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ORIGINAL PAPER

# Column Fractionation of Canola Oil Deodorizer Distillate Using Supercritical Carbon Dioxide

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**Abstract** Semi-continuous column fractionation of canola oil deodorizer distillate using supercritical $CO_2$ ($SCCO_2$) was carried out to determine the feasibility of value-added processing of this feed material for the recovery of bioactive components such as sterols and tocopherols and to determine the effect of operating conditions [pressure (20, 25 MPa using a temperature gradient of 70–100 °C), temperature (70, 100 °C) and a linear temperature gradient (70–100 °C at 25 MPa)] on extract yield and separation efficiency. Total extract yield increased significantly ($p \leq 0.05$) with pressure, whereas at isobaric conditions (25 MPa) the highest yield was obtained at the lowest temperature tested (70 °C). Fractionation efficiency was reflected in the composition of fractions and was affected by operating conditions. Residue composition was determined by extract yield in addition to selectivity. Use of the thermal gradient (70–100 °C) decreased the content of volatiles, free fatty acids and tocopherols while increasing sterol content significantly ($p \leq 0.05$) to a level of 40% (GC area %) in the residue obtained at 25 MPa. The findings indicate the potential of canola oil deodorizer distillate as a source of sterols and warrant further research on the countercurrent column fractionation to improve the separation efficiency.

**Keywords** Canola · Column fractionation · Deodorizer distillate · Supercritical carbon dioxide · Sterol · Tocopherol

Vegetable oil deodorizer distillate, which is a by-product of the conventional refining process, is a very complex mixture containing aldehydes, ketones, free fatty acids (FFA), glycerides and bioactive components such as sterols and tocopherols and has been used commercially for the production of these components [1, 2]. Value-added processing of canola oil deodorizer distillate will benefit the canola oil processing industry in Canada considerably as canola is the leading oilseed produced and processed in Canada [3] where canola oil deodorizer distillate is either added back to canola meal for use as animal feed or sold to leading manufacturers of sterols and tocopherols for further processing.

Supercritical carbon dioxide ($SCCO_2$) has been widely used in the last decade for the processing of fats and oils for fractionation purposes [4]. Although the concentration of sterols and tocopherols from soybean deodorizer distillate [5–8] and squalene from olive oil distillate [9] using $SCCO_2$ have been investigated, fractionation of canola oil distillate has not been reported. Ramamurthi et al. [10] and Ramamurthi and McCurdy [11] studied the esterification and subsequent processing (vacuum distillation) of soybean and canola oil deodorizer distillates and noted that canola oil distillate can be used as a cheap source of fatty acids (FA) due to its high FFA and low sterol and tocopherol contents.

Therefore, $SCCO_2$ column fractionation of canola oil deodorizer distillate was carried out to determine the feasibility of this process for the concentration of sterols and tocopherols and to determine the effect of operating conditions (temperature and pressure) on extract yield and fractionation efficiency.

## Experimental Procedures

### Materials

Canola oil deodorizer distillate was supplied by CanAmera Foods (Wainwright, AB, Canada). All lipid standards used

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for gas chromatography (GC) analysis were from Sigma Chemical Co. (Oakville, ON, Canada) and were 99% pure unless otherwise noted. HPLC grade hexane and chloroform and ACS grade pyridine were obtained from Fisher Scientific (Fair Lawn, NJ, USA). The derivatization agent, Sylon BFT (*N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA): Trimethylchlorosilane (TMCS), 99:1) was from Supelco Inc. (Bellefonte, PA, USA) and ethanol (100%) was supplied by Commercial Alcohols Inc. (Winnipeg, MB, Canada). Liquid $CO_2$ (bone dry) used in the fractionation experiments and GC gases, air, helium, hydrogen and nitrogen were from Praxair Canada Inc. (Mississauga, ON, Canada).

## $SCCO_2$ Fractionation Column

A $SCCO_2$ fractionation column built in-house was used for the fractionation experiments (Fig. 1). The column (3.1 m) consists of five sections (2.54 cm (1″) o.d., 1.75 cm (0.688″) i.d.), which are connected through cross or tee fittings. The 0.635 cm (1/4″) processing lines for feed, $CO_2$ introduction and sampling are connected to the column through these fittings. Each section is heated by two band heaters wrapped around the column wall and connected to a separate temperature controller, which reads the temperature from a thermocouple (Type J) placed on the outer wall in the middle of each section. This allows the temperature of each section to be controlled independently and a temperature gradient maintained along the column. An internal reflux can thus be created by establishing an increasing temperature profile along the column height provided that the solubilities of some of the feed components decrease with increasing temperature in the experimental range. The overall effect of an isobaric increase in temperature on solubility is determined by the interplay of two competing effects: a decrease in solvent density and an increase in solute vapor pressure with temperature. $CO_2$, feed and sampling lines and the expansion valves can be heated using Glasrope heaters connected to rheostats to avoid condensation on the inside walls of the tubing and freezing at the expansion valves. Two sampling lines are connected at the top and bottom of the column to collect extract and raffinate samples, respectively. The sampling lines contain on/off valves, whereas a metering valve is also added to the extract line for manual control of the flow rate. A pressure gauge is used at the top of the column to monitor column pressure. All column components (rated at 68.8 MPa) were manufactured by Autoclave Engineers Inc. (Erie, PA, USA) and purchased from Zimco Valves and Tubings (Calgary, AB, Canada). All electrical/heating components were supplied by Tru-temp Electric Heat Ltd. (Edmonton, AB, Canada). The column is packed with 0.41 cm (0.16″) stainless steel

