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Supercritical Carbon Dioxide Extraction of Corn Distiller's Dried Grains with Solubles: Experiments and Mathematical Modeling

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ABSTRACT: Corn distiller's dried grains with solubles (DDGS) is a byproduct of the ethanol industry and has potential as a source of valuable compounds. In this study, corn DDGS was extracted using supercritical carbon dioxide (SC-CO_2) at 50−70 °C, 34.5−49.6 MPa, and constant CO_2 flow rate of 1 L/min (measured at ambient conditions). The highest yield of total lipids (9.2%, w/w) was obtained at 49.6 MPa/70 °C. Apparent solubility of corn DDGS lipids ranged between 0.010 kg/kg CO_2 at 34.5 MPa/50 °C and 0.026 kg/kg CO_2 at 49.6 MPa/70 °C. The extract contained 107 mg/kg carotenoids, 1538 mg/kg tocochromanols, and 15904 mg/kg phytosterols at 49.6 MPa/70 °C. The Sovova model and Chrastil model were successfully used to describe the extraction of total lipids and apparent solubility of total and minor lipids, respectively. The study revealed that DDGS is a good inexpensive source of lipids and valuable minor lipid components and that SC-CO_2 extraction can be used as a "green" process to add value to corn DDGS by recovering such high-value lipids.

KEYWORDS: *corn distiller's dried grains with solubles, supercritical carbon dioxide extraction, mathematical modeling, minor lipids, solubility*

INTRODUCTION

Increasing interest in ethanol as a biofuel has driven the growth of the ethanol industry in North America in recent years. Because construction of wet-milling plants is complex and capital-intensive, nearly all ethanol currently produced in the United States is made from corn based on dry-milling. Corn distiller's dried grains with solubles (DDGS) and carbon dioxide (CO_2) are coproducts of the dry-milling ethanol process. DDGS is the dried residue remaining after the starch fraction is fermented to produce ethanol and CO_2. The alcohol is then separated by distillation, and the stillage is centrifuged to separate coarse grains from solubles. The solubles are concentrated by evaporation and dried together with coarse grain to produce DDGS.[1]

At present, the most common utilization of DDGS is as livestock feed. However, its real value is underestimated when its composition is considered. Major components of corn DDGS, based on a dry matter basis, are 31.3% protein, 11.9% fat, 10.2% crude fiber, and 5.1% starch.[2] Finding alternative uses of DDGS is necessary to add value to this abundant and inexpensive (ca. $270/ton) byproduct. In recent years, the biorefinery concept has attracted much attention to maximize the utilization of biomass to produce biofuels and other food and industrial products through a number of separation and conversion routes.

Lipid recovery from corn DDGS is an alternative way of utilization because its fat content is beyond what is needed in livestock feed.[3] The lipid content of corn DDGS is higher than that of corn, and lipids in DDGS are not different from those in corn. DDGS lipids contain not only triacylglycerols (TAG) but also other valuable minor lipid components such as carotenoids, tocochromanols (tocols), and phytosterols.

Supercritical fluid technology has proven itself as an efficient and environmentally friendly technology and has found use in the extraction of lipids from a variety of natural materials. CO_2 is the most widely used supercritical fluid due to its advantages of nontoxicity, nonflammability, low cost, availability in large quantities, tunable solvent properties, and moderate critical temperature and pressure (31.1 °C and 7.4 MPa, respectively). Supercritical CO_2 (SC-CO_2) has been widely used as a "green" solvent for the extraction of oils from different sources such as canola,[4] olive husk,[5] and rice bran.[6] However, literature data about SC-CO_2 extraction of corn DDGS are limited. Wang et al.[7] recovered high-value components from corn DDGS using supercritical fluids including CO_2, super- or subcritical water, and ethanol, whereas Winkler et al.[1] studied the extraction of phytochemicals in corn DDGS using SC-CO_2.

Investigation of the SC-CO_2 extraction mechanism of corn DDGS is crucial for its efficient utilization. The economic value of corn DDGS can be increased by recovery of a fraction rich in desired minor lipid components and understanding the SC-CO_2 extraction mechanism. For instance, oil extracted from DDGS can be utilized for biodiesel production; however, separation of minor lipid components from TAG before conversion of extracted oil to biodiesel will produce a high-value fraction while increasing the efficiency of the biodiesel production process. However, such an approach has not been evaluated previously.

Therefore, the main objective of this research was to extract the lipids of corn DDGS using SC-CO_2. The specific objectives were (a) to study the effects of extraction parameters, namely, temperature and pressure, on the recovery of corn DDGS lipids; (b) to determine the composition of lipid extracts in terms of phytosterol, carotenoid, and tocol contents in an effort to develop fractional extraction protocols; (c) to evaluate the

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apparent solubility of total lipids and minor lipid components in SC-CO_2; and (d) to apply a mathematical model to describe extraction kinetics.

MATERIALS AND METHODS

Materials. Corn DDGS was kindly provided by Sanimax (Hamilton, ON, Canada) and stored sealed at −20 °C until used. CO_2 (99.8% bone dry, water level < 3 ppm) was obtained from Praxair Canada Inc. (Mississauga, ON, Canada). Pyridine and Sylon BFT (*N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA)/trimethylchlorosilane (TMCS), 99:1) were purchased from Supelco Inc. (Bellefonte, PA, USA). Carotenoid, tocol, and phytosterol standards were purchased from Sigma-Aldrich (Oakwille, ON, Canada). All other reagents and solvents were of analytical or chromatographic grade.

Proximate Analysis of DDGS Samples. Coarse DDGS samples were ground using a coffee grinder. Moisture contents of DDGS samples were determined by drying in an oven at 105 °C for 2 h. Ash content was determined according to AOAC Official Method 942.05,[8] using a muffle furnace (model F-A1730, Thermolyne Corp., Dubuque, IA, USA) set at 525 °C overnight. Nitrogen content was determined using a nitrogen analyzer (model fp-428, Leco Instruments Ltd., Mississauga, ON, Canada), which was then converted to protein content using a conversion factor of 6.25. Crude lipid content was determined by Soxhlet extraction using petroleum ether for 5 h followed by solvent removal under vacuum at 40 °C. Carbohydrate content was estimated by difference between 100 and the sum of the percentages of ash, moisture, lipid, and protein. Each proximate analysis determination was carried out in triplicate.

SC-CO_2 Extraction. SC-CO_2 extractions were carried out in a laboratory-scale supercritical fluid extraction system (Newport Scientific, Inc., Jessup, MD, USA) described previously.[9] The system was operated in a semicontinuous mode by pumping SC-CO_2 through a fixed bed of DDGS particles, depressurizing the CO_2 to precipitate the lipid extract after the extraction cell, and releasing the expanded CO_2 to the atmosphere. For each run, approximately 82 g of ground DDGS sample was loaded into a basket, and the basket was placed in the 300 mL extraction cell. CO_2 was compressed to the desired pressure by using a diaphragm compressor with a maximum rating of 69 MPa. Pressure was controlled (±1 MPa) by a back pressure regulator and monitored by a pressure gauge. The extraction vessel was heated with an electrical heating jacket. The temperature of the vessel wall was monitored by a type K thermocouple and controlled by a temperature controller (United Electric Control Co., model D921, Watertown, MA, USA) with an accuracy of ±1 °C. The flow rate of the CO_2 was maintained constant by a heated micrometering valve. The flow rate and total volume of CO_2 used in each run were measured at ambient conditions by a flow meter and a dry gas meter, respectively.

