J Am Oil Chem Soc (2009) 86:277–282
DOI 10.1007/s11746-008-1340-9

ORIGINAL PAPER

Counter-Current Carbon Dioxide Purification of Partially Deacylated Sunflower Oil

F. J. Eller · S. L. Taylor · J. A. Laszlo · D. L. Compton · J. A. Teel

Received: 3 October 2008 / Revised: 26 November 2008 / Accepted: 4 December 2008 / Published online: 30 December 2008

Abstract A liquid carbon dioxide counter-current fractionation method was developed to remove by-product fatty acid propyl esters (FAPEs) from the reaction mixture after the partial deacylation of sunflower oil. The fractionation column was 1.2 m long and separations were done at 25 °C and 8.27 MPa. Several solvent to feed ratios (S:FR) (i.e., 7.6, 15.2, 30.3 and 60.6 g/g) and feed rates (FR) (i.e., 1, 2, 2.5, 3 and 4 mL/min) at a constant S:FR of 15.2 were examined. Raffinate purity (i.e., percentage glycerides) and extract purity (i.e., percentage FAPEs) were both monitored. Percentage glycerides in both the raffinate and the extract increased with S:FR. The raffinate contained ca. 83, 97, 100 and 100% glycerides at S:FRs of 7.6, 15.2, 30.3 and 60.6, respectively. The percentage glycerides in the extracts were ca. 3, 4, 8 and 17%, respectively. With the S:FR held constant at 15.2, the raffinate purity peaked at ca. 99% glycerides at a FR of 2.5 mL/min and the extract at this FR contained ca. 96% FAPEs. The optimal S:FR and FR determined from the experimental assays was applied to large batches in both a semi-continuous feed mode, and later in a continuous feed mode, which gave raffinates of 99.3 and 98.1% glycerides, respectively, and extracts of 97.3 and 97.2% FAPEs, respectively.

Keywords Liquid carbon dioxide · Continuous counter-current fractionation · Sunflower oil · Fatty acid propyl ester · SoyScreen™

Introduction

An enzymatic conversion of soybean oil (SBO) triacylglycerides (TAGs) to feruloylated acylglycerols called SoyScreen™ has been previously described [1–3] and patented [4]. These feruloylated acylglycerols are believed to have great potential as natural sunscreens and as antioxidants for both the cosmetic and the food industry [5].

Recently, it was reported that it is advantageous to first deacylate the TAGs to form diacylglycerols (DAGs), prior to the subsequent biocatalytic esterification of the DAGs with the feruloyl moiety [6]. In this case, high oleic sunflower oil (HOSO) was partially deacylated by enzyme-catalyzed propanolysis to form 1,2-diacyl-*sn*-glycerols (1,2-DAG) which are regiospecific intermediates in the synthesis of structured feruloylated acylglycerols for cosmeceutical and nutraceutical applications [7]. The crude reaction mixture from the propanolysis results in a mixture of mono- (MAGs), di-, and TAGs, as well as by-product fatty acid propyl esters (FAPEs). Before the reaction of the partially deacylated sunflower oil (PDSO) with ethyl ferulate to yield the final product, feruloylated acylglycerols, the by-product FAPEs must be removed.

The process for developing supercritical gas counter-current separations has been discussed in detail [8]. Supercritical carbon dioxide fractionations have been used to separate a variety of lipids, including: fish oil fatty acid

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name USDA implies no approval of the product to the exclusion of others that may also be suitable.

F. J. Eller (✉) · S. L. Taylor · J. A. Laszlo · D. L. Compton · J. A. Teel
New Crops & Processing Technology Research,
National Center for Agricultural Utilization Research,
Agricultural Research Service,
United States Department of Agriculture,
1815 North University Street, Peoria, IL 61604, USA
e-mail: Fred.Eller@ARS.USDA.gov

ethyl esters [9–12], a mixture of MAGs, DAGs and TAGs [13], free fatty acids in rice bran oil [14] and a mixture of squalene/methyl oleate [15, 16]. The reported solubilities of methyl oleate, monoolein, diolein, and triolein in CO_2 indicate that CO_2 could be used to effectively fractionate a mixture of these compounds [17]. It has been demonstrated that liquid carbon dioxide (L-CO_2) can be used in both batch [3] and, more recently, in a continuous counter-current fractionation mode [18] to effectively remove both by-product fatty acid ethyl esters (FAEEs) and unreacted ethyl ferulate from a crude SoyScreen™ reaction mixture.

