Supercritical Fluid Fractionation of Thermally Oxidized Canola Oil

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Supercritical fluid extraction procedures were developed to fractionate thermally oxidized canola oil. Canola oil was heated in a sealed glass ampoule for 72 h at 200°C in a forced-convection oven. After heating, the percentages of triacylglycerol (TAG) trimer, dimer, monomer and lower-molecular weight (LMW) compounds in the heated oil sample (as determined by high-performance size-exclusion chromatography) were 3.0, 8.4, 81.3 and 7.3%, respectively. The LMW compounds included diacylglycerols, monoacylglycerols and free fatty acids. Fractions of TAG monomer of approximately 98% purity were isolated after extraction at 408 atm for 45 min. After a second extraction at 442 atm for 45 min, a TAG dimer fraction of 95% purity was isolated. Recoveries of TAG monomer and dimer were 96 and 87%, respectively.

KEY WORDS: Heated oil, high-performance size-exclusion chromatography, light-scattering detector, oxidation, polymerization, supercritical fluid extraction and fractionation.

Numerous heating/oxidation studies have been conducted on different types of food oils, including soybean (1-5), corn (6,7), palm (3,8), olive (3,6,9) and marine oils (10,11). Three major types of reactions have been characterized during heating, namely thermolytic, oxidative and polymerization reactions. The major nonvolatile products produced from these reactions are nonpolar and polar polymeric triacylglycerols (TAGs) (12,13), particularly TAG dimers (14). High-performance size-exclusion chromatography (HPSEC) has been used to quantitate TAG polymers in heated oils (2,15-20). A TAG polymer content of 20% corresponds to approximately 27% polar compounds (16), and a concentration of 27% polar material in heated oil has been suggested as the point to which the oil has deteriorated and where it is no longer considered useful (21).

The use of supercritical (SC) carbon dioxide (CO_2), instead of an organic solvent for extraction, has advantages that include inertness, nonflammability, environmental compatibility, easy removal of the "solvent" from the analyte (22,23) and reduced cost (depending upon CO_2 purity) (22-24). However, SC CO_2 is a nonpolar solvent, which makes it a poorer solvent for slightly or moderately polar analytes, as compared to more polar SC fluids, such as ammonia or nitrous oxide. The presence of polar functional groups, such as hydroxyl, carbonyl and carboxylic acid groups and even double bonds, will reduce analyte solubility in CO_2 (24). Increasing the pressure or the addition of a modifier will alter the solubility properties of SC CO_2 (22-24). Other factors affecting extraction efficiency include the analyte molecular weight (MW), polarity, volatility, thermal stability, pKa, solubility and concentration in the sample matrix (22,23). The sample matrix and its physical and chemical characteristics, such as chemical composition, particle size, homogeneity, amount, porosity and density, are also important factors (22). Liquids, such as oils, must be loaded onto a solid support to allow the extraction or fractionation process to occur, otherwise the oil could be swept out of the extractor.

Currently, the only other effective method to fractionate monomeric and dimeric TAG for further analysis is preparative HPSEC. Supercritical fluid technology has been applied extensively to extraction, both on an analytical scale and a processing scale. Only limited work has been published on the use of supercritical fluid technology for fractionation (25-27) on an analytical scale.

The objective of this research was to develop the methodology for fractionating TAG monomers and dimers by supercritical fluid extraction (SFE) into relatively pure fractions (>90%) in a relatively short time (15-45 min) with good recoveries (90-100%).

EXPERIMENTAL PROCEDURES

Oil sample preparation. Approximately 4 mL canola oil (Crisco Puritan; Procter & Gamble, Cincinnati, OH) was placed in a glass ampoule (approximately 20 mL) and sealed. The ampoule was wrapped in aluminum foil and heated at 200°C for 72 h in an oven (Type OVE-100 230Y; Sanyo Gallenkamp PLC, Loughborough, Leicestershire, England).

HPSEC. The HPSEC system consisted of an HP solvent delivery system (Rainin Instrument Co., Woburn, MA), electronic pressure module with a dual-chamber Dynamax dynamic mixer, prime-purge valve, 7030 Rheodyne (Coati, CA) switching valve, a 7125 Rheodyne injection valve with 20-μL sample loop and a 7161 Rheodyne position sensing switch. The compounds were separated with four Phenogel (Phenomenex, Torrance, CA) columns with a particle size of 5μ. The first column was 500 × 8.0 mm with a 500 Å pore size; the next two columns were 500 × 8.0 mm with a 100 Å pore size and the last column was 300 × 7.8 mm with a pore size of 50 Å. The columns were protected with a Phenogel 5 guard column (50 × 7.8 mm). The columns were connected in-line to a evaporative light-scattering detector (Model ELSD IIA; Varex Corp., Burtonsville, MD).

