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ORIGINAL PAPER

Effect of Operating Parameters on Oil and Phenolic Extraction Using Supercritical CO_2

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Abstract Supercritical fluid extraction (SFE) with carbon dioxide was used to extract oil from canola press cake. Different operating conditions, e.g. pressure, temperature, and co-solvent % were investigated to optimize extraction parameters to yield canola meal with <4% oil. The residual oil content in the extracted canola meal reduced to 2.1–2.9% in our experimental trials. Residues of the optimum conditions based on oil yield were compared for the total phenolic content and the main phenolic compounds. Sinapine (the choline ester of sinapic acid) was the major phenolic constituent in both the SFE and *n*-hexane extracted canola meals and press cakes. *n*-Hexane extracted residues showed the retention of the highest sinapic acid, sinapine, sinapoyl glucose and total phenolic contents (mg/g) while the SF-extracted residues showed the lowest values for these compounds.

Keywords Supercritical fluid extraction · Canola press cake · Extraction parameters · Oil yield · *n*-Hexane extraction · Phenolic content

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Introduction

Canola, developed from traditional rapeseed by Canadian plant breeders during the 1970s, is the largest oilseed crop in Canada [1]. The production of canola oil involves two main processes: mechanical pressing and extraction, and further processing to remove impurities. Canola press cake, the by-product of canola oil processing, is used as a high-protein feed ingredient in the poultry, swine, cattle and fish industries [2, 3]. Due to its high oil content, the press cake is further extracted using solvent extraction (typically *n*-hexane) to retrieve much of the residual oil.

In the cold pressing process, oil is primarily removed through mechanical pressing at a relatively low temperature (typically near 60 °C). The cold pressing process results in only partial recovery of oil from the seed which usually yields canola meal of about 15–25% in oil content; therefore, pressing of seeds is often followed by *n*-hexane extraction at higher temperatures to achieve an approximately 96% recovery efficiency [4]. However, extraction requires large amounts of solvent and is very time-consuming [5]. Additional processing (desolventisation/toasting stage) is needed with an operating temperature of 100–110 °C to evaporate the solvent (*n*-hexane) from the meal [6]. Some compounds in the oil may undergo thermal degradation under solvent extraction conditions at high temperatures [7]. There is an increasing demand for new extraction techniques, which have shorter extraction times, no chemical residues, and a safer operation environment, to replace *n*-hexane extraction.

Supercritical fluid extraction has received increasing attention over the past two decades, including its use as a viable alternative to solvent extraction from oilseeds [8–12]. Supercritical fluid extraction utilizes the

combination of temperature and pressure to alter certain fluids to become excellent solvents for particular solutes. A supercritical state for a solvent is achieved when the temperature and pressure of the solvent is raised above its critical points. A supercritical fluid has the characteristics of both gases and liquids.

Supercritical CO_2 extraction of canola seeds and meals has been reported [11, 13–18]. Several researchers studied the supercritical extraction of compounds, such as tocopherols, from rapeseed and canola seed and meals [14, 15]. Other studies focused on the effect of supercritical extraction on extracted oil properties, such as, fatty acid composition, oxidative stability [11, 16–18].

Some recent studies involving supercritical CO_2 extraction of lipids focused on extracting oil from dried distillers grains [5], and grape seeds [19]. While there has been a considerable amount of investigation on supercritical extraction of oil from canola seed and meals, literature is lacking on optimizing extraction conditions, such as, temperature and pressure, to achieve maximum oil extraction from the substrate. The first objective of this work was to investigate the extraction of oil from canola press cake using supercritical CO_2 extraction under different operating conditions, to optimize extraction parameters to yield canola meal with <4% residual oil. The yield of supercritical carbon dioxide extraction was compared with a traditional solvent extraction method (*n*-hexane extraction). The second objective was to examine the retention of phenolic components in the residues under the optimum SFE conditions.

