Concentration of Tocopherols from Soybean Sludge by Supercritical Carbon Dioxide

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A supercritical fluid extraction method has been applied to test the feasibility of tocopherol concentration from soybean sludge with carbon dioxide at temperatures and pressures ranging from 35 to 70°C and 200 to 400 bar, respectively. The supercritical solubility of the esterified soybean sludge was over 4–6 times greater than that of the original soybean sludge. By a simple batch-type one-stage method the tocopherols in the esterified soybean sludge could be concentrated up to 40 wt%. The overall results of the present study show that soybean sludge initially containing about 13–14 wt% tocopherols may require a countercurrent multistage column to be highly and effectively concentrated.

KEY WORDS: Extraction, solubility, soybean oil, supercritical carbon dioxide, tocopherols.

Supercritical fluid extraction (SFE) has been widely applied as a promising alternative to current extraction processes used in the food and pharmaceutical industries. SFE has some advantages over conventional separation processes such as liquid-liquid extraction, distillation, and adsorption. The most important advantage of utilizing SFE is the easy separation of the solvent from the extracted material without solvent residue. Further, supercritical fluids provide lower mass transfer resistance than those in conventional separation processes. A number of references dealing with the basic principles and applications of SFE are now available (1–3).

Several studies for directly extracting oil from soybeans by means of supercritical carbon dioxide (SC-CO_2) have been reported (4,5), but a SFE study for concentrating tocopherols from the soybean sludge, which is a by-product produced in the deodorization of soybean oil, has not yet been available in the literature. Among the components contained in the soybean sludge the tocopherols are of most interest because of their vitamin E activity and their application as an antioxidant. A conventional method has been widely used in order to obtain tocopherol concentrate from soybean sludge by molecular or vacuum distillation after removing the sterols via alcohol recrystallization. However, this process requires several steps such as solvent recovery and purification and further requires copious amounts of organic solvents and energy.

The objective of this study is therefore to examine the feasibility and technical merits of SFE as a potential alternative to molecular distillation for enriching tocopherols effectively from soybean sludge. In this regard, the solubilities of both sterol-removed soybean sludge and esterified soybean sludge in SC-CO_2 were determined by a flow-through SFE system at temperature and pressure ranges of 35–70°C and 200–400 bar, respectively. The tocopherol content in the extracts collected continuously at equilibrium conditions up to the approximate 70 wt% extraction rate of the feed was checked by high-performance liquid chromatography (HPLC) at each interval. The results of these initial feasibility experiments could provide fundamental information for the suitable application of a continuous-type SFE for concentrating tocopherols.

Soybean deodorized sludge containing about 13–14 wt% tocopherols was obtained from Cheil Sugar Co. (Inchon, Korea). The sterol mixture was then removed from the soybean deodorized sludge by recrystallization in n-hexane and 50% (v/v) methanol solution held at low temperature of 4°C. The sterol-removed sludge (810 g) was dissolved in methanol (1.7 L) and 27% (v/v) HCl (17 mL), and refluxed for 1 hr to drive the transesterification to completion. The resulting mixture was neutralized with 10 N NaOH solution and evaporated under a reduced pressure to remove methanol and salt solution. After removing sterols the esterified soybean sludge contained about 18 wt% tocopherols. The HPLC analysis showed that the tocopherol mixture consisted of 10% α-tocopherol, 63.2% γ-tocopherol, 26.8% δ-tocopherol and the trace amount of β-tocopherol.

A continuous flow-through SFE system shown schematically in Figure 1 was used for the tests. Carbon dioxide was supplied from a gas cylinder and was directed to an electrically driven diaphragm-type compressor (Model 554-2121, Nova Werke Ag., Effretikon, Switzerland). The line-filter (Model 60-51HF2, High Pressure Equipment Co., Erie, PA), incorporating 10 μm filter-discs, was used to remove contaminants contained in the carbon dioxide. After compression, the carbon dioxide was introduced into a surge vessel to dampen the fluctuations generated by the compressor. In order to maintain a constant pressure within the system, a back pressure regulator (Model 26-1700, Tescom Co., Elk River, MN) with a stated accuracy of + 1% of the relief pressure range was employed. The equilibrium cell is a 200-cm^3 high-pressure stainless steel vessel packed with 3-mm glass beads. To increase the extraction efficiency between carbon dioxide and the soybean sludge, a metal filter was installed at the end of the inlet tube. The temperature inside the air bath was controlled to within ±0.1°C by using a proportional temperature controller (Model 4202PC2, Omega Engineering Inc., Stamford, CT). The carbon dioxide-solute mixture leaving the top of the extractor was expanded to atmospheric pressure through a micrometering valve into cold traps where the solute has condensed. The flow rate and volume of carbon dioxide were measured by a flow meter and a dry test meter (Model 63115, Precision Scientific, Inc., Chicago, IL). The dry test meter was equipped with gauges to measure flow temperature and pressure. The use of low solvent flow rates (between 0.1 and 0.5 std. L/min) guarantees the attainment of equilibrium conditions at the extractor's outlet. The extracts from the SFE experiments were immediately dissolved with ethyl acetate,

