EXTRACTION OF OIL AND MINOR LIPIDS FROM COLD-PRESS RAPESEED CAKE WITH SUPERCRITICAL CO$_2$

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Abstract - This study examines the extraction of oil from cold-press rapeseed cake using Supercritical CO$_2$ (SC-CO$_2$). The effects of pressure (20, 30, and 40 MPa), temperature (40, 50, and 60 ºC), and extraction time (60, 90, and 120 min) on oil yield and composition (tocopherols and carotenoids) were studied using response surface design. The results indicated that pressure influenced the most the yield of oil, followed by temperature and extraction time. Extraction time had no effect on oil composition. Extraction pressure and temperature did not affect the tocopherol concentration of the oil to a great extent, whereas temperature had no affect in its carotenoid concentration. A comparison was made between the relative qualities of oil extracted with SC-CO$_2$ at 40 MPa and 60 ºC and with n-hexane. Neither solvent affected the unsaponifiable matter content or the composition of phytosterols (mainly β-sitosterol, campesterol and brassicasterol) of the oils, although there was a significant difference (p<0.05) in tocopherol. Extraction with SC-CO$_2$ at 40 MPa and 60 ºC is recommended to obtain rapeseed-oil enriched with tocopherols and carotenoids as important functional components.
Keywords: Cold-press cake; Minor lipids; Oil extraction; Rapeseed; Supercritical CO$_2$.

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 (Johnson, 1997). The residual press cake (as well as the oil in low-oil seeds), in turn, is traditionally extracted using nonpolar solvents, commonly n-hexane (Johnson, 1997). However, the United States Environmental Protection Agency listed n-hexane among 187 hazardous air pollutants in the 2002 National-Scale Air Toxics Assesments because of its toxic nature (United States Environmental Protection Agency, 2011). Carbon dioxide (CO$_2$) has emerged as a substitute for n-hexane for the extraction not only of vegetable oils but also other nonpolar solutes in plants and other biological substrates. CO$_2$ is the solvent most commonly used in supercritical fluid extraction processes because it has a relatively low critical temperature (31.1 ºC) and moderate critical pressure (7.39 MPa) and is non-toxic, inert, non-flammable,

non-corrosive, and inexpensive. Further, after the extraction, the system solute+CO_2 is descompressed and CO_2 is eliminated as gas. Thus, the extract is obtained free of residues of solvent and can be used without additional treatments. Traces of organic solvent (e.g. hexane) can cause degradation in components of the oil. This is avoided by extraction with SC-CO_2. Supercritical CO_2 (SC-CO_2) allows fast and efficient extractions of oils that deteriorate less and demand less refinement to remove undesirable contaminants than oils extracted using conventional nonpolar solvents. Brazilian Journal of Chemical Engineering has pusblished a series of articles on the use of the technology for the extraction of vegetable oil from rice bran (Sarmento *et al*., 2006), wheat germ (Zacchi *et al*., 2006) and macadamia nuts (Silva *et al*., 2008).

Rapeseed (*Brassica napus*) press cake contains oils rich in linolenic (omega 3) fatty acid, as well as minor lipids of great functional value as pro-vitamins, antioxidant, and anticholesterolemic agents such as carotenoids, tocopherols, and phytosterols (Przybylski *et al*., 1998). Indeed, the unsaponifiable fraction represents about 2% of crude rapeseed and contains approximately 40% phytosterols, 10% tocopherols, and 1% carotenoids (Przybylski *et al*., 1998). 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. This refining sequence may negatively affect minor lipids in oils, as exemplified by the reduction in phytosterol content in corn (from 85 to 73 mg/kg oil), rapeseed (from 82 to 77), sunflower (from 43 to 35 mg/kg), and soybean (from 35 to 26 mg/kg) oils (Fernándes and Cabral, 2007). SC-CO_2 extraction is an alternative technique for the extraction of edible oils that can maximize the retention of the bioactive components in crude oils and make possibile their use as functional oils.

The main independent variables in SC-CO_2 extractions include substrate pretreatment and particle size, extraction pressure and temperature, co-solvent addition and concentration, CO_2 flow rate, and extraction time. SC-CO_2 extraction of oilseeds is typically carried out at 30 MPa and 20-80 °C (Eggers, 1996). An effective technique to evaluate the effect of some of these variables on the extraction yield and extract composition, which allows identification of the main effects with limited experimental work, is response surface analysis of results of carefully planned experiments, or so-called response surface design (RSD) of experiments. Although there are reports on the SC-CO_2 extraction of rapeseed oil,

most work refers to seeds that are not pre-pressed, but subjected to alternative treatments such as grinding (Przybylski *et al*., 1998). An exception is the work of del Valle *et al*. (2006), who extracted pre-pressed rapessed cake with CO_2 at 30 MPa and 40 ºC, but did not evaluate the quality of the extracted oils.

The main objective of this study was to analyze the effects of pressure (20-40 MPa), temperature (40-60 ºC), and extraction time (60-120 min) on the extraction yield and composition of SC-CO_2–extracted oil from cold-press rapeseed cake using RSD. As a complementary objective, we compared the composition of fatty acids and minor lipids and the oxidative stability of rapeseed oils extracted with hexane (conventional extraction) and SC-CO_2 under relevant conditions determined from the analysis of results from the RSD.

MATERIALS AND METHODS

Oil Extraction

The substrate was cold-press rapeseed (*Brassica napus*) cake acquired from Oleotop (Freire, Chile) that was ground in a disc mill (model 4E, The Straub Company, PHILA, USA) and stored at 5 ºC in polyethylene bags until analysis. The substrate had a moiture content of 9.9 ± 0.1 g/100 g dry substrate (determined gravimetrically by drying in an oven for 15 h at 102 ℃) Untreated and spent substrates were finely ground with a mortar and pestle prior to analyzing moisture and oil content. Oil content of the substrate was 160 ± 2 g/kg dry substrate (determined gravimetrically by extraction with technical grade hexane in a Soxhlet apparatus for 10 h at 70 °C). The particle size distribution of the milled substrate was determined in a Ro-Tap test sieve shaker (model RX-29-10, W.S. Tyler, Mentor, OH) using 80, 48, 35, 28, 24, 20, 16, 12 and 6 mesh Tyler screens. The Sauter mean diameter of substrate was 0.848 mm.