Experimental Procedures

Canola Press Cake Sample Preparation

Samples of canola (Nexera^{TM}) press cake were obtained from Dow AgroSciences (Indianapolis, IN). Press cake was ground using a Retsch PM100 Planetary Ball Mill running at 450 RPM for 5 min to reduce and homogenize the particle size to an average of 473.9 μm. The ground sample was then dried in a freeze dryer (FreeZone Plus 6 Liter Cascade Console Freeze Dry System, Lanconco) at −54 °C and 0.030 mBar for 48 h to bring down the moisture content from 6.29 to 1.64%. The dried sample was then used as the starting material for the supercritical fluid extraction (SFE) and *n*-hexane extraction. The sample was kept at −20 °C in the dark before use for extraction and analysis.

Supercritical Fluid Extraction (CO_2)

Supercritical fluid extraction was carried out in a lab scale unit (SFE 2000, Thar Technologies Inc., Pittsburgh, PA) equipped with 1,000 mL extraction vessel. The supercritical fluid extraction system was operated by passing supercritical state CO_2 (with or without co-solvent) through a fixed bed of sample particles, precipitating liquid extract in cyclone separators and finally releasing CO_2 to the ambient surroundings. A high pressure pump (P-50, Thar Technologies, Inc., Pittsburgh, PA) was pre-cooled to 4 °C using a cooling heat exchanger (Fig. 1) in order to

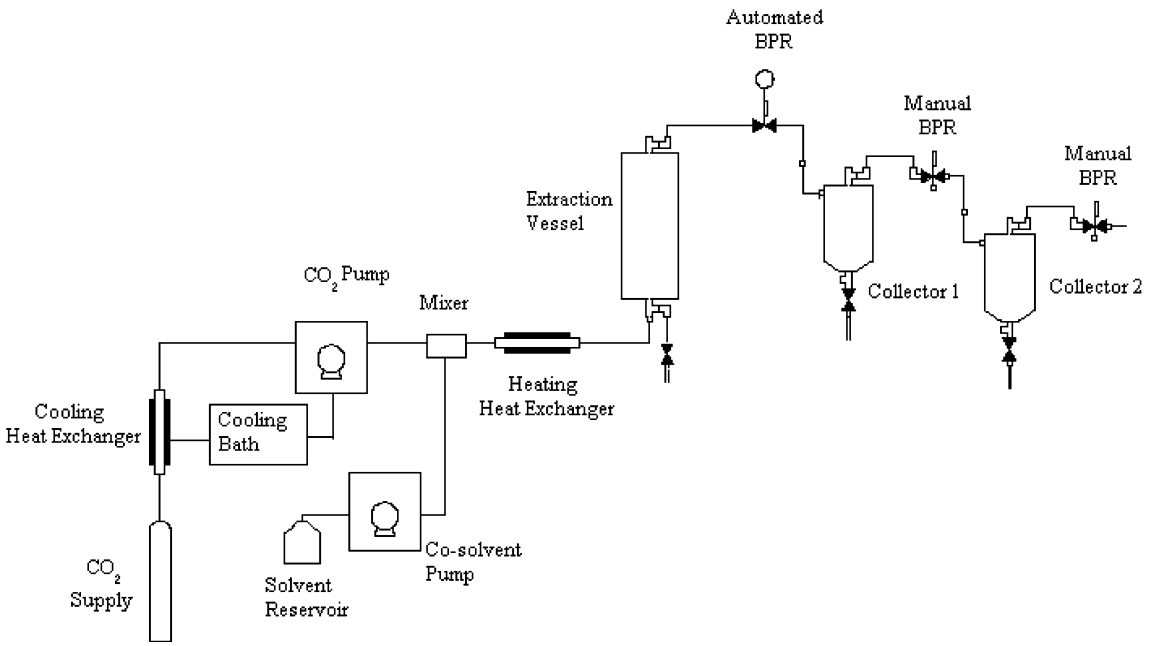

Fig. 1 Schematic diagram of a pilot scale supercritical fluid extraction system (Developed by RCFFN and Thar Technologies Inc.)

deliver liquid CO_2 from a storage cylinder (Medigas Manitoba Ltd., Winnipeg, MB) to the extraction vessel efficiently. The purity of the CO_2 used is around 99%. A second P-50 pump was used as a modifier pump to deliver co-solvent (optional) to a mixer where the co-solvent mixed with CO_2 before entering into the extraction vessel.