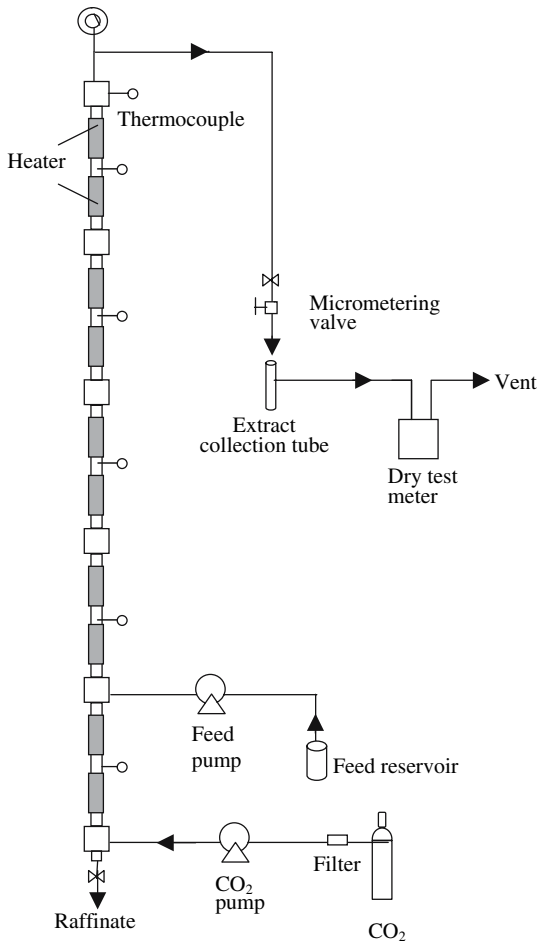


**Fig. 1** Schematic diagram of $SCCO_2$ fractionation column

Propak packing material (Cannon Instrument Company, State College, PA, USA). An ISCO dual pump system consisting of two syringe pumps coupled to a single controller (260D, Perkin Elmer Life and Analytical Sciences, Woodbridge, ON, Canada) was used to deliver $CO_2$ continuously to the column from the bottom zone.

## Experimental Design and Operating Protocols

Semi-continuous fractionation of canola oil deodorizer distillate was carried out to determine the effect of pressure (20, 25 MPa using a temperature gradient of 70–100 °C), temperature (70, 100 °C) and a linear temperature gradient (70–100 °C at 25 MPa) on extract yield and separation efficiency. In a typical run, the column was heated and pressurized with $CO_2$ after 25 mL of melted feed material was introduced into the column using a peristaltic pump (Watson-Marlow 320 F, Watson-Marlow Bredel Inc., Wilmington, MA, USA). The amount of feed introduced into the column corresponded to approximately 1/5 of the internal void volume of one column section. Therefore, no

carry over of the feed material due to $CO_2$ flow was expected.

After the desired temperature and pressure conditions were achieved, the extract valve was opened and $CO_2$ flow thus started. $CO_2$ stream leaving the column at the top was expanded to atmospheric pressure through a heated valve while the extract was collected into side-armed glass tubes immersed in an ice water bath. $CO_2$ passed through a gas meter to measure its volume and was then vented. $CO_2$ flow rate was maintained at $3 \pm 0.3$ L/min (measured at ambient conditions) using the micrometering valve. Six fractions were collected in each run (1 fraction/30 min), which lasted a total of 3 h. However, as no sample was collected in the first half hour in any of the runs, data for five fractions were reported (such that the first fraction corresponds to 1 h of fractionation). This time lag is attributed to the residence time of $CO_2$ in the column, which is dependent on $CO_2$ flow rate and column volume (determined by column dimensions). It is estimated that the amount of $CO_2$ pumped through the column over a 30 min period corresponded to 40–60% of the internal void volume of the column depending on the operating conditions.

The yield of each fraction was determined gravimetrically. The fractions were transferred into a sample vial using hexane, which was evaporated under a gentle flow of $N_2$ after the transfer. After the last fraction was collected, the column was depressurized and the raffinate/residue was collected using the raffinate valve at the bottom of the column during depressurization. After each run, the column was cleaned by pumping ethanol (200 mL) first and then by pumping $CO_2$ at 20–25 MPa and 70 °C with an additional 30 mL ethanol for 3 h.