The objective of this research was to develop a continuous L-CO_2 counter-current fractionation method to separate the FAPEs from the crude mixture resulting from the enzymatic reaction of *n*-propyl alcohol and high-oleic sunflower oil. Factors such as solvent to feed ratio (S:FR) and feed rate (FR) were investigated to optimize the separation. Subsequently, scaled up batches of the crude reaction mixtures were fractionated using the optimized parameters.

Experimental

Partially Deacylated High Oleic Sunflower Oil

The feed solution consisted of a mixture of MAGs, DAGs, TAGs and by-product FAPEs (ca. 3.5, 27.6, 35.7, and 33.2%, respectively) and was prepared as previously described [7]. The density of this solution was determined to be ca. 0.903 g/mL (STP) using a mini weight per gallon cup (Paul N. Gardner Co. Inc., Pompano Beach, FL, USA).

Fractionation Apparatus

The basic design of the fractionation system has been described [14]. Although the previous work did not utilize a reduced pressure receiver for collecting the extract and recycling the CO_2, the system was modified to include recycling the CO_2 for this study. A schematic of the complete apparatus used for this L-CO_2 counter-current fractionation research is shown in Fig. 1.

The stainless steel fractionation column was 120 cm high with an internal diameter of 1.75 cm (ca. 288 mL volume) and was packed with protruded stainless-steel packing (0.16 in Pro-Pak, Scientific Development Company, State College, PA) with a 94% void volume. The column was held at 25 °C and all extractions were done at 8.27 MPa (CO_2 density ca. 0.796 g/mL). Welding-grade CO_2 (i.e., solvent) (Airgas Inc., Radnor, PA, USA) was fed from a cylinder through a mass flow meter (model D6, Micro Motion, Inc., Boulder, CO, USA) to a Haskel model AG-30-C gas booster pump (Haskel International Inc., Burbank, CA, USA). Liquid CO_2 entered from the bottom of the column, moved up through the column, out the top of

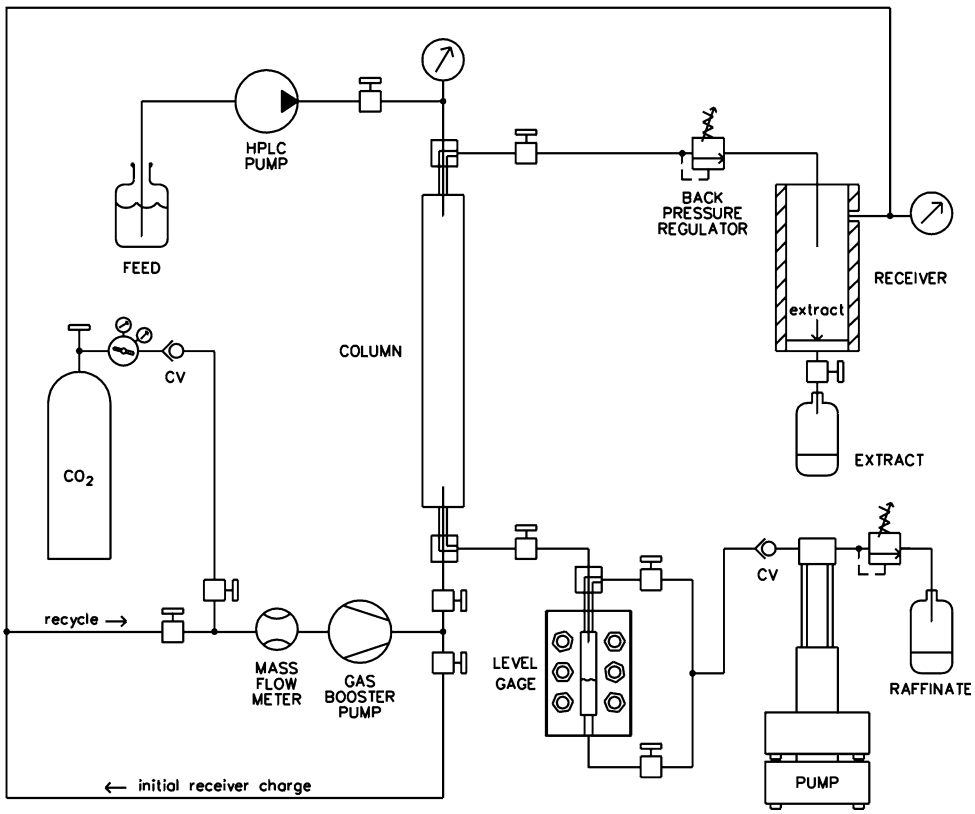

Fig. 1 Schematic of the liquid carbon dioxide counter-current fractionation system

the column and into a reduced pressure receiver held at 30–35 °C and 4.82–5.17 MPa where the extract was collected. These conditions allowed the desired separation and prevented liquid CO_2 from forming in the receiver. The gaseous CO_2 from the receiver was subsequently recompressed through the gas booster pump and recycled.

