Supercritical Carbon Dioxide Extraction of Cottonseed with Co-Solvents[1]

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Extraction of cottonseed lipids with supercritical carbon dioxide (SC-CO_2) was conducted with and without a co-solvent, ethanol or 2-propanol (IPA). At 7000 psi and 80°C, the reduced pressure, temperature and density of SC-CO_2 was at 6.5, 1.17 and 1.85, respectively; the specific gravity was 0.87. Under these conditions, CO_2 is denser than most liquid extraction agents such as hexane, ethanol and IPA. The extraction of cottonseed with SC-CO_2 gave a yield of more than 30% (moisture-free basis). This is comparable to yields obtained by the more commonly used solvent, hexane. The crude cottonseed oil extracted by SC-CO_2 was visually lighter than refined cottonseed oil. This was substantiated by colorimetric measurements. No gossypol was detected in the crude oil. However, crude oil extracted by SC-CO_2, to which less than 5% of ethanol or IPA as co-solvent was added, contained *ca.* 200 ppm of gossypol, resulting in the typical dark color of cottonseed crude oil with gossypol. CO_2 extracted a small amount of cottonseed phosphatides, about one-third of that extracted by pure ethanol, IPA or hexane. A second extraction with 100% ethanol or IPA after the initial SC-CO_2 extraction produced a water-soluble lipid fraction that contained a significant amount of gossypol, ranging between 1500 and 5000 ppm. Because pure gossypol is practically insoluble in water, this fraction is believed to be made up of gossypol complexed with polysaccharides and phosphatides.

KEY WORDS: Carbon dioxide, co-solvent, cottonseed lipids, ethanol, extraction, gossypol, hexane, phosphatides, polysaccharides, supercritical fluid.

Supercritical fluid (SCF) has versatile extraction capability, which comes from the innate solvent power that originates from the molecular association of the fluid in its supercritical domain (1). For a normally incompressible liquid, such as hexane, the extraction capability depends upon the thermal energy levels of the constituting molecules and is less affected by pressure exerted by the surrounding molecules. However, once liquid molecules are placed at a temperature and pressure higher than their critical point, the intermolecular distances and forces can be closely controlled by independently controlling either pressure or temperature. This control is exploited in supercritical fluid extraction (SFE) (2).

Cottonseed contains proteins, triglycerides and their degenerated species, phosphatides, polysaccharides and biphenyls and other minor compounds. Although solvent extraction of cottonseed aims at recovering triglycerides, the recovered oil contains other undesired compounds. A powerful solvent such as hexane is industrially used for the exhaustive recovery of triglycerides. Because of the exhaustive nature of extraction, the mixture of cottonseed crude oil and solvent extracts cottonseed lipid components other than triglycerides, such as gossypol.

In this investigation, supercritical carbon dioxide (SC-CO_2) was used to extract cottonseed flakes with and without ethanol and 2-propanol (IPA) as co-solvents. It was aimed at testing whether SC-CO_2 is capable of extracting only triglycerides. After the initial extraction, the remaining compounds in the cottonseed were extracted again with the alcohols to produce a gossypol-rich fraction. This gossypol-rich extract was separated into two fractions, one water-soluble and the other water-insoluble. The gossypol content in these two fractions was examined to investigate the possible relationship of gossypol with nontriglyceride cottonseed lipids.

MATERIALS AND METHODS

Newly harvested, delinted and hulled cottonseed was conditioned without cooking to *ca.* 9.5% moisture and was then flaked to 0.25 mm. Research-grade CO_2 (Matheson, Montgomeryville, PA) was compressed and heated above the critical point of CO_2 (Fig. 1) to produce SC-CO_2. U.S.P.-grade anhydrous ethanol (USI Chemicals, Cincinnati, OH) and IPA (Fisher Scientific, Fairlawn, NJ) were used as co-solvents without further purification. A commercially available fluid extractor, SFX-10 (Isco, Lincoln, IA), equipped with a 10-mL SFE vessel, was used without modification. Three sytems of SCF (SC-CO_2, SC-CO_2 with ethanol and SC-CO_2 with IPA) were tested for SFE.