SC-CO_2 extraction was carried out in a Spe-ed SFE unit (Applied Separations, Allentown, PA) by loading 27.1 g of ground rapessed press cake (24.7 g dry substrate) in a 50 cm^3 extraction vessel (14 mm inner diameter) and extracting with 4 L NPT/min of CO_2 (Aga S.A., Santiago, Chile) (equivalent to 7.2 g/min). The extraction pressure (20, 30, or 40 MPa) was manually controled with an air-actuated booster pump. The temperature of the air bath (oven) containing the extraction vessel (40, 50, or 60 ºC) was, in turn, automatically controlled. The extract in the CO_2 stream leaving the extraction vessel came

out of solution following an expansion valve kept at 110 ºC and was collected in pre-weighed glass vials (60 cm³ capacity). Recovered extract at pre-set extraction times (60, 90, or 120 min) was assessed gravimetrically by difference with cleaned and dried vials. Oil in vials was dissolved in isopropyl alcohol p.a. (Caledon Laboratories Ltd., Georgetown, Ontario, Canada) and diluted to 50 cm³ in volumetric flasks prior to further analysis.

To evaluate the relative quality of the oil extracted with SC-CO₂, conventional extraction was performed in a Soxhlet apparatus with hexane, as described previously, using 60 g of substrate. Hexane was mostly recovered in a Janke & Kunkel rotary evaporator (model RVO5-ST, IKA Laboratories, Staufen, Germany) using a Welch vacuum pump (model 2522C-02, Thomas Compressors & Vacuum Pumps, Skokie, IL). Residual solvent traces were then removed in a Memmert oven (model UM-400, WTB Binder, Tutlingen, Germany) at 80 ºC for 2 h.

Oil Analysis

The tocopherol and carotenoid contents in isopropyl alcohol solutions of the oil samples were assessed using UV spectrophotometry in a SP-2000 UV apparatus (Bausch-Lomb, USA). Total tocopherol content was quantified at 520 nm in terms of α-tocopherol equivalents using the method of Wong *et al*. (1988). Total carotenoid content was quantified at 452 nm in β-carotene equivalents using an adaptation of the method of the Malaysian Palm Oil Board (2005). The standards of α-tocopherol and β-carotene used for establishing calibration curves were from Sigma-Aldrich (St. Louis, MO).

Additional oil quality characteristics were evaluated in selected extracts as well as in hexane-extracted oil using standard methods of the American Oil Chemists' Society (1993): unsaponifiable matter expressed as percentage (Ca 6a-40), peroxide value expressed as milli-equivalents (meq) of peroxide per 1000 g of oil (Cd 8-53), p-anisidine value (Cd 18-90), and induction time (IT) for oxidation (Cd 12b-92). IT was measured using a Rancimat Oxidative Stability Instrument (Metrohm Ltda, Herisau, Switzerland) employing standard Rancimat tubes containing 10-g samples of oil. Samples were heated to 110 ± 1 ºC while passing 20 L NPT/h of filtered air and the gases released during oxidation were led into a conductivity cell containing water. IT corresponds to the time when the conductivity of the aqueous solution in the cell exhibits an inflection point.

The fatty acid composition in the selected oil samples was determined by Gas Chromatography (GC) as the methyl ester derivatives (Asociación Española de Normalización, 1999). A Hewlett Packard 5890 chromatograph (Hewlett-Packard, Palo Alto, CA, USA) with a flame ionization detector (FID) equipped with a 50 m (length) × 0.32 mm (inner diameter) × 0.25 μm (film thickness) fused silica BPX70 (70% bis-cyanopropilsiloxane) coated-capillary column (SGE, Incorporated Austin, TX, USA) was used. Separation was carried out using 2.0 mL NPT/min of hydrogen (Indura S.A., Santiago, Chile) as carrier gas. Oven temperature increased from 160 to 230 ºC at a rate of 2 ºC/min; the injector was kept at 230 ºC, and the detector at 240 ºC. Reference fatty acid methyl esters (FAME) from Merck (Merck KGaA, Darmstadt, Germany) were used as standards for identification and quantitation purposes.

Phytosterol composition was determined using the official method of the European Union Commission (1991) that considers four steps: 1) saponification of the oil using KOH (J.T. Baker, Mexico City, Mexico) in ethanol (Caledon Laboratories Ltd., Ontario, Canada) solution; 2) isolation of the unsaponified fraction with ethyl ether (J.T. Baker, Mexico City, Mexico); 3) concentration of the unsaponified fraction by washing with water and drying with anhydrous sodium sulfate (Scharlau Chimie S.A., Barcelona, Spain); and, 4) separation of sterols from the nonsaponified fraction by thin layer chromatography (TLC) on silica gel plates 60 F₂₅₄ (Merck KGaA, Darmstadt, Germany). The band of sterols was scraped off the TLC plate and treated with (99:1) bis-trimethylsilyl-trifluoroacetamide (Sigma-Aldrich, Steinheim, Germany) and chlorotrimethylsilane (Merck Schuchardt OHG, Hohenbrunn, Germany) as derivatizing reagent and analyzed by GC on the Hewlett Packard 5890 chromatograph equipped with a 30 m × 0.32 mm × 0.25 μm fused silica DB-17 (95% dimethylpolysiloxane–5% diphenyl) capillary column (Agilent Technologies, Palo Alto, CA, USA). Separation was carried out using 1.0 mL NPT/min of hydrogen (Indura S.A., Santiago, Chile) as carrier gas. The injector (split ratio 1:50), oven, and detector were kept at 300 ºC, 275 ºC, and 300 ºC, respectively. Quantification was done using α-cholestanol (Sigma-Aldrich, St. Louis, MO) as internal standard.