Extractions were performed at temperatures of 50, 60, and 70 °C and at pressures of 34.5, 42.1, and 49.6 MPa at a constant CO_2 flow rate of 1 L/min (measured at ambient conditions). The extracted lipid fractions were collected over 30 min intervals for a total extraction time of 340 min. Extracts were collected in glass vials held in a cold trap at −20 °C after the depressurization valve. The amount of each fraction was determined gravimetrically, and the oil yield was expressed as a percentage of the mass of DDGS used as feed. Extracted lipid samples were stored at −20 °C until analyzed.

Analysis of Minor Components. *Phytosterol Analysis.* Phytosterol content and composition in the DDGS extracts were determined by a gas chromatograph (GC) according to the method of Verleyen et al.[10] after saponification. Briefly, 50 mg of DDGS extract and 50 μL of α-cholestane (internal standard, 156 mg/mL) were added into test tubes and saponified with 10% KOH in methanol for 18 h at room temperature in a dark place. Then, 16% NaCl solution was added and unsaponifiables were extracted with hexane. The extracted fraction was concentrated by evaporating hexane under a mild stream of nitrogen. Acetone and anhydrous sodium sulfate were added into the concentrated mixture and filtered through a 0.5 μm filter. Then, acetone in the filtrate was evaporated under nitrogen to dryness.

Dry residues were derivatized prior to injection into the GC to increase the volatility of the components. Derivatization was done using the method of Verleyen et al.[10] with some modification. Dry residue was dissolved in 0.5 mL of pyridine, and 0.5 mL of BSTFA containing 1% TMCS solution was added as silylating agent. Then, test tubes were placed in an oven at 70 °C for 20 min for completion of the silylation reaction. Samples were further diluted with 1 mL of chloroform. Finally, 1 mL of derivatized sample was transferred into a GC vial for injection into the GC.

Derivatives of the phytosterols were separated on an automatic cool on column injection GC (Varian 3400, Varian Inc., Walnut Creek, CA, USA) equipped with a DB-5 capillary column (25 m × 0.20 mm × 0.33 μm; J&W Scientific, Folsom, CA, USA) and a flame ionization detector. An aliquot (1 μL) of sample was injected, and hydrogen was used as carrier gas at a head pressure of 155 kPa. Column temperature was programmed with an initial hold at 60 °C for 1 min, followed by an increase to 140 °C at 30 °C/min and then to 235 °C at 5 °C/min, held for 7 min, increased to 340 °C at 15 °C/min, and held for 15 min. The detector temperature was set at 360 °C. Phytosterols were identified by comparison of the retention times to those of authentic standards.

Carotenoid Analysis. Carotenoids in the SC-CO_2 extracts were analyzed according to the method of Breithaupt and Schlatterer[11] with some modifications. DDGS extracts were saponified following the same procedure used for saponification of phytosterols as described above. Apo-carotene was used as internal standard. Saponified extracts were dissolved in dichloromethane and analyzed for their carotenoid composition by reversed phase high-performance liquid chromatography (HPLC). The HPLC system consisted of a quadratic pump (model LC-20AT; Shimadzu, Columbia, MD, USA), a column (Supelcosil LC-18, 250 × 4.6 mm i.d., 20−40 μm, Supelco) with an accompanying guard column (5 × 4.6 mm i.d.), and a photodiode array detector (model SPD-M20A, Shimadzu). A gradient system of solvent A (methanol/methyl *tert*-butyl ether/water, 90:7:1, v/v/v) and solvent B (methyl *tert*-butyl ether/methanol/water, 90:7:1, v/v/v) was used as the mobile phase at a flow rate of 1 mL/min. The gradient elution profile started with 95% A and 5% B and changed to 65% A and 35% B in 30 min, to 60% A and 40% B in 10 min, to 5% A and 95% B in 10 min, and to 95% A and 5% B in 10 min, which was held for 5 min. The column temperature was set at 35 °C. Elution was monitored at 450 nm. Carotenoids were identified by comparison of the retention times to those of authentic standards.

Tocol Analysis. Tocols were analyzed according to the method of Kramer et al.[12] Extracted DDGS lipids were dissolved in hexane (5 mg/mL) and separated using HPLC (Varian, Prostar, model 325; Walnut Creek, CA, USA) with a fluorescence detector set for excitation at 295 nm and emission at 325 nm. An aliquot of sample (10 μL) was injected onto a normal-phase Supelcosil LC-Diol column (25 cm × 4.6 mm, 5 μm particle size; Supelco). The mobile phase was 1% propan-2-ol in hexane at a flow rate of 1 mL/min. Standards of tocopherol and tocotrienol isomers were used for identification by comparing retention data. Quantification was based on external calibration for each isomer separately.

Statistical Analysis. Data are presented as the mean ± standard deviation (SD) based on triplicate experiments. Statistical analysis of the data was performed by ANOVA and least-squares difference (LSD) post hoc test using SPSS (version 17.0) software package at 95% confidence interval.

Mathematical Modeling. *Modeling of Apparent Solubility.* The apparent solubility of total lipids and minor lipid components was determined from their corresponding extraction curves. According to mass transfer kinetics, extraction curves consist of three regions. Region I or the constant extraction rate period is the linear part where easily accessible solute is extracted and mass transfer is controlled by the resistance in the solvent phase. In region II, the extraction rate decreases rapidly due to diminishing availability of the easily accessible solutes. In region III, the extraction rate is very slow because less accessible solutes are extracted and mass transfer is diffusion controlled. When the extraction curve is plotted as (g extract) versus (g CO_2), the slope of the linear region I corresponds to "apparent

solubility" provided that two conditions are met. First, the CO_2 flow rate has to be low enough to provide sufficient contact time to approach equilibrium. Second, there has to be a sufficient quantity of solute to saturate the CO_2. The apparent solubility of total lipids and minor components was obtained from the slope of the region I on their respective extraction curves.

Experimental apparent solubility data of total lipids and minor lipid components (carotenoids, phytosterols, and tocols) were correlated using the Chrastil model.[13] The Chrastil model relates the solubility of a solute to the density and absolute temperature of the solvent fluid, as shown in eq 1, and is based on the assumption that a solvato-complex is formed between solute and solvent molecules.

$$S = \rho^k \exp\left(\frac{a}{T} + b\right) \tag{1}$$

S is the solubility of the solute in the supercritical solvent (kg/m^3), ρ is the density of pure solvent (kg/m^3), T is the operating temperature (K), and k (association number) is the average number of fluid molecules forming the solvato-complex. Parameter a is dependent on the total heat of reaction, and b is dependent on the molecular weights of the solute and solvent and the association constant. Equation 1 can be expressed as a linear relationship as follows:

$$\ln(S) = k \ln(\rho) + \frac{a}{T} + b \tag{2}$$

Model parameters were estimated using SigmaPlot curve fitter (Marquardt–Levenberg algorithm) via an iterative least-squares procedure that gives the best fit between the equation and the data by minimizing the sum of the squared differences.