## Compositional Analysis

Compositional analyses of the feed, residue and extract fractions obtained at different operating conditions were carried out using a gas chromatograph (Varian 3600, Varian Canada Inc., Mississauga, ON, Canada) equipped with a capillary DB-5 HT column (30 m, 0.25 mm i.d., 0.1 μm film thickness; J&W Scientific/Agilent Technologies, Palo Alto, CA, USA) and flame ionization detector. A GC method reported by Verleyen et al. [12] was adopted after modifications to ensure the elution of triglycerides. The parameters of the GC method were: initial column temperature: 60 °C for 2 min, ramp rate 30 °C/min to 140 °C, ramp rate at 5 °C/min to 235 °C, held at 235 °C for 7 min, ramp rate at 5 °C/min to 350 °C, held at 350 °C for 3 min, ramp rate at 10 °C/min to 380 °C, held at 380 °C for 14 min; initial injector temperature: 70 °C for 0.1 min, ramp rate 150 °C/min to 370 °C, held at 370 °C for 69 min; and detector temperature = 370 °C. This method enabled the analysis of all the components of interest (FFA, mono-, di- and triglycerides (MG, DG, TG), sterols and tocopherols). Fatty acid composition of the canola oil deodorizer distillate was determined using a SP 2560 capillary column (100 m, 0.25 mm i.d., 0.2 μm film thickness; Supelco, Bellefonte, PA, USA) according to procedures outlined by Yurawecz et al. [13]. FFA content of the feed was determined by titration using AOCS Method Ca 5a-40 [14].

## Preparation of Samples and Standards

Samples and standards were derivatized with Sylon BFT prior to GC analysis to increase their volatility. An aliquot of sample (20 mg) was weighed into a test tube and dissolved in 0.5 mL pyridine. After addition of 0.5 mL Sylon BFT, the tube was placed in an oven at 70 °C for 20 min for the completion of the derivatization reaction. Individual standards (10 mg) were derivatized using the same procedure. The mixture was then diluted with the addition of 9 mL chloroform. Standard solutions were prepared by transferring 0.05 mL aliquots of each standard into a GC vial and adding 1 mL chloroform. Sample (0.5 mL) was transferred to a GC vial for analysis where it was diluted by the addition of 0.5 mL chloroform.

## Qualitative Analysis

Qualitative analysis was carried out by comparing the retention times of standards with those of mixture components. Standard mixtures were analyzed in parallel with every batch of sample (12 vials/batch). Typical chromatograms for the standard mixture and the sample are given in Fig. 2. Deodorizer distillate is a complex mixture of various lipid classes such as FFA, MG, DG, TG, tocopherols and sterols [12]. Every major lipid class in turn contains a range of components, usually varying in carbon number, which further increases the complexity of the mixture. Due to the wide range in their volatility, peaks of components belonging to different lipid classes overlap, complicating the analysis. Therefore, qualitative analysis of the mixture using single standards as representatives of each lipid class is not sufficient. Information on the FA composition of the mixture (Table 1) can be used to determine the standards to be used for the qualitative analysis. In light of these results, C16 and C20 glycerides (Nu-Chek-Prep Inc., Elysian, MN, USA) were used to identify the FFA and glyceride (MG, DG, TG) groups. A sterol standard mixture containing campesterol, stigmasterol, sitosterol and brassicasterol (Matreya Inc., Pleasant Gap, PA, USA); $\alpha$-, $\beta$-, $\gamma$- and $\delta$-tocopherol and squalene

