evident from Figs. 4-7. They are reflected in the SFE model parameters
10 listed in Tables 2, 3. More severe extraction conditions applied (increasing the pressure and, except for
11 the cross-over region at low pressures, also increasing the temperature) lead to an increase of: the
12 solubility of triglycerides in scCO₂, the relative solubility of carotenoids, and the partition coefficient.

13 In contrast, the adsorption capacity of microalga decreases. Thus, the extraction of carotenoids is
14 enhanced not only by its increasing solubility but also by weakening interaction with matrix.

15 *Oil solubility.* As C18 followed by C16 fatty acids prevail in fatty acid profiles of examined
16 microalgae, the solubility of triglycerides adjusted as a model parameter could be compared with the
17 solubility of vegetable oils, w_{oil} , calculated according to del Valle et al. [41]. The Y_s/w_{oil} values in
18 Table 2 indicate similarity of both solubilities. However, the oil solubility determined from the data of
19 Millao and Uquiche (2016) was, on the average, almost twice as high as the solubility of vegetable
20 oils. We suppose therefore that this oil contained a large percentage of more soluble partial glycerides
21 and free fatty acids, perhaps as a result of hydrolysis taking place in pelletized microalgae.

22 23 4.1.3. Flow pattern and effects of microalgae pretreatment

24 *Flow pattern.* Except for one set of the modelled experimental data, the amounts of microalgae in
25 extractors ranged between 5 and 25 g, sufficient to suppress the impact of increased solvent mixing at
26 inlet and outlet areas. As the 1.25 g of microalga extracted by Nobre et al. [27] was mixed with glass
27 beads, this effect was suppressed also in these experiments. The number of stages in the model was
28 therefore in all calculations fixed at 5, which corresponds to plug flow with small axial mixing.

Another type of flow inhomogeneity is, however, demonstrated in the kinetic data obtained by Machmudah et al. [12]. First, the oil solubility values determined from initial slopes of extraction curves were approximately twice lower than those calculated according to del Valle et al. [41], w_{oil} . Second, in the graph of extraction yield against the solvent-to-feed ratio, the shape of extraction curves strongly varied with the flow rate and the apparent solubility was increasing with increasing flow rate. In order to simulate roughly this inhomogeneity, two parallel streams of solvent with different flow rates were assumed, each in one half of the extractor cross-section, and the extraction yield was calculated separately for both halves and then summarized. The solubility was assumed to be equal to w_{oil} and the flow asymmetry was characterized by additional model parameter f_{as} ,

$$Q'_1 = f_{as}Q'/2, Q'_2 = (2 - f_{as})Q'/2 \quad (13)$$

The flow asymmetry was considerable in most of the modelled extraction runs, as indicated in Table 3. Except for one, these extractions were carried out at the flow rate 3 ml/min. Though a part of data published by Machmudah et al. [12] seems to be rather scattered, it represents an important source of information on SFE from microalga because it covers much higher extraction pressures than other published data.

Mass transfer resistance. The characteristic time of mass transfer resistance in experiments published by Nobre et al. [27] was estimated as $t_{MT} = 0.6$ min by fitting the model simultaneously to extraction curves measured for two different flow rates at 30 MPa and 40 °C. The effect of this small resistance on extraction rate was almost negligible. In modelling of the other extraction experiments with milled or crushed microalgae listed in Tables 2 and 3, $t_{MT} = 1$ min was applied with a similarly small effect. As shown in Table 4, the mass transfer resistance was an order of magnitude higher in the extraction from whole microalgae; however, the main effect of milling or crushing was the disruption of microalgae cells, which increased the amount of available extract. The latter is evident in Figure 8, which shows the effect of microalga pretreatment measured by Mendes et al. [47] for three conditions: whole, slightly crushed, and well crushed material; a large increase in the content of available extract, after at least a slight crushing, is evident; While, on the other hand, the change of model parameters from slightly crushed to well crushed cells consists mainly of an increase of mass transfer resistance, possibly because of sticking of fine microalga particles together, and of an almost lost ability of

microalga surface to bound the extract. This suggests that a further research on techniques decreasing the adsorption capacity of microalgae could lead to an increase of SFE efficiency.