Modeling of Extraction Curves. The Sovova model,[14] which is an extended Lack's plug flow model, was applied to model the SC-CO_2 extraction curves of corn DDGS. This model considers the resistance to mass transfer both in the solvent phase and in the solid phase while neglecting the accumulation of solute in the fluid phase. It also assumes that the pressure, temperature, and solvent velocity are constant throughout the extraction and that the particle size and initial solute distributions in the solid bed are homogeneous.

Equations of the Sovova model that give the amount of solute extracted during the three regions described above are

$$e = \begin{cases} qy_r[1 - \exp(-Z)] & q < q_m \\ y_r[q - q_m \exp(z_w - Z)] & q_m \le q < q_n \\ x_0 - \dfrac{y_r}{W} \ln\left\{1 + \left[\exp\left(\dfrac{Wx_0}{y_r}\right) - 1\right] \exp[W(q_m - q)]\dfrac{x_k}{x_0}\right\} & q_n \le q \end{cases} \tag{3}$$

where

$$q_m = \frac{x_0 - x_k}{y_r Z} \tag{4}$$

$$q_n = q_m + \frac{1}{W} \ln\left[\frac{x_k + (x_0 - x_k)\exp\left(\frac{Wx_0}{y_r}\right)}{x_0}\right] \tag{5}$$

$$\frac{z_w}{Z} = \frac{y_r}{Wx_0} \ln\left[\frac{x_0 \exp[W(q - q_m)] - x_k}{x_0 - x_k}\right] \tag{6}$$

$$Z = \frac{k_f a_0 \rho}{\dot{q}(1 - \varepsilon)\rho_s} \tag{7}$$

$$W = \frac{k_s a_0}{\dot{q}(1 - \varepsilon)} \tag{8}$$

$$1 - f_k = \frac{x_k}{x_0} \tag{9}$$

e is the amount of extract (kg extract/kg solute-free feed), q is the specific mass of the solvent passed through the extractor (kg solvent/kg solute-free feed), y_r is the solubility of the solute in the solvent (kg solute/kg solvent), q_m is the q value at the end of region I, q_n is the q value at the end of region II, x_0 is the initial concentration of solute in the solid particles (kg solute/kg solute-free feed), x_k is the concentration of difficultly accessible solute inside the solid particles (kg solute/kg solute-free feed), Z and W are dimensionless mass transfer parameters in the solvent and solid phases, respectively, k_s and k_f are solvent phase and solid phase mass transfer coefficients (m/s), respectively, a_0 is the specific interfacial area (m^2/m^3), ρ_s and ρ are densities of solid and solvent (kg/m^3), respectively, ε is the bed porosity, $\dot{q}$ is the mass flow rate of the solvent (kg solvent/s per kg solute-free feed), z_w is the dimensionless axial coordinate between fast and slow extraction, and f_k is the fraction of the solute directly exposed to the solvent. The overall mass transfer coefficients in the fluid and solid phases are $K_f = k_f a_0$ and $K_s = k_s a_0$, respectively.

Model parameters Z, z_w, W, and x_k were estimated by fitting the Sovova model to experimental data by minimizing the average absolute relative deviation (AARD) (eq 10) using the solver program of Microsoft Excel.

$$\text{AARD}\,(\%) = \frac{1}{N}\sum\left|\frac{e^{\text{calcd}} - e^{\text{exptl}}}{e^{\text{exptl}}}\right| \times 100 \tag{10}$$

N is the number of experimental data points, e^{calcd} is the calculated yield, and e^{exptl} is the experimentally measured yield.

RESULTS AND DISCUSSION

Extraction of Total Lipids. The proximate composition of DDGS samples used in this study is given in Table 1. The lipid content of DDGS was 11.2% after 5 h of Soxhlet extraction using petroleum ether. Winkler et al.[1] reported the oil content of corn DDGS as 12.7% for 24 h hexane extraction and 12.5%

Table 1. Proximate and Minor Lipid Composition of Corn DDGS[a]

proximate (%, w/w)	
moisture	9.4 ± 0.1
ash	2.0 ± 0.1
protein	33.7 ± 0.3
lipid	11.2 ± 0.1
carbohydrate	43.6 ± 0.1
minor lipids (mg/kg extract)	
total carotenoids	89 ± 16.7
β-carotene	2 ± 0.1
cryptoxanthin	4 ± 0.9
lutein	47 ± 9.5
zeaxanthin	35 ± 6.3
total tocochromanols	1265 ± 156.3
α-tocopherol	111 ± 14.6
α-tocotrienol	123 ± 12.4
γ-tocopherol	687 ± 72.5
γ-tocotrienol	116 ± 42.7
δ-tocotrienol	228 ± 102.8
total phytosterols	12418 ± 900.3
campesterol	3033 ± 177
stigmasterol	1148 ± 87.6
β-sitosterol	7909 ± 753.3
Δ^5-avenasterol	328 ± 58.7

[a]Values are reported as the mean ± standard deviation based on triplicate extractions.

for SC-CO_2 extraction, where 2.5 g of sample was extracted at 55 MPa/80 °C at a CO_2 flow rate of 2 L/min (measured at ambient conditions) for 60 min after an initial static hold of 1 min. It is important to specify the source of DDGS for better comparison of the data because some plants may use a mixture of grains to produce ethanol. As well, the polarity of the solvents used for Soxhlet extraction should be taken into consideration, because the solubility of polar minor lipid compounds will increase with solvent polarity.

Apparent Solubility of Total Lipids. Experimental and modeled extraction curves of total lipids are given in Figure 1. As expected, higher yields of total lipids were obtained at higher pressures at all temperatures. The highest yield (9.2%) was obtained at 49.6 MPa and 70 °C, whereas the lowest (7.1%) was obtained at 34.5 MPa and 50 °C at a CO_2 to oil-free DDGS mass ratio of 8.4. The highest total lipid yield obtained by SC-CO_2 extraction was approximately 82% of that obtained by Soxhlet extraction, which would be mainly due to the differences in the solubility of polar lipids. The apparent solubility of corn DDGS lipids ranged between 0.022 kg/m^3 (0.010 kg/kg CO_2) (34.5 MPa/50 °C) and 0.058 kg/m^3 (0.026 kg/kg CO_2) (49.6 MPa/70 °C). The solubilities of corn and sunflower oil in SC-CO_2 at 35 MPa/60 °C were reported as 0.0114 and 0.0098 kg/kg CO_2, respectively.[15]

Apparent solubility data of corn DDGS lipids correlated with CO_2 density using the Chrastil model are shown in Figure 2a, and the estimated model parameters using eq 2 are presented in Table 2. The increase in total lipid yield with pressure was due to an increase in the density of CO_2 at higher pressures, which in turn increased its dissolving capacity.[16] Temperature had a more complicated effect on the extraction of DDGS lipids. As presented in Figure 2b, apparent solubility increased with temperature at higher pressures, but decreased slightly at 34.5 MPa as a result of the crossover behavior. Crossover behavior of solubility isotherms is due to two opposing factors: increased solubility with temperature due to increased solute vapor pressure, and decreased solubility with temperature due to decreased solvent density.[16] Increase in apparent solubility with pressure becomes steeper at higher temperatures. A higher amount of CO_2 is needed at lower temperatures and pressures for the extraction of total lipids of corn DDGS (Figure 1). The lowest solvent consumption (4.49 kg CO_2/kg DDGS) for the extraction of the highest amount of total lipids was observed at 49.6 MPa and 70 °C.