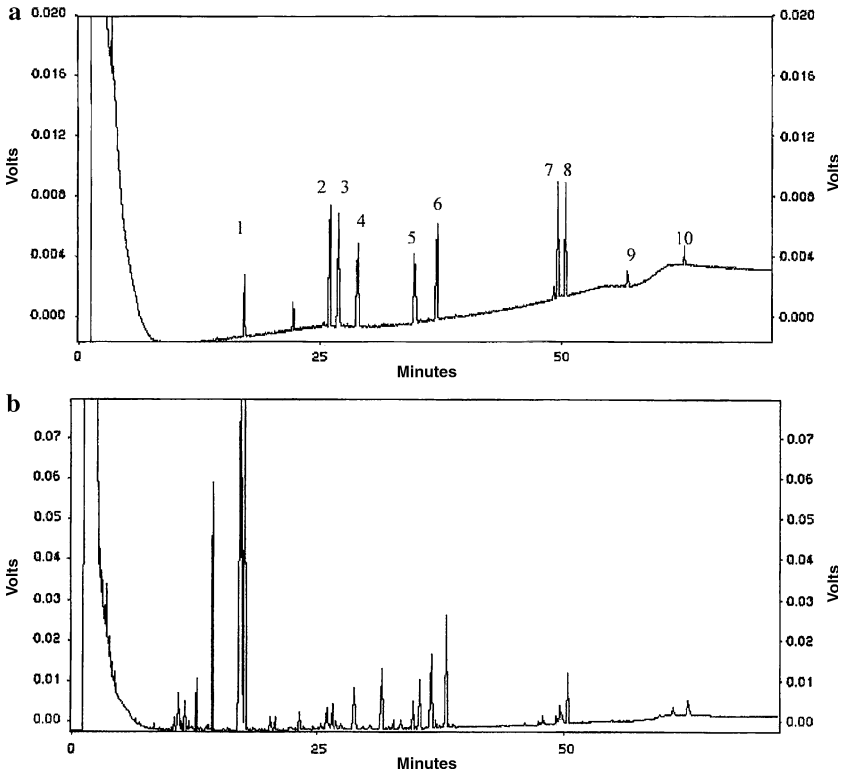


**Fig. 2** **a** A typical GC chromatogram of the derivatized standard mixture (*1* oleic acid, *2* monoolein, *3* squalene, *4* δ-tocopherol, *5* α-tocopherol, *6* stigmasterol, *7* diolein, *8* internal standard, *9* cholesterol stearate, *10* triolein). **b** A typical GC chromatogram of the derivatized sample

standards were also used for the qualitative analysis of the minor components. The unidentified components with retention times less than that of C16 FFA were grouped and reported as "volatiles". Using this approach, 95–97% of sample components were accounted for.

## Quantitative Analysis

The compositions of the feed, extract fractions and residue were reported as GC area %. Quantification of sterols and tocopherols in the feed was carried out using an internal standard (IS) method. A standard solution containing known amounts of oleic acid, monoolein, diolein, cholesterol stearate, triolein, α- and δ-tocopherols (95 and 90% pure, respectively) and stigmasterol (95% pure), and C13 triglyceride (tridecanoin, Nu-Chek-Prep Inc., Elysian, MN, USA) as an internal standard was analyzed and relative response factors (RRF) were calculated for sterols and tocopherols using the formula:

$$\mathrm{RRF} = \frac{(\text{weight of standard})\ (\text{area of IS})}{(\text{weight of IS})\ (\text{area of standard})} \quad (1)$$

Stigmasterol standard was used to calculate the RRFs for the sterols, whereas RRFs were calculated for both α- and δ-tocopherol and an average of these RRFs were used

**Table 1** Composition of the canola oil deodorizer distillate

| Component | Concentration (%) |
|---|---|
| Tocopherols[a] | |
| α- | 1.44 ± 0.08 |
| γ- | 3.88 ± 0.20 |
| δ- | 2.78 ± 0.15 |
| Total | 8.11 ± 0.43 |
| Sterols[a] | |
| Brassicasterol | 2.86 ± 0.15 |
| Campesterol | 4.69 ± 0.25 |
| Stigmasterol | 0.37 ± 0.02 |
| Sitosterol | 6.54 ± 0.34 |
| Total | 14.73 ± 0.38 |
| Free fatty acids[b] | 50.39 ± 1.13 |
| Fatty acids[c] | |
| C16:0 | 13.97 ± 0.16 |
| C18:0 | 22.29 ± 0.15 |
| C18:1 | 52.73 ± 0.37 |
| C18:2 | 7.17 ± 0.06 |
| C18:3 | 2.46 ± 0.04 |
| C20:0 | 0.85 ± 0.06 |
| C20:1 | 0.53 ± 0.01 |

[a] % w/w

[b] % as oleic acid

[c] GC area %

for the remaining tocopherols. RRFs were determined to be 0.80 (±0.002) for α-tocopherol, 0.83 (±0.057) for δ-tocopherol and 0.86 (±0.035) for stigmasterol. A known amount of IS (0.4 mL) was also added to the sample to be analyzed and the weight of components of interest in the sample was calculated as

$$\text{Weight}_x = (\text{RRF}_x)\ (\text{weight of IS})\ (\text{area}_x)/(\text{area of IS}) \quad (2)$$

### Statistical Analysis

All fractionation experiments were replicated. GC analysis of each fraction obtained in each run was carried out in duplicate. Analysis of variance (ANOVA) was carried out using the General Linear Model Procedure of SAS Statistical Software [15] to test the effect of temperature and pressure on the total extract yield and residue composition. Multiple comparison of the means was carried out using the pdiff option of the LSMEANS (Least Squares Means) statement at $\alpha = 0.05$.

## Results and Discussion

Physical and chemical properties of mixture components and the effect of operating conditions on yield and selectivity of the fractionation process were considered while choosing the experimental conditions to be tested. Pressure affects the feasibility of the process through its effect on selectivity and yield. Yield of the extract increases, whereas that of raffinate decreases with increasing pressure at a set temperature. In general, selectivity of the separation decreases with pressure [5, 16]; however, it has also been shown to increase with pressure [17]. The net pressure effect is determined by mixture composition and targeted separation. The lower operating pressure was chosen as 20 MPa, considering the yield or solvent loading and its effect on residue composition as well as fractionation time. The effect of yield on residue composition is determined by the amount of feed introduced into the column, whereas yield at a given time is also affected by column volume and $CO_2$ flow rate. The highest pressure was chosen as 25 MPa, as lower selectivity was expected at higher pressures for the targeted separation [16]. The lowest temperature limit was chosen to ensure that the feed would be in the liquid phase during the fractionation experiments to avoid any potential plugging of the column upon its solidification. The higher temperature limit was set considering the heat sensitivity of the mixture components to avoid any degradation.

