Supercritical carbon dioxide extraction and fractionation of rapeseed cake oil
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ABSTRACT
Minor lipids co-extracted with oil from seeds are partially responsible for desirable antioxidant effects that protect against degradation and impart functional value to the oil. Fractionation effects during extraction that may increase the concentration of these minor lipids in the oil are desirable, and this work explored the possibility of using SuperCritical carbon dioxide (SC-CO₂) at high temperature (40 °C) and pressure (≥40 MPa) to achieve fractionation of prepressed rapeseed (Brassica napus) oil. For this purpose cold-pressed rapeseed cake was extracted using SC-CO₂ at 30-50 MPa, 40 or 80 °C, and a superficial velocity of 1 mm/min. The weight and concentration of minor lipids (sterols, tocopherols, carotenoids) in oil fractions collected during the first 60 min of extraction were recorded and analyzed. Cumulative oil yield increased with extraction pressure, and with extraction temperature at ≥40 MPa, but was lower at 80 than 40 °C at ≤35 MPa, which is consistent with a solubility-controlled process and a crossover pressure between 35 and 40 MPa. Differences in solubility between the oil and minor lipids explained fractionation effects that were small for tocopherols. Unlike tocopherols, that are more soluble in SC-CO₂ than the oil, sterols and carotenoids are less soluble than the oil, and their concentration increased in the later stages of the extraction process, particularly at ≥40 MPa, when there was no enough oil to saturate the CO₂ phase. Consequently, this study suggests that SC-CO₂ extraction can be used to isolate vegetable oil fractions having increased functional value.

Keywords: Cold-press cake; minor lipids; oil extraction; rapeseed; supercritical CO₂.

INTRODUCTION
High-oil seeds are commonly pressed in the vegetable oil industry, to obtain a high-value crude oil (e.g., extra virgin olive oil) and a residual press cake. The residual press cake (as well as the oil in low-oil seeds), in turn, is traditionally extracted using nonpolar solvents, commonly n-hexane. Rapeseed (Brassica napus) press cake contains oils rich in linolenic (omega 3) fatty acid, as well as minor lipids of great functional value such as carotenoids, tocopherols, and phytosterols. The refining sequence applied to crude oils to upgrade their quality and/or applicability includes neutralizing, bleaching, and deodorisation steps that are normally conducted at elevated temperatures. In this context, SC-CO₂ extraction is an alternative technique for the extraction of edible oils that may maximize the retention of the bioactive components in crude oils, and possibilitate their use as functional oils.

Residual press cake contains minor lipids such as phytosterols, tocopherols, and carotenoids (Przybylski et al., 1998) of great functional value as natural antioxidants (Tuberoso et al., 2007). In a companion study, Uquiche et al., submitted used response surface analysis to explore the effect of extraction conditions on the recovery of triglycerides and minor lipids from cold-pressed rapeseed (Brassica napus) cake by applying SuperCritical Fluid Extraction (SCFE) using SC-CO₂. Their work was limited to moderate pressures (20–40 MPa) and temperatures (40–60 °C), thus requiring moderate-to-large extraction times (≥60 min). In this work we explored the possibility of selectively recovering minor lipids from cold-pressed rapeseed cake in short (≥60 min) SC-CO₂ extractions carried out at higher pressures (≥50 MPa) and temperature (80 °C).

Few studies have reported on the fractionation of oil from rapeseeds using SC-CO₂ (Przybylski et al., 1998; Jachmanían et al. 2006). Fractionation of SC-CO₂ extracts takes advantage of differences in solubility between extract components with system (pressure, temperature, and composition of CO₂-rich phase) conditions, and can be carried out using staged extractions, fractional separations, or over time. Fractionation can be carried out over time by retrieving fractions of progressively decreasing solubility in SC-CO₂ taking advantage of the dynamic nature of a SCFE process carried out under constant conditions; the compositions
of the substrate and SC-CO₂ phases that equilibrate with each other change as extraction proceeds (Güçlü-Ustündag & Temelli, 2004). The objective of this study was to identify the effects of various combinations of medium to high pressures and temperatures and short times on the yield of oil and the concentration of minor lipid in fractions of SC-CO₂-extracted cold-pressed rapeseed cake, with the aim of separating fraction enriched in minor lipids and with increased antioxidant activity.