Pressure in the extraction vessel was controlled by an automatic back pressure regulator and the pressures in the cyclone separators were maintained by a manual back pressure regulator. The back pressure regulators were usually heated to 40 °C to avoid freezing upon depressurization. The temperature of the extraction vessel and the cyclone separators was maintained by separate heaters. Thermocouples were used for instant feedback in all the temperature zones. The extraction parameter (temperature, pressure etc.) was controlled by the Thar software on the computer. The exiting fluid of CO_2 from the separator was expanded to the atmosphere through an outlet at the back of the separator pressure controller when the controller was opened manually at the end of the extraction process.

For each run, a total of 150 g canola press cake sample was loaded into the extraction vessel. Extraction temperature was set at 40, 50 or 60 °C. The extraction pressure was set at 300, 400 or 500 bar. The total flow rate of CO_2 was set at 70 g/min.

Co-solvent was added by either pumping it from the solvent supply bottle at a certain flow rate (10%) or by pouring a certain volume (2.3%) directly into the sample extraction vessel before each extraction process (Table 1). Food-grade ethyl alcohol (Commercial Alcohols, Brenntag Canada) was used as the co-solvent in these experiments. The composition of the ethyl alcohol used was 95% EtOH and 5% distilled H_2O. Table 1 shows the detailed extraction parameters. For each parameter, extraction was conducted in duplicate and therefore there were two extracted samples used for each analysis. For each collected sample, oil content analysis and phenolic compounds analysis were performed in tandem.

n-Hexane Extraction of Canola Press Cake

n-Hexane extraction was conducted to obtain a comparison with SF-extraction. A laboratory scale Soxtec unit (Soxtec™ 2050, Foss, Eden Prairie, MN) was used to extract oil from canola press cake using *n*-hexane. Fifteen grams of ground canola press cake (sieved through 14 mesh or 1,410 μm screen) was extracted with 80 mL of *n*-hexane for 95 min. Extraction was repeated twice for each sample and then the extracted oil and defatted residues were cooled down to room temperature in the fume hood. *n*-Hexane in the oil was removed using a rotary evaporator R-205 (BÜCHI Labortechnik AG, Switzerland) and dried under nitrogen (N_2) to a negligible amount and the oil was used for further studies.

Oil Content Analysis for SFE

The SF-extracted press cake was spread overnight in the fume hood to remove the residual ethanol prior to determination of the oil content. The initial and final oil contents of the canola press cake samples before and after

Table 1 Supercritical fluid extraction parameters and concentration of residual oil in the extracted cake for each sample trial

Parameter [a,b]	Total flow rate (CO_2) (g/min)	Co-solvent %	Extraction vessel Temperature (°C)	Operating pressure (bar)	Cyclone separator pressure (bar)	Concentration of residual oil in the extracted sample (g/100 g ± SD)[e,d]
EP 1	70	10[b]	40	300	60	10.3 ± 0.8a
EP 2	70	10[b]	40	300	70	6.7 ± 0.1b
EP 3	70	10[b]	50	400	50	8.2 ± 0.07c
EP 4	70	2.3[c]	50	300	60	8.7 ± 0.03c
EP 5	70	2.3[c]	50	400	60	2.9 ± 0.2d
EP 6	70	2.3[c]	60	400	60	2.4 ± 0.03d
EP 7	70	2.3[c]	50	500	60	2.1 ± 0.05d

[a] EP, extraction parameter; the total flow rate of CO_2 was kept at 70 g/min, the collector temperature was maintained at 40 °C, and the extraction time was 4 h long for all the above experimental runs

[b] Trials with 10% co-solvent were carried out as follows: co-solvent was run only for the first 1 h then shut off and CO_2 was run for another 3 h; co-solvent 10% means a co-solvent flow rate of 8 g/min, it was calculated based on the total flow rate

[c] Co-solvent 2.3% means 500 mL ethanol, it was calculated based on the total weight of CO_2 consumed in the 4 h extraction process

[d] The initial oil content of canola press cake was 21.06% ± 0.1 (g/100 g); the concentration of oils in the extracted sample (g/100 g)

[e] Values are means of four replications for each extraction parameter. Means ($n = 4$) with different letters are significantly different by LSD ($p = 0.05$)

supercritical CO_2 extraction were determined using the traditional Soxhlet method (ISO/DIS 659: 2007) to compare the efficiency among the different extraction parameters. The initial oil content of the canola press cake was analyzed twice and the reported data was the mean of the two replications. To determine oil content after SFE, replications were done for each sample, and thus the reported yield was the mean of four replications for each extraction parameter. Residual oil percentages in the extracted sample with different extraction parameters were compared and statistically analyzed by least significance difference method (LSD).

