Short communication

Supercritical fluid chromatographic analysis for on-line monitoring of hexane removal from soybean oil miscella using liquid carbon dioxide

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Abstract

Liquid carbon dioxide (L-CO$_2$) can be used to separate hexane from hexane/soybean oil (SBO) mixtures (i.e., miscella). An on-line supercritical fluid chromatographic (SFC) method was developed to monitor this separation. L-CO$_2$ (25 °C and 9.31 MPa) was passed through 50 mL of a 25% (w/w) hexane miscella and then directed on-line through a SFC injector. After passing 300-L expanded CO$_2$, the hexane concentrations in the L-CO$_2$ were 0.05% and 0.04% for *n*-hexane and isohexane, respectively and the residual hexane concentrations in the SBO were 3.8 and 3.3 ppm, respectively. This technique provided real time on-line monitoring of the hexane separation process.
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1. Introduction

Soybean oil (SBO) is currently extracted on an industrial scale by hexane and the largest operating cost in this process is the separation of hexane from the extracted oil [1]. The removal of hexane from the oil is critical because of hexane's demonstrated toxic effects [2] and hexane is listed as a hazardous air pollutant by the U.S. Environmental Protection Agency. With conventional methods, the concentration of hexane in SBO after the first stripper is typically 1000 ppm, with 500 ppm the minimum that can be achieved [3]. It has been demonstrated that supercritical carbon dioxide (SC-CO$_2$) (12 MPa, 40–60 °C) could be used to remove hexane from a hexane/SBO mixture using a counter-current packed tower. Residual hexane levels as low as 20 ppm were obtained [4].

Because capital and operating costs are proportional to the pressures and temperatures used in critical fluid extractions, our laboratory investigated the use of liquid carbon dioxide (L-CO$_2$)

(i.e., lower pressure and temperature) in place of SC-CO$_2$ to reduce the costs associated with the use of supercritical CO$_2$ to separate hexane from SBO [5]. In our initial study, we found that 200 L CO$_2$ (expanded gas) was sufficient to decrease the concentration of *n*-hexane in 50 mL of a 25% solution of *n*-hexane in SBO (i.e., 250,000 ppm) to less than 20 ppm. Residual *n*-hexane levels in the extracted SBO (i.e., the raffinate) were determined using ISO Method 9832:2002 [6]. This method, however, is very tedious and requires sample preparation times of well over an hour as well as an analysis of a large series of standard solutions.

Previous researchers have coupled SC-CO$_2$ extractions on-line with supercritical fluid chromatography (SFC) [7–9] to analyze extracts. However, in these cases, the extractions and the subsequent SFC are somewhat discontinuous in that the extraction is run for a limited time, stopped and then the SFC analysis is performed or the extract is concentrated on the head on an adsorbent column or cryofocusing trap then eluted onto the SFC column. The purpose of this study was to develop an on-line SFC analysis method that would allow real time determination of the concentrations both *n*-hexane as well as isohexane over the course of L-CO$_2$ extractions of hexane/SBO mixtures.

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2. Experimental

2.1. Determination of hexane calibration curves

Calibration curves for *n*-hexane and isohexane in L-CO$_2$ were determined using concentrations of 0.01, 0.03, 0.1, 0.3, 1.0 and 3.0% (v/v) of hexane in L-CO$_2$. A Rheodyne Model 3725-038 sample injector (Chrom Tech, Inc., Apple Valley, MN, USA) with a 10 mL sample loop was placed on the inlet side of an Isco Model 260D (Isco Inc., Lincoln, NE, USA) syringe pump. The syringe pump's outlet tubing was connected to the sample inlet of the SFC injection valve. Varying volumes of *n*-hexane or isohexane were loaded into the sample loop and then introduced into the syringe pump during CO$_2$ filling by turning the injector to the inject position. After the hexane was introduced into the syringe pump, the pump was pressurized to 9.3 MPa and then depressurized to ca. 5.5 MPa twice to uniformly mix the hexane with the L-CO$_2$. The pump was then pressurized to 9.3 MPa, the outlet valve was opened and hexane/CO$_2$ mixture was pumped through the injector of the SFC. The hexane/CO$_2$ flow was allowed to equilibrate for ca. 40 min, then the SFC program was initiated and run for 10 cycles of 4 min each. The FID counts for the six concentrations of *n*-hexane and isohexane were recorded and analyzed separately to find mathematical equations that described the hexane concentration/FID response data using TableCurve 2D curve-fitting software (Systat Software Inc., Richmond, CA, USA).