4.1.4. Relative solubility of carotenoids

In preliminary calculations of extraction curves, we adjusted two values of parameter S , one for the initial extraction of free solution and the other one for the extraction of oil adsorbed on microalgal matrix. However, it was evident at a very early stage that these values are close to each other; hence, one value of S can be applied for the whole experimental run with only a small decrease in accuracy. Thus, S was maintained constant when modelling an extraction run, which enabled us also to model experiments carried out at mild conditions where all oil was adsorbed on matrix.

As carotenoids are less soluble in scCO₂ than triglycerides, the S values are lower than 1 and increase with more severe extraction conditions because, generally, the selectivity decreases with increasing solubility. When plotted against scCO₂ density, the relative solubility of carotenoids increased with temperature (Fig. 9). The figure combines the modelling results of all the above listed experiments with the exception of those published by Millao and Uquiche [19], where the S values were relatively low at 60 °C. It is our belief that the latter is a result of the substitution of triglycerides by partial glycerides and/or free fatty acids and, hence, the carotenoids solubility would be different. It is evident from the figure why high extraction temperatures and pressures should be applied to extract carotenoids from microalgae effectively with pure CO₂, unless a high carotenoid-to-oil ratio in alga, like in *H. pluvialis*, compensates for low value of S . However, there is a danger of carotenoid degradation at high temperatures, as evident from at least one too small value of S at 70 °C (at CO₂ density 905 kg/m³).

4.2. VLE of the system TAG+CAR+CO₂

To model the liquid-vapor phase behavior of the (triolein+astaxanthin+CO₂) system we have chosen the predictive Soave-Redlich-Kwong (PSRK) cubic EoS [49]. In PSRK EoS a dimensionless quantity α , defined as $\alpha = a/bRT$ is introduced, which allows replacement of the classical van der Waals

mixing rule for the cohesion term, and thus determination of a set of binary interaction parameters is avoided:

$$\alpha = \sum_{i=1}^{N_c} x_i \cdot \alpha_i + \frac{1}{A} \left(\frac{g^E}{RT} + \sum_{i=1}^{N_c} x_i \ln \frac{b_m}{b_i} \right) \quad (14)$$

where A is equal to -0.64663.

The excess energy term is calculated from the UNIFAC model applying the temperature dependent parameters, fitted directly for the PSRK [50]. The calculation of the alpha term is performed either using the form with Mathias-Copeman parameters [51], when information on the vapor pressure of the constituent compounds is available, or instead, as in our case, the generalized form with the acentric factors.

Once the values of triolein and astaxanthin critical parameters were estimated and known, the equilibrium concentrations of the two model compounds in the liquid and vapor phases, at a given temperature and pressure, were determined by solving the system of non-linear equations given by Eq. 3.

The calculations were carried out for pairs of mixture composition as shown in Table 5: the first one at the beginning of an extraction run and the second one at the end of free oil extraction, when the oil concentration in microalga, X_{TG} , approaches the adsorption capacity, X_{AC} . The good agreement between the equilibrium fluid phase concentrations, calculated using PSRK, and the model for SFE kinetics is illustrated by Figs. 10, 11 for extractions from microalgae differing in their content of carotenoids. Mass fractions y_{TG}^+ and y_{CAR}^+ from Table 5, converted to $\text{mg g}(\text{CO}_2)^{-1}$ units, are practically equal to Y_{TG}^+ and Y_{CAR}^+ , respectively. The only exception was at 80 °C and 55 MPa, where astaxanthin solubility in the end of free oil extraction (see last line of Table 5) increased to 6.27 $\text{mg g}(\text{CO}_2)^{-1}$ according to the results of PSRK calculations, while maintaining a constant S value in the model for SFE leads to underestimated astaxanthin solubility (93%). Evidently, the use of the SFE model with S values independent of the changes in liquid phase composition, as presented in section 3.2, should not be extended to higher astaxanthin concentrations.

5. Conclusions

In this work, the hypothesis on the extraction of carotenoids from microalgae with supercritical CO₂ as an extraction from their solution in microalgal oil was proposed and proved by modelling literature data. The relative carotenoids solubility used in the kinetic model for supercritical fluid extraction is consistent and agrees well with the results of the vapor-liquid equilibrium calculations for the system where carotenoids and oil are represented by astaxanthin and triolein, respectively.