For the S:FR study and the FR study, an ISCO model 260D syringe pump (ISCO Inc., Lincoln, NE, USA) was used for the feed pump. Because the syringe pump required periodic refilling, the column could only be run in a semi-continuous feed mode in this configuration. However, in later experiments, a HPLC pump (model Series 1, Scientific Systems, Inc., State College, PA, USA) was substituted for the syringe pump to provide uninterrupted continuous feed flow. In both cases, the feed mixture was pumped into the top of the fractionation column and the mixture flowed down through the column.

The extracted feed material (i.e., the raffinate) exited the bottom of the fractionation column through a level gage (Jerguson model 11-R-32, Clark-Reliance Corp., Strongsville, OH, USA) and was drawn into a second ISCO syringe pump (raffinate collection pump). The level gage was used to maintain an optimized raffinate removal rate by adjusting the flow rate of the raffinate collection pump. When the raffinate collection pump was full (i.e., ca. 260 mL), it automatically reversed direction and quickly forced the collected raffinate through a back pressure relief valve into the raffinate collection bottle. After emptying the raffinate collection pump (i.e., piston at the top of the cylinder), the syringe pump resumed collecting fresh raffinate for another cycle.

Solvent to Feed Ratio and Feed Rate Studies

The treatment descriptions for the S:FR study are shown in Table 1. In the S:FR study, the solvent (i.e., CO_2) flow was held constant at 13.6 g/min while the feed rate was varied from 0.23 to 1.81 g/min. The S:FR was thus varied from 60.6 to 7.6 g/g. The treatment descriptions for the FR study are described in Table 2. In the FR study, both the solvent flow and the feed rate were varied together to maintain a constant S:FR of 15.2 g/g. Each experimental treatment was replicated twice.

Table 1 Treatment descriptions for liquid carbon dioxide countercurrent fractionation study: solvent to feed ratio study

Feed rate (g/min)	Solvent (CO_2) rate (g/min)	Solvent:Feed ratio (g/g)
0.23	13.6	60.6
0.45	13.6	30.3
0.91	13.6	15.2
1.81	13.6	7.6

Table 2 Treatment descriptions for liquid carbon dioxide countercurrent fractionation study: feed rate study

Feed rate (g/min)	Solvent (CO_2) rate (g/min)	Solvent:Feed ratio (g/g)
0.90	13.6	15.2
1.81	27.3	15.2
2.26	34.1	15.2
2.71	40.9	15.2
3.61	54.5	15.2

Scaled-up Fractionations

Based on the results obtained from the S:FR and FR studies, a larger batch of ca. 1.75 L (i.e., ca. 7 syringe pump fills) of the crude propanolysis of HOSO mixture was fractionated in a semi-continuous mode using the syringe pump for the feed. The conditions used were as follows: 25 °C, 8.27 MPa, FR of 2.5 mL/min, and a S:FR of ca. 20 g/g. Samples of both the raffinate and extract were taken from their respective collection bottles at the end of the run as well as a few during the fractionation run for compositional analyses.

Subsequently, the syringe pump, which only allowed discontinuous feed flow, was replaced with the HPLC pump, which allowed for continuous feed flow. In addition, to maintain a constant CO_2 flow, independent of system pressure variations, a programmable-timed solenoid switching assembly was added between the air drive regulator and the air drive inlet port of the gas booster pump. The assembly included two normally open air solenoid valves, one to pressurize, and the other to exhaust the air drive port. They were actuated through a single-pole, double-throw (SPDT) relay driven by a GraLab model 451 electronic timer/intervalometer (GraLab Corp., Centerville, OH, USA), which provided a timing cycle such that when one solenoid was open, the other was closed. Each open-closed cycle provided one gas booster pump stroke. In this configuration, the fractionation column was run continuously and used to purify a total of 20 kg of the crude PDSO. The conditions used were as follows: 25 °C, 8.27 MPa, FR of 2.5 mL/min, and a S:FR of ca. 20 g/g.