MATERIALS & METHODS

The substrate was cold-pressed rapeseed cake from Oleotop (Freire, Chile) that was ground in a disc mill to a mean Sauter diameter of 0.848 mm and stored at 5 °C in polyethylene bags until analysis. The milled cold-pressed rapeseed cake contained 99 ± 0.1 g water/kg dry substrate, 160 ± 2 g oil/kg dry substrate. SCFE was carried out in a Spe-ed SFE unit (Applied Separations, Allentown, PA) as done by Uquiche et al. (submitted). Samples of 28.5 g ground rapeseed (W_s = 25.9 g dry substrate) were placed in a 50 cm³ vessel (14 mm inner diameter) and extracted with 3.1-4.1 L NPT/min of 99.95%-pure CO₂, corresponding to a single superficial velocity of 1 mm/s. Extractions were carried out at 30, 35, 40, 45, or 50 MPa and at 40 or 80 °C. In all cases, a 20-min static extraction period was followed by a 60-min dynamic extraction when the expansion valve (kept at 120 °C) was opened. The extract (oil) in the CO₂ stream leaving the expansion valve was sampled every 15-min (four fractions) in pre-weighed glass vials (60 cm³ capacity). Recovered oil (M_i) was assessed gravimetrically by difference with cleaned and dried vials after removing co-extracted water in a dessicator with silica gel, and cumulative oil yields (Y_i) were estimated using equation 1.

\[ Y_i = \frac{1}{W_s} \sum_{j=1}^{i} M_j \]

where “i” represents the fraction number (0-to-15 min for fraction 1, 15-to-30 min for fraction 2, 30-to-55 min for fraction 3, and 45-to-60 min for fraction 4).

For comparison purposes, oil was also extracted by extraction to exhaustion (10 h) of a 60-g sample of finely ground (mortar and pestle) rapeseed press cake using technical grade hexane in a Soxhlet apparatus operating at 70 °C as done by Uquiche et al. (submitted). Oil in vials was dissolved in chloroform p.a. (Merck, Darmstadt, Germany) and flashed to 50 cm³ in volumetric flask prior quantification of sterols, tocopherols, and carotenoids by UV spectrophotometry in a SP-2000 UV apparatus (Bausch-Lomb, USA). Total sterol concentrations were quantified at 640 nm and expressed as stigmasterol equivalents using the method of Sabir et al. (2003). Total tocopherol concentrations were quantified at 520 nm in α-tocopherol equivalents using the method of Wong et al. (1988). Finally, total carotenoid concentrations were quantified at 452 nm in β-carotene equivalents using an adaptation of the method of the Malaysian Palm Oil Board (2005). The standards for the analysis of sterols (stigmasterol), tocopherols (α-tocopherol), and carotenoids (β-carotene) were all from Sigma-Aldrich (St. Louis, MO). Cumulative yields of minor lipids (X_i) were estimated using equation 2.

\[ X_i = \frac{1}{W_s} \sum_{j=1}^{i} M_j x_i \]

where X represents sterol, tocopherol, or carotenoid yield; x, the corresponding concentration of the component; and, “i”, the fraction number.