The results of modelling the kinetics data from different literature sources show how microalgae pretreatment and extraction conditions are reflected in model parameters. The extraction is found to be controlled primarily by phase equilibrium, which is, in a later phase of the process, expressed in terms of the Henry's adsorption isotherm.

The extraction can be facilitated by higher extraction pressures and temperatures and higher carotenoids-to-oil ratios in microalga. The well-known positive effect of addition of a modifier were not examined in this study, however, the results of modelling suggest that a similar extraction efficiency could be achieved in the extraction with pure CO₂ if the adsorption capacity of microalga is decreased by an appropriate pretreatment.

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Nomenclature

A, B, C parameters in the model of Méndez-Santiago and Teja

b_i - volume parameter of component i , dm³·mol⁻¹

e extraction yield, g·kg⁻¹

f fugacity, MPa

f_{as} parameter of flow asymmetry, -

g^E excess Gibbs energy, J·mol⁻¹

1	K	partition coefficient, $\text{kg}(\text{microalga}) \cdot \text{kg}(\text{CO}_2)^{-1}$
2	m	number of experimental points, -
3	M	CO_2 in the space between particles, kg
4	n	number of ideally mixed stages, -
5	N	feed of microalga, kg
6	N_c	number of components, -
7	P	pressure, MPa
8	Q'	mass flow rate, $\text{kg} \cdot \text{s}^{-1}$
9	R	gas constant, $\text{J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$
10	S	relative solubility of carotenoids in scCO_2 , -
11	t	time, s
12	t_{MT}	characteristic time of mass transfer resistance, s
13	T	temperature, K
14	w_{oil}	vegetable oil solubility in scCO_2 according to [41], $\text{g} \cdot \text{kg}^{-1}$
15	x	concentration in the liquid phase, mole fraction
16	x^+	equilibrium liquid phase composition calculated using PSRK EoS, mass fraction
17	X	concentration of extractable solutes in microalga, $\text{g} \cdot \text{kg}^{-1}$
18	X_{AC}	adsorption capacity of microalga, $\text{g} \cdot \text{kg}^{-1}$
19	X_u	content of extractable solutes in pre-treated microalga, $\text{g} \cdot \text{kg}^{-1}$
20	y	concentration in the fluid phase, mole fraction
21	y^+	equilibrium vapor phase composition calculated using PSRK EoS, mass fraction
22	Y	concentration in the fluid phase, $\text{g} \cdot \text{kg}^{-1}$
23	Y^+	equilibrium fluid phase concentration at interface with oil in open cells, $\text{g} \cdot \text{kg}^{-1}$
24	Y_s	solubility of TG in scCO_2 , $\text{g} \cdot \text{kg}^{-1}$
25	Greek letters	
26	α	– a dimensionless quantity in eq. (14)
27	α_i	– attractive parameter of component i , $\text{dm}^6 \cdot \text{bar} \cdot \text{mol}^{-2}$
28	Δ	curve fitting objective function, -

1	φ	fugacity coefficient, -
2	ρ	CO ₂ density, kg m ⁻³
3	Superscripts	
4	L	liquid phase
5	V	vapor phase
6	Subscripts	
7	<i>c</i>	critical point
8	<i>calc</i>	calculated
9	<i>CAR</i>	carotenoids
10	<i>exp</i>	experimental
11	<i>i</i>	component
12	<i>j</i>	stage in the model for SFE
13	<i>m</i>	corresponding to a mixture
14	<i>OIL</i>	oil
15	<i>TG</i>	oil without carotenoids

16

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1 Figure captions

2 Figure 1. Molecular structure of selected carotenoids.

3 Figure 2. Pressure and temperature dependence of the solubility of ◆ astaxanthin [4], ■ lycopene [4],
4 and ▲ β -carotene [38] in scCO_2 .

5 Figure 3. Isotherms of triolein-enhanced solubility of β -carotene in scCO_2 at temperatures ◆ 40 °C, ■
6 50 °C, and ▲ 60 °C and different pressures [38] in dependence on the solubility of vegetable oil in
7 scCO_2 , w_{oil} , at identical conditions.

