Supercritical Carbon Dioxide Extraction of *Dimorphotheca pluvialis* Oil Seeds

F. Petrus Cuperus*, Gerard Boswinkel, Bart G. Muuse, and Johannes T.P. Derksen
Agrotechnological Research Institute (ATO-DLO), P.O. Box 17, NL-6700 AA Wageningen, The Netherlands

ABSTRACT: The possibility of extraction of *Dimorphotheca* oil with supercritical carbon dioxide is demonstrated in this article. Before extraction, the seeds have to be pretreated to improve extraction yield. Experiments showed that the best pretreatment procedure for *Dimorphotheca* was heating the seeds under reduced pressure to 100°C for 60 min, followed by flaking or milling. To give an impression about the efficiency of the supercritical extraction, a mathematical model has been developed to estimate the overall mass transfer coefficient (A_pK). Also, an empirical relation between A_pK and the interstitial velocity has been found. The physical properties of the supercritical *Dimorphotheca* oil are in good agreement with those of conventionally extracted oil, except for a lower phospholipid content. It is expected that further refining of supercritical *Dimorphotheca* oil will be marginal.

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KEY WORDS: Carbon dioxide, *Dimorphotheca* oil, extraction, mass transfer coefficient, oil quality, seed pretreatment, supercritical.

The oil of *Dimorphotheca pluvialis* seeds (DMO) contains the optically active hydroxy fatty acid *S*(+) 9-hydroxy-*trans*-*trans*-10,12 octadecadienoic acid, or β-dimorphecolic acid (1,2). This highly reactive hydroxydiene-containing moiety may be an interesting synthon for the oleochemical industry (3,4). Its chiral center, combined with two conjugated double bonds, offers versatile reactivity for the synthesis of high-added value compounds. Compared to other hydroxy fatty acids from, e.g., *Ricinus* (Castor) (ricinoleic acid) or *Lesquerella* oil (lesquerolic acid), dimorphecolic acid is more reactive. Therefore, the recovery of DMO and its major fatty acid should be executed at mild conditions and with great care (4,5).

During extraction of oil from seeds, oil has to diffuse from the small oil bodies in the seed to the outer surface. To avoid mass transfer problems during extraction, the use of nascent seeds is normally prohibiting, and pretreatments are used to macerate the seed hulls and cell walls. Additionally, thermal treatments are used to prevent enzymatic deterioration of oils during extraction. The extraction of *D. pluvialis* seeds is not yet practiced commercially at any scale. Nevertheless, from preliminary experiments, conventional pretreatments appear to be crucial for oil quality (4). Hence, for efficient extraction, current industrial pretreatment procedures should be adapted.

To complicate matters further, *D. pluvialis* produces two types of seeds: cone- and wing-shaped seeds (referred to as cones and wings), which contain approximately 20 and 10 wt% of DMO, respectively.

To facilitate the introduction of DMO in agricultural and industrial practice, several oil production techniques have been evaluated (6). So far, extraction with supercritical carbon dioxide (SC-CO_2) appears to offer the best possibilities for the extraction of DMO with intact dimorphecolic acid and relatively few contaminants, such as gums and pigments.

In this paper, we are reporting our investigations on the extraction of oil from *Dimorphotheca* seeds with SC-CO_2. In addition, a mathematical model is presented to estimate the overall mass transfer coefficients by matching the calculated and experimental extraction curves.

EXTRACTION THEORY

For optimized pretreatments, it is generally assumed that mass transfer from the seed to the bulk extraction phase is rate-determining. For this situation, a model (7,8) was evaluated, and specific characteristics for *Dimorphotheca* seed extraction by SC-CO_2 in a fixed bed were determined. In principle, these parameters can be directly used for scale-up of the process.

The solvent's transport through the extractor bed is assumed to be in plug-flow mode. For the mathematical treatment, the solvent in the extractor bed is divided into small segments in which the concentration of oil in a solvent element is constant. The mass balance over a bed segment with height δh yields:

$$\frac{\partial}{\partial t}(A\,\delta h\,\varepsilon\rho y) = \rho U A\,\frac{\partial y}{\partial h}\,\delta h + A_p A\,\delta h\,K(y^* - y) \qquad [1]$$

It is assumed that the axial dispersion of the oil is negligible and that an overall mass transfer coefficient can be used. When solvent density and solvent flow are assumed to be