Phenolic Content of SFE Extracted Residue

The extracts were left overnight in the fume hood, evaporated the next day using the rotary evaporator, followed by drying under nitrogen (N_2) to remove the residual ethanol prior to determination of phenolics. As per the oil content analysis after SFE, the reported yield for phenolic content (Table 3) was the mean of four replications of the two samples for each extraction parameter. The total phenolic content was determined by Folin-Ciocalteu's reagent method according to Swain and Hillis [20] and Schanderl [21] with some modifications. Aliquots (0.2 mL) of extracts were diluted to 0.5 mL with distilled water, and Folin–Ciocalteau's phenol reagent (0.5 mL) was added. After 3 min, 19% sodium carbonate (1 mL) was added. After 60 min, the absorption was measured at 750 nm using the DU 800 UV/Visible Spectrophotometer (Beckman Coulter Inc., Mississauga, ON, Canada). Sinapic acid (Sigma) was used for the calibration, and the results of duplicate analyses were expressed as sinapic acid equivalents (SAE). The main phenolic components were estimated according to Khattab et al. [22] using the reversed-phase DAD-HPLC (Ultimate 3000; Dionex, Sunnyvale, CA, USA). A gradient elution was performed using water/methanol (90:10) with 1.2% o-phosphoric acid as solvent A, and methanol (100%) with 0.1% o-phosphoric acid as solvent B, using C18 column (Synergi 4μ Fusion-RP 80 A°; 150 × 4.0 mm 4 micron; Phenomenex, Canada). The column was maintained at 25 °C and different the flow rate was 0.8 mL/min. Chromatograms were acquired at 230, 275 and 330 nm and data were analyzed using the Chromeleon software (Version 6.8). Sample chromatograms were compared with authentic standards of sinapic acid, sinapine and sinapoyl glucose. Identity and purity of the phenolic compounds peaks were monitored through the 3d chromatograms, in addition to monitoring retention times and the symmetry of the peak. The method was validated according to the ICH guidelines [23]. Total phenolics and main phenolic compounds (sinapic acid, sinapine, sinapoyl glucose) in canola press cake residues extracted with Soxtec and SFE were compared and statistically analyzed by LSD (Table 3).

Results and Discussion

Effects of SFE Parameters on Oil Yield of Canola Press Cake

In order to improve the extraction efficiency, the press cake was subjected to freeze drying and fine milling before supercritical CO_2 extraction. Moisture interferes with the efficiency of SFE of oil, in part by inhibiting contact between the supercritical CO_2 and the sample [24]. High moisture content of the sample acts as a barrier to diffusion of supercritical CO_2 into the press cake as well as diffusion of oil out of the press cake [11]. Removal of water by freeze drying prior to supercritical extraction has been recommended [25]. Freeze drying and fine milling will also increase the surface area and therefore improve the diffusion of the extraction fluid through the matrix [25]. For commercial purposes, additional research will be required to determine whether supercritical fluid extraction immediately following cold pressing is as efficient as drying and fine milling of press cake prior to extraction.

Extraction yield of oil using SFE is affected by two factors: (a) the properties and stability of the substrate, and (b) the solvent effect of the supercritical fluid, which is a function of operating parameters of the SFE unit. The SFE operating parameters impact fluid density and solubility and these include pressure, temperature and choice of co-solvent [26–30]. The solvent power (and hence control of selectivity) of a supercritical fluid can be adjusted by these parameters [2].

The last column of Table 1 shows the residual oil remaining in the canola press cake following supercritical fluid extraction using the corresponding parameter combinations outlined in the table. Many combinations of temperatures, pressures, CO_2 flow rates, and co-solvent flow rates were initially evaluated for extraction yield (data not shown), but the parameter combinations shown in Table 1 are indicative of the range of extraction efficiency for canola oil from press cake.