Modeling of Extraction. The Sovova model described the extraction curves of total corn DDGS lipids well (Figure 1). The parameters of the Sovova model are presented in Table 3. As indicated above, y_r values increased with increasing CO_2 density at higher pressures due to the increasing solvating power of CO_2. Therefore, q_m values decreased at higher pressures due to faster extraction of free oil. q_m is also directly proportional to the amount of ruptured cells, which depends on the grinding process and particle size of the DDGS. The overall mass transfer coefficient in the fluid phase (K_f) and solid phase (K_s) were calculated using the dimensionless parameters Z and W. Higher K_f values than K_s values indicate that the convection mechanism was more effective than diffusion inside the particles. K_f and K_s values were different because K_f depends on pressure, temperature, and the hydrodynamics of the extraction bed, whereas K_s depends on the diffusion of the unreleased oil in the particles and properties of the DDGS particles. Except for 50 °C, K_f decreased with increasing pressure due to the decrease in the solvent velocity, which in turn increased mass transfer resistance. Rezaei and Temelli[17] have reported that the diffusion coefficients of several fatty acids, fatty acid esters, and glycerides in SC-CO_2 decreased with increasing pressure. On the contrary, K_s increased with increasing pressure, which means resistance in the solid phase decreased. f_k values ranging between 0.66 and 0.75 showed that the fraction of the lipids directly exposed to the solvent was

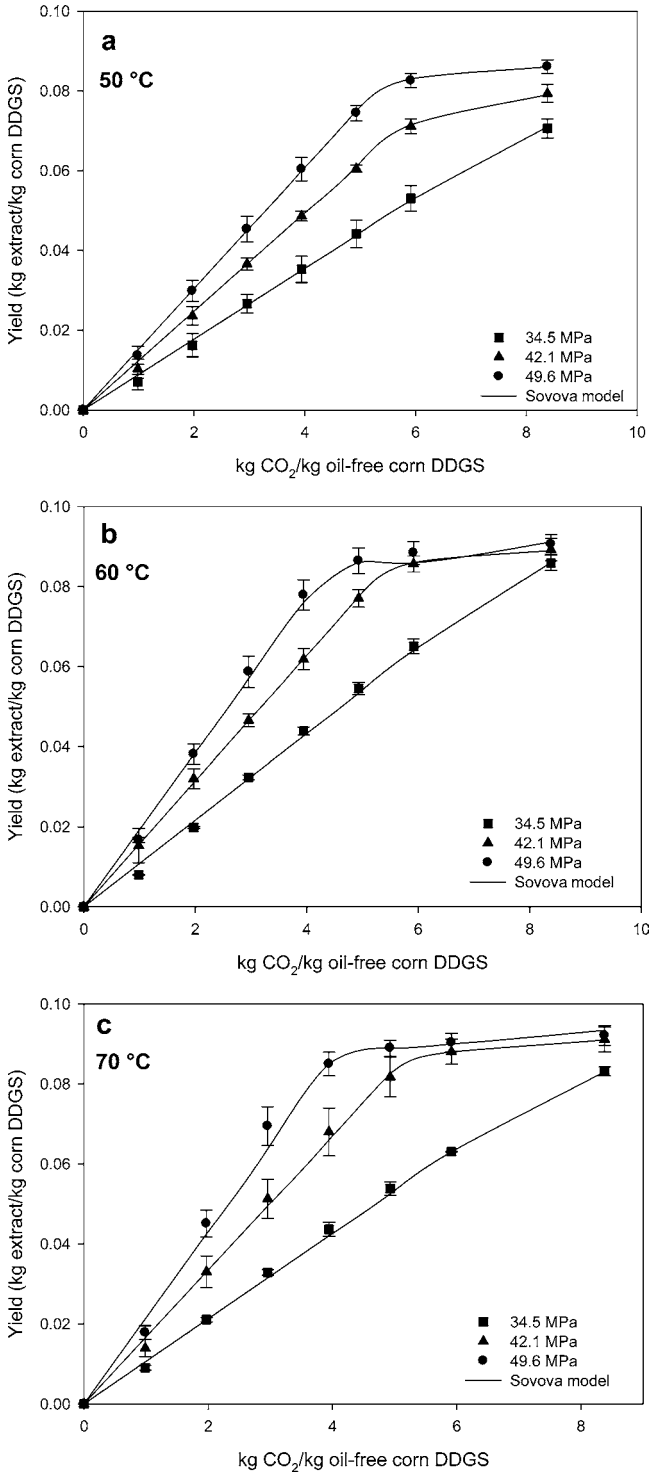

Figure 1. Experimental and modeled extraction curves for corn DDGS: (a) 50 °C; (b) 60 °C; (c) 70 °C. Symbols represent experimental data obtained at different pressures, and the curves are based on Sovova model predictions.

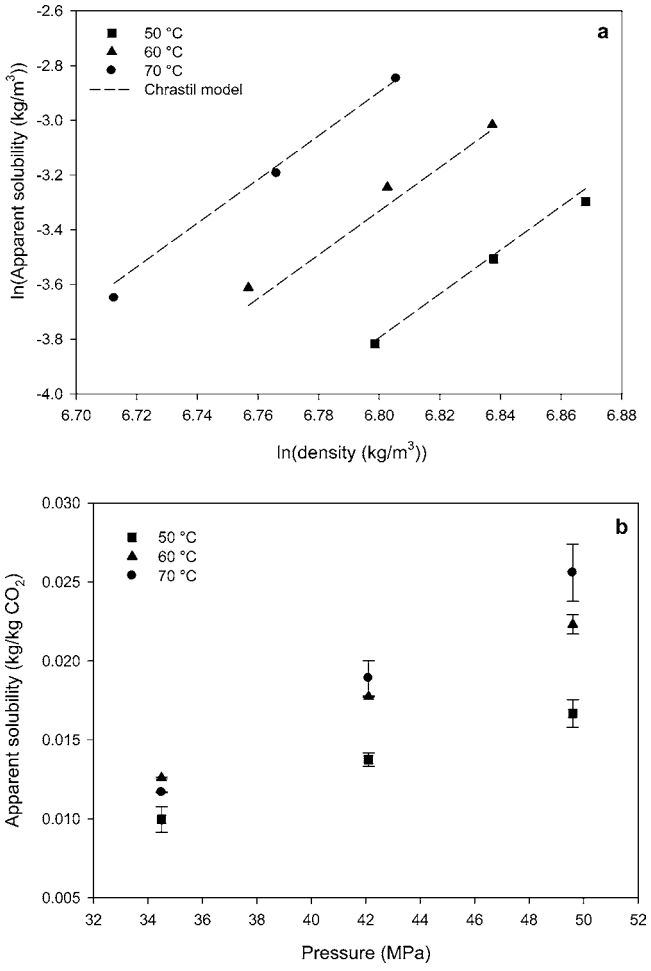

Figure 2. (a) Apparent solubility data of corn DDGS lipids correlated with CO_2 density using the Chrastil model; (b) apparent solubility of corn DDGS lipids at different pressures and temperatures.