RESULTS & DISCUSSION

Oil extraction

Cumulative extraction plots (g oil/kg dry substrate) of cold-pressed rapeseed at 40 °C and 80 °C versus specific solvent consumption (kg CO₂/kg dry substrate) (figures not showed) shows that there is a pronounced increase in extraction rate with system pressure in the 30-50 MPa range that is explained by the increase in the solvation power of SC-CO₂ with its density. The effect of system temperature on extraction rate is more complex. At ≤35 MPa extraction rate decreases as temperature increases from 40 to 80 °C, but at ≥40 MPa extraction rate increases as temperature increases. A decrease in extraction rate with temperature at low pressures is possible if the extraction process is under solubility-controlled conditions, which is facilitated by the initial 20-min period of static extraction, because of retrograde condensation phenomena.
Under these retrograde condensation conditions, the decrease with increasing temperature in the density of CO₂ (at 35 MPa, it decreases 15.6% from 935 kg/m³ at 40 ºC to 789 kg/m³ at 80 ºC, NIST) and the associated decrease in its solvent power cannot be compensated by the accompanying increase in the vapor pressure and volatility of the oil, with the end result of a decrease in solubility when increasing the temperature isobarically. This does not occur at higher pressures because, being CO₂ less compressible, the decrease in the density (at 40 MPa, it decreases only 13.9% from 956 kg/m³ at 40 ºC to 823 kg/m³ at 80 ºC, NIST) and solvent power of the CO₂ is fully compensated by the increase in vapor pressure and volatility of the oil.

Figure 1 plots cumulative oil yield versus specific solvent consumption to unveil the relationship between extraction rate and oil solubility based on the correlation proposed by del Valle et al. (submitted) for the solubility of vegetable oils in SC-CO₂. The specific solvent consumption is multiplied by the solubility estimated of the oil using the correlation of del Valle et al. (submitted). Thus, extraction time is presented in units of grams of oil per kilogram of dry substrate, but in this case the amount of oil represents all the oil that would have been carried out by the SC-CO₂ if it had reached saturation conditions. In Figure 1, the 45º line by the origin represents extractions carried out under the solubility-controlled conditions hypothesized for the initial stages of the SC-CO₂ extraction process. It appear as if extractions are solubility-controlled as long as the total amount of oil extracted is limited to ca. 25-40% of the total (40-60 g oil/kg dry substrate), and that the correlation of del Valle et al. (submitted) provides good estimates of rapeseed oil solubility within the experimental region. Besides a solubility-controlled initial period, SC-CO₂ extraction curves consider subsequent periods where extraction rate is controlled by external mass transfer mechanisms, inner mass transfer mechanisms, and desorption mechanisms that are markedly dependent on the substrate pretreatment and particle size (Güçlü-Üstündag & Temelli, 2004).

Time-fractionation of minor lipids on SC-CO₂ extracts

The composition of minor lipids in SC-CO₂ oil fractions as a function of extraction conditions were determined. Sterol concentration in the oil extracted at 40 ºC increased slightly along extractions carried out at 30 or 35 MPa (3.04-8.47 g/kg oil) and then progressively more pronouncedly along those performed at increasingly higher pressures up to 50 MPa (58.73 g/kg oil). This resulted in an increase in the average concentration of sterols in the oil by a factor of about 2.3 up to about 5.2. Similar trends were observed at 80 ºC, but at this higher temperature there was a decrease with extraction pressure in the sterol content in the initial fractions of the oil (proportionally larger in oils extracted at higher pressures), which resulted in a decrease in the average concentration of sterols in the oil by a factor of about 1.9 down to a minimal of about 2.0 at 50 MPa.
Unlike in the case of sterols, there were limited variations in the concentration of tocopherols in SC-CO₂ extracted oil, particularly at 40 ºC. Overall, the average concentration of tocopherols in the oil was not affected to a great extent (about 1.1 to 1.5 g/kg) by extraction pressure at 40 ºC. On the other hand, tocopherol concentration in the oil extracted at 80 ºC remained constant along extractions carried out at 30 and 35 MPa (1.47-1.75 g/kg oil) but increased during extractions performed at 40 (0.44-5.41 g/kg oil) and 50 MPa (0.44-12.95 g/kg oil), with this increase being limited mainly to the last fraction. However, the same as in the case of sterols, there was a decrease with extraction pressure in the tocopherol content in the initial fractions of the oil extracted at 80 ºC, which resulted in a decrease in the average concentration of sterols in the oil by a factor of about 3 down to a minimal of about 0.6 g/kg at 50 MPa.