Experimental Design

RSD was used to evaluate the effects of the coded independent variables pressure (X_1, Eq. (1)), where P is pressure in MPa), temperature (X_2, Eq. (2)), where T is temperature in ºC), and extraction time (X_3, Eq. (3)), where t is extraction time in min), all expressed in dimensionless units, on the response variables: oil

extraction yield (Y_{oil}), and concentrations of tocopherols (C_{toc}) and carotenoids (C_{car}) in the oil.

$$X_1 = \frac{P - 30}{10} \tag{1}$$

$$X_2 = \frac{T - 50}{10} \tag{2}$$

$$X_3 = \frac{t - 90}{30} \tag{3}$$

The experimental design (Table 1) was based on a face centered (FCD), three-variable (independent), three-level (coded values -1, 0, and +1), 2^3 fractional factorial design with star points and triplicates at the center. Experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous factors. A second-order polynomial equation was used to express each response variable Y as a function of the independent variables,

$$Y = \sum_{i=0}^{3} \sum_{j=0}^{3} A_{ij}^{y} X_i X_j \tag{4}$$

where X_0 (dummy variable) equals one; A_{00} is a constant; A_{01}, A_{02}, and A_{03} are linear coefficients; A_{12}, A_{13}, and A_{23} are cross-product coefficients; A_{11}, A_{22} and A_{33} are quadratic coefficients, and the supraindex y defines the dependent variable (y = 1 for oil yield, y = 2 for concentration of tocopherols, and y = 3 for

concentration of carotenoids). The goodness of fit of the model was evaluated by the coefficient of determination r^2 and the analysis of variance (ANOVA). The coefficients of the response surface equation were estimated by using Design-Expert Software, version 6.0.1 (Stat-Ease, Inc., Minneapolis, MN). The statistical significance was based on the total error criteria with a confidence level of 95%.

In selected experiments, oil samples were analyzed for the concentration of tocopherols, unsaponifiable matter, peroxide value, p-anisidine value, IT, fatty acid composition, and phytosterol composition.

RESULTS AND DISCUSSION

Table 1 summarizes the experimental results for oil extraction yield and concentration of tocopherols and carotenoids in the oil as a function of system pressure and temperature and extraction time for the 17 experiments in the fractional factorial experimental design. These experimental results were fitted to second order response surface models for oil extraction yield, concentration of tocopherols in the oil, and concentration of carotenoids in the oil. Only significant ($p < 0.05$) terms were kept in each response surface equation (*cf.* Eq. (4)), and for this purpose, the coefficient of the least significant term (having the largest p-value) in each equation was set to zero and the corresponding model refitted to the experimental data up to the point where the p-values of all terms in the simplified equation were below 0.05 (all terms significant at the 5% level).

Table 1: Experimental design and results for the supercritical CO_2 extraction of oil from cold-pressed rapeseed cake.

| Pressure (P, MPa)	Temperature (T, ºC)	Extraction time (t, min)	X_1 (-)	X_2 (-)	X_3 (-)	Y_{oil} (g oil / kg dry substrate)	C_{toc} (mg tocopherol/kg oil)	C_{car} (mg carotenoid//kg oil)
20	40	60	-1	-1	-1	69.9	863	138
20	40	120	-1	-1	1	104.6	791	109
20	50	90	-1	0	0	66.3	1034	124
20	60	60	-1	1	-1	50.4	901	106
20	60	120	-1	1	1	68.8	914	169
30	40	90	0	-1	0	128.8	806	147
30	50	60	0	0	-1	110.4	847	148
30	50	90	0	0	0	124.1 ± 4.4	822 ± 33	199 ± 23
30	50	120	0	0	1	130.2	853	170
30	60	90	0	1	0	134.8	738	257
40	40	60	1	-1	-1	130.0	784	199
40	40	120	1	-1	1	133.5	858	318
40	50	90	1	0	0	131.8	903	340
40	60	60	1	1	-1	131.5	945	293
40	60	120	1	1	1	136.9	953	263

Table 2 summarizes the statistical parameters for the three response surface models that are deemed adequate because of non-significance (p < 0.05) of the lack of fit relative to the pure error for all dependent (response) variables, high coefficients of determination ($r^2 > 0.90$) for all response surface models, and high signal-to-noise (> 4) for all responses, which indicates that the models can be used to navigate the design space. This statistical indicators are complemented in Figure 1 by scatter plots demonstrating the correlation between predicted and experimental responses (data points are close to the line representing equality between the two) for the dependent variables oil extraction yield and concentration of tocopherols. The plot of predicted versus observed values for the concentration of carotenoids (C_{car}) shows a more limited correlation, probably because the quadratic equation (significant at p<0.05) considered only the effect of the linear term of pressure on C_{car}. However, we considered it appropriate to our approach, according to the significant model (p<0.05), a coefficient of determination of 0.91, a signal/noise ratio of 8.6 and lack of fit of the non-sgnificant model.

Table 2: Statistical indicators of appropriateness of the response surface models selected in this work.

Statistical indicator	Response variable		
	Y_{oil} (g oil / kg dry substrate)	C_{toc} (mg tocopherol / kg oil)	C_{car} (mg carotenoid / kg oil)
F-value	42.4*	60.1*	7.76*
r^2	0.982	0.987	0.909
Lack of fit	2.04[ns]	5.12[ns]	3.93[ns]
Signal-to-noise ratio	19.68	26.88	8.62

*   Significant (p <0.01); and,
[ns]   Non-significant (p > 0.1).

(a)

(b)