The basic extraction procedure is as follows: *ca.* 5 g of preconditioned cottonseed flakes were packed in a cylindrical stainless-steel SFE vessel with glasswool placed at both ends. The vessel was placed in an isothermal chamber at the extraction temperature. The vessel was charged with SC-CO_2 by a positive-displacement pump. The extraction temperature and pressure, 80°C and 7000 psi, were selected so that the molar density of SC-CO_2 was slightly denser than that of the industrial extraction sol-

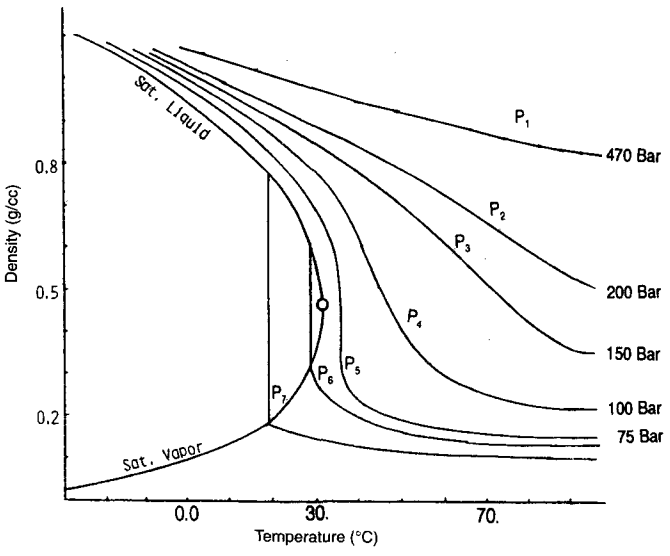

FIG. 1. Supercritical fluid density map. P_1–P_7 = isobaric curves; sat., saturated.

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vent of cottonseed, hexane. It was found that an extraction time of 60 min removed more than 98% of cottonseed lipid by the SC-CO_2 used in this investigation. The extractant and extract were collected in a vial filled with aqueous methanol, and CO_2 was vented out to ambient air. The amount of SC-CO_2 used for SFE runs was recorded. In the case of SFE with co-solvent, cottonseed flakes and the co-solvent were charged together in the SFE vessel. The charged amount of co-solvent was limited to 5 vol% of SC-CO_2, which was determined from the prior SFE runs without the co-solvent. Extracted cottonseed flakes were collected for further lipid extraction with the alcohols to determine the residual oil content and gossypol. In addition to the three types of cottonseed oils produced by SFE, an additional cottonseed crude oil was produced with ethanol. The AOCS Method Aa 4-38 (3), which is used for determining cottonseed oil content, was modified to produce the ethanol-extracted crude oil. Two modifications were made in this AOCS method: a Soxhlet extractor was used in place of a Butt-type, and petroleum ether was replaced by ethanol.

Crude oils were analyzed for color determination with a colorimeter (Colorscan; Tintometer, Salisbury, United Kingdom), triglyceride profiles with a high-temperature capillary gas chromatograph, gossypol and free fatty acid by AOCS methods (3), phosphorus content by induction-coupled plasma analysis, and CO_2-extracted meal for amino acid profiles by high-performance liquid chromatography (HPLC).

RESULTS AND DISCUSSION

Figure 1 shows the density map of SC-CO_2. The critical density of CO_2 is 0.466 (circle on saturated liquid–vapor curve). The SC-CO_2 density increases with pressure but decreases with temperature in the upper-right quadrant of the density map. The region of the map that is bounded by isobaric curves P_1 and P_3 and by temperatures between 40 and 100°C is the most investigated area. This is because the primary physical property, fluid density, in this region is comparable to that of more commonly used liquid extraction agents. The SC-CO_2 has other advantageous properties in this region, namely lower viscosity and higher diffusivity (1,2).

An extraction temperature of 80°C was selected in this investigation, because most cottonseed glycerides, including all high-melting-point lipids, exist in their liquid form at this temperature (4). The selected extraction pressure was 7000 psi (P_1 in Fig. 1). The fluid density is *ca.* 0.87 at the extraction temperature and pressure, about 30% heavier than hexane at its normal boiling point. At this condition, SC-CO_2 is expected to extract the neutral cottonseed lipids as efficiently as hexane does.

The crude cottonseed oils, obtained by extraction in each of four systems (pure SC-CO_2, SC-CO_2 plus co-solvents, and pure ethanol), were colorimetrically examined and compared with refined cottonseed oil. The AOCS color measurements of these oils, expressed in terms of the AOCS-Tintometer color scale (3), are presented in Table 1. As shown in Table 1, the SC-CO_2-extracted crude has a much lighter color compared with that produced with SC-CO_2 plus a co-solvent, or with 100% ethanol. It was even lighter than refined cottonseed oil. The color of refined cottonseed oil, which was produced by ethanol extraction, was slightly lighter than hexane-extracted oil (5).