Compositional Analyses

The compositions of both the extract as well as the raffinate were determined by off-line supercritical fluid chromatography (SFC). The SFC analyses were conducted with a Series 4000 SFC (Selerity Technologies, Inc., Salt Lake City, UT, USA) equipped with a flame ionization detector

(FID) held at 350 °C. SFC/SFE-grade carbon dioxide (Airgas Inc., Radnor, PA, USA) was used as the carrier fluid. A 3B-Methyl-100 capillary column (10 m × 50 μm i.d., 0.25 μm film thickness) (Selerity Technologies, Inc., Salt Lake City, UT, USA) was used with a program of: 100 °C isothermal, 10.1 MPa hold for 5 min, and a ramp of 1.5 MPa/min to 31.4 MPa. A solution containing ca. 5 mg/mL was injected into the SFC (500 nL loop) and the relative amounts were determined from the FID area percentages. A single SFC analysis was performed on each sample.

Analyses of variance (ANOVA) were conducted on the percentage data using Statistix 7 software (Analytical Software, Tallahassee, FL, USA). Main effects of S:FR were tested using *F*-tests and means were compared using least significant difference (LSD). Mathematical equations that described the data were determined using TableCurve™ 2D curve-fitting software (Systat Software Inc., Richmond, CA, USA).

Results and Discussion

The mean percentage glycerides in the raffinate and the mean percentage FAPEs in the extract as a function of the S:FR are shown in Fig. 2. ANOVA indicated that there were highly significant effects of S:FR on percentage glycerides in the raffinate ($F_{3,4} = 98.0$, $p = 0.001$) as well as FAPEs in the extract ($F_{3,4} = 51.8$, $p = 0.0003$). The percentage raffinate glycerides (i.e., product purity) increased with S:FR and the raffinate was relatively pure (i.e., ca. 83%) even at the lowest S:FR of 7.6. At a S:FR of 15.2, the raffinate was ca. 97% glycerides and at S:FR of 30.3 or 60.6, the raffinate was essentially pure glycerides. Conversely, the percentage FAPEs in the extract decreased as S:FR increased. The percentage FAPEs in the extract was quite high (i.e., ca. 96%) at the two lowest S:FRs (i.e., 7.6 and 15.2). The percentage FAPEs were ca. 92% and 83% at S:FR of 30.3 and 60.6, respectively. Although the purities of the glycerides in the raffinate and the FAPEs in the extract are inversely proportional, the S:FR of 15.2 gave both a raffinate of 97% glycerides and an extract of 96% FAPEs.

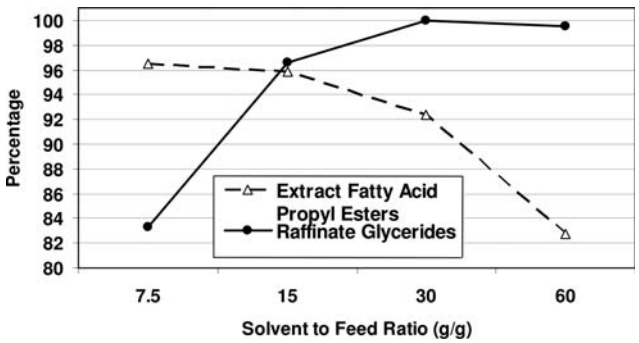

Fig. 2 Percentage glycerides in the raffinate and FAPEs in the extract as a function of solvent to feed ratio

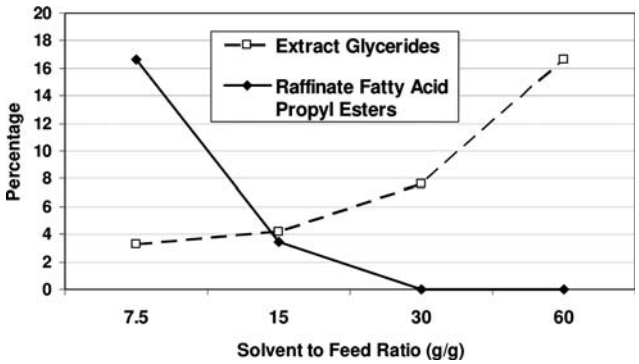

Fig. 3 Percentage glycerides in the extract and FAPEs in the raffinate as a function of solvent to feed ratio

The mean percentage glycerides in the extract and the mean percentage FAPEs in the raffinate as a function of the S:FR are shown in Fig. 3. ANOVA indicated that there was a significant effect of S:FR on percentage glycerides in the extract ($F_{4,5} = 8.90$, $p = 0.02$) as well as FAPEs in the raffinate ($F_{4,5} = 6.98$, $p = 0.03$). The percentage FAPEs in the raffinate (i.e., contaminants) decreased as S:FR increased, while the percentage glycerides in the extract increased as S:FR increased. There is a trade-off between the purity of the raffinate product and the purity of the extract.