(c)
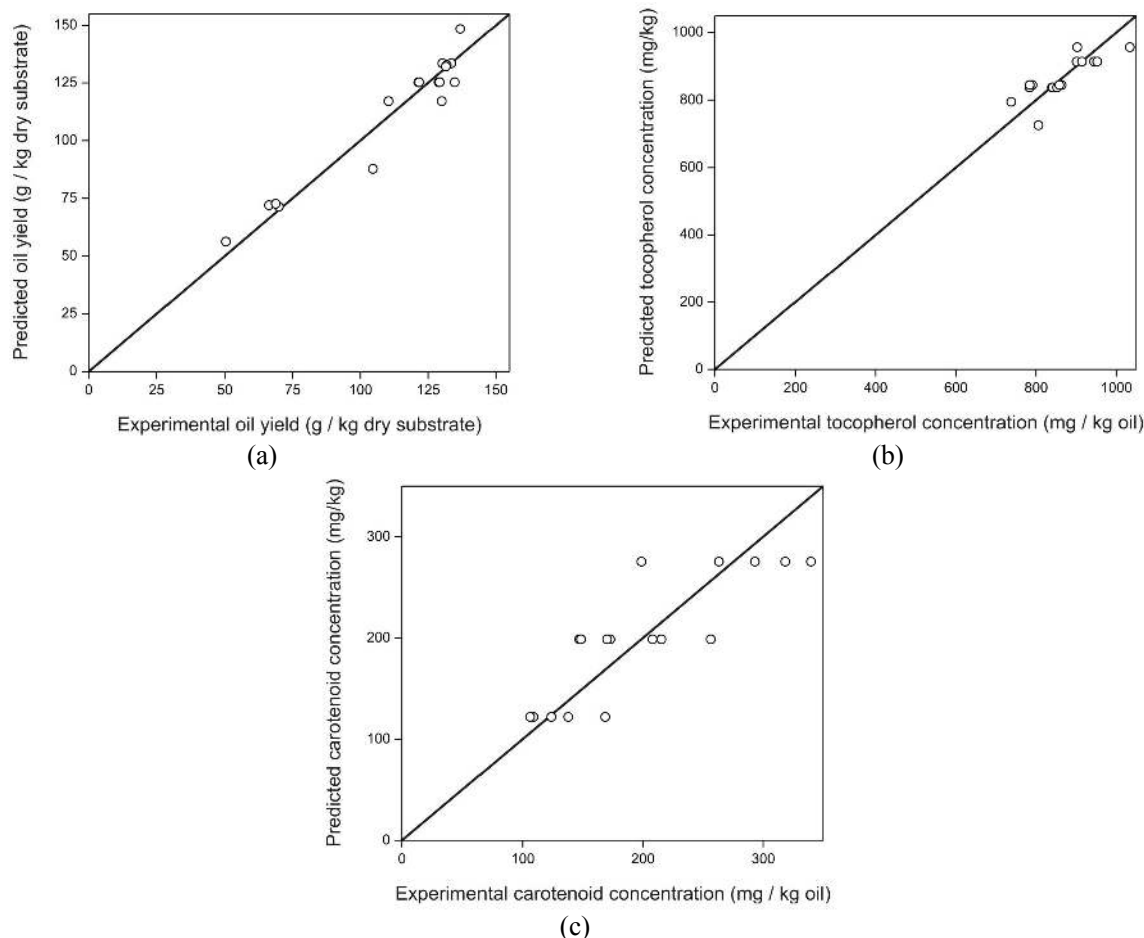
Figure 1: Differences between experimentally measured values and values predicted by the response surface models for (a) oil yield, (b) tocopherol content in the oil, and (c) carotenoid content in the oil.

This section concludes with the analysis of differences in quality attributes between the hexane-extracted oil and the CO_2-extracted oil under the best conditions suggested by the analysis of response surfaces.

Oil Extraction Yield

Equation (5) predicts the oil extraction yield (Y_{oil}, in grams of oil per kilogram of dry substrate) as a function of extraction pressure (P, MPa), temperature (T, ºC), and extraction time (t, min), with all independent variables expressed in coded, dimensionless form (*cf.* Eqs. (1)-(3)).

$$Y_{oil} = 125.2 + 30.37\left(\frac{P-30}{10}\right) + 8.180\left(\frac{t-90}{30}\right) - \\ 22.83\left(\frac{P-30}{10}\right)^2 + 7.525\left(\frac{P-30}{10}\right)\left(\frac{T-50}{10}\right) \quad (5)$$

Because all terms in Equation (5) are significant ($p < 0.05$), this equation represents the relevant effects of extraction pressure, temperature, and time on oil extraction yield when carrying out extractions in the selected experimental region ($20 \leq P \leq 40$ MPa; $40 \leq T \leq 60$ ºC; and $60 \leq t \leq 120$ min). Coded variables are important because they give a direct, quantitative indication of the effect of the independent variables on any dependent variable as a funtion of the selected experimental region. The term $A_{03}{}^1$ that multiplies the linear term X_3 indicates that the oil extraction yield increases 8.18 g oil/kg dry substrate when the extraction time increases 30 [=0.5 (120 – 30)] min while operating at 30 MPa ($X_1 = 0$) and 50 ºC ($X_2 = 0$). Furthermore, given that this term is the only one containing X_3, this effect of extraction time on oil extraction yield does not depend on either extraction pressure or temperature.

It is possible to rewrite Equation (5) in a different way so as to show the effect of extraction temperature (Eq. (5a)) on oil yield more clearly, as follows:

$$Y_{oil} = \alpha(X_1, X_3) + \beta(X_1)\left(\frac{T-50}{10}\right), \quad (5a)$$

where $\beta(X_1) = 7.525\left(\frac{P-30}{10}\right)$.

Equation (5a) states that the oil extraction yield increases with temperature as long as $P \geq 30$ MPa (condition that determines $\beta \geq 0$). A decrease in yield

with extraction temperature at low pressures is possible if the extraction process is under solubility-controlled conditions because of retrograde condensation phenomena. Under these retrograde condensation conditions, the decrease in the density and solvent power of CO_2 when increasing the temperature of highly compressible CO_2 under near-critical (low-pressure) conditions cannot be compensated by the accompanying increase in the vapor pressure and volatility of the oil, the end result being a decrease in solubility upon increasing the temperature isobarically. This does not occur in this example at high pressure ($P \geq 30$ MPa) because CO_2 is less compressible and the decrease in its density and solvent power is fully compensated by the increase in vapor pressure and volatility of the oil. In their correlation study for the solubility of vegetable oils in SC-CO_2, del Valle *et al.* (2007) observed an increase in the crossover pressure with temperature within the experimental region, from 28.0-30.0 MPa at 40 ºC to 35.4-37.0 MPa at 60 ºC. Boutin and Badens (2008) experimentally observed crossover phenomena at 30 MPa in the SC-CO_2 extraction of rapeseed oil.

The effect of extraction pressure on the oil extraction yield is more clearly shown by rewriting Equation (5) as follows:

$$Y_{oil} = \alpha(X_3) + \beta(X_2)\left(\frac{P-30}{10}\right) - 22.83\left(\frac{P-30}{10}\right)^2, (5b)$$

where $\beta(X_2) = 30.37 - 7.525\left(\frac{T-50}{10}\right)$.

Equation (5b) represents a parabola having a maximum for $X_1 = \beta(X_2) / 45.66$. These maximal values decrease from 35.0 MPa at 40 ºC to 31.7 MPa at 60 ºC. It is not reasonable to expect the oil extraction yield to decrease as pressure increases within the experimental region, but the response surface for Y_{oil} at pressures near $P \approx 40$ MPa (or $X_1 \approx 1$) is fairly flat, as depicted in Figure 2. This is to be expected when the extraction time is sufficient to remove all available oil and its solubility is sufficiently large.