TABLE 1

AOCS-Tintometer Color Scale of Crude and Refined Cottonseed Oil Extracted by SC-CO_2 and Alcohols

Oil	Extraction solvent	AOCS-Tintometer color[a]
Crude	SC-CO_2 only	0.2 R/1.0 Y
Crude	SC-CO_2 with ethanol	2.8 R/23.8 Y
Crude	SC-CO_2 with IPA	3.6 R/26.4 Y
Crude	Ethanol	56.0 R/70.0 Y
Refined	Ethanol	1.1 R/6.1 Y

[a]AOCS Official Method Cc 13b-45 (Ref. 3) measured with 10-mm path cube. R = red; Y = yellow; SC-CO_2 = supercritical carbon dioxide; IPA = 2-propanol.

Table 2 shows the yield data of cottonseed crude oil and the content of gossypol and phosphorus extracted by SC-CO_2 with and without co-solvents. The threshold amount of CO_2 required to produce the yield was *ca.* 35 g of SC-CO_2 (*ca.* 40 mL) at the condition of SFE. The free gossypol in the raw flakes was relatively high, amounting to 1.4% [AOCS method (3)], which was attributed to the seed preparation without cooking. Even with high content of "unbound" gossypol in the raw flakes, SC-CO_2 at ϱ_r of 0.9 extracted *ca.* 200 ppm of gossypol. From the data in Table 2, it may be inferred that the SC-CO_2, with a molecular volume about 50 mL/mol (equivalent to specific gravity of 0.87) or larger, does not extract gossypol from cottonseed lipids, although gossypol existed in the "unbound" form in the extraction source.

Although SC-CO_2 was unable to transfer gossypol into the pool of crude oil from the oilseed matrix, it extracted 270 ppm of phosphorus compounds, *ca.* one-third of what would be produced by 100% ethanol, IPA or even hexane. Because of the presence of the fatty acid moieties in the cottonseed phosphatides, the phosphorus compounds may be more easily tied to SC-CO_2 than gossypol.

The triglyceride profiles of the CO_2-extracted crude and of that produced with 100% ethanol are shown in Figures 2 and 3, respectively. The two chromatograms of

TABLE 2

Crude Oil Yield and Gossypol and Phosphorus Content in Cottonseed[a] Crude Oils Extracted by SC-CO_2 and Co-Solvents

Extraction solvent	Gossypol[b] (%)	Phosphor[c] (ppm)	Crude oil yield[d,e] (%)
SC-CO_2	0.0	270	30.5–31.1
SC-CO_2/ethanol	0.0228	570	33
SC-CO_2/IPA	0.0207	573	32
Ethanol	1.37	876	31
IPA	1.33	880	30.5

[a]The raw flakes contained 1.51% total gossypol, 1.4% free gossypol [AOCS Official Method Ba 7p-58 (Ref. 3)]. See Table 1 for abbreviations.
[b]AOCS Official Method Ba 8-78 (Ref. 3).
[c]Determined by Induction-Coupled Plasma Method after Micro-Kjeldahl digestion.
[d]Wt% without moisture.
[e]With a range of ±0.5%.

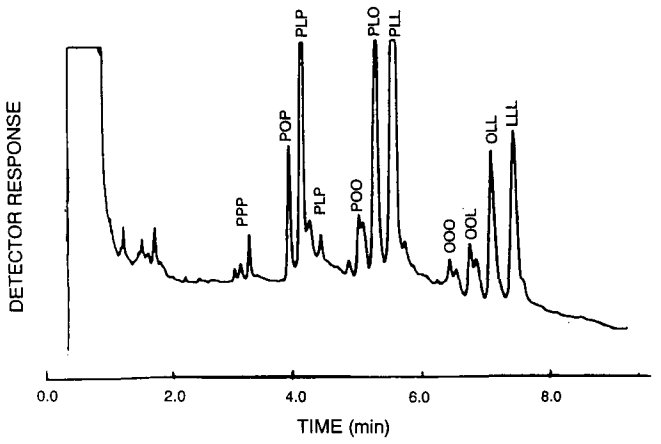

FIG. 2. Supercritical carbon dioxide-extracted cottonseed triglycerides. Acids: P, palmitic; O, oleic; L, linoleic.