Compositions of the feed material and the fractions obtained under investigated conditions are shown in Tables 1 and 2, respectively. Sterol and tocopherol contents of the canola oil deodorizer distillate used in this study were higher than those reported by Ramamurthi et al. [10]. This is an expected result because of the inherent variability in oil composition and the dependence of distillate composition on deodorization and storage conditions [2].

### Effects of Fractionation Conditions on Total Extract Yield

Total extract yield is defined as the cumulative weight of the extract fractions collected from the top of the column at a certain time during the fractionation. It is determined by loading of the solvent and thus the solubility behavior under the investigated conditions. Total extract yield increased significantly ($p \leq 0.05$) with pressure (Table 3). An increase in yield with pressure is expected due to an increase in the solvent power of $SCCO_2$ and has been reported in both phase equilibrium and fractionation studies of multicomponent lipid mixtures such as deodorizer distillates [5–9, 16–18] and vegetable oils [19, 20].

At isobaric conditions (25 MPa), a slightly higher yield was obtained at the lowest temperature tested (70 °C), whereas the yield obtained using a temperature gradient was between those obtained under isothermal conditions of 70 and 100 °C; however, these differences were not significant ($p > 0.05$) (Table 3). Decreasing extract yields with increasing temperature had also been reported for esterified olive oil deodorizer distillate (at 13–15 MPa and 40–60 °C) [9]. A similar temperature effect had been reported for the pseudobinary solubility behavior (determined by treating the mixture as a single component) of deacidified palm oil enriched with sterols or carotene (26 MPa, 50–100 °C) and palm oil deodorizer distillate [16, 17]. Pseudobinary solubility of soybean oil deodorizer distillate showed a minimum with temperature at 90 °C or decreased with temperature for different tocopherol concentrations [18]. While pseudobinary solubility data of sterol removed soybean distillate pointed to a crossover of isotherms at approximately 30 MPa, solubility of the distillate that was esterified to convert fatty acids to fatty acid esters decreased with temperature in the range of 35–70 °C at 20–30 MPa [21].

The decrease in extract yield with time reflects the changing composition of the distillate due to the batch nature of the processing protocol applied and occurs due to the depletion of the more soluble components of the mixture.

**Table 2** Composition of feed, fractions and residues of $SCCO_2$ column fractionation of canola oil deodorizer distillate