8 Figure 4. Modelling of extraction yields published by Mendes et al. [14]. Experimental data: ◆ 20
9 MPa, 40 °C; ▲ 20 MPa, 55 °C; ■ 35 MPa, 40 °C; + 35 MPa, 55 °C. — SFE model with parameters
10 listed in Table 2.

11 Figure 5. Modelling of extraction yields published by Nobre et al. [27]. Experimental data: ◆ 12.5
12 MPa, 40 °C; ▲ 20 MPa, 40 °C; ■ 30 MPa, 40 °C. — SFE model with parameters listed in Table 2.

13 Figure 6. Modelling of extraction yields published by Millao and Uquiche[19]. Experimental data: ◆
14 31.7 MPa, 40 °C; ■ 38.1 MPa, 40 °C; ▲ 46.4 MPa, 60 °C; ✕ 54.3 MPa, 60 °C. — SFE model with
15 parameters listed in Table 2.

16 Fig. 7. Modelling of extraction yields published by Machmudah et al. [12]. Experimental data: ◆ 55
17 MPa, 40 °C; ■ 30 MPa, 70 °C; ▲ 55 MPa, 60 °C; ✕ 55 MPa, 80 °C. — SFE model with parameters
18 listed in Table 3.

19 Figure 8. The effect of microalgae crushing according to Mendes et al. [47]. Experimental data at 35
20 MPa and 55 °C: ◆ whole alga; ■ slightly crushed alga, ▲ well crushed alga. — SFE model with
21 parameters listed in Tables 2, 4.

22 Figure 9. Pressure and temperature dependence of S , relative solubility of carotenoids extracted with
23 scCO_2 from microalgae (combined results based on different literature data).

24 Figure 10. Evolution of equilibrium fluid phase concentrations at 40 °C and 35 MPa in the experiment
25 of Mendes et al. [14]. Thermodynamic model: ▲ triglycerides, ■ carotenoids. Model for extraction
26 kinetics: — triglycerides, --- carotenoids.

1 Fig. 11. Evolution of equilibrium fluid phase concentrations at 60 °C and 55 MPa in the experiment of
2 Machmudah et al. [12]. Thermodynamic model: ▲ triglycerides, ■ carotenoids. Model for extraction
3 kinetics: — triglycerides, --- carotenoids.

4

5

Figure 1. Molecular structure of selected carotenoids.

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Figure 3. Isotherms of triolein-enhanced solubility of β -carotene in $scCO_2$ at temperatures $\blacklozenge$ 40 °C, $\blacksquare$ 50 °C, and $\blacktriangle$ 60 °C and different pressures [38] in dependence on the solubility of vegetable oil in $scCO_2$, w_{oil} , at identical conditions.

Figure 4. Modelling of extraction yields published by Mendes et al. [14]. Experimental data: $\blacklozenge$ 20 MPa, 40 °C; $\blacktriangle$ 20 MPa, 55 °C; $\blacksquare$ 35 MPa, 40 °C; $+$ 35 MPa, 55 °C. — SFE model with parameters listed in Table 2.

Figure 5. Modelling of extraction yields published by Nobre et al. [27]. Experimental data: ◆ 12.5 MPa, 40 °C; ▲ 20 MPa, 40 °C; ■ 30 MPa, 40 °C. — SFE model with parameters listed in Table 2.

Figure 6. Modelling of extraction yields published by Millao and Uquiche[19]. Experimental data: ◆ 31.7 MPa, 40 °C; ■ 38.1 MPa, 40 °C; ▲ 46.4 MPa, 60 °C; × 54.3 MPa, 60 °C. — SFE model with parameters listed in Table 2.

Fig. 7. Modelling of extraction yields published by Machmudah et al. [12]. Experimental data: ◆ 55 MPa, 40 °C; ■ 30 MPa, 70 °C; ▲ 55 MPa, 60 °C; × 55 MPa, 80 °C. — SFE model with parameters listed in Table 3.

Figure 8. The effect of microalgae crushing according to Mendes et al. [47]. Experimental data at 35 MPa and 55 °C: ◆ whole alga; ■ slightly crushed alga, ▲ well crushed alga. — SFE model with parameters listed in Tables 2, 4.