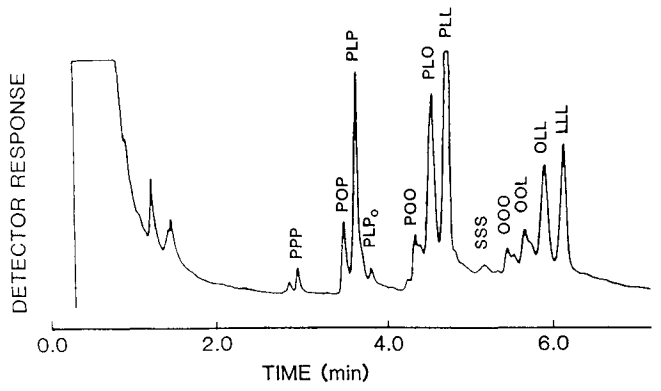

FIG. 3. Ethanol-extracted cottonseed triglycerides. See Figure 2 for other abbreviations. S, stearic acid.

cottonseed triglycerides were almost identical; the difference is that the chromatogram of SC-CO_2 crude displays an extra component in the group of triglycerides with 48 carbons. These triglycerides with 48 carbons are the peaks shown prior to the component labeled "PPP" in Figure 2 (PPP stands for tripalmitin). These chromatograms indicated that SC-CO_2 tends to dissolve smaller triglycerides than the conventional counterpart, hexane, does. This observation was also made elsewhere in a fish oil extraction by SC-CO_2 (6). From the oil yield data and the analysis of extracted components, one may conclude that SC-CO_2 at a molecular volume of *ca.* 50 mL/mol had as much attraction force as hexane or the alcohols to extract molecules with the glyceride structure but not enough to extract gossypol molecules.

Subsequent to the SC-CO_2 extraction of triglycerides, an attempt was made to recover the residual free gossypol that was left intact in the flakes. Because more than 98% of the triglycerides were extracted by SC-CO_2, the subsequent extraction produced an extremely gossypol-rich fraction. This gossypol-rich lipid was composed of two fractions, one water-soluble and the other water-insoluble. The water-soluble fraction contained *ca.* 1500 to 5000 ppm of free gossypol. Because free gossypol is practically insoluble in water (7), the water-soluble fraction must consist of gossypol–polysaccharide or gossypol–phosphatide complexes. Previous studies (8) have shown that minor polysaccharides, extracted from cottonseed by aqueous alcohol, along with phosphatides, form a variety of complexes with gossypol (9). The characteristics of gossypol complexes, especially those in the water-soluble fraction, have not been well investigated.

The water-insoluble fraction contained 4.4 to 5.4% gossypol, an unusually high concentration. This high concentration of gossypol was expected because of minimum presence of triglycerides in the secondary extraction. The water-insoluble fraction contained the residual triglycerides and phosphatides, which amounted to about 1% of the raw flakes. The water-insoluble fraction did not contain polysaccharides but did contain the unhydratable phosphatides. Cottonseed phosphatides have been suspected to form complexes with gossypol (9). Quantitation and characterization of the phosphatides in the water-insoluble fraction, along with determination of the gossypol that is bound to the phosphatides, may bring some elucidation on the complex between gossypol and the unhydratable phosphatides. Because gossypol and its derivatives are the cause of the color fixation problem (9) in cottonseed crude oil, further investigation of gossypol complexes obtained from the water-soluble and water-insoluble fractions may be useful to develop preventive measures for the problem.

To examine quality of the cottonseed meal produced by SC-CO_2, an amino acid analysis of the meal was conducted by HPLC, and its results are presented in Table 3. The amino acid profile was similar to that obtained by hexane, indicating that SC-CO_2 extraction produced a cotton meal similar to that produced by conventional extraction (10). The amino acid analysis given in Table 3 is a fair representative profile of SC-CO_2-extracted cottonseed meal.

In conclusion, we demonstrated that SC-CO_2 may be utilized not only to extract the desired cottonseed lipids (mainly triglycerides) without including deleterious compounds such as gossypol but also to produce a lipid fraction made up of phosphatide–gossypol and polysaccharide–gossypol complexes.

TABLE 3

Comparison of Amino Acid Profile[a] of Cottonseed Meal Extracted with SC-CO_2 and Hexane

Amino acid	SC-CO_2 extract	Hexane[b] extract
Arginine	53.6	47.0
Histidine	13.2	17.2
Lysine	20.6	23.4
Tyrosine	14.3	9.4
Phenylalanine	25.5	34.4
Methionine	6.8	7.8
Threonine	16.0	17.2
Serine	19.6	28.1
Leucine	26.7	34.4
Isoleucine	22.7	28.1
Valine	4.5	7.8
Glutamic	90.2	106.0

[a]Per 100 grams of feed nitrogen. See Table 1 for abbreviation.
[b]From Baumgarten *et al.* (10).

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