The mobile phase flow rate was 1.0 mL/min tetrahydrofuran (THF) (Optima; Fisher Scientific, Fair Lawn, NJ). It was filtered with 0.45-μm HV discs (Millipore Corp., Bedford, MA) and degassed prior to use. The THF was kept under a constant nitrogen gas purge while in use. No butylated hydroxytoluene (BHT) was added as an antioxidant.

The ELSD II A detector was operated at the following optimal conditions—adjusted temperature, 100°C; heater temperature, 98.4°C; exhaust temperature, 60.0°C; gas flow pressure, 39 mm; gas pressure, 11 psi; range, 20; and time constant, 1.0. Ultra-high purity nitrogen (99.999%) gas was used as the carrier gas.

Polypropylene glycol (PPG) MW standards of 4000, 3000 and 2000 (Aldrich Chemical Co., Milwaukee, WI) and triolein (MW = 885.4), diolein (MW = 621.0) and monoolein (MW = 356.5) (Sigma Chemical Co., St. Louis, MO) were used to estimate the canola oil fraction MWs

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TABLE 1

High-Performance Size-Exclusion Chromatography Retention of MW Standards[a]

MW standards	Average retention volume (mL)	Log MW
4000 (PPG)	32.50	3.6021
3000 (PPG)	34.33	3.4771
2000 (PPG)	37.83	3.3010
885.4 (Triolein)	39.72	2.9471
621.0 (Diolein)	41.33	2.7931
356.5 (Monoolein)	44.39	2.5521

[a]Polypropylene glycol (PPG) 4000, 3000 and 2000 (Aldrich Chemical Co., Milwaukee, WI) and triolein (MW = 885.4), diolein (MW = 621.0) and monoolein (MW = 356.5) (Sigma Chemical Co., St. Louis, MO) were used as molecular weight (MW) standards.

based on retention volume (V_r) (Table 1). Triolein, diolein and monoolein were ≥99% pure. The MW standards and canola oil sample were prepared at approximately 20 mg/mL with THF, filtered with 0.45-μm HVHP discs (Millipore Corp.) and stored in amber vials at approximately 2°C. The log of the MW standards was plotted vs. V_r (Fig. 1). The MW of each heated canola oil component was estimated from Equation 1.

$$\log MW = 6.6346 - (9.1850 \times 10^{-2})V_r \quad [1]$$

The detector response to the analyte varies depending upon the size and shape of the analyte molecule. The response factors (R_f) were determined by dividing the area by the concentration and then plotting the R_f as a function of the MW of each standard (Table 2). Equation 2 was derived from the plot.

$$R_f = 4.32 \times 10^4 + 7.16 \times MW - 6.37 \times 10^{-3} \times MW^2 + 7.50 \times 10^{-7} \times MW^3 \quad [2]$$

The R_f values obtained were used for quantitation of the heated oil components. The V_r for each canola oil component was used in Equation 1 to determine the MW for each fraction. The R_f for each canola oil component was determined from Equation 2. Table 3 summarizes the V_r, MW and R_f ratios of the heated canola oil sample. The percentage of each fraction (Table 4) was determined by dividing the average area by the R_f ratio. The adjusted area for each fraction was divided by the total areas and multiplied by 100. The HPSEC system was controlled, and the data was collected and analyzed with the Dynamax Method Manager Software, Version 1.3.1 (Rainin Instrument Co.). All analyses were conducted in triplicate.

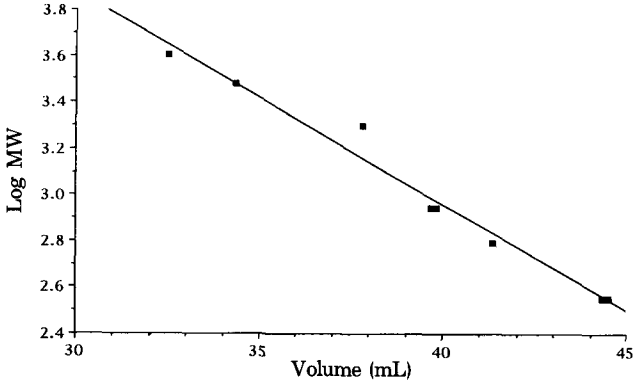

FIG. 1. Calibration curve of molecular weight (MW) standards.

TABLE 2

Response Factors (R_f) for the Standards[a]

MW Standards	Average area (μVs)	Conc. (mg/mL)	Average R_f (μVs/mg/mL)	Normalized R_f
4000 (PPG)	417,897	24.72	16,905	0.3408
3000 (PPG)	640,263	20.16	31,759	0.6402
2000 (PPG)	745,047	23.30	31,976	0.6446
885.4 (triolein)	1,034,790	21.48	48,175	0.9711
621.0 (diolein)	1,020,970	20.58	49,610	1.0000
356.5 (monoolein)	903.997	22.30	40,538	0.8171

[a]See Table 1 for other abbreviations; μVs, microvolt seconds.