Figure 2 shows the effect of extraction pressure and temperature on the oil extraction yield for an extraction time of 90 min ($X_3 = 0$); within the selected experimental region, the largest positive effect is that due to the increase in pressure from 20 to 40 MPa. As indicated above, increasing extraction time moves the surface upwards, but to a low extent (16.4 g oil/kg dry substrate) when going from a 1 h

to a 2 h-extraction, which corresponds to only an 11% displacement along the vertical axis of the plot. Boutin and Badens (2008) also concluded that pressure was the most influential factor in the SC-CO₂ extraction of rapeseed oil within their experimental regions (15–45 MPa, 35–75 ºC, 20–120 min extraction).
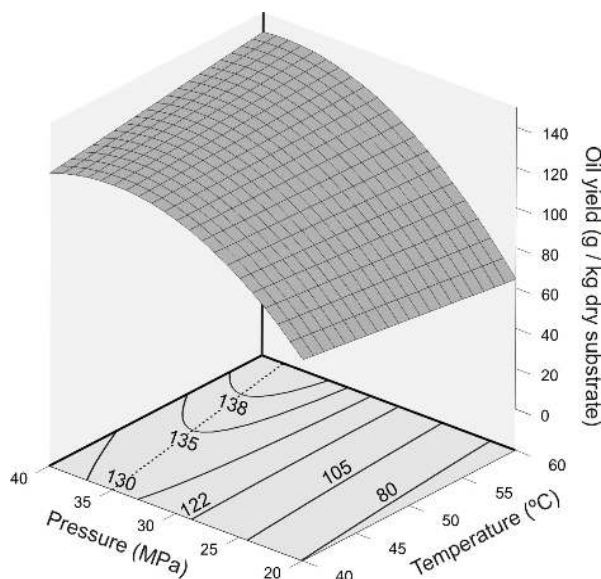
Figure 2: Surface plot of oil yield as a function of extraction pressure and temperature for an extraction time of 90 min. The plot includes level lines for the noted yields (in g oil/kg substrate), as well as the steepest ascent direction (segmented line).

Figure 2 also includes level curves (continuous lines) giving the combinations of extraction pressure (P) and temperature (T) that result in identical yields of oil (Y_{oil}), as well as the direction of steepest ascent in Y_{oil} as a function of P and T (segmented line). Clearly, the level curves correspond to parabolas.

Concentration of Tocopherols

Equation (6) predicts the total concentration of tocopherols (C_{toc}, in milligrams of tocopherol per kilogram of oil) as a function of extraction pressure (P, MPa) and temperature (T, ºC), with all independent variables expressed in coded, dimensionless form (*cf.* Eqs. (1) and (2)).

$$C_{toc} = 837.6 + 34.79\left(\frac{T-50}{10}\right) + \\ 119.0\left(\frac{P-30}{10}\right)^2 - 77.29\left(\frac{T-50}{10}\right)^2 \quad (6)$$

Extraction time (t, min) did not affect the concentration of tocopherols in the extracted oil and the response surface for $C_{toc}(P,T)$ is the same regardless of the value of t.

It is possible to rewrite Equation (6) in different ways so as to show more clearly the effects of extraction pressure (Equation (6a)) or extraction temperature (Equation (6b)) on concentration of tocopherols in the oil, as follows:

$$C_{toc} = \alpha\left(X_2\right) + 119.0\left(\frac{P-30}{10}\right)^2, \text{ or} \quad (6a)$$

$$C_{toc} = \alpha\left(X_1\right) + 34.79\left(\frac{T-50}{10}\right) - 77.29\left(\frac{T-50}{10}\right)^2 \quad (6b)$$

Equation (6a) represents a series of up-going parabolas (the cuts of the response surface at different temperatures) having maximal values for P = 30 MPa, whereas Equation (6b) represents a series of down-going parabolas (the cuts of the response surface at different pressures) having minimal values for T = 52.3 ºC. Overall, the response surface (Figure 3) resembles a saddle, having an inflection point at P = 30 MPa and T = 52.3 ºC and for $C_{toc} = 841.5$ mg tocopherol/kg oil.
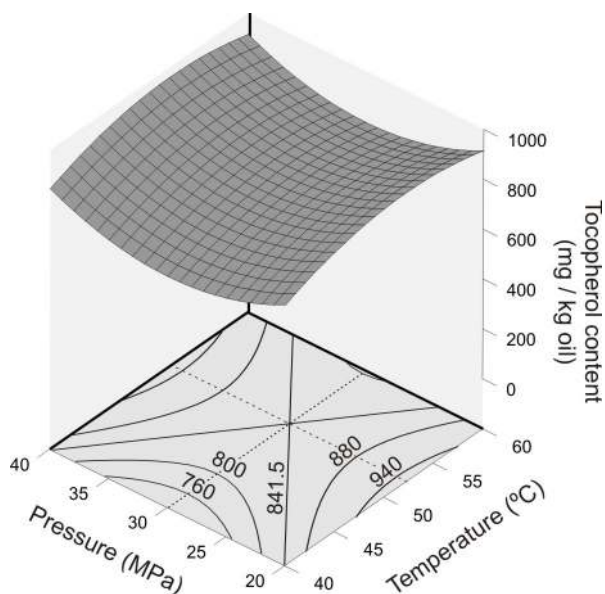
Figure 3: Surface plot of tocopherol concentration in the oil as a function of extraction pressure and temperature for any extraction time. The plot includes level lines for the noted concentrations (in mg tocopherol/kg oil), as well as the steepest ascent directions (segmented lines).

Figure 3 shows the complex influence of extraction pressure and temperature on the concentration of tocopherols in the oil for any extraction time ($60 \leq t \leq 120$ min). Figure 3 also includes level curves (continuous lines, hyperbolas), giving the combinations of extraction pressure (P) and temperature (T) that result in identical concentrations of tocopherols (C_{toc}), as well as the directions of steepest ascents in C_{toc} as a function of P and T (segmented lines). The concentration of tocopherols in the oil goes up as the temperature approaches an intermediate level of 52.3 ºC and as the pressure moves down or up from an intermediate level of 30 MPa. However, it is apparent from Figure 3, whose C_{toc}-axis starts at 0 mg tocopherol/kg oil, that, within the selected experimental region, the response surface is fairly flat, i.e., the concentration of tocopherols in the oil does not depend on extraction conditions to a great extent going from a lower limit value of 726 mg tocopherol/kg oil to an upper limit value of 961 mg tocopherol/kg oil. Consequently, when we fitted a response surface model to tocopherol extraction yield (the product $Y_{oil}C_{toc}$), we obtained an equation that was qualitatively similar to Equation (5), indicating that the tocopherol extraction yield depended highly on the oil extraction yield because of the small changes in tocopherol content in the oil as a function of extraction conditions (results not shown). To the best of our knowledge, there is no information on the effect of SC-CO_2 extraction conditions on the yield of rapeseed tocopherols, but Ge *et al*. (2002) reported that the tocopherol yield from wheat germ increased with system pressure.