| | GC area (%) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Volatiles | FFA | MG | Sq | Tocopherols | | | | Sterols | | | | | DG | TG |
| | | | | | δ- | γ- | α- | Total | Brassica- | Campe- | Stigma- | Sito- | Total | | |
| Feed | 7.10 | 61.52 | 4.78 | 0.43 | 2.27 | 3.18 | 1.21 | 6.66 | 2.29 | 3.77 | 0.31 | 5.16 | 11.53 | 2.42 | 1.86 |
| 25 MPa at 70 °C | | | | | | | | | | | | | | | |
| F1 | 33.08 | 50.62 | 3.53 | 0.55 | 2.00 | 1.21 | 0.62 | 3.82 | 0.72 | 1.05 | 0.08 | 1.35 | 3.20 | 0.43 | 0.24 |
| F2 | 20.69 | 61.84 | 4.45 | 0.62 | 2.43 | 1.56 | 0.77 | 4.76 | 0.77 | 1.11 | 0.09 | 1.39 | 3.36 | 0.46 | 0.14 |
| F3 | 10.77 | 68.94 | 5.00 | 0.59 | 2.55 | 1.89 | 0.95 | 5.39 | 0.95 | 1.38 | 0.12 | 1.77 | 4.21 | 0.76 | 0.28 |
| F4 | 5.60 | 72.62 | 5.08 | 0.47 | 2.57 | 2.37 | 1.15 | 6.08 | 1.26 | 1.86 | 0.16 | 2.32 | 5.60 | 1.04 | 0.29 |
| F5 | 3.18 | 71.08 | 5.35 | 0.37 | 2.24 | 3.01 | 1.37 | 6.62 | 1.85 | 2.67 | 0.23 | 3.46 | 8.21 | 1.56 | 0.36 |
| Residue | 5.14 | 61.10 | 4.52 | 0.38 | 2.87 | 3.22 | 1.25 | 7.34 | 2.59 | 4.26 | 0.34 | 5.92 | 13.11 | 2.80 | 2.00 |
| 25 MPa at 70–100 °C | | | | | | | | | | | | | | | |
| F1 | 29.06 | 51.47 | 4.38 | 0.62 | 2.40 | 1.45 | 0.74 | 4.59 | 1.04 | 1.64 | 0.11 | 2.18 | 4.97 | 0.59 | 0.16 |
| F2 | 17.52 | 63.66 | 5.78 | 0.74 | 2.98 | 1.58 | 0.83 | 5.39 | 0.73 | 1.01 | 0.07 | 1.27 | 3.08 | 0.25 | 0.01 |
| F3 | 8.52 | 72.64 | 5.44 | 0.61 | 3.40 | 1.83 | 0.97 | 6.21 | 0.75 | 0.97 | 0.07 | 1.16 | 2.95 | 0.32 | 0.13 |
| F4 | 3.60 | 76.41 | 5.45 | 0.51 | 3.52 | 2.23 | 1.16 | 6.91 | 0.87 | 1.11 | 0.09 | 1.36 | 3.43 | 0.39 | 0.09 |
| F5 | 1.70 | 75.21 | 6.43 | 0.42 | 2.44 | 2.92 | 1.46 | 6.82 | 1.33 | 1.70 | 0.14 | 2.00 | 5.18 | 0.51 | 0.01 |
| Residue | 1.89 | 29.89 | 2.58 | 0.15 | 1.20 | 3.28 | 0.91 | 5.39 | 7.12 | 12.73 | 0.99 | 19.56 | 40.41 | 5.24 | 9.16 |
| 25 MPa at 100 °C | | | | | | | | | | | | | | | |
| F1 | 33.97 | 45.83 | 3.41 | 0.58 | 2.84 | 1.39 | 0.65 | 4.88 | 1.08 | 1.99 | 0.12 | 2.52 | 5.71 | 0.58 | 0.95 |
| F2 | 23.76 | 57.40 | 5.08 | 0.83 | 3.36 | 1.73 | 0.85 | 5.93 | 0.72 | 1.01 | 0.08 | 1.26 | 3.06 | 0.31 | 0.22 |
| F3 | 13.92 | 65.43 | 5.79 | 0.87 | 4.10 | 1.94 | 0.99 | 7.03 | 0.74 | 1.03 | 0.08 | 1.28 | 3.13 | 0.22 | 0.04 |
| F4 | 8.19 | 70.30 | 5.94 | 0.72 | 3.68 | 2.22 | 1.17 | 7.08 | 0.86 | 1.17 | 0.08 | 1.45 | 3.56 | 0.27 | 0.08 |
| F5 | 5.84 | 70.17 | 6.27 | 0.55 | 3.22 | 2.62 | 1.42 | 7.26 | 1.17 | 1.54 | 0.12 | 1.92 | 4.75 | 0.70 | 0.42 |
| Residue | 8.55 | 52.15 | 5.03 | 0.51 | 3.08 | 3.07 | 1.34 | 7.49 | 2.56 | 4.20 | 0.33 | 5.94 | 13.04 | 3.16 | 3.24 |
| 20 MPa at 70–100 °C | | | | | | | | | | | | | | | |
| F1 | 44.74 | 40.04 | 3.43 | 0.72 | 2.27 | 1.03 | 0.54 | 3.84 | 0.61 | 0.95 | 0.05 | 1.22 | 2.82 | 0.37 | 0.31 |
| F2 | 37.98 | 47.21 | 4.65 | 0.92 | 3.45 | 0.88 | 0.48 | 4.81 | 0.23 | 0.24 | 0.00 | 0.30 | 0.77 | 0.00 | 0.05 |
| F3 | 26.58 | 56.08 | 6.13 | 1.18 | 3.67 | 1.11 | 0.60 | 5.38 | 0.24 | 0.23 | 0.00 | 0.27 | 0.74 | 0.00 | 0.05 |
| F4 | 16.58 | 63.44 | 6.76 | 1.31 | 4.51 | 1.31 | 0.75 | 6.57 | 0.26 | 0.26 | 0.00 | 0.29 | 0.81 | 0.00 | 0.15 |
| F5 | 11.22 | 68.79 | 7.03 | 1.15 | 5.17 | 1.45 | 0.81 | 7.42 | 0.27 | 0.24 | 0.00 | 0.29 | 0.80 | 0.00 | 0.09 |
| Residue | 2.59 | 65.41 | 5.34 | 0.31 | 2.87 | 2.71 | 1.07 | 6.66 | 2.42 | 3.84 | 0.31 | 5.32 | 11.89 | 2.11 | 1.94 |

*Sq* Squalene

## Effects of Fractionation Conditions on the Composition of Extract Fractions and Residue

At every condition tested, the volatiles content of the fractions decreased whereas that of FFA, tocopherols and sterols increased with fractionation time/fraction number (Figs. 3, 4, 5; Table 2). Increasing the fractionation pressure increased the content of all of the fraction components except volatiles, which points to decreasing selectivity in the separation of volatiles from the rest of the mixture components. The composition of the fractions was also affected by temperature (Figs. 3–5; Table 2). While the tocopherol content followed the order 100 °C > 70–100 °C > 70 °C; for sterols the order was reversed after 1.5 h. The effect on FFA content was 70–100 °C > 70 °C > 100 °C, whereas for volatiles it was 100 °C > 70 °C > 70–100 °C.