Table 2. Parameters of the Chrastil Model for Total Lipids and Minor Lipid Components of Corn DDGS

	Chrastil model			
	k	a	b	R^{2a}
total lipids	8.0	−4973.4	−42.8	0.98*
total carotenoids	15.3	−9703.8	−88.8	0.95*
β-carotene	10.9	−4179.3	−79.1	0.90*
cryptoxanthin	12.0	−4863.9	−84.2	0.91*
lutein	14.5	−6074.2	−94.9	0.90*
zeaxanthin	13.8	−5435.7	−92.6	0.87*
total tocols	6.2	−5667.0	−35.4	0.93*
α-tocopherol	6.9	−6980.0	−38.3	0.92*
α-tocotrienol	8.3	−6946.7	−48.2	0.92*
γ-tocopherol	10.2	−8809.2	−53.7	0.87*
γ-tocotrienol	10.3	−4902.5	−68.4	0.88*
δ-tocotrienol	7.5	−7056.0	−41.9	0.75*
total phytosterols	11.7	−10396.0	−56.2	0.85*
campesterol	11.9	−9292.6	−62.9	0.86*
stigmasterol	9.1	−5951.7	−54.5	0.81*
β-sitosterol	8.0	−7044.1	−41.9	0.96*
Δ^5-avenasterol	5.7	−5956.1	−32.8	0.77*

[a]An asterisk (*) indicates $P < 0.05$.

Table 3. Parameters of the Sovova Model for Extraction Curves of Total Lipids

P (MPa)	T (°C)	y_r (kg/kg)	q_m (kg/kg)	$K_f \times 10^4$ (s^{-1})	$K_s \times 10^6$ (s^{-1})	f_k
34.5	50	0.010	5.32	13.05	6.29	0.75
42.1	50	0.014	4.44	14.87	8.46	0.66
49.6	50	0.017	4.44	21.38	11.75	0.71
34.5	60	0.012	5.32	10.25	4.09	0.73
42.1	60	0.018	4.44	9.66	10.48	0.73
49.6	60	0.021	1.77	6.07	16.65	0.68
34.5	70	0.012	5.32	9.47	4.07	0.74
42.1	70	0.019	4.44	10.22	8.38	0.75
49.6	70	0.025	1.77	8.03	11.76	0.72

similar at all conditions. This is because x_k is related to particle size,[18] and the average particle sizes of the DDGS samples extracted under different conditions in this study were similar (1.6 mm).

Extraction of Minor Lipid Components. Isolation of minor lipid components such as carotenoids, tocols, and phytosterols from complex lipid mixtures is receiving increased attention due to their biological activity and health benefits.[16] Therefore, extraction of those minor lipid components was one of the goals of this study. Minor lipid components constituted 1.38% of the Soxhlet-extracted lipids of corn DDGS (Table 1), whereas they comprised 0.98 and 1.75% of the SC-CO_2 extracts obtained at 34.5 MPa/50 °C and 49.6 MPa/70 °C, respectively.

Minor lipids in the Soxhlet extract were composed of 0.6% of carotenoids, 9.2% of tocols, and 90.2% of phytosterols (Table 1). These values were 0.6% of carotenoids, 8.8% of tocols, and 90.6% of phytosterols for the SC-CO_2 extract obtained at 49.6 MPa and 70 °C. The extraction curves for the three classes of minor lipids are presented in Figure 3. The slopes of the linear

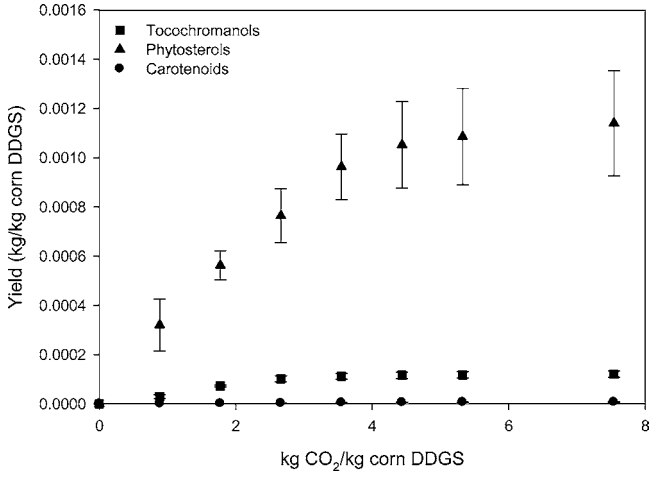

Figure 3. Experimental extraction curves of minor lipid components at 49.6 MPa and 70 °C.

region I were used to determine apparent solubility. Figure 4 shows the apparent solubility of minor lipids of corn DDGS correlated with CO_2 density using the Chrastil model, and the model parameters are given in Table 2. The highest apparent solubility was observed for phytosterols, whereas the lowest was for carotenoids. The solubility behavior of pure minor lipid components in SC-CO_2 was discussed in detail by Güçlü-Üstündağ and Temelli[16] on the basis of their structural differences. The order of k values for the minor components

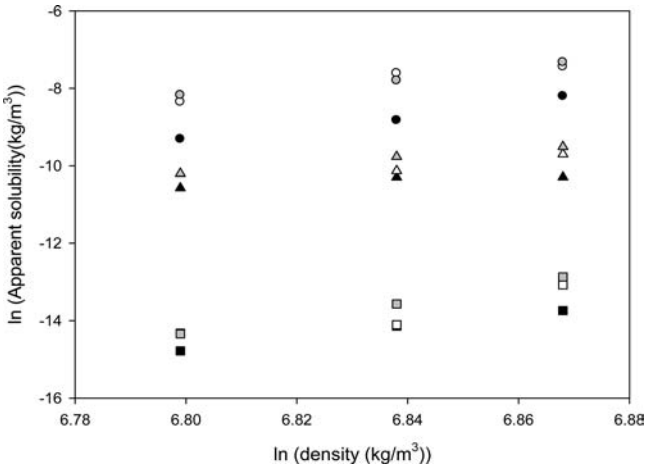

Figure 4. Apparent solubility data of minor lipid components of corn DDGS correlated with CO_2 density using the Chrastil model: (squares) carotenoids; (triangles) tocols; (circles) phytosterols; (black) 50 °C; (white) 60 °C; (gray) 70 °C.

was carotenoids > phytosterols > tocols, which is in agreement with the findings of Güçlü-Üstündağ and Temelli.[16] The apparent solubility of all minor lipids increased with pressure at all temperatures. The most pronounced effect of pressure on the apparent solubility was observed for phytosterols when the pressure was increased from 34.5 to 42.1 MPa.