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3 Figure 9. Pressure and temperature dependence of S_r , relative solubility of carotenoids extracted with
 4 scCO₂ from microalgae (combined results based on different literature data).

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7 Figure 10. Evolution of equilibrium fluid phase concentrations at 40 °C and 35 MPa in the experiment
 8 of Mendes et al. [14]. Thermodynamic model: ▲ triglycerides, ■ carotenoids. Model for extraction
 9 kinetics: — triglycerides, - - - carotenoids.

Fig. 11. Evolution of equilibrium fluid phase concentrations at 60 °C and 55 MPa in the experiment of Machmudah et al. [12]. Thermodynamic model: ▲ triglycerides, ■ carotenoids. Model for extraction kinetics: — triglycerides, --- carotenoids.

1 Appendix

2 Example of the calculation of extraction yields using the model for extraction kinetics

3

4 General model equations (4)-(8) were re-written for the extraction of two components, TG and CAR.

5 Then, their mass balance equations are:

$$\frac{dY_{TG,j}}{dt} + \frac{N}{M} \frac{dX_{TG,j}}{dt} + n \frac{Q'}{M} (Y_{TG,j} - Y_{TG,j-1}) = 0 \quad \text{for } j=1,2,\dots,n \quad (\text{A1})$$

$$\frac{dX_{TG,j}}{dt} = -\frac{M}{N} \frac{Y_{TG,j}^+ - Y_{TG,j}}{t_{MT}} \text{ if } Y_{TG,j}^+ > Y_{TG,j}, \text{ otherwise } \frac{dX_{TG,j}}{dt} = 0 \quad \text{for } j=1,2,\dots,n \quad (\text{A2})$$

$$\frac{de_{TG}}{dt} = \frac{Q'}{N} Y_{TG,n} \quad (\text{A3})$$

$$\frac{dY_{CAR,j}}{dt} + \frac{N}{M} \frac{dX_{CAR,j}}{dt} + n \frac{Q'}{M} (Y_{CAR,j} - Y_{CAR,j-1}) = 0 \quad \text{for } j=1,2,\dots,n \quad (\text{A4})$$

$$\frac{dX_{CAR,j}}{dt} = -\frac{M}{N} \frac{Y_{CAR,j}^+ - Y_{CAR,j}}{t_{MT}} \quad \text{for } j=1,2,\dots,n \quad (\text{A5})$$

$$\frac{de_{CAR}}{dt} = \frac{Q'}{N} Y_{CAR,n} \quad (\text{A6})$$

6 with boundary conditions:

$$Y_{TG,j-1} = Y_{CAR,j-1} = 0 \text{ for } j = 0 \quad (\text{A7})$$

7 The initial conditions correspond to the mass balance in the extractor at the start of the solvent flow:

$$X_{u,TG} = X_{TG0} + \frac{M}{N} Y_{TG0}, Y_{TG,j} = Y_{TG0}, X_{TG,j} = X_{TG0} \text{ for } j=1,2,\dots,n, e_{TG} = 0 \text{ for } t = 0 \quad (\text{A8})$$

$$X_{u,CAR} = X_{CAR0} + \frac{M}{N} Y_{CAR0}, Y_{CAR,j} = Y_{CAR0}, X_{CAR,j} = X_{CAR0} \text{ for } j=1,2,\dots,n, e_{CAR} = 0 \text{ for } t = 0 \quad (\text{A9})$$

8 Equilibrium concentrations:

$$Y_{TG,j}^+ = Y_s \text{ for } X_{TG,j} > X_{AC}, Y_{TG,j}^+ = KX_{TG,j} \text{ for } X_{TG,j} \leq X_{AC}, \text{ for } j = 1,2,\dots,n \quad (\text{A10})$$

$$Y_{CAR,j}^+ = SY_{TG,j}^+ \frac{X_{CAR,j}}{X_{TG,j}} \text{ for } j = 1,2,\dots,n \quad (\text{A11})$$

9 Total oil yield is equal to the sum of the yields of CAR and TG, see eq. (11), and the curve fitting

10 objective function is calculated according to eq. (12).