In this study, the total CO_2 flow rate was held constant at 70 g/min. This was the minimum flow rate of CO_2 that would adequately pressurize the extraction vessel. The press cake was soaked in the EtOH at room temperature/pressure for 1 h before the extraction process. The collection vessel temperature was also held constant at 40 °C and the run time was terminated after 4 h (Table 1). Higher oil extraction yield does not appear to be correlated with higher CO_2 flow rate [31, 32]. The main impact of flow rate does appear to be a reduction in extraction time (see Table 2).

Table 2 Comparisons of different operating parameters for supercritical CO_2 extraction of oil from canola

Method	Materials	Extraction pressure (bar)	Extraction temperature (°C)	Extraction time (h)	CO_2 flow rate	Mass ratio (g of CO_2/g of sample)	Use of co-solvent (wt%)	Initial oil content %[b]	Yield%	Residual oil %
Current RCFFN trial[e]	Ground canola press cake	400	50	4	70 g/min	112.0	2.3% ethanol[a]	21.1a	18.2b	2.9c
	Ground canola press cake	500	50	4	70 g/min	112.0	2.3% ethanol[a]		19.0b	2.1c
Stahl et al. [3]	Ground rapeseeds	320	17	3	N/A	N/A	None	40.1	38.2	4.5
		350	40	3	N/A	N/A	None		39.3	3.9
Dunford and Temelli [11]	Cooked flaked canola seed	414	75	3	2.5 g/min	12.9	None	N/A	11.4	32.6
		414	55	3	2.5 g/min	12.9	None		10.9	33.1
	Preheated flaked canola seed	414	75	3	2.5 g/min	12.9	None		11.1	29.3
		414	55	3	2.5 g/min	12.9	None		8.0	32.4
Taylor et al. [37]	Canola flakes	680	80	1.5	5 L/min (9.9 g/min)[c]	254.6	None	40.5	39.8	N/A
Temelli [8]	Canola flakes	620	70	5	0.65 L/min (1.29 g/min)[c]	7.7	5 g ethanol (1.3% ethanol)[a,c]	N/A	28	N/A
	Canola press cake	620	70	5	0.65 L/min (1.29 g/min)[c]	7.7	5 g ethanol (1.3% ethanol)[a,c]		22	N/A
Barthet and Daun [38]	Ground canola seed (*Brassica napus*)	517	100	0.5	2 mL/min (0.004 g/min)[c]	0.07[d]	None	47.26	40.32	N/A
		517	100	1	2 mL/min (0.004 g/min)[c]	0.14[d]	15% ethanol		46.27	N/A
	Ground Canola seed (*Brassica rapa*)	517	100	0.5	2 mL/min (0.004 g/min)[c]	0.07[d]	None	44.03	38.32	N/A
		517	100	1	2 mL/min (0.004 g/min)[c]	0.14[d]	15% ethanol		42.34	N/A

[a] The percentage of ethanol used for our experiment and the percentage calculation of ethanol for Temelli [8] were calculated based on the total weight of CO_2

[b] The initial oil content was measured using *n*-hexane extraction

[c] The conversion of the volume (L) to Mass (g) of CO_2 and mass (g) to wt% of ethanol was calculated based on their density at standard temperature and pressure

[d] Sample weight of 2 g was used to calculate mass ratio for Barthet and Daun [38]

[e] Means ($n = 2$) without letters in common differ significantly by LSD ($p = 0.05$) for the initial oil content %, means ($n = 4$) without letters in common differ significantly by LSD ($p = 0.05$) for the Yield% and Residual oil%