Based on the review of Güçlü-Üstündağ and Temelli (2000) on the solubility of major plant lipids (including triglycerides) in SC-CO_2, it can be estimated that the solubility of triolein (a representative component in rapeseed oil) in CO_2 at 30 MPa and 50 ºC is 6.44 g/kg. This value is 3.5 times lower than the solubility of α-tocopherol in CO_2 under equivalent conditions (22.4 g/kg) estimated using the relationship proposed by Güçlü-Üstündağ and Temelli (2000), who reviewed the solubility behaviour of minor plant lipids such as phytosterols, tocopherols, and carotenoids in SC-CO_2. Within the experimental region we studied, the estimated solubility of α-tocopherol in SC-CO_2 is always larger (between 1.9 and 9.4 times larger) than the solubility of triolein, with the differences decreasing with CO_2 pressure and increasing with CO_2 temperature. In the 60 min extraction of 0.0271 kg of rapeseed at 30 MPa and 50 ºC using 7.20 g CO_2/min, or a specific CO_2 mass of 15.9 kg/kg dry

substrate, we obtained Y_{oil} = 110 g/kg dry substrate of oil having C_{toc} = 0.847 g/kg oil (Y_{toc} = 93.5 mg/kg dry substrate) of tocopherols. The ratio between the yields of extract or tocopherols and the specific solvent mass results in an "apparent" solubility of 6.90 g/kg CO_2 of extract (close to the thermodynamic solubility of triolein reported above of 6.44 g/kg) and 5.86 mg/kg CO_2 of tocopherols (much lower than the thermodynamic solubility of α-tocopherol reported above of 22.4 g/kg). Thus, we conclude that the 60 min SC-CO_2 extraction experiment at 30 MPa and 50 ºC is a solubility-controlled extraction, as expected for short extractions under low solubility (low pressure and high temperature) conditions, and that there are no limitations to the extraction of tocopherols other than those imposed by their limited content in cold-press rapeseed cake.
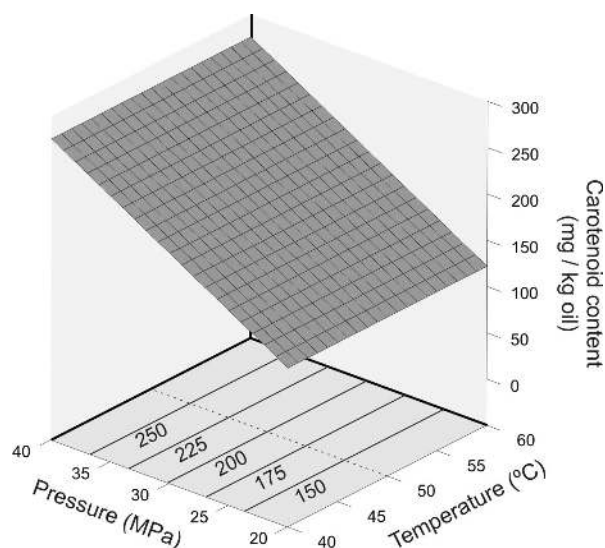
Concentration of Carotenoids

Equation (7) predicts the total concentration of carotenoids (C_{car}, in milligrams of carotenoids per kilogram of oil) as a function of the extraction pressure (P, MPa) expressed in coded, dimensionless form (*cf*. Eq. (1)).

$$C_{car} = 198.7 + 76.78\left(\frac{P-30}{10}\right) \tag{7}$$

Neither extraction temperature nor extraction time affected the concentration of carotenoids in the extracted oil and the response surface for $C_{toc}(P,T)$ (Fig. 4) is flat and does not depend on the value of t. Figure 4 also includes level curves (continuous lines, straight lines) that give the values of the extraction pressure (P) that correspond to the desired concentrations of carotenoids in the oil (C_{toc}), as well as the direction of steepest ascent in C_{car} as a function of P and T (segmented line). The concentration of carotenoids in the oil goes up as pressure increases, ranging from 137 mg carotenoids/kg oil at 20 MPa to 275 mg carotenoids/kg oil at 40 MPa. Furthermore, despite the lower concentrations of carotenoids than tocopherols in the oil (*cf*. Figure 3 and Figure 4), extraction conditions (pressure) affect the concentration of carotenoids more than the concentration of tocopherols. These results agree with those of Martínez *et al*. (2008) for the SC-CO_2 extraction of walnut oil, who found that, under isothermal conditions, an increase in operating pressure above 20 MPa caused a significant increase in β-carotene yield.

Figure 4: Surface plot of carotenoid concentration in the oil as a function of extraction pressure and temperature for any extraction time. The plot includes level lines for the noted concentrations (in mg carotenoid/kg oil), as well as the steepest ascent direction (segmented line).

When we fitted a response surface model to the carotenoid extraction yield (the product $Y_{oil}C_{car}$), we obtained an equation that was qualitatively similar to Equation (7), indicating that the carotenoid extraction yield depended highly on the concentration of carotenoids in the oil (results not shown). The carotenoid content in the oil changed by a factor slightly above 3 within the experimental region, a change that did not differ much from that of the oil extraction yield (slightly below 3), but was considerably larger than the 1.4-fold change in the concentration of tocopherols in the oil. These differences may help explain the qualitative differences in the response surfaces for carotenoid extraction yield and tocopherol extraction yield (results not shown).