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*To whom correspondence should be addressed at Agrotechnological Research Institute (ATO-DLO), P.O. Box 17, NL-6700 AA Wageningen, The Netherlands.

constant, Equation 1 turns into Equation 2:

$$\varepsilon\rho \frac{\partial y}{\partial t} = -\rho U \frac{\partial Y}{\partial h} + A_p K(y^* - y) \qquad [2]$$

A mass balance over the seed bed yields

$$(1 - \varepsilon)\rho_s \frac{\partial x}{\partial t} = -A_p K\,(y^* - y) \qquad [3]$$

Boundary conditions for Equations 2 and 3 are

$$t = 0 \qquad 0 < h < \mathrm{H} \qquad x = x_0$$

$$t > 0 \qquad h = 0 \qquad y = 0$$

Basic equations (2 and 3) for the extraction can only be solved when the equilibrium relation between oil in the seed and oil in the solvent is known. It can be determined *via* model experiments. When it is assumed that, for a given temperature and pressure, $y^* = f(x)$, the only unknown operational parameters in Equations 2 and 3 are A_p and K. Together, A_pK, they are the crucial parameters that determine mass transfer rate in a leaching bed, usually called "volumetric mass transfer parameter." This value is of great importance for the scale-up of this extraction process. The only factors that influence the value of A_pK are the velocity of the supercritical gas through the extractor bed and the size and shape of the seed particles. In the equations the nomenclature is A_p: specific area of seeds (m^2/m^3 of bed); A_pK: overall volumetric mass transfer coefficient (g CO$_2$/m^{3}s); H; h: (total) height of bed (mm); t: time (s); v: superficial velocity (mm/s); x: oil concentration in seeds (kg oil/kg oil free seeds); x_0: initial oil content of seeds (kg oil/kg oil free seeds); y^*: saturation oil concentration (kg oil/kg CO$_2$); y: oil concentration (kg oil/kg CO$_2$); ε: void fraction in the bed (–); ρ: density of solvent phase (kg/m^3); ρ_s: density of seeds (kg/m^3).

EXPERIMENTAL PROCEDURES

The effect of pretreatment on mass transfer was studied by using different heat, flaking, and milling treatments. Heat treatments were done in an oven under reduced pressure below 1 mbar. Flakes were made in a flaking apparatus with the shafts calibrated at 0.25 mm, and milling was done in an edge runner mill for 5 min.

Pretreated seeds were extracted in a SITEC (Maurizurich, Switzerland) extraction apparatus (Fig. 1) with two condensors. The CO$_2$ flow was varied between 4 and 12 kg/h. The extraction temperature was 45°C, extraction pressure 300 bar. During seed extraction, CO$_2$ was recycled and reused. To determine the solubility of *Dimorphotheca* oil, SC-CO$_2$ was led single-pass with a slow CO$_2$ flow of 1 kg/h through a bed of carborundum beads that were impregnated with *Dimorphotheca* oil. Equilibrium experiments were done in the temperature and pressure intervals of 25–55°C and 200–300 bar, respectively.

RESULTS AND DISCUSSION

From preliminary experiments, it became clear that *Dimorphotheca* seed needed pretreatment to avoid mass transfer problems during extraction. Because the dimension of the wings does not permit seed-flaking, a milling procedure was considered. Eventually, a toasting step (100°C) under reduced pressure, followed by a flaking (cones) or a milling (wings) procedure, was selected. Higher temperatures led to enhanced degradation, while lower temperatures were not sufficient to inactivate the enzymes present in the seeds. The aim of toasting the seeds is, on the one hand, to rupture the cells of the seeds and to make the oil particles available for extraction. Hence, the extraction rate will be improved. On the other hand, the toasting step is used to inactivate any enzyme activity present in the seeds. However, when seeds were pretreated