TABLE 3

Response Factors (R_f)[a] for the Heated Canola Oil

Fraction	Average retention volume (mL)	Average MW	Average R_f[a] (μVs/mg/mL)	Normalized R_f
Trimer	35.00	2629	31,627	0.6989
Dimer	36.67	1847	39,413	0.8709
Monomer (TAG)	39.67	979	44,796	0.9899
DAGs	42.94	490	45,254	1.0000
FFAs	47.72	178	44,264	0.9781

[a]$R_f = 4.32 \times 10^4 + 7.16 \times MW - 6.37 \times 10^{-3} \times MW^2 + 7.50 \times 10^{-7} \times MW^3$. The formula was developed based upon the molecular weight (MW) standards in Table 2. TAG, triacylglycerol; DAG, diacylglycerols; FFA, free fatty acids.

TABLE 4

Composition of the Heated Canola Oil

Fraction	Average area (μVs)	Normalized R_f	Area[a] (μVs)	Composition (%)
Trimer	182,517	0.6989	261,149	3.02
Dimer	633,970	0.8709	727,948	8.42
Monomer (TAG)	6,953,740	0.9899	7,024,689	81.27
DAGs	581,130	1.0000	581,130	6.72
FFAs	48,120	0.9781	49,197	0.57

[a]Adjusted for R_f; see Table 3 for abbreviations.

SFE/HPSEC. All samples were dynamically and sequentially extracted with an SFE System 2100 (Isco Inc., Lincoln, NE) comprised of an SFX 2-10 heated extractor and two model 100DX syringe pumps. The samples were extracted at 60°C with the capillary restrictor heater temperature held at 80°C. The capillary restrictor was a piece of fused silica tubing, 27.7 cm in length and 50 μm i.d. A 10-mL stainless-steel extraction cell (cartridge) with 2.0-μm frits was filled with 3.0-mm glass beads (Kimble Glass Inc., Vineland, NJ). The glass beads were used as the support matrix from which the oil could be extracted (28). Previous extractions with sodium sulfate as the support matrix had poor supercritical fluid (SCF)

flow rates, recoveries and fraction purities. SFC-grade CO_2 (Scott Specialty Gases, Plumbsteadville, PA) was used as the extracting fluid. The collection vessels were glass test tubes of 20-cm i.d. × 150-cm length with Teflon caps and contained 10 mL THF.

Approximately 50 mg of heated oil was placed on top of the glass beads in the cartridge. The weight of the glass beads plus cartridge was recorded before the oil sample was loaded, after the oil was loaded, and after each extraction or fractionation step. The supercritical carbon dioxide flow rate was determined by the difference in the amount of carbon dioxide remaining in the pump before and after the extraction, divided by the extraction time. As a safety precaution, test tubes containing the extracted fractions should be vented 3–5 min after extraction to prevent excessive CO_2 pressure, which could result in explosive rupture of the test tubes. Fractions (filtered with 0.45-μm HVHP discs) were either analyzed with HPSEC or stored in a refrigerator (0–4°C) until further analysis. Only single replicates (SFE and HPSEC analysis) were run until the conditions were optimized (≥90% recovery and purity for both monomer and dimer). The samples were then analyzed in triplicate under the optimized SFE conditions.

The percent purity was determined from the HPSEC analysis results by means of the R_f adjusted areas (Table 4). The percent recovery of each fraction is equal to the extracted sample weight multiplied by the percent purity and divided by the initial sample weight multiplied by the component percentage of the oxidized oil. For example, a typical calculation for the monomer fraction appears in Equation 3.

$$(40 \text{ mg} \times 90\%)/(50 \text{ mg} \times 75\%) \times 100\% = 96\% \quad [3]$$

where 50 mg is the original sample weight, 40 mg is the weight of material extracted, 90% is the percent of monomer in the extracted fraction as determined by HPSEC, 75% is the percent monomer in the original sample and 96% is the percent recovery. The total percent recovery is the difference between the cartridge weight before and after extraction divided by the sample size and multiplied by 100%.

RESULTS AND DISCUSSION

HPSEC for the heated canola oil sample is shown in Figure 2. The MWs of the TAG monomer and dimer were approximately 979 and 1847, respectively, based on HPSEC of the standards. The calculated average MW for the monomeric TAG was 882, and the dimeric TAG was 1762–1778, based on the constituent atoms. Due to the use of MW standards, PPG, that are linear molecules for the polymer portion of the calibration curve, the MWs of the TAG monomer and dimer were overestimated. The calculated average MW of canola oil used was 8% 18:0 (MW = 284.47) and 92% 18:1 (MW = 282.45) (due to hydrogenation). The glycerol backbone added 41.04 g. The MW range for the dimer was due to the type of linkage between the monomers (-C-C- or -C-O-C-).