Based on the review of Güçlü-Üstündağ and Temelli (2000) on the solubility behaviour of minor plant lipids in SC-CO2, it can be estimated that the solubility of β-carotene in CO2 at 30 MPa and 50 ºC is 4.99 mg/kg. This value is 1300 times lower than the solubility of triolein in CO2 under equivalent conditions (6.44 g/kg) estimated above. Within the experimental region we studied, the estimated solubility of β-carotene in SC-CO2 is always smaller (between 390 and 3400 times smaller) than the solubility of triolein, with the differences increasing with CO2 pressure and decreasing with CO2

temperature. The extract from 0.0271 kg of rapeseed in the 60 min experiment at 30 MPa and 50 ºC using 15.9 kg/kg dry substrate contained C_{car} = 0.148 g/kg oil (Y_{car} = 16.4 mg/kg dry substrate) of carotenoids. The ratio between the yield of carotenoids and the specific solvent mass resulted in an "apparent" solubility of 1.03 mg/kg CO2 of carotenoids, which is slightly (about 5 times) lower than the thermodynamic solubility of β-carotene reported above. Thus, we conclude that, unlike in the case of tocopherols, in the 60 min SC-CO2 extraction experiment at 30 MPa and 50 ºC, the extraction of carotenoids was to an extent solubility-controlled and this helps explain the large differences between the effects of extraction pressure (large; at 60 ºC β-carotene solubility in CO2 increases from 3.63 mg/kg at 20 MPa to 13.0 mg/kg at 40 MPa) and temperature (smaller; at 20 MPa β-carotene solubility in CO2 increases from 1.67 mg/kg at 40 ºC to 3.63 mg/kg at 60 ºC) on concentration of carotenoids in the oil.

For the purpose of comparing SC-CO2-extracted and conventionally-extracted rapeseed oil, we selected operational conditions where the yield is high and solubility-controlled. These can be identified by estimating the "apparent" solubilities as done before for oil (6.90 g/kg CO2), tocopherols (5.86 mg/kg CO2), and carotenoids (1.03 mg/kg CO2) in the 60 min extraction at 30 MPa and 50 ºC. The highest "apparent" solubilities we experimentally measured were 8.25 g oil/kg CO2, 7.80 mg tocopherols/kg CO2, and 2.42 mg carotenoids/kg CO2 in the 60 min extraction at 40 MPa and 60 ºC, which constitutes a high density and temperature combination within our experimental region.

Characteristics of Rapeseed Oil

Table 3 compares the chemical characteristics of rapeseed oils and suggests a higher susceptibility to oxidation of the one extracted with SC-CO2 at 60 ºC and 40 MPa for 60 min than with hexane. The tocopherol content was significantly ($p<0.05$) lower in SC-CO2 than in hexane-extracted oil. List and Friedrich (1985) also found that the concentration of tocopherols in soybean, corn, and cottonseed oils extracted with SC-CO2 was lower than in the oils removed by pressing or extraction with hexane.

Unsaponifiable matter was approximately the same in SC-CO2 and hexane-extracted oil. The unsaponifiable matter of vegetable oils comprises those constituents which, after saponification of the oil, have low solubility in water and high solubility in organic (lipophilic) solvents. Exhaustive

extraction of full-fat soybean flakes with SC-CO$_2$ at 34.5 MPa and 50 ºC yielded an oil having a comparable percent unsaponifiables to hexane-extracted oil (List and Friedrich, 1989). Jenab *et al*. (2006) also found no significant differences in the amounts of unsaponifiable matter in rapeseed oils extracted by SC-CO$_2$ at 40 ºC and 34 MPa and an organic solvent.

Table 3: Chemical characteristics of rapeseed oil extracted using hexane (extraction to exhaustion in a Soxhlet apparatus) or Supercritical CO$_2$ (SC-CO$_2$) at 40 MPa and 60 ºC (60 min extraction).

Indicator	n-Hexane-extracted oil	SC-CO$_2$-extracted oil
Tocopherol content (g / kg oil)[*]	1.290 ± 0.011	0.945 ± 0.004
Carotenoid content (mg / kg oil)*	432 ± 18.6	88.5 ± 4.9
Unsaponifiable matter (g/100 g oil)	1.45 ± 0.07	1.40 ± 0.05
Peroxide value (meq O$_2$ / kg oil)[*]	2.5 ± 0.2	3.8 ± 0.1
p-Anisidine value (arbitrary units)[*]	6.9 ± 0.7	20.7 ± 0.6
Induction time (h)[*]	12.6 ± 0.5	0.30 ± 0.0

[*] Significant (p <0.05) difference between hexane- and SC-CO$_2$-extracted oil.

The peroxide value (PV) measures the amount of peroxides in the oil, which are important intermediates in oxidations because they decompose into highly reactive free radicals via transition metal catalyzed reactions at elevated temperatures. The PV was significantly higher (p < 0.05) in oil extracted with SC-CO$_2$ than with hexane; however, the PV of both oil samples was below the limit for edible oils of 10 meq O$_2$/kg oil. On the other hand, Friedrich and List (1982) claimed comparable PVs of oils from soybean flakes fully extracted with SC-CO$_2$ at 34.5 MPa and 50 ºC or with hexane (0.2 versus < 0.1 meq O$_2$/kg oil).

The p-anisidine test provides useful information on non-volatile carbonyl compounds formed in oils during processing and is often used to quantify secondary oxidation products. Although SC-CO$_2$-extracted rapeseed oil has a significantly (p > 0.05) higher p-aniside value than the hexane-extracted oil (Table 3), both had values above four, the acceptable level for good quality refined oils, making them both highly susceptible to oxidative deterioration.

The induction time (IT) characterizes the ability of the oil to resist oxidation and is an important parameter in identifying conditions that preserve oil

quality. The oil extracted with SC-CO$_2$ (IT = 18 min) had a significantly lower (p < 0.05) oxidative stability than the one extracted with hexane (IT = 12.6 h). Martínez *et al*. (2008) reported IT values ranging from 18 to 47 min for walnut oil extracted with SC-CO$_2$ at 20–40 MPa and 50–70 ºC.

Table 4 shows no difference in fatty acid composition between rapeseed oils extracted with SC-CO$_2$ and hexane. In order of decreasing importance, the most abundant acids in the oils are oleic, linoleic, and linolenic acids, which are all unsaturated fatty acids having 18 carbon atoms. These results are consistent with those of Jenab *et al*. (2006), who evaluated oils from genetically similar variants of rapeseed. The oil had 92.0% unsaturated fatty acids, 32.0% of which were of the poly-unsaturated type. Consequently, SC-CO$_2$ extracted rapessed oil had a 11.5 ratio of unsaturated-to-saturated fatty acids, and a 3.68 ratio of polyunsaturated-to-saturated fatty acids. Very little difference in fatty acid composition was found between rapeseed oil extracted with SC-CO$_2$ and using hexane (except in the composition of fatty acids C18:0, C18:1, n9 trans, C18:1 n9 cis, C18:2), suggesting that the fatty acid content does not depend on extraction method (del Valle *et al*., 2000). Several works have reached a similar conclusion, including the classical one of Friedrich and List (1982) for soybean oil.