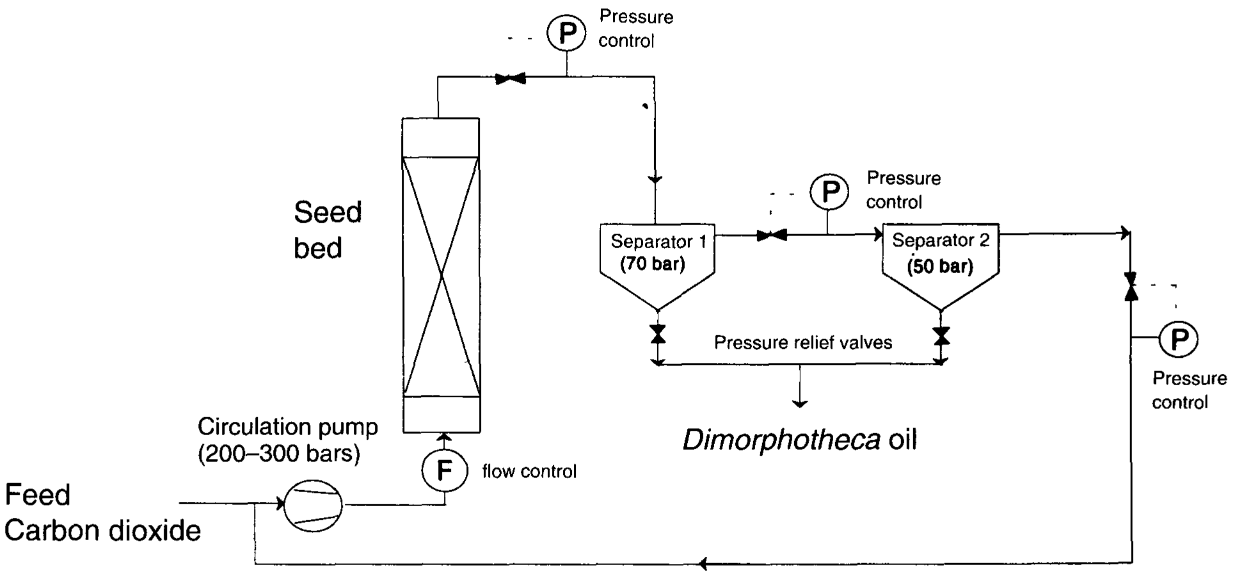

FIG. 1. Schematic of the SITEC apparatus (Maurizurich, Switzerland) used. The pressure was relieved in two stages; in stage 2, a liquid CO$_2$ level was present.

in the described way and stored afterward at room temperature for some weeks, it was observed that during extraction free fatty acids almost exclusively were extracted from the seeds. This was probably caused by remaining lipase activity, and therefore, pretreated seeds should either be processed further immediately or be stored below 0°C.

The oil content of the *D. pluvialis* seeds was used about 20 wt% (dry weight) and 10% (2,3) for cones and wings, respectively. It appeared that DMO is extracted slowly when a crushing step without thermal treatment was used. A heating step at 100°C under reduced pressure for 60 min, before crushing, strongly increases mass transfer, as shown in Figure 2. Temperatures of less than 100°C resulted in a higher free fatty acid content of the oil. Probably, the lipases still active in the seeds generate free fatty acids during the comminution procedure.

With the optimized conditions (cones: 100°C, flaking; wings: 100°C, milling), the experimental data initially fall on a straight line, due to a constant rate of oil extraction. The slope of the line is directly related to the solubility of DMO in SC-CO_2. It was postulated that, during the first period, the external surface of the seeds is completely covered with oil, which is realized by the pretreatment of the seeds. This results in a constant extraction rate, directly related to saturation concentration of the oil in the SC-CO_2 and the CO_2-flow. The first phase is followed by a transition period, during which the extraction rate drops substantially, probably due to depletion of the oil layer on the external surface of the seeds. When all the oil on the external surface has been extracted, a further decrease of the extraction rate occurs. During this final period, the oil has to diffuse from the interior of the seed particles to the surface before it dissolves in the SC-CO_2. As a consequence, this extraction rate is low compared to the initial rate.