The concentration of carotenoids in SC-CO₂ extracted oil increased with extraction temperature, extraction pressure (≥40 MPa), and time. Carotenoid concentration in the oil extracted at 40 ºC remained constant along extractions carried out at 30 or 35 MPa but increased markedly as extractions at 40–50 MPa proceeded. A similar trend was observed at 80 ºC, but in this case the average concentration of carotenoids in the oil (e.g., about 125 mg/kg or ppm at 45 MPa) was higher than at 40 ºC (about 35 ppm at 45 MPa).

To better understand the fractionation of minor lipids in SC-CO₂, yields of sterols, tocopherols, and carotenoids can be plotted against the yield of oil. Reference lines representing the concentration of the particular component in conventionally extracted oil (using hexane as the solvent) can be included in such plots. Experimental points above the reference line indicate enrichment of the oil in the particular component as compared to conventional oil. Time-fractionation effects, on the other hand, are seen as departures from straight lines by the origin with an upward trend indicating progressive enrichment of the particular component as the residual oil content in the press cake diminishes. This type of plot allows isolating the relative effects of oil yield and component concentration in the oil on the actual recovery of the component, because large increases in concentration do not amount to a desirable fractionating effect if they are not accompanied by a large recovery of oil in the particular fraction.

The fractionation of sterols during SC-CO₂ extraction of cold-pressed rapeseed oil at 40 ºC and 80 ºC (data not showed) shows that sterols are not well extracted from cold-pressed rapeseed by SC-CO₂, especially at high pressures (45 and 50 MPa) and 80 ºC. Based on the review of Güçlü-Üstündağ & Temelli (2004) on the solubility in SC-CO₂ of minor plant lipids, it can be estimated that the solubility of stigmasterol in CO₂ within our experimental region increases with system pressure (from 165 ppm at 30 MPa to 249 ppm at 50 MPa, at 40 ºC) and specially with system temperature (from 249 ppm at 40 ºC to 1920 ppm at 80 ºC, at 50 MPa). The percent increase in the solubility of stigmasterol with pressure in the 30 to 50 MPa range is larger at 80 than 40 ºC. The ratio between the sterol yield (g/kg dry substrate) and the specific solvent mass (kg CO₂/kg dry substrate) for short (15-min) extractions represents the “apparent” solubility of sterols in CO₂. The apparent solubilities of rapeseed sterols in CO₂ at 40 ºC increased from 17 ppm at 30 MPa to 44 ppm at 50 MPa, ca. 9-17% of the corresponding thermodynamic solubilities of pure stigmasterol. Analogously, the apparent solubilities of rapeseed sterols at 80 ºC were 1.1-2.4% of the corresponding thermodynamic solubilities of stigmasterol. As it will be expanded later, this may be explained by several factors, including a limited content of sterols in the substrate and interactions with other solutes in the oil and the solid matrix.