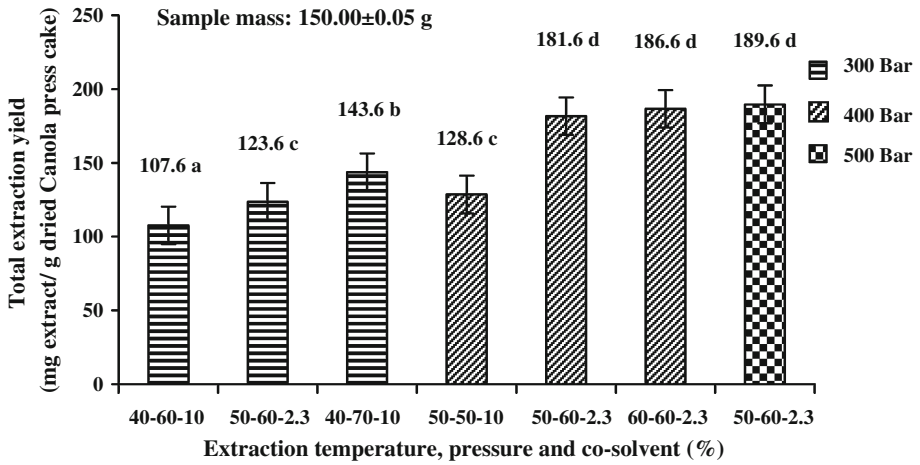

Fig. 2 Effect of extraction pressure and temperature, collection vessel pressure and co-solvent on the yield of oil during supercritical CO_2 extraction of canola press cake. The *symbols* under each *bar* indicate the actual extraction parameters. The first two digits indicate the extraction temperatures (40, 50, 60 °C); the third and fourth digits indicate pressure (50, 60, 70 bar) in the first collection vessel; and the last two digits indicate the use of co-solvent (2.3 and 10%, respectively). Example: 40–60–10 = 40 °C, 60 bar, 10% co-solvent. *Bars* with *different letters* are significantly different by LSD ($p = 0.05$)

The concentration of residual oil in the press cake was reduced with increasing extraction vessel temperature (Table 1). Setting the extraction vessel temperature to 50 °C and operating pressure to 500 bar resulted in the lowest level of residual oil (2.1 g/100 g press cake) remaining in the press cake following extraction (Table 1 Treatment EP7). Increasing the extraction vessel temperature by 10 °C (60 °C) and reducing operating pressure to 400 bar (Treatment EP6) left a slightly higher level of residual oil remaining in the press cake (Table 1).

The impact of supercritical fluid extraction parameters on yield of oil are shown in Fig. 2. Increasing extraction the vessel operating temperature resulted in higher oil yields. For example, 50 °C increased the extraction yield relative to 40 °C. As in the case of pressure, extraction yield increase with an increase in the extraction temperature for the temperature range studied. This temperature effect has been reported in several instances [17, 33–36].

The oil yield was further enhanced by increasing the pressure in the first collection vessel. To be consistent throughout, pressure in the second collection vessel was not measured, as the heater and the pressure of the second collection vessel were not turned on. No oil was collected in this vessel. The yield reported was based on the amount of extract collected in the first collection vessel only. Increasing extraction pressure increases the density of the supercritical CO_2 which in turn increases its solvating power. Pressure impacts the rate of extraction and extraction time. Typically, increasing pressure increases extraction yield and reduces extraction time necessary to reach optimum yield [11, 13, 29]. It is possible that higher extraction levels could be achieved with elevated operating pressure in our study, but due to equipment limitations higher pressures could not be explored at this time.

Adding ethanol as a co-solvent (2.3%) directly to the press cake in the extraction vessel was typically more efficient than pumping 10% ethanol for 1 h into the extraction vessel (Fig. 2), especially when the operating pressure was increased from 300 to 400 bar. At 300 bar, co-solvent at 10% was more efficient. Increasing the operating pressure from 400 bar to 500 bar had very little impact on oil yield. Oil yield increased by only 3 mg/g of dried press cake. 500 bar pressure is at the upper limit of operating pressure for the Thar unit. In conclusion, optimum oil yields were obtained with extraction vessel temperatures of 50 or 60 °C, a collection vessel pressure of 60 bar and an operating pressure of 400 bar in our experiments.

Comparison of Different Supercritical CO_2 Oil Extraction Parameters for Canola

Table 2 provides comparative data for different operating parameters for supercritical CO_2 extraction of oil from canola from published sources as compared to our results. Extraction pressure and temperature have been shown to influence oil extraction yield. Table 2 indicated that there appeared to be a trend to higher oil yield with increased extraction pressure and temperature [11, 13]. The experimental result from Barthet and Daun [38] indicated that extraction oil yield could also be improved by elevating total mass ratio.