The percentages of glycerides in the raffinate as a function of FR are shown in Fig. 4. Initially, only FRs of 1, 2, 3, and 4 mL/min (i.e., 0.90, 1.81, 2.71, and 3.61 g/mL, respectively) were planned; however, the resulting data suggested that a feed rate of 2.5 mL/min (i.e., 2.26 g/mL) might yield a very high raffinate purity and it was therefore added. A second degree polynomial (see Fig. 4) fit the percentage raffinate glycerides as a function of FR data very well and gave an *R*-squared of 0.86 ($p < 0.001$). At a

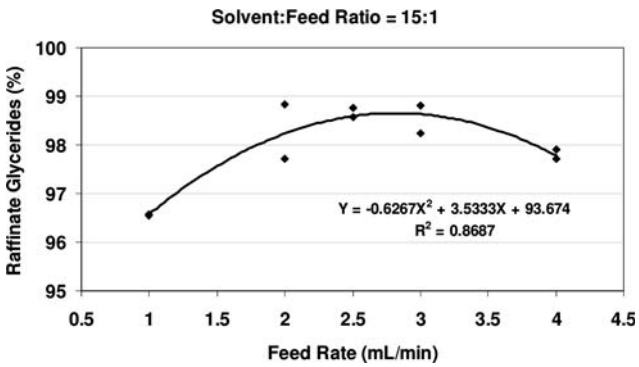

Fig. 4 Percentage glycerides in the raffinate as a function of feed rate with a constant solvent to feed ratio

FR of 2.5 mL/min, a raffinate of ca. 99% purity was obtained.

The results from the previous S:FR and FR experiments were used to choose optimized parameters (i.e., S:FR of ca. 20 and FR of 2.5 mL/min) to fractionate a large batch (ca. 1.75 L) of the crude propanolysis of HOSO mixture in a semi-continuous mode. This separation gave a raffinate product of ca. 99.3% glycerides and an extract of ca. 97.3% FAPEs. Although the percentage glycerides in the raffinate from the semi-continuous fractionation was similar to that observed in previous experiments, the FAPEs in the extract were slightly higher than anticipated. The substitution of the continuous feed pump and the subsequent elimination of the disruptions that occurred during the semi-continuous fractionations may have allowed better separation of the components.

Prior to the substitution of the HPLC pump to provide continuous feed flow and the addition of the gas booster pump timed solenoid assembly to minimize flow fluctuations, fractionation runs were conducted during regular working hours and required frequent checks and adjustments to the system. The modified system allowed unattended overnight runs and greatly enhanced our capacity to purify the 20 kg of crude reaction mixture from the propanolysis of the HOSO. This continuous feed system yielded a raffinate which contained ca. 98.1% glycerides and an extract which contained ca. 97.2% FAPEs. The raffinate obtained from this separation was used in the subsequent preparation of feruloylated HOSO intended for potential commercial manufacturers.

This research demonstrates that L-CO_2 can be used in a continuous counter-current fractionation system to effectively remove by-product FAPEs from a mixture of TAGs, DAGs, MAGs and FAPEs. The resulting glycerides can subsequently be used as the starting material to produce feruloylated glycerides. The purity of the glyceride raffinate product increased with S:FR, however, the extract purity (i.e., FAPEs) decreased as S:FR increased. Therefore, operating parameters can be chosen to optimize the relative purities of the raffinate and/or extract as desired. Both the product glyceride fraction (i.e., raffinate) as well as the FAPE fraction (i.e., extract) were both quite pure (i.e., 98.1% and 97.2%, respectively) under certain conditions (i.e., S:FR of ca. 20 and FR of 2.5 mL/min). The FAPE byproduct resulting from the partial deacylation (i.e., propanolysis) of HOSO has garnered interest as a potential cosmeceutical ingredient as well.

There is interest in controlling acyl migration during the synthesis and purification of structured acylglycerols [19, 20]. In a comparison of L-CO_2 fractionation and molecular distillation methods, the L-CO_2 fractionation afforded a more efficient removal of FAPEs (96% versus 77%, respectively) [7]. In addition, the L-CO_2 fractionation method caused no appreciable acyl migration in the product glycerides, and did not adversely affect the acid value nor the Lovibond colors of the partially deacylated oils or the FAPE as did the molecular distillation method [7]. Because of these benefits, this continuous counter-current L-CO_2 separation fractionation technique may be useful to those interested in synthesizing and purifying specifically structured lipids.