2.2. Hexane soybean oil mixtures

The hexane/SBO mixtures were prepared by mixing HPLC grade *n*-hexane (Fisher Scientific, Fair Lawn, NJ, USA) or isohexane (Conoco-Phillips, Houston, TX, USA) and SBO purchased locally (Aldi Inc., Batavia, IL, USA) to give solutions containing 25% (w/w) *n*-hexane or isohexane, respectively. The densities of the 25% (w/w) solutions were determined to be 0.844 g/mL and 0.841 g/mL, respectively using a mini weight per gallon cup (P.N. Gardner Co. Inc., Pompano Beach, FL, USA). These mixtures were placed in glass bottles filled to the rim, sealed and held at −70 °C until used in experiments.

2.3. Fractionation column

The L-CO$_2$ extractions were performed in a column previously described [5]. A schematic of the fractionation column modified with the SFC is shown in Fig. 1. The column has an internal diameter of 1.43 cm, a height of 253.2 cm, an internal volume of 412 mL and was packed with protruded stainless-steel packing (0.41 cm Pro-Pak) (Cannon Instrument Co., State College, PA, USA) with a 94% void volume. The column was held at 25 °C by being wrapped with silicone tubing that was attached to a Neslab Model RTE-220 refrigerated circulating bath (Neslab Instruments, Inc., Newington, NH, USA). Glas Col heating mantles enclosed the column and were used to heat the column during the post-extraction cleanup.

Fifty mL of the 25% (w/w) solutions of hexane/SBO (i.e., 42.20 g and 42.05 g of the *n*-hexane and isohexane solutions,
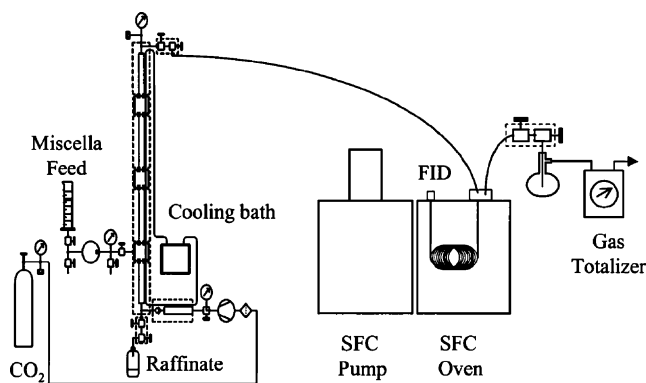

Fig. 1. Schematic of fractionation column and supercritical fluid chromatograph.

respectively) were introduced into the column using a Haskel Model MS-188 liquid metering pump (Haskel International Inc., Burbank, CA, USA) connected to a stroke counter controller. Mixtures were fed into the column above the first zone. Welding-grade carbon dioxide (Airgas Inc., Radnor, PA, USA) was fed from a cylinder and passed through a filter containing alumina C to a Haskel Model AG-30 gas booster pump (Haskel International Inc., Burbank, CA, USA). The column was pressurized to 9.3 MPa and equilibrated for 10 min before the extraction commenced.