Table 3 Individual and total phenolic compounds of canola extracts

	Phenolic compounds (mg/g)				
	Sinapic acid	Sinapine	Sinapoyl glucose	Total phenolics (SAE)	
				Folic Cio	HPLC
Soxtec extracted	0.32 ± 0.00a	10.39 ± 0.01a	1.48 ± 0.02a	20.96 ± 0.33a	13.14 ± 0.09a
SFE 1 (EP2+)	0.18 ± 0.00b	8.05 ± 0.00b	1.18 ± 0.02b	12.67 ± 0.20b	10.10 ± 0.00b
SFE 2 (EP3+)	0.27 ± 0.02c	7.03 ± 0.00c	2.21 ± 0.15c	12.62 ± 0.11b	9.48 ± 0.63c
SFE 3 (EP4+)	0.31 ± 0.00a	7.88 ± 0.04d	2.43 ± 0.01d	14.15 ± 0.09c	10.67 ± 0.12d

Values are the means of four replications ± standard deviation (SD), values followed by different letters in the same column are significantly different by LSD ($p = 0.05$)

SAE sinapic acid equivalents; *Folic Cio* Folin-Ciocalteu's reagent method; *HPLC* high performance liquid chromatography; *EP* extraction parameter, see Table 1 for SFE parameters of EP2, EP3, and EP4

Although the initial oil contents of samples were different in the trials listed in Table 2, the efficiency of supercritical CO_2 oil extraction could be evaluated by the following two aspects: (1) oil yield of SFE versus that of *n*-hexane extraction; (2) the residual oil left in the extracts. Most of the experimental results in Table 3 indicated that canola samples were successfully extracted with supercritical CO_2 at a comparable oil level to that obtained with standard *n*-hexane extraction procedure. Most of the trials shown in Table 3 did not aim at lowering residue oil level, except Stahl et al. [13] who reported low residual oil content at 3.9%. Our trials revealed that the lowest residual oil values of 2.1 and 2.9% were achieved with operating pressures of 400 and 500 bar, respectively. In addition, our extraction vessel temperatures were comparable to many previous studies and our extraction pressures were within the upper and lower limits reported in Table 2. Table 2 also indicates that the consumption of CO_2 in our experiments were considerably higher than most trials focused on oil extraction efficiency, which is mainly due to the high capacity of our extraction unit.

Phenolic Compounds in Supercritical CO_2 and *n*-Hexane Extracted Residues

Seventy percent aqueous methanolic extracts were evaluated for the retention of the phenolics in the residues of the SFE and *n*-hexane extracted press cake. Figure 3 shows the HPLC chromatogram of canola extract. To demonstrate the method specificity, the peak purity tests were performed and the diode array analysis showed that each chromatographic peak was attributable to a single component (graph B in Fig. 3). Contents of the total phenolics in canola extracts are illustrated in Table 3. It was found that sinapine (the choline ester of sinapic acid) was the major phenolic constituent in both the SF and *n*-hexane extracted canola meals and press cakes.

In addition to sinapine, residues of the press cake after SFE- and Soxtec-extraction indicated the presence of sinapic acid, sinapoyl glucose as the main phenolic compound. Four other compounds were present which could not be identified. Soxtec-extracted residues showed the retention of the highest sinapic acid, sinapine, sinapoyl glucose and total phenolic contents (mg/g) as indicated in Table 3. The SF-extracted residues showed the lowest values for these compounds. The difference in the individual compounds as well as in the total phenolic contents between the *n*-hexane and the SFE-defatted substrate could be attributed to the use of added ethanol, which is a polar organic solvent. Ethanol aided SF-extraction was capable of extracting the polar phenolic compounds as compared to *n*-hexane extraction. These results agree with previous work reported by Sun et al. [12] who found that the total phenolic contents of the SC-CO_2 + ethanol-defatted canola meal were significantly ($p \leq 0.05$) lower than those of the *n*-hexane-defatted one indicating that SC-CO_2 + ethanol extraction removed 37.9% of the phenolic compounds from the meal. They also stated that the content of soluble tannins in the SC-CO_2 + ethanol extract was significantly higher than the amount retained in the meal as the ethanol was able to break the protein- and carbohydrate-tannin complexes and release some tannins into the extract. Thus, ethanol enhanced the extraction of phenolics. Furthermore, hexane-extracted press cakes as compared to SFE/EtOH extracted press cakes are potential sources of the phenolic compounds. More studies are needed to examine the retention of individual phenolics using ethanol and SFE.