Figure 2 shows that tocopherols are well extracted from cold-pressed rapeseed by SC-CO₂ and that there is a limited time-fractionation effect. An exception occurs at 80 ºC and very high pressures (45 and 50 MPa) (Fig. 2B). Within the experimental region, the solubility of α-tocopherol in CO₂ estimated using the correlation of Güçlü-Üstündağ & Temelli (2004) also increases with system pressure (from 19.2 g/kg at 30 MPa to 28.8 g/kg at 50 MPa, at 40 ºC) and system temperature (from 28.8 g/kg at 40 ºC to 60.9 g/kg at 80 ºC, at 50 MPa), with the pressure-increasing effect being more pronounced at high temperature, and the positive effect of the increase in temperature improving with system pressure. These thermodynamic solubilities are ca. 2.1 to 5.3 larger than the corresponding solubilities of vegetable oils at 30-50 MPa and 80 ºC, and 1.7 to 2.4 times larger than the corresponding solubilities of vegetable oils at 40 ºC, with the differences between the two decreasing as pressure increases from 30 to 50 MPa at the two temperatures. These differences between the solubilities in SC-CO₂ of tocopherols and vegetable oils cannot explain the fractionation effects observed at 45 or 50 MPa and 80 ºC (Fig. 2B).
The extraction of tocopherols, the same as the one of sterols, is not determined by their solubility in SC-CO₂, specially when carrying SCFE out at high pressure and temperature. Because there is not enough tocopherol within the substrate loaded in the extraction vessel (32.3 mg, considering that rapessed samples contain 1.24 mg/g dry substrate of tocopherols) to fully saturate the SC-CO₂ at 50 MPa and 80 °C during the 20-min static extraction, it is reasonable to expect much lower apparent than thermodynamic solubilities at 50 MPa and 80 °C as experimentally observed under these conditions.

Figure 2. Cumulative tocopherol yield versus cumulative oil yield at (A) 40 °C and (B) 80 °C at (—) 30 MPa, (—) 35 MPa, (—) 40 MPa, (—) 45 MPa, or (—) 50 MPa. Figure 2B also includes results for the two fractions collected in separate 60-min experiments at (△) 35 MPa, (○) 40 MPa, or (▼) 45 MPa and 80 °C.

The fractionation of carotenoids during SC-CO₂ extraction of cold-pressed rapeseed oil at 40 °C and 80 °C (data not showed) shows that carotenoids are poorly extracted by SC-CO₂, and that a time-fractionation effect occur at ≥40 MPa, specially at 80 °C, when oil yield increases slightly above 125-135 g/kg dry substrate. Within our experimental region, the solubility of β-carotene in CO₂ estimated using the correlation of Güçlü-Üstündağ & Temelli (2004) increases with system pressure (from 2.76 ppm at 30 MPa to 4.70 ppm at 50 MPa, at 40 °C) and specially with system temperature (from 4.70 ppm at 40 °C to 53.4 ppm at 80 °C, at 50 MPa). These values are 270-550 times smaller than the corresponding solubilities of vegetable oils at 30-50 MPa and 80 °C, and 2900-3600 times smaller than the corresponding solubilities of vegetable oils at 40 °C. These trends in relative solubilities explain the effect on time-fractionation of rapeseed carotenoids of system
pressure at 40 °C or 80 °C, the differences observed between the two temperatures, and the differences observed between carotenoides and other minor lipids.

The same as for sterols and tocopherols, thermodynamic solubilities in binary CO₂ + carotene systems only partially explain the cumulative extraction plots for carotenoids. Besides thermodynamic solubilities of the pure compounds, additional factors such as availability, interactions with other minor lipids and triglycerides in the extract, and interactions with the solid matrix of rapeseed carotenoids should be all accounted for (del Valle & Urrego, submitted).

CONCLUSIONS

SC-CO₂ extraction of rapeseed oil appears to be a solubility-controlled partially dependent on residual oil concentration in the prepressed seeds. The effects of extraction pressure (30–50 MPa) and temperature (40 or 80 °C) on cumulative oil yield are consistent with a cross-over pressure between 35 and 40 MPa. Differences in solubility between the oil and minor lipids (sterols, tocopherols, carotenoids) also explain fractionation effects. These fractionation effects are small for tocopherols that are more soluble in SC-CO₂ than the oil. On the other hand, there is an increase in concentration of sterols and carotenoids in the later stages of the extraction process, when there is no enough oil to saturate the CO₂ phase, because these two components are less soluble in SC-CO₂ than the oil.

REFERENCES