Phytosterols. The Soxhlet extract of corn DDGS contained 12418 mg/kg of total phytosterols, consisting of campesterol, stigmasterol, β-sitosterol, and avenasterol (Table 1). Phytosterols constituted 1.24% of the total lipids in the Soxhlet extract. Moreau et al.[19] reported that total phytosterols constituted 2–3% of the corn kernel oil and 10–15% of the corn fiber oil. Among individual phytosterols, β-sitosterol was the major phytosterol at 7909 mg/kg, making up 64% of total phytosterols. β-Sitosterol was followed by campesterol (24%), stigmasterol (9%), and avenasterol (3%). SC-CO_2 extracts of corn DDGS contained the same phytosterols, varying from 8592 mg/kg at 34.5 MPa/50 °C to 15904 mg/kg at 49.6 MPa/70 °C (Table 4). The total phytosterol content increased with pressure at all temperatures. SC-CO_2 extracts contained 21–23% campesterol, 10–12% stigmasterol, 63–64% β-sitosterol, and 3% avenasterol. These results show that the selectivities of hexane and SC-CO_2 under their respective conditions toward individual phytosterols are similar; however, at high pressures and temperatures SC-CO_2 has the ability to extract more phytosterols than hexane. Kuk and Dowd[20] found that the amount of sterols extracted by hexane was similar to that by SC-CO_2 at 48.3 MPa and 80 °C, but increased 2-fold when SC-CO_2 conditions were changed to 62 MPa and 100 °C.

The Chrastil model described the apparent solubility of the minor lipid components of corn DDGS well. In the temperature range of 50–60 °C, increasing pressure from 34.5 to 42.1 MPa increased the apparent solubility significantly ($P < 0.05$); however, a further increase did not cause a significant increase in the apparent solubility ($P > 0.05$). At 70 °C, increasing pressure increased the apparent solubility significantly within the range of pressures studied ($P < 0.05$). Solubility parameters for phytosterols are scarce in the literature. The k value estimated for stigmasterol (9.1) in this study was higher than the range reported in literature (4.9–8.0).[21]

Carotenoids. Lutein was the predominant carotenoid in the Soxhlet (Table 1) and SC-CO_2 extracts (Table 5), and it constituted approximately 52% of the total carotenoids. Lutein was followed by zeaxanthin (40%), and the rest was composed of cryptoxanthin and β-carotene. The percentage of each carotenoid in the extract stayed almost constant at all pressures and temperatures. The highest amount of total carotenoids was obtained at 49.6 MPa/60 °C (108 mg/kg) and 49.6 MPa/70 °C (107 mg/kg), whereas the lowest (25 mg/kg) was obtained at 34.5 MPa/50 °C. Moreau et al.[22] reported the highest amounts of total carotenoids as 307.5 mg/kg for ethanol-extracted unrefined corn kernel oil, 118.3 mg/kg for hexane-extracted unrefined corn fiber oil, and 3.4 mg/kg for commercial corn oil. The contents of the individual carotenoids reported by Moreau et al.[22] for hexane-extracted unrefined corn fiber oil were 20.3 (17.2%), 45.7 (38.7%), 25.0 (21.1%), and 27.3 (23.1%) mg/kg of β-carotene, cryptoxanthin, lutein, and zeaxanthin, respectively. As seen in Table 5, whereas lutein and zeaxanthin contents were between the values reported for corn kernel oil and corn fiber oil, β-carotene and cryptoxanthin contents of the SC-CO_2-extracted oil were far below the reported values. The contents of carotenoids in the extract obtained at 49.6 MPa/70 °C were 3 (2.8%), 5 (4.7%), 57 (53.2%), and 42 (39.3%) mg/kg of β-carotene, cryptoxanthin, lutein, and zeaxanthin, respectively. The qualitative compositions of both SC-CO_2 and Soxhlet extracts were found to be similar.

No crossover behavior was observed for the apparent solubility isotherms of carotenoids within the range of pressures studied (Figure 4). When literature data are reviewed,[16] it

Table 4. Phytosterol Content of the Total SC-CO_2 Extract Obtained over 3 h from Corn DDGS

		content[a] (mg/kg extract)				
P (MPa)	T (°C)	campesterol	stigmasterol	β-sitosterol	avenasterol	total
34.5	50	1821 ± 424 a	1046 ± 258 ab	5433 ± 465 a	292 ± 65 ab	8592 ± 566 a
42.1	50	3310 ± 371 cd	1373 ± 96 bc	8529 ± 333 bc	389 ± 8 c	13601 ± 600 cd
49.6	50	1542 ± 220 a	1066 ± 102 ab	7004 ± 894 ab	245 ± 42 a	9857 ± 1053 ab
34.5	60	1804 ± 306 a	1082 ± 47 ab	7180 ± 1081 ab	276 ± 48 ab	10342 ± 870 ab
42.1	60	2241 ± 223 ab	1054 ± 73 ab	7590 ± 502 ab	313 ± 22 abc	11198 ± 331 abc
49.6	60	3240 ± 249 bcd	1110 ± 177 ab	8858 ± 429 bc	363 ± 47 bc	13572 ± 403 cd
34.5	70	2385 ± 523 abc	1017 ± 70 a	7033 ± 90 ab	343 ± 45 bc	10778 ± 638 abc
42.1	70	2469 ± 622 abc	1129 ± 107 ab	8185 ± 906 bc	278 ± 29 ab	12061 ± 1663 bc
49.6	70	3628 ± 654 d	1640 ± 149 c	10151 ± 2165 c	485 ± 13 d	15904 ± 2981 d

[a]Values are reported as the mean ± standard deviation based on triplicate extractions and analyses. Means within a column with different letters are significantly different ($P < 0.05$).

Table 5. Carotenoid Content of the Total SC-CO_2 Extract Obtained over 3 h from Corn DDGS

		content[a] (mg/kg extract)				
P (MPa)	T (°C)	lutein	zeaxanthin	cryptoxanthin	β-carotene	total
34.5	50	13 ± 2 a	10 ± 1 a	1 ± 0 a	1 ± 0 a	25 ± 1 a
42.1	50	38 ± 18 bc	28 ± 11 bc	3 ± 1 de	2 ± 1 cd	72 ± 6 c
49.6	50	39 ± 2 bc	31 ± 1 c	4 ± 0	2 ± 0 bc	76 ± 0 c
34.5	60	24 ± 5 ab	18 ± 3 ab	3 ± 1 bc	2 ± 0 bc	46 ± 8 b
42.1	60	37 ± 4 bc	27 ± 2 bc	3 ± 0 cd	2 ± 0 bc	68 ± 6 c
49.6	60	58 ± 2 d	42 ± 1 d	5 ± 0 f	2 ± 0 bc	108 ± 3 d
34.5	70	19 ± 2 a	14 ± 1a	2 ± 0 b	1 ± 0 ab	37 ± 3 ab
42.1	70	40 ± 2 c	30 ± 2 c	3 ± 0 cd	2 ± 0 bc	76 ± 5 c
49.6	70	57 ± 8 d	42 ± 6 d	5 ± 1 ef	3 ± 0 d	107 ± 15 d

[a]Values are reported as the mean ± standard deviation based on triplicate extractions and analyses. Means within a column with different letters are significantly different ($P < 0.05$).