To conclude our findings, in the press cake substrates tested oil content could be further reduced to <4% using SFE. Retention of phenolic compounds can be influenced

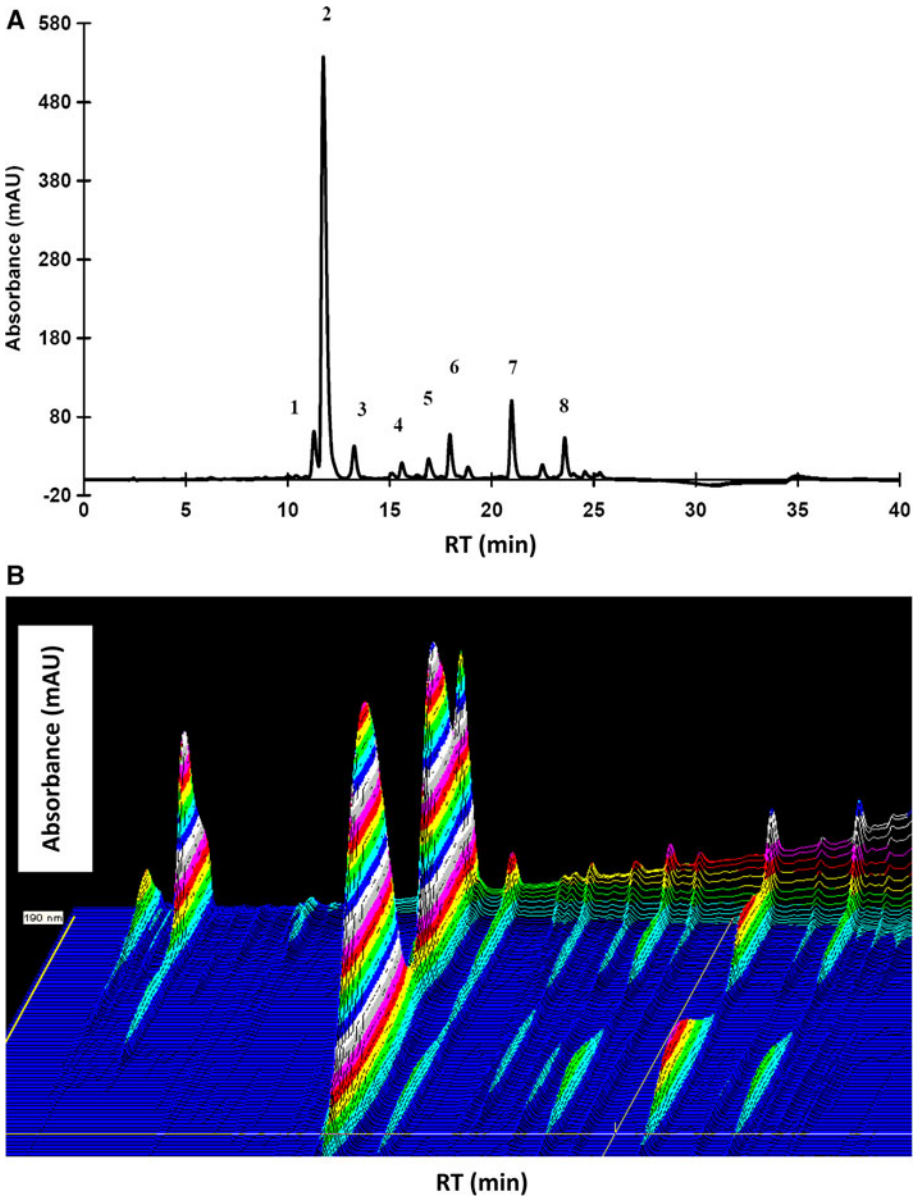

Fig. 3 **a** Typical elution profile including retention times (RT) of phenolics of canola extract; **b** 3d HPLC chromatogram [*x* axis: RT, *y* axis: Absorbance (mAU)]. **a** *1* sinapoyl glucose, *2* sinapine, *4* sinapic acid, *3*, *5*, *6*, *7* and *8* other unidentified sinapic acid derivatives

by the use of ethanol as a co-solvent. HPLC-DAD results indicate that sinapine, sinapic acid and sinapoyl glucose are present in the residues.

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