References

1. Compton DL, Laszlo JA, Berhow MA (2000) Lipase-catalyzed synthesis of ferulate esters. J Am Oil Chem Soc 77:513–519
2. Compton DL, King JW (2001) Lipase-catalyzed synthesis of triolein-based sunscreens in supercritical CO_2. J Am Oil Chem Soc 78:43–47
3. Laszlo JA, Compton DL, Eller FJ, Taylor SL, Isbell TA (2003) Packed-bed bioreactor synthesis of feruloylated monoacyl- and diacylglycerols: clean production of a "green" sunscreen. Green Chem 5:382–386
4. Compton DL, Laszlo JA (2000) Sunscreens from vegetable oil and plant phenols. US Patent 6346236
5. Warner K, Laszlo JA (2005) Addition of ferulic acid, ethyl ferulate, and feruloylated monoacyl- and diacylglycerols to salad oils and frying oils. J Am Oil Chem Soc 82:647–652
6. Laszlo JA, Compton DL (2006) Enzymatic glycerolysis and transesterification of soybean oil for enhanced production of feruloylated mono- and diacylglycerols. J Am Oil Chem Soc 83:765–770
7. Compton DL, Laszlo JA, Eller FJ, Taylor SL (2008) Purification of 1,2-diacylglycerols from vegetable oils: comparison of molecular distillation and CO2 extraction. Ind Crops Prod 28:113–121
8. Brunner G (1998) Industrial process development: countercurrent multistage gas extraction (SFE) processes. J Supercrit Fluids 13:283–301
9. Nilsson WB, Gauglitz EJ Jr, Hudson JK, Stout VF, Spinelli J (1988) Fractionation of menhaden oil ethyl esters using supercritical fluid CO2. J Am Oil Chem Soc 65:109–117
10. Perretti G, Motori A, Bravi E, Favati F, Montanari L, Fantozzi P (2007) Supercritical carbon dioxide fractionation of fish oil fatty acid ethyl esters. J Supercrit Fluids 40:349–353
11. Vazquez L, Torres CF, Fornari T, Senorans FJ, Reglero G (2007) Recovery of squalene from vegetable oil sources using countercurrent supercritical carbon dioxide extraction. J Supercrit Fluids 40:59–66
12. Vazquez L, Fornari T, Senorans FJ, Reglero G, Torres CF (2008) Supercritical carbon dioxide fractionation of nonesterified alkoxyglycerols obtained from shark liver oil. J Agric Food Chem 56:1078–1083
13. King JW, Sahle-Demessie E, Temelli F, Teel JA (1997) Thermal gradient fractionation of glyceride mixtures under supercritical fluid conditions. J Supercrit Fluids 10:127–137
14. Dunford NT, Teel JA, King JW (2003) A continuous countercurrent supercritical fluid deacidification process for phytosterol ester fortification in rice bran oil. Food Res Inter 36:175–181
15. Ruivo R, Couto R, Simoes PC (2008) Supercritical carbon dioxide fractionation of the model mixture squalene/oleic acid in a membrane contactor. Sep Purif Technol 59:231–237
16. Ruivo R, Cebola MJ, Simoes PC, Nunes da Ponte M (2001) Fractionation of edible oil model mixtures by supercritical carbon

dioxide in a packed column. Part I. Experimental results. Ind Eng Chem Res 40:1706–1711
17. Nilsson WB, Gauglitz E Jr, Hudson JK (1988) Solubilities of methyl oleate, oleic acid, oleyl glycerols, and oleyl glycerol mixtures in supercritical carbon dioxide. J Am Oil Chem Soc 68:1087–1091
18. Eller FJ, Taylor SL, Compton DL, Laszlo JA, Palmquist DE (2008) Counter-current liquid carbon dioxide purification of a model reaction mixture. J Supercrit Fluids 43:510–514
19. Compton DL, Vermillion KE, Laszlo JA (2007) Acyl migration kinetics of 2-monoacylglycerols from soybean oil via 1H NMR. J Am Oil Chem Soc 84:343–348
20. Laszlo JA, Compton DL, Vermillion KE (2008) Acyl migration kinetics of vegetable oil 1,2-diacylglycerols. J Am Oil Chem Soc 85:307–312