Table 6. Tocol Content of the Total SC-CO_2 Extract Obtained over 3 h from Corn DDGS

		content[a] (mg/kg extract)					
P (MPa)	T (°C)	α-tocopherol	α-tocotrienol	γ-tocopherol	γ-tocotrienol	δ-tocotrienol	total
34.5	50	164 ± 67 b	136 ± 14 bc	746 ± 41 bc	116 ± 14 abc	137 ± 9 a	1134 ± 51 ab
42.1	50	115 ± 4 ab	122 ± 1 ab	717 ± 47 abc	140 ± 67 bc	154 ± 100 ab	1133 ± 78 ab
49.6	50	95 ± 2 a	109 ± 6 ab	613 ± 27 ab	165 ± 4 c	110 ± 36 a	997 ± 1 a
34.5	60	110 ± 20 ab	124 ± 23 ab	657 ± 112 abc	80 ± 10 a	269 ± 45 cd	1130 ± 190 ab
42.1	60	95 ± 8 a	95 ± 3 a	591 ± 23 a	77 ± 1 a	209 ± 42 abc	972 ± 70 a
49.6	60	124 ± 4 ab	133 ± 1 bc	758 ± 32 c	97 ± 3 ab	286 ± 27 cd	1274 ± 64 b
34.5	70	126 ± 16 ab	125 ± 5 ab	715 ± 7 abc	91 ± 3 ab	247 ± 24 bc	1177 ± 32 ab
42.1	70	120 ± 1 ab	131 ± 1 bc	747 ± 23 bc	98 ± 5 ab	278 ± 22 cd	1253 ± 48 b
49.6	70	144 ± 20 ab	163 ± 30 c	889 ± 82 d	112 ± 12 abc	374 ± 36 d	1538 ± 160 c

[a]Values are reported as the mean ± standard deviation based on triplicate extractions and analyses. Means within a column with different letters are significantly different ($P < 0.05$).

appears that the crossover pressure of carotenoids is lower than the minimum pressure employed in this study. The composition of the carotenoid mixture determines the crossover behavior of carotenoids.[16] Crossover pressure for synthetic *trans*-carotene was reported to be between 15 and 17 MPa in the temperature range of 40−50 °C,[23] whereas it was around 30 MPa for the natural carotene from *Dunalliella salina*, which is a mixture of *cis* and *trans* isomers.[24]

The lowest apparent solubility of carotenoids was 3.8×10^{-7} kg/m^3 at 34.5 MPa and 50 °C, whereas the highest was 25.7×10^{-7} kg/m^3 at 49.6 MPa and 70 °C. When calculated in terms of mole fraction using the average molecular weight of carotenoids in the Soxhlet-extracted corn DDGS oil, apparent solubility values of the total carotenoids were in the range of 1.6×10^{-8} and 1.1×10^{-7}. The solubility of pure β-carotene was reported as $(4.3–9.4) \times 10^{-7}$ mole fraction at 12−20 MPa and temperatures of 40 and 50 °C in SC-CO_2 using the quartz crystal microbalance technique by Saldaña et al.[25] However, when Saldaña et al.[25] determined β-carotene solubility in SC-CO_2 in a complex system based on the dynamic extraction of carrots, the apparent solubility was $(2.4–24) \times 10^{-8}$ mole fraction at 12−20 MPa and temperatures of 40 and 50 °C. That study showed that the apparent solubility of a particular compound in a complex matrix depends also on its location and interactions with the other matrix components.

As shown in Table 2, β-carotene had a lower k value compared to the other carotenoids, whereas the k value for lutein was higher. Lutein was the major carotenoid component in both the Soxhlet (Table 1) and SC-CO_2 (Table 5) extracts. However, it has been reported that lutein has a lower solubility in SC-CO_2 compared to the other carotenoids due to its polar nature.[25] The contents of β-carotene and cryptoxanthin in the corn DDGS extracts were very low. It is important to note that sufficient amounts of the investigated components should be present in the extracted material to saturate the CO_2 to make a satisfactory solubility comparison. The calculated k value of β-carotene in this study is in agreement with the k value of 9.7 determined for β-carotene by Sovova et al.[26] Güçlü-Üstündağ and Temelli[16] reported a k value for β-carotene in the range of 4.9−10.6 using the Chrastil equation. The location and interactions of the components of interest in the solid matrix of natural materials should also be considered in the study of solubility and process behavior.[16]

Tocols. Tables 1 and 6 show the composition and content of tocols detected in the Soxhlet and SC-CO_2 extracts of corn DDGS, respectively. γ-Tocopherol was the main tocol in all extracts. Among all isomers, the most substantial increase was observed in the δ-tocotrienol content when the extraction conditions were changed from 34.5 MPa/50 °C to 49.6 MPa/70 °C. Whereas the percentage of all isomers in the total tocols decreased at all conditions, the percentage of δ-tocotrienol increased from 12.1 to 24.3%. The extract obtained at 49.6 MPa/70 °C contained the highest amount of tocols (1538 ± 160 mg/kg). The total amount of tocols in all of the SC-CO_2 extracts was similar to that of Soxhlet extract (1265 mg/kg) ($P > 0.05$). Winkler et al.[1] also obtained similar amounts of tocols regardless of the extraction solvent used (0.23 mg/kg for Soxhlet extraction with hexane or ethanol vs 0.21 mg/kg for SC-CO_2 extraction). Tocols are nonpolar molecules due to their long side chain and aromatic rings and, therefore, have good solubility in both *n*-hexane and SC-CO_2. Most notably, the tocotrienol contents of all the extracts obtained in this study

were higher than what was reported for oils extracted from corn germ or commercial corn oil.[27] Tocopherol and tocotrienol contents of corn fiber were higher than those of corn kernel and corn germ oils.[28] Therefore, it can be stated that corn DDGS samples contain a high portion of fiber, and the tocotrienols are derived from the fiber portion of the corn kernel.

Increasing pressure increased the apparent solubility of tocols within the temperature range studied. As seen in Figure 4, no crossover was observed for tocol solubility isotherms within the range of pressures studied. The tocopherol solubility data of Johannsen and Brunner[29] showed a crossover between 40 and 60 °C isotherms at around 30 MPa and between 60 and 80 °C isotherms at around 35 MPa. The apparent solubility of the tocols at 34.5 MPa/50 °C was 2.6×10^{-5} kg/m^3 (1.41×10^{-5} kg/kg CO_2), whereas it increased to 7.4×10^{-5} kg/m^3 (4.1×10^{-5} kg/kg CO_2) at 49.6 MPa/70 °C. Johannsen and Brunner[29] reported the solubility of α-tocopherol as 0.0062 kg/kg CO_2 at 19.9 MPa/80 °C and 0.0339 kg/kg CO_2 at 34.9 MPa/60 °C. As shown in Table 2, k values of γ-tocopherol (10.2) and γ-tocotrienol (10.3) were the highest, whereas α-tocopherol had the lowest k value (6.9). Güçlü-Üstündağ and Temelli[16] reported the k value for α-tocopherol as 5.7 using the Chrastil equation. Puah et al.[30] reported the solubility of tocols in the SC-CO_2 density range of 740−860 kg/m^3 in the following order: γ-tocotrienol > α-tocotrienol > α-tocopherol > δ-tocotrienol. It should be noted that correlation coefficients (R^2) of the solubility models have to be considered for the evaluation of k values. In this study, the R^2 of δ-tocotrienol was relatively low (R^2 = 0.753) for the Chrastil model (Table 2).

In conclusion, the highest yield of total lipids (9.2%) from corn DDGS was obtained using SC-CO_2 at 49.6 MPa/70 °C. Minor lipid components (carotenoids, tocols, and phytosterols) made up 1.75% of the extract. The Sovova model and Chrastil model were used successfully to describe the extraction kinetics and apparent solubility of total and minor lipids, respectively. These models can be used for the optimization of SC-CO_2 processes. DDGS was demonstrated to be a good source of lipids and valuable minor lipid components, which can be extracted using SC-CO_2. Further value addition to DDGS is beneficial for the sustainability of corn-based ethanol plants.