While the concentrations of sterols, di- and triglycerides in the extract fractions were lower than the respective feed concentrations under all the investigated conditions, the distribution of other components was affected by fractionation time, temperature and pressure (Table 2; Figs. 3–5). The volatiles were enriched up to a factor of 6.3, but the decrease in volatile content with time resulted in concentrations lower than the feed values in the latter fractions. Contrary to the results for the volatiles, FFAs were enriched in the latter fractions relative to their concentration in the feed but to a lesser extent. The tocopherol concentration of the initial fractions was in the range of 3.8–4.9% and increased with

**Table 3** Total fractionation yields and yields of collected fractions of $SCCO_2$ column fractionation of canola oil deodorizer distillate

| | Temperature effect at 25 MPa | | |
|---|---|---|---|
| | Extract yield (g) | | |
| | 70 °C | 100 °C | 70–100 °C |
| Fraction 1 | 0.47 | 0.47 | 0.48 |
| Fraction 2 | 1.16 | 1.01 | 1.11 |
| Fraction 3 | 1.59 | 1.12 | 1.35 |
| Fraction 4 | 1.86 | 1.05 | 1.20 |
| Fraction 5 | 1.36 | 0.99 | 0.98 |
| Total[1] | 6.43 ± 1.6[a] | 4.64 ± 0.06[a] | 5.13 ± 0.49[a] |
| | Pressure effect at 70–100 °C | | |
| | Extract yield (g) | | |
| | 20 MPa | 25 MPa | |
| Fraction 1 | 0.277 | 0.48 | |
| Fraction 2 | 0.436 | 1.11 | |
| Fraction 3 | 0.435 | 1.35 | |
| Fraction 4 | 0.366 | 1.20 | |
| Fraction 5 | 0.356 | 0.98 | |
| Total[1] | 1.87 ± 0.05[a] | 5.13 ± 0.49[b] | |

[1] Mean ± standard deviation

[ab] Mean in the same row with different letters are significantly different ($p \leq 0.05$)

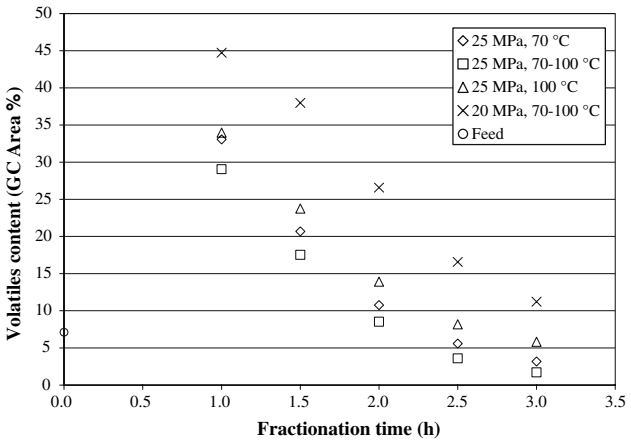


**Fig. 3** Volatile content of the fractions obtained by $SCCO_2$ column fractionation of canola oil deodorizer distillate

fractionation time leading to slight enrichment in the latter fractions.

The separation behavior of the volatiles and sterols (i.e. enrichment in the extract and raffinate phases, respectively) can be to a certain extent predicted by the results of phase equilibrium measurements [16, 18], extraction [21, 22] and fractionation [5] studies of deodorizer distillates. However, as also demonstrated in this study, the behavior of

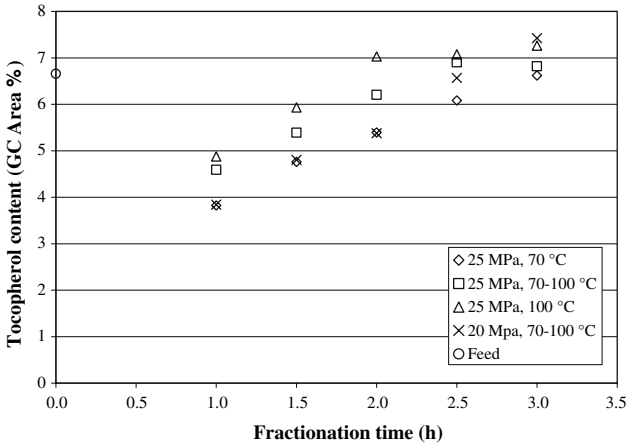


**Fig. 4** Total tocopherol content of the fractions obtained by $SCCO_2$ column fractionation of canola oil deodorizer distillate