Liquid CO$_2$ entered the precooler, passed through the hexane/SBO mixture and up through the column. The hexane-loaded L-CO$_2$ flowed out of the top of the column and directly through the SFC injector via a stainless steel tube (3 m × 1.59 mm o.d. × 0.76 mm i.d.) (Alltech Associates, Inc., Deerfield, IL, USA). The CO$_2$ flowed through the sample loop (200 nL) of the SFC injector and exited at a flow rate of ca. 3–4 L/min (expanded gas) across a micro-metering valve, allowing the extract to be collected in a tared 100-mL round-bottomed flask. The expanded CO$_2$ passed through a dry test meter to measure the total gas volume and was then vented to the atmosphere. The extracted SBO (i.e., raffinate) was then collected from the bottom of the column through the valve system. Residual hexane levels in the SBO raffinate were determined by headspace GC analysis using ISO Method 9832:2002 [6]. Three replications were performed for both *n*-hexane and isohexane.

After each extraction, the column was cleaned using SC-CO$_2$ to prevent contaminants from being carried over to subsequent runs. The 25 °C refrigerated circulating bath was turned off, the column was heated to 100 °C and pressurized to 58.6 MPa and SC-CO$_2$ (800 L expanded) was run through the column at 5 L/min for cleanup.

2.4. Supercritical fluid chromatography

SFC analyses of *n*-hexane and isohexane standard solutions in L-CO$_2$ as well as the hexane/SBO solutions in L-CO$_2$ were conducted with a Lee Scientific Series 600 SFC (Dionex Corp., Salt Lake City, UT, USA) equipped with a flame ionization detector held at 350 °C. SFC/SFE-grade carbon dioxide (Airgas Inc., Radnor, PA, USA) was used as the carrier fluid. A SB-Octyl-50 capillary column (10 m × 50 μm i.d., 0.25 μm film thickness)
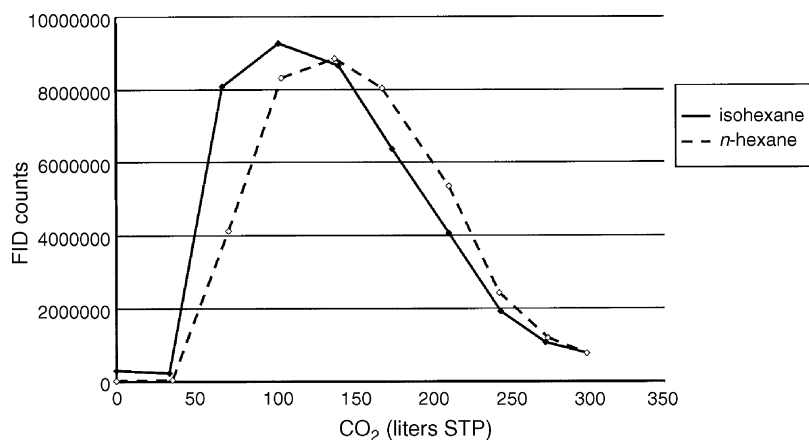
Fig. 2. FID counts vs. liters of CO_2 for liquid CO_2 separation of *n*-hexane and isohexane from soybean oil.

(Dionex Corp., Salt Lake City, UT, USA) held at 80 °C and 21.3 MPa for 10 min was used. Injections were made as timed-split injections with a Valco valve for 0.5 s with a 200 nL loop. Chromatographic data was quantified using a Data-Jet integrator (Spectra Physics Inc., Fremont, CA, USA).

3. Results and discussion

Relatively simple equations were found which fit the hexane concentration/FID count data very well. The equation for *n*-hexane was: concentration (%) = FID count/(2.097 × FID count + 14224963) and the equation for isohexane was: concentration (%) = FID count/(−2.082 × FID count + 22234891) with R^2 values of 0.972 and 0.988, respectively. These equations were compared statistically using SAS software (SAS Institute, Inc. Cary, NC, USA) to determine if a combined equation might be used instead of two separate equations, however, the two lines were found to be significantly different ($F_{2,118} = 22.65$, $P \ll 0.01$). Subsequently, the individual equations relating FID counts to hexane concentration were used to determine unknown hexane concentrations in the L-CO_2 stream after passing through the hexane/SBO mixtures.