11 The calculation is demonstrated on the data on the SFE from freeze-dried *Chlorella vulgaris* at 35

12 MPa a 55 °C published by Mendes et al. [14]. The model input data for the extraction from crushed

13 alga are listed in Table A1.

Table A1. Input data for modeling the SFE from crushed alga at 35 MPa and 55 °C [14]

Experimental characteristics		Experimental yields		
N , g (feed)	5	t , min	e_{OIL} , g·kg ⁻¹	e_{CAR} , g·kg ⁻¹
M , g (interparticle CO ₂)	4	25	42.1	0.041
Q' , g·min ⁻¹ (flow rate)	0.78	78	80.5	0.164
		130	102.1	0.268
Extractables in alga, g·kg ⁻¹		187	108.9	0.327
$X_{u,TG}$	124.1	262	118.1	0.423
$X_{u,CAR}$	0.90	337	123.4	0.486
Initial concentrations, g·kg ⁻¹		Estimated model parameters		
X_{TG0}	115.1	n (number of stages)	5	
Y_{TG0}	11.2	t_{TM} , min (mass transfer resistance)	1	
X_{CAR0}	0.884	Y_S , g·kg ⁻¹ (TG solubility in scCO ₂)	11.2	
Y_{CAR0}	0.020	X_{AC} , g·kg ⁻¹ (adsorption capacity)	60	
		K , g·g ⁻¹ (partition coefficient of TG)	0.05	
		S (rel. solubility of CAR in scCO ₂)	0.23	

The feed of alga is known, the mass of inter-particle CO₂ was estimated, and the volumetric flow rate was converted to mass flow rate using the density of CO₂ at standard gas conditions 1.95 g·kg⁻¹. The experimental yields were read from graphs in [14]. The number of stages in the model was adjusted to 5, which corresponds to the plug flow with a medium axial dispersion. The characteristic time of mass transfer, t_{MT} , was estimated as 1 min, slightly more than the value of t_{MT} determined from experiments carried out with another freeze-dried and ground alga at two different flow rates [27]. The content of TG in alga, available from crushed cells, could be roughly estimated as the asymptote of extraction yield; it was then fine-tuned to satisfy the model for all extraction runs from crushed alga shown in the

paper. A similar procedure was performed to estimate extractable CAR. As the solutes dissolve in scCO₂ already before the flow out of the extractor begins, their fluid phase concentration at $t = 0$ is in the range from zero to equilibrium concentration. Due to the low mass transfer resistance, equilibrium was assumed to be established at $t = 0$ and the initial concentrations X_{TG0} , Y_{TG0} , X_{CAR0} , and Y_{CAR0} , were calculated using equations (A8), (A9), and equilibrium relationships with equilibrium parameters Y_s , X_{AC} , K , S obtained by fitting the calculated extraction curves to experimental yields. The integration of mass balance equations was carried out using the Euler's method with integration step 0.5 min. Table A2 shows the calculated concentrations in the first and last (fifth) model stage, and calculated extraction yields at the sampling times.

Table A2. Selected results of modeling the SFE from crushed *C. vulgaris* at 35 MPa and 55 °C [14]

t , min	$X_{TG,1}$ mg·g ⁻¹	$Y_{TG,1}$ mg·g ⁻¹	$X_{TG,5}$ mg·g ⁻¹	$Y_{TG,5}$ mg·g ⁻¹	$X_{CAR,1}$ mg·g ⁻¹	$Y_{CAR,1}$ mg·g ⁻¹	$X_{CAR,5}$ mg·g ⁻¹	$Y_{CAR,5}$ mg·g ⁻¹	e_{OIL} mg·g ⁻¹	e_{CAR} mg·g ⁻¹
25	46.64	1.193	106.6	10.11	0.717	0.0042	0.880	0.0204	42.87	0.080
78	16.68	0.427	52.66	2.296	0.565	0.0033	0.782	0.0084	86.11	0.194
130	5.911	0.151	37.58	1.527	0.445	0.0026	0.755	0.0080	101.9	0.262
187	1.942	0.050	23.23	0.891	0.344	0.0020	0.721	0.0075	112.5	0.330
262	0.446	0.011	10.76	0.390	0.245	0.0014	0.666	0.0067	119.7	0.413
337	0.102	0.003	4.472	0.155	0.174	0.0010	0.607	0.0060	122.8	0.487