Table 4: Composition of fatty acids (%) in rapeseed oil extracted using hexane (extraction to exhaustion in a Soxhlet apparatus) or Supercritical CO$_2$ (SC-CO$_2$) at 40 MPa and 60 ºC (60 min extraction).

Fatty acid	n-Hexane-extracted oil	SC-CO$_2$-extracted oil
C14:0	nd	0.08 ± 0.01
C16:0	5.24 ± 0.16	4.95 ± 0.11
C16:1	0.41 ± 0.01	0.39 ± 0.01
C18:0	2.17 ± 0.01	2.82 ± 0.11
C18:1 n9 trans	0.56 ± 0.01	2.00 ± 0.13
C18:1 n9 cis	56.4 ± 1.1	55.7 ± 1.2
C18:1 n7 cis	5.22 ± 0.11	4.68 ± 0.08
C18:2	nd	0.75 ± 0.01
C18:2n6	19.7 ± 1.1	18.1 ± 1.2
C20:0	0.58 ± 0.01	0.65 ± 0.01
C18:3n3	9.71 ± 0.76	9.27 ± 0.62
C22:0	nd	0.35 ± 0.01
C24:0	nd	0.22 ± 0.01

nd, not detected

Table 5 shows that the concentration and composition of phytosterols were approximately the

same in SC-CO$_2$ and hexane-extracted rapeseed oil. In decreasing orden of importance, the main phytosterols were β-sitosterol, campesterol, and brassicasterol, and there were no significant differences (p > 0.05) in phytosterol composition between the oils obtained using the two extraction methods. These results are consistent with those of Przybylski *et al*. (1998).

Table 5: Composition of sterols (%) in rapeseed oil extracted using hexane (extraction to exhaustion in a Soxhlet apparatus) or Supercritical CO$_2$ (SC-CO$_2$) at 40 MPa and 60 ºC (60 min extraction).

Sterol	n-Hexane-extracted oil	SC-CO$_2$-extracted oil
Cholesterol	0.4 ± 0.0	0.4 ± 0.0
Brassicasterol	12.3 ± 0.3	12.1 ± 0.2
24-Metilencholesterol	0.7 ± 0.1	0.7 ± 0.1
Campesterol	33.0 ± 2.2	32.8 ± 1.7
Campestanol	0.2 ± 0.0	0.2 ± 0.0
Estigmasterol	0.4 ± 0.1	0.4 ± 0.0
δ-7-Campesterol	0.7 ± 0.1	0.7 ± 0.1
Clerosterol	0.5 ± 0.0	0.5 ± 0.0
β-Sitosterol	48.8 ± 3.1	49.1 ± 1.5
Sitostanol	0.2 ± 0.1	0.1 ± 0.0
δ-5-Avenasterol	2.2 ± 0.1	2.3 ± 0.1
δ-5,24-Estigmastadienol	0.4 ± 0.1	0.5 ± 0.1
δ-7-Estigmastenol	0.1 ± 0.0	0.1 ± 0.0
δ-7-Avenasterol	0.1 ± 0.0	0.1 ± 0.0
Total sterols (g/kg oil)	13.4	13.5

Because the negligible differences in the amount and composition of fatty acids and phytosterols in the rapessed oils cannot explain the differences in the oil stability index (IT), we can asume than the differences in IT in favor the hexane-extracted as compared to the SC-CO$_2$-extracted oil are due to differences in minor components of the oils (Pekkarinen *et al*., 1998) such as tocopherols, that perform well as antioxidants. Differences in concentrations of antioxidant compounds other than tocopherols (e.g., phospholipids, phenolic compounds) between the two types of oil may also help explain differences in their oxidative stabilities.

Phospholipids present in significant amounts in conventional crude oils are essentially absent from SC-CO$_2$-extracted corn germ, soybean, and cottonseed oils (List and Friedrich, 1989), or rapeseed oil (Buskov *et al*., 1997). The low extraction efficiency for polar and heavy phospholipids can be attributed to their low solubility in SC-CO$_2$. List and Friedrich (1989) claimed that the absence of

phospholipids was partially responsible for the rapid deterioration of the SC-CO$_2$-extracted corn germ, soybean, and cottonseed oils and their failure to exhibit the normal induction period observed with their conventional expeller and solvent-extracted counterparts. Phospholipids may chelate metal ions in canola oil and may function as peroxy radical scavengers and thereby improve the oxidative stability of the oils (Pekkarinen *et al*., 1999).

Buskov *et al*. (1997) reported that the rapeseed oil extracted with SC-CO$_2$ had a much lower phenol content than the oil extracted with an organic solvent, with sinapic acid being the most concentrated phenolic compound. A linear relationship has been reported between the total phenol content and oxidative stability by Rancimat in sunflower and palm oil (Salta *et al*., 2007). Rapeseeds are very rich in phenolics, mainly sinapic acid (Tuberoso *et al*., 2007), and this may explain the improved oxidative stability of oil extracted with hexane.

CONCLUSIONS

This work investigated the effects of extraction pressure, temperature, and time on oil yield and the concentration of tocopherols and carotenoids in the SC-CO$_2$ extraction of cold-pressed rapeseed cake. It was demonstrated that SC-CO$_2$ can extract the oil with minor lipids, and that pressure within the 20-40 MPa range was the most important parameter affecting oil yield and composition. The RSD revealed that extraction with SC-CO$_2$ at 40 MPa and 60 ºC is recommendable to obtain high yields of oil having high contents of tocopherols and carotenoids. The unsaponifiable fraction and the composition of free fatty acids and phytosterols in the oil extracted with SC-CO$_2$ at 40 MPa and 60 ºC were similar to those of oil extracted by hexane. However, the oxidative stability of the SC-CO$_2$-extracted oil was lower than that of the hexane-extracted oil, presumably due to the presence of antioxidant compounds such as tocopherols, phospholipids, and/or phenolics that are better co-extracted using hexane as the solvent.

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