Fig. 2 shows the mean FID counts for *n*-hexane and isohexane as a function of the volume of L-CO_2 passed through the fractionation column. For both solvents, the FID counts initially increase with the amount of L-CO_2, reach a maximum at ca. 100–150 L CO_2 then decrease with increasing amounts of L-CO_2. After 300 L expanded CO_2 has passed through the hexane/SBO mixture, the FID counts are quite low. The peak FID count for isohexane was slightly earlier than that for the *n*-hexane. TableCurve 2D curve-fitting software (Systat Software Inc., Richmond, CA, USA) was used to derive equations relating FID count versus volume of CO_2 for both *n*-hexane as well as isohexane, resulting in equations with R^2 values of 0.98 and 0.91, respectively. From these equations, function FID maxima were determined as well as the corresponding CO_2 volumes associated with the FID maxima. The CO_2 volumes and 95% confidence intervals (CI) were determined to be 119.1 (95% CI 118.2, 120.0) and 107.4 (95% CI 106.8, 108.0) L, for *n*-hexane and isohexane, respectively. Isohexane appears to be more read-ily removed from the hexane/SBO mixture than does *n*-hexane perhaps because it has a lower boiling point, higher vapor pressure and thus higher solubility in L-CO_2.

Conversion of the FID counts to hexane concentrations indicated that the hexane concentrations in the L-CO_2 after 300 L expanded CO_2 were very low, with mean (SD) values of 0.05% (0.01) and 0.04% (0.01) for *n*-hexane and isohexane, respectively. The subsequent headspace GC analysis of residual hexane in the extracted SBO (i.e., raffinate) after 300 L expanded CO_2 also showed that the SBO had extremely low levels of hexane. The mean (SD) residual *n*-hexane and isohexane values were 3.8 ppm (3.3) and 3.3 ppm (1.8), respectively. Considering that the *n*-hexane and isohexane were initially 25% (w/w) (i.e., 250,000 ppm), these residual hexane values represent a greater than 99.998% removal of the hexanes from the hexane/SBO mixtures. These residual hexane values are also well below the values of 500–1000 ppm typically obtained by the first stage stripper using conventional extraction methodology [3].

Previous research indicated there was a slight co-extraction of SBO with *n*-hexane when L-CO_2 is used to separate hexane from a hexane/SBO mixture [5]. This co-extraction of SBO by the L-CO_2 was also observed in this study. The SFC retention times for the hexane and co-extracted SBO triglycerides in the L-CO_2 stream were 0.8 min and 4.9 min, respectively of the 10 min analysis. The SFC FID counts for the SBO present in the L-CO_2 indicated that the SBO concentration began to increase after ca. 100 L expanded CO_2 and then leveled off after ca. 150 L expanded CO_2. The mean (SD) carry-over of SBO after 300 L expanded CO_2 was 0.63 g (0.06) and 0.50 g (0.05) for *n*-hexane and isohexane, respectively. This represents ca. 2.0% and 1.6% of the total amount of SBO placed in the column for *n*-hexane and isohexane, respectively. Our laboratory is investigating means to limit this co-extraction of SBO when using L-CO_2 to separate hexane from hexane/SBO mixtures.

4. Conclusions

Liquid CO_2 readily removes both *n*-hexane and isohexane from hexane/SBO mixtures and by correlating the SFC FID data of hexane in the L-CO_2 stream to the residual hexane in SBO, it

can be seen that hexane removal is complete when the hexane in L-CO_2 percentage is below 0.1%. Both *n*-hexane and isohexane in SBO mixtures are reduced from 250,000 ppm to ca. 3 ppm with 300 L of expanded CO_2. SFC provides a useful tool for real time in-line monitoring of hexane removal from hexane/SBO mixtures. This technique allows a quick determination of the progression of the hexane removal process using L-CO_2. This in-line coupling of L-CO_2 extraction on-line with SFC analysis is inherently different from previous coupling methods in that the extraction is continuous without a concentration step and repeated SFC analyses can be made to monitor the course of the extraction.

Acknowledgment

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