Since, the equilibrium parameters at 35 MPa and 55 °C were now known, the modeling of another experiment carried out under identical extraction conditions but with whole alga, as described in [14], was possible. To achieve the lower experimental extraction yields from whole alga, lower contents of extractable solutes in alga and substantially higher mass transfer resistance were used in the model. Their values obtained by minimizing the curve fitting objective function were $t_{MT} = 8$ min, $X_{u,TG} = 51$ g·kg⁻¹, and $X_{u,CAR} = 0.25$ g·kg⁻¹. (The content of TG was determined to satisfy all experimental data concerning the extraction from whole alga, reported in [14].) Due to the high mass

transfer resistance, no dissolution was assumed before $t = 0$ and therefore the modelled initial concentrations were $X_{TG0} = X_{u,TG}$, $X_{CAR0} = X_{u,CAR}$, and $Y_{TG0} = Y_{CAR0} = 0$, respectively. Also the adsorption capacity of alga decreased to $X_{AC} = 19.2 \text{ g}\cdot\text{kg}^{-1}$. The other input data listed in Table A1 were not changed.

As evident from Table A3, the agreement between the experimental and calculated extraction yields is good except for the second sampling point. Table 4, showing the calculated concentrations in the first and last model stages, indicates a relatively flat solid phase concentration profile along the extractor height, typical for an extraction controlled by mass transfer resistance.

Table A3. Experimental and calculated extraction yields from whole *C. vulgaris* at 35 MPa and 55 °C [14]

t	$e_{OIL,exp}$	$e_{OIL,calc}$	$e_{OIL,calc} - e_{OIL,calc}$	$e_{CAR,exp} \times 10^2$	$e_{CAR,calc}$	$e_{OIL,calc} - e_{OIL,calc}$
min	$\text{mg}\cdot\text{g}^{-1}$	$\text{mg}\cdot\text{g}^{-1}$	$\text{mg}\cdot\text{g}^{-1}$	$\text{mg}\cdot\text{g}^{-1}$	$\text{mg}\cdot\text{g}^{-1}$	$\text{mg}\cdot\text{g}^{-1}$
24	15.5	16.7	1.2	2.22	2.17	-0.05
68	29.1	34.0	4.9	4.44	5.49	1.05
111	38.2	36.8	-1.4	6.26	6.27	0.01
180	40.5	40.4	-0.1	7.47	7.45	-0.02
257	44.2	43.3	-0.9	8.15	8.67	0.52
353	46.5	45.9	-0.6	-	10.1	-
399	48.3	46.8	-1.5	-	10.7	-

Table A4. Selected results of modeling the SFE from whole *C. vulgaris* at 35 MPa and 55 °C [14]

t	$X_{TG,1}$	$Y_{TG,1}$	$X_{TG,5}$	$Y_{TG,5}$	$X_{CAR,1}$	$Y_{CAR,1} \times 10^2$	$X_{CAR,5}$	$Y_{CAR,5} \times 10^2$
min	$\text{mg}\cdot\text{g}^{-1}$	$\text{mg}\cdot\text{g}^{-1}$	$\text{mg}\cdot\text{g}^{-1}$	$\text{mg}\cdot\text{g}^{-1}$	$\text{mg}\cdot\text{g}^{-1}$	$\text{mg}\cdot\text{g}^{-1}$	$\text{mg}\cdot\text{g}^{-1}$	$\text{mg}\cdot\text{g}^{-1}$
24	27.51	1.274	35.60	5.077	0.216	0.225	0.229	0.775
68	15.80	0.105	17.73	0.451	0.190	0.029	0.196	0.118

111	12.65	0.084	15.37	0.379	0.181	0.028	0.190	0.113
180	8.860	0.059	12.16	0.287	0.167	0.025	0.181	0.106
257	5.980	0.040	9.305	0.210	0.152	0.023	0.171	0.099
353	3.647	0.024	6.579	0.141	0.136	0.021	0.159	0.090
399	2.870	0.019	5.541	0.116	0.129	0.020	0.153	0.087

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