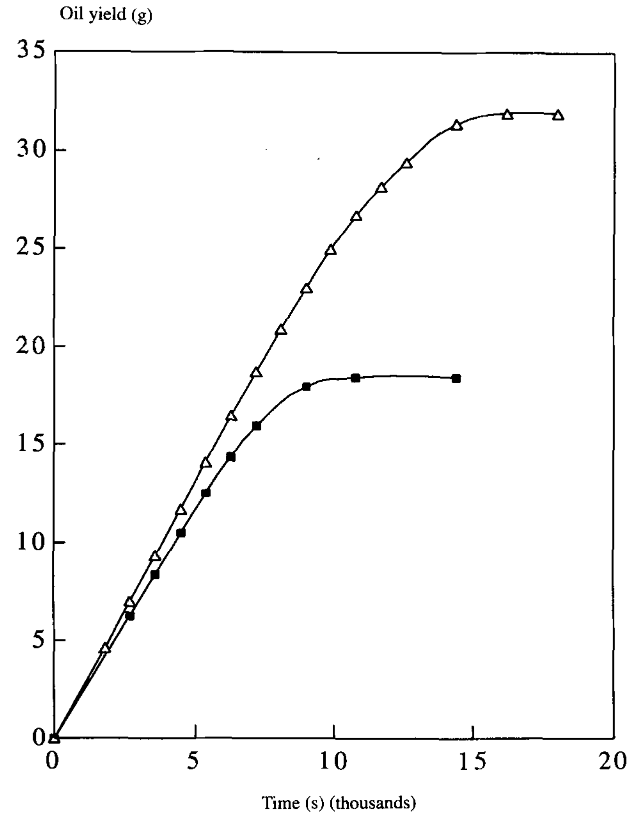

FIG. 2. Time course of supercritical extractions of flaked *Dimorphotheca* seeds (cones) at a CO_2 flow-rate of 10 kg/h. The lines represent the values predicted by the proposed model. The two curves show the influence of pretreatment of conical *Dimorphotheca* seeds on the recovery of the oil during supercritical extraction. (Δ) Seeds heated to 100°C under reduced pressure for 60 min, followed by flaking. (■) Cone-shaped seeds that were only flaked.

The solubility of DMO was determined from the extraction rate of the oil from the carborundum bed. It was assumed that, at a low flow rate (1 kg/h), the residence time of the CO_2 in the extractor was sufficient to reach equilibrium between the impregnated beads and the extractant. At lower flow velocity (0.5 kg/h), the concentration of DMO in the CO_2 did not change, which confirmed that indeed equilibrium was reached. The saturation concentration of DMO was found to be 0.1% (w/w) and did not change much in the temperature and pressure intervals considered. Eggers and Sievers (9) reported a theoretical saturation concentration for triglycerides of 0.9%, which is in reasonable agreement with experimental data found by other authors (8,10). However, the observed

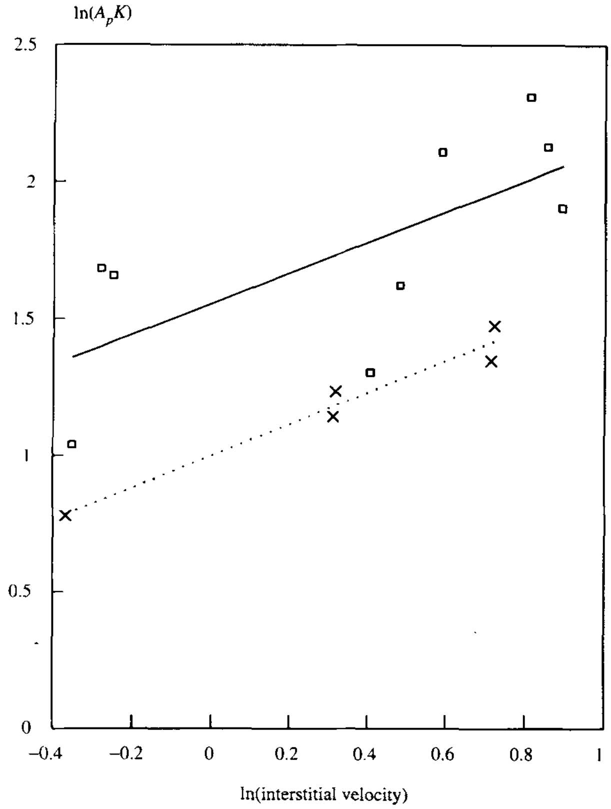

FIG. 3. Volumetric mass transfer coefficient (A_pK) as a function of interstitial velocity (v) of cone (shaded box)- and wing (x)- shaped *Dimorphotheca* seeds.

low saturation concentration of DMO in SC-CO_2 could possibly be explained by the relatively polar character of DMO caused by the dimorphecolic acid present in the triglyceride moiety (11). Further evidence that dimorphecolic acid is responsible for the low saturation concentration was given by Muuse *et al.* (4), who compared traditionally extracted DMO with supercritically extracted DMO. They observed that especially di- and tridimorphecolin were present in supercritically extracted DMO in significantly lower amounts than in traditionally extracted DMO. Such triglycerides can thus be fractionated with supercritical extraction (SCE). The saturation concentration could possibly be improved by extracting DMO at higher pressures as was demonstrated with castor oil (11). However, for castor oil, an improvement of only approximately 0.5 to 2.8 wt% was achieved when the pressure was increased from 344 to 690 bar.