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REFERENCES

(1) Winkler, J. K.; Rennick, K. A.; Eller, F. J.; Vaughn, S. F. Phytosterol and tocopherol components in extracts of corn distiller's dried grain. *J. Agric. Food Chem.* **2007**, *55*, 6482−6486.

(2) Belyea, R. L.; Rausch, K. D.; Tumbleson, M. E. Composition of corn and distillers' dried grains with solubles from dry grind ethanol processing. *Bioresour. Technol.* **2004**, *94*, 293−298.

(3) Stallings, C. C. *Distiller's Grains for Dairy Cattle and Potential Environmental Impact*; Virginia Cooperative Extension (http://pubs.ext.vt.edu/404/404-135/404-135.html) (accessed March 26, 2012).

(4) Sun, M.; Xu, L.; Saldaña, M. D. A.; Temelli, F. Comparison of canola meals obtained with conventional methods and supercritical CO_2 with and without ethanol. *J. Am. Oil Chem. Soc.* **2008**, *85*, 667−675.

(5) Esquivel, M. M.; Bernardo-Gil, M. G. Extraction of olive husk oil with compressed carbon dioxide. *J. Supercrit. Fluids* **1993**, *6*, 91−94.

(6) Shen, Z.; Palmer, M. V.; Ting, S. S. T.; Fairclough, R. J. Pilot scale extraction and fractionation of rice bran oil using supercritical carbon dioxide. *J. Agric. Food Chem.* **1997**, *45*, 4540−4544.

(7) Wang, L.; Weller, C. L.; Schlegel, V. L.; Carr, P.; Cuppett, S. L. Comparison of supercritical CO_2 and hexane extraction of lipids from sorghum distillers grains. *Eur. J. Lipid Sci. Technol.* **2007**, *109*, 567−574.

(8) *Official Methods of Analysis*, 18th ed.; AOAC Press: Gaithersburg, MD, 2006; Method 942.05.

(9) Bozan, B.; Temelli, F. Supercritical CO_2 extraction of flaxseed. *J. Am. Oil Chem. Soc.* **2002**, *79*, 231−235.

(10) Verleyen, T.; Verhe, R.; Garcia, L.; Dewettinck, K.; Huyghebaert, A.; De Greyt, W. Gas chromatographic characterization of vegetable oil deodorization distillate. *J. Chromatogr., A* **2001**, *921*, 277−285.

(11) Breithaupt, D. E.; Schlatterer, J. Lutein and zeaxanthin in new dietary supplements- analysis and quantification. *Eur. Food Res. Technol.* **2005**, *220*, 648−652.

(12) Kramer, J. K. G.; Blais, L.; Foucharda, R. C.; Melnyka, R. A.; Kallury, K. M. R. A rapid method for the determination of vitamin E forms in tissues and diet by high-performance liquid chromatography using a normal-phase diol column. *Lipids* **1997**, *32*, 323−330.

(13) Chrastil, J. Solubility of solids and liquids in supercritical gases. *J. Phys. Chem.* **1982**, *86*, 3016−3021.

(14) Sovová, H. Rate of vegetable oil extraction with supercritical CO_2-modelling of extraction curves. *Chem. Eng. Sci.* **1994**, *49*, 409−414.

(15) Soares, B. M. C.; Gamarra, F. M. C.; Paviani, L. C.; Goncalves, L. A. G.; Cabral, F. A. Solubility of triacylglycerols in supercritical carbon dioxide. *J. Supercrit. Fluids* **2007**, *43*, 25−31.

(16) Güçlü-Üstündağ, Ö.; Temelli, F. Correlating the solubility behavior of minor lipid components in supercritical carbon dioxide. *J. Supercrit. Fluids* **2004**, *31*, 235−253.

(17) Rezaei, K. A.; Temelli, F. Using supercritical fluid chromatography to determine diffusion coefficients of lipids in supercritical CO_2. *J. Supercrit. Fluids* **2000**, *17*, 35−44.

(18) Papamichail, I.; Louli, V.; Magoulas, K. Supercritical fluid extraction of celery seed oil. *J. Supercrit. Fluids* **2000**, *18*, 213−226.

(19) Moreau, R. A.; Powell, M. J.; Hicks, K. B. Extraction and quantitative analysis of oil from commercial corn fiber. *J. Agric. Food Chem.* **1996**, *44*, 2149−2154.

(20) Kuk, M. S.; Dowd, M. K. Supercritical CO_2 extraction of rice bran. *J. Am. Oil Chem. Soc.* **1998**, *75*, 623−628.

(21) Wong, J. M.; Johnston, K. P. Solubilization of biomolecules in carbon dioxide based supercritical fluids. *Biotechnol. Prog.* **1986**, *2*, 29−39.

(22) Moreau, R. A.; Johnson, D. B.; Hicks, K. B. A comparison of the levels of lutein and zeaxanthin in corn germ oil, corn fiber oil, and corn kernel oil. *J. Am. Oil Chem. Soc.* **2007**, *84*, 1039−1044.

(23) Mendes, R. L.; Nobre, B. P.; Coelho, J. P.; Palavra, A. F. Solubility of β-carotene in supercritical carbon dioxide and ethane. *J. Supercrit. Fluids* **1999**, *16*, 99−106.

(24) Mendes, R. L.; Nobre, B. P.; Palavra, A. F. Supercritical CO_2 extraction of β-carotene from *Dunaliella salina*. In *Proceedings of the Fifth International Symposium on Supercritical Fluids*, Atlanta, GA; Teja, A., Eckert, C., Eds.; International Society for Advancement of Supercritical Fluids (ISASF): Nancy, France, 2000.

(25) Saldaña, M. D. A.; Sun, L.; Guigard, S. E.; Temelli, F. Comparison of solubility of β-carotene in supercritical CO_2 based on a binary and a multicomponent complex system. *J. Supercrit. Fluids* **2006**, *37*, 342−349.
(26) Sovova, H.; Stateva, R. P.; Galushko, A. A. Solubility of β-carotene in supercritical CO_2 and the effect of entrainers. *J. Supercrit. Fluids* **2001**, *21*, 195−203.
(27) Moreau, R. A.; Hicks, K. B. The composition of corn oil obtained by the alcohol extraction of ground corn. *J. Am. Oil Chem. Soc.* **2005**, *82*, 809−815.
(28) Moreau, R. A.; Hicks, K. B. A reinvestigation of the effect of heat pretreatment of corn fiber on the levels of extractable tocopherols and tocotrienols. *J. Agric. Food Chem.* **2006**, *54*, 8093−8102.
(29) Johannsen, M.; Brunner, G. Solubilities of the fat-soluble vitamins A, D, E, and K in supercritical carbon dioxide. *J. Chem. Eng. Data* **1997**, *42*, 106−111.
(30) Puah, C. W.; Choo, Y. M.; Ma, A. N.; Chuah, C. H. Solubility of tocopherol and tocotrienols from palm oil in supercritical carbon dioxide. *J. Food Lipids* **2007**, *14*, 377−385.