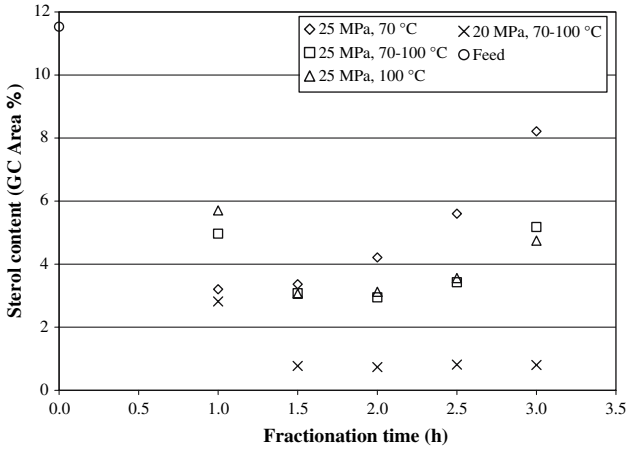


**Fig. 5** Total sterol content of the fractions obtained by $SCCO_2$ column fractionation of canola oil deodorizer distillate

tocopherols was affected by processing conditions and process design to a greater extent such that tocopherol enrichment in both extract [8, 23] and raffinate [5, 16, 18, 21, 22] phases has been observed. The dependence of separation behavior on mixture composition was observed for palm oil deodorizer distillate [17], where distribution coefficient of linoleic acid increased (reversing its enrichment behavior) with increasing concentration of tocopherols in the mixture.

Temperature had a significant effect on the composition of the residues obtained under different conditions (Table 4). Increasing the temperature from 70 to 100 °C increased the volatile content significantly ($p \leq 0.05$), whereas FFA, tocopherol and sterol contents were not affected. Use of the thermal gradient decreased the content of volatiles, FFA and tocopherols although the decrease in volatiles was not significant ($p > 0.05$). On the other hand, sterol content increased significantly ($p \leq 0.05$) when a

**Table 4** Residue compositions of $SCCO_2$ column fractionation of canola oil deodorizer distillate

| | Residue composition (GC area%) | | | |
|---|---|---|---|---|
| | Volatiles | FFA | Tocopherols | Sterols |
| 25 MPa | Temperature effect | | | |
| 70 °C | 5.14[a] | 61.10[a] | 7.34[a] | 13.11[a] |
| 70–100 °C | 1.89[a] | 29.89[b] | 5.39[b] | 40.41[b] |
| 100 °C | 8.55[b] | 52.15[a] | 7.49[a] | 13.04[a] |
| 70–100 °C | Pressure effect | | | |
| 20 MPa | 2.59[x] | 65.41[x] | 6.66[x] | 11.89[x] |
| 25 MPa | 1.89[x] | 29.89[y] | 5.39[y] | 40.41[y] |

[ab] Mean in the same column with different letters are significantly different ($p \leq 0.05$)

[xy] Mean in the same column with different letters are significantly different ($p \leq 0.05$)

temperature gradient was applied, reaching a level of 40%. Pressure did not have a significant effect on volatiles, while increasing the sterol and decreasing the tocopherol and FFA contents. It should be noted that the reported compositions are for the residue collected upon depressurization of the column (0.04–3.45 g) and does not represent the composition of the remaining raffinate. At the end of each run, the raffinate is expected to be distributed along the column height with a concentration gradient. The reported residue compositions are indicative of the separation efficiency for the studied mixture.

## Fractionation Efficiency

The distribution of mixture components between raffinate and extract streams, hence the fractionation efficiency, was affected by the fractionation time and operating conditions. While the solubility behavior of mixture components under the investigated conditions was the main determinant of extract composition, the composition of the residue was also affected by the extraction yield. For example at 20 MPa, although sterols were not extracted to any appreciable extent, no enrichment could be achieved in the raffinate (Table 2). The higher selectivity achieved for sterol separation at this pressure is thus not reflected in the composition of the residue as the yields of extracted components at this pressure were substantially lower than the amount of raffinate.

An inverse correlation between solvent power, hence extraction yield, and selectivity, which results in a decrease in selectivity with pressure, has been observed during $SCCO_2$ fractionation of various mixtures [5, 8] and offers a processing challenge as high solvent power and selectivity values are required for an efficient process. An increase in selectivity values with pressure hence solvent power has also been reported although for a small number of systems such as palm oil deodorizer distillate (tocopherols/FFA) [17]. Therefore, in a complex mixture the effect of pressure on selectivity may depend on the particular separation targeted.

Although canola oil deodorizer distillate has previously been regarded as a FFA source, the results of this study using $SCCO_2$ technology show the potential of this raw material as a sterol source. Semi-continuous processing of the distillate at 25 MPa and 70–100 °C yielded a residue containing 40% sterols. It should be noted that the efficiency of this separation can be further improved by changes in the process design such as continuous processing of the distillate, by using a reflux pump to generate an external reflux, by increasing the height of the column or by using multiple columns.

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