Typical extraction curves of the total amount of oil extracted vs. total CO_2 used are shown in Figure 2. The extraction curve calculated by the proposed mathematical model is also shown. The experimental data are in good agreement with the calculated values, indicating that the model simulates the extraction process well. Simulation of the extraction process also results in an estimate for the volumetric mass transfer parameter A_pK. This value can be of great importance for the scale-up of this extraction process. The calculated A_pK values for the extraction of cones and wings at different circumstances are shown in Figure 3. The curve shows that the A_pK value rises with progress of the interstitial velocity of the SC-CO_2 through the oil-bearing seeds. Following traditional leaching extraction engineering (12), the relation between A_pK and the interstitial velocity of the SC-CO_2 through the extractor bed is represented by:

$$A_pK = C \cdot v^p \qquad [4]$$

The values for C are 4.7 (cones) and 3.1 (wings), and for p 0.56 (cones) and 0.58 (wings) (see also Fig. 3).

By taking the experimental error into consideration, the exponents (p) of the calculated equations for wings and cones are of the same order of magnitude. This indicates that no difference can be observed when cones or wings are being extracted by SC-CO_2. Moreover, Lee *et al.* (8) calculated an exponent of 0.54 for the SCE of canola oilseeds. Despite being another species of seed, this value is in good agreement with our results. However, the pre-exponential factor (C) for the extraction of canola seeds is approximately one order of magnitude higher than that found for *Dimorphotheca.* This difference in the pre-exponential factor is mainly due to the higher saturation concentration of canola oil in SC-CO_2.

The color of the supercritically extracted oil strongly depends on the ripeness of the seeds. When ripe seeds are used, a light yellow to light brown oil is extracted. However, when unripe seeds are extracted, a dark green oil was collected. This is probably caused by the co-extraction of large amounts of chlorophyll.

One of the reasons for application of SCE with CO_2 in DMO recovery is the mild extraction conditions due to the low critical temperature of CO_2, which can reduce the generation of side-products. Whether or not side-products are produced during the applied pretreatment and the SCE of the seeds can only be checked by comparing the extracted oil with traditionally extracted DMO.

In Table 1, the physicochemical parameters of DMO obtained by pressing, by hexane and pentane extraction, and by SCE are given. A remarkable low value of the phospholipid content was found in the supercritically extracted DMO, suggesting that this oil will probably need little further refining. This is in striking contrast with earlier experiments, where conventional refining resulted in deterioration of dimorphecolic acid (13). This deterioration led to trienes and oligomers of dimorphecolic acid and of dimorphecolic acid-containing triglycerides (5). On the other hand, the peroxide value of the supercritically extracted DMO after four-month storage increased substantially, possibly due to low concentrations of phospholipids and antioxidants, causing an increase in the oxidative sensitivity of the oil.

For scaling-up the SCE of *Dimorphotheca* seed oil, it should be taken into consideration that the absolute saturation concentration of this oil in SC-CO_2 is low. To prevent a low production rate of DMO, the supercritical production facility should be equipped with three separate extractors that are connected in a parallel mode. Thus, it becomes possible

TABLE 1
Physicochemical Quality Parameters of *Dimorphotheca pluvialis* Seed Oils

	Hexane	Pentane	SCE A[a]	SCE B[b]	Press oil
Phospholipids (mg/kg)	210	190	n.d.[c]	11	95
Viscosity (in cP at 25°C)	750	430	n.d.[c]	815	960
p-Anisidine value	8 (18)[d]	9	9	7 (29)[d]	10 (18)[d]
Color (red/yellow/blue)	0:400:2	0:700:2	1:35:0	1:35:0	0:600:20
Peroxide value (meq/kg)	18 (15)[d]	21	15 (72)[d]	25 (52)[d]	13 (5)[d]
Free fatty acid (% oleic acid)	6	6	n.d.[c]	3	4

[a] SCE A, oil from a short (single pass) supercritical carbon dioxide extraction.

[b] SCE B, oil from a continuous supercritical carbon dioxide extraction for 1.5 h.

[c] n.d., Not determined.

[d] Number in parentheses indicates the value for oil, stored for four months at room temperature in the dark.

to extract the content of two extractors while the third one is loaded and unloaded (6).

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