Preliminary SFE analyses investigations included both microcrystalline cellulose and magnesium sulfate as potential support matrices. However, sufficient and consistent flow rates were difficult to obtain. Subsequent

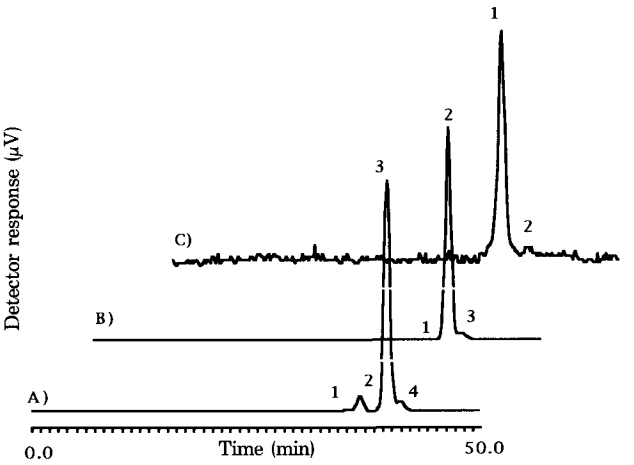

FIG. 2. A) High-performance size-exclusion chromatograph (HPSEC) of canola oil heated for 72 h at 200°C: 1) triacylglycerol (TAG) trimer, 2) TAG dimer, 3) TAG monomer and 4) lower-molecular weight (LMW) compounds such as diacylglycerols (DAGs), monoacylglycerols (MAGs) and free fatty acids (FFAs). B) HPSEC of 72-h heated (200°C) canola oil that was supercritical-fluid fractionated (SFF) at 408 atm for 45 min: 1) TAG dimer, 2) TAG monomer and 3) LMW compounds such as DAGs, MAGs and FFAs. C) HPSEC of 72-h heated (200°C) canola oil that was sequentially SFF at 442 atm for 45 min (following SFF at 408 atm for 45 min): 1) TAG dimer and 2) TAG monomer. μV, microvolts.

analysis and experience indicated that restrictor length, restrictor i.d., support matrix type and sample size were all important parameters affecting the fractionation. The fractionation was affected by sample composition, pressure, extraction cell temperature, capillary restrictor temperature, flow rate, time and the addition of modifier (22). Therefore, based on preliminary analyses, a set of conditions was established, namely a set restrictor length of 27.7 cm and 50 μm i.d., a 50-mg sample and a support matrix of 3-mm glass beads (11.5–12.0 g). The restrictor capillary tubing length should be kept to a minimum to maximize the flow rate and to minimize the extraction time. However, it should be of sufficient length to deposit the sample on the bottom of the collection tube.

The percentages of dimer and monomer concentrations for the heated canola oil sample were 8.42 and 81.27%, respectively. The other component percentages are listed in Table 4. The LMW compounds diacylglycerols, monoacylglycerols and free fatty acids are included with the TAG monomer fractions in calculating the percent recovery because there was no attempt to remove the LMW compounds from the TAG monomer fraction.

Figure 2 is the HPSEC chromatogram of the fractionated heated canola oil, which contained predominantly monomer. The purity achieved was approximately 98%, while the recovery was 96% for the monomer after extraction at 408 atm for 45 min (Table 5). Further supercritical-fluid fractionation of the oil sample produced a dimer fraction of approximately 95% purity with a recovery of approximately 87% (Fig. 2).

Preliminary analyses indicated that complete extraction of the monomeric components is required to maximize purity of the dimer fraction. The monomer and dimer begin to dissolve in SF CO_2 at their miscibility pressures,

TABLE 5

Supercritical Fluid Fractionation of Canola Oil (heated for 72 h at 200°C)

Sample	Pressure (atm)	Time (min)	% Purity	% Recovery	Total recovery (%)	Supercritical CO_2 flowrate (mL/min)
Monomer	408	45	98 ± 0.5[a]	96 ± 4		1.23 ± 0.07
Dimer	442	45	94 ± 2	87 ± 17	94 ± 5	1.28 ± 0.02

[a]Average of three replicates ± standard deviation.

which can be technique-dependent (29). Monomer and dimer fractions can have close miscibility pressures, and hence, it will be difficult to achieve purities greater than 95% in those cases. Extraction conditions (time and pressure) may have to be reoptimized for each oil sample type, due to the possibility of overlapping fractionation ranges, depending upon the chemical nature and amount of the components to be fractionated. Relatively pure monomer and dimer fractions from a thermally oxidized oil can be obtained with SFE.

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