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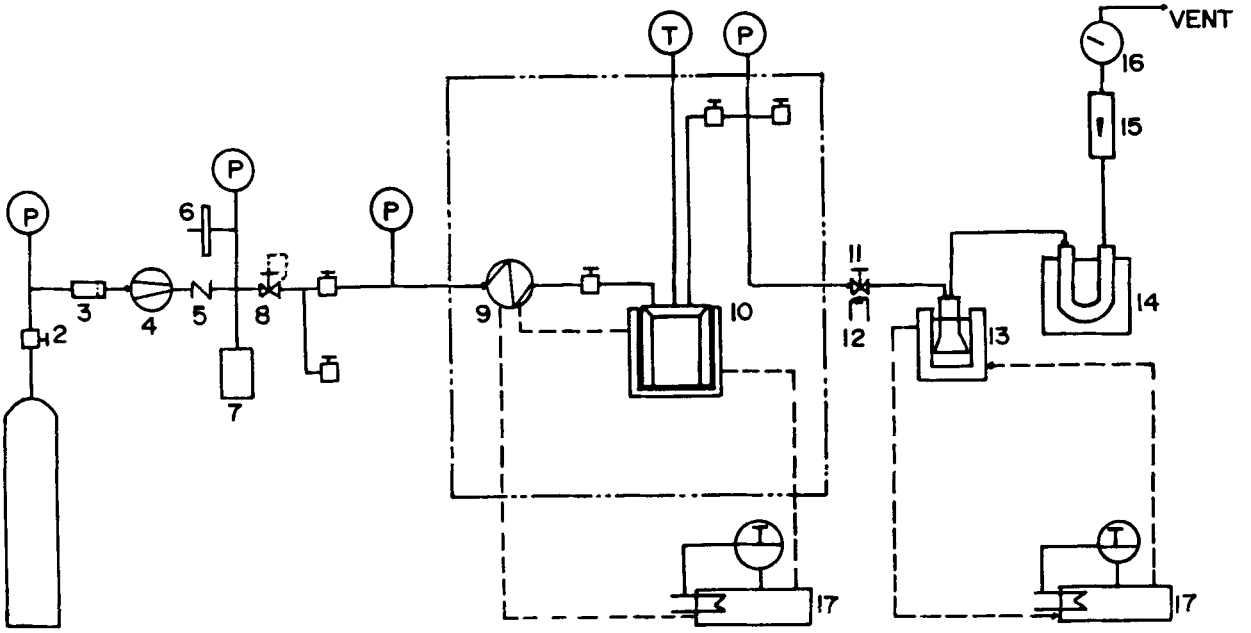

1. CO_2 cylinder
2. High pressure valve
3. Filter
4. Compressor
5. Check valve
6. Rupture disk
7. Damper
8. Pressure regulator
9. Preheater
10. Extractor
11. Pressure control valve
12. Heater
13. Separator/ collector
14. U-tube in cooling bath
15. Rotameter
16. Dry gas meter
17. Constant temp. circulator

FIG. 1. Schematic diagram of the experimental supercritical fluid extraction system used in this study.

and then analyzed by HPLC (UV8010, Tosoh Co., Tokyo, Japan) by eluting with a mobile phase of 96% methanol, 3.8% H_2O and 0.2% acetic acid at a flow rate of 1 mL/min. The compounds were detected by absorbance at 290 nm with an ultraviolet (UV) detector. Under these conditions, the retention times of α-, γ-, and δ-tocopherols were found to be 11.3, 9.7 and 8.3 min, respectively.

RESULTS AND DISCUSSION

The solubilities of the sterol-removed soybean sludge in SC-CO_2 were measured at temperatures of 45, 55, and 70°C and pressures of 200–400 bar and are presented in Table 1. The solubilities of esterified soybean sludge in SC-CO_2 were determined at temperatures of 35, 45, and 70°C and pressures of 200–300 bar to elucidate the effect of the soybean sludge on extraction efficiency (Table 1). The results indicate that esterified soybean sludge has 4–6 times higher solubility in SC-CO_2 than the sterol-removed soybean sludge. The relationships between soybean sludge solubilities established from the mass of oil extracted and the mass of CO_2 consumed during the first sampling interval (30 min), and the extraction pressures can be known from Table 1 for two different experiments. Table 1 indicates that, as the pressure of the CO_2 increases, the soybean sludge solubility also increases for all the temperatures studied. While a cross-over point appeared at approximately 300 bar for the sterol-removed soybean sludge in SC-CO_2, no cross-over point was found in the esterified soybean sludge in SC-CO_2. It has been established (6) that the solvation power of a supercritical solvent is directly linked to its corresponding density. This

TABLE 1

Experimental Solubility Data of Sterol-Removed Soybean Sludge and Esterified Soybean Sludge in SC-CO_2

T (°C)	P (bar)	Density (g/cm^3)	Sterol-removed oil solubility (g oil/g CO_2)	Esterified oil solubility (g oil/g CO_2)
35	200	0.8668		0.1240
	250	0.9024		0.1681
	300	0.9302		0.2379
45	200	0.8131	0.0188	0.1012
	250	0.8577	0.0287	0.1495
	300	0.8909	0.0384	0.2238
	400	0.9404	0.0511	
55	200	0.7550	0.0154	
	250	0.8112	0.0272	
	300	0.8508	0.0386	
	400	0.9240	0.0610	
70	200	0.6599	0.0095	0.0361
	250	0.6734	0.0218	0.0878
	300	0.7885	0.0361	0.1743
	400	0.8574	0.0720	

suggests the possible existence of a relationship between the density of the fluid and the solubility of soybean sludge in the supercritical fluid phase. A semilogarithmic relationship was found to exist between oil solubility and SC-CO_2 density.

The continuously extracted oil concentrations (g oil/g CO_2) are plotted against the extraction rate (% oil extracted) at various temperature and pressure conditions in Figure 2. As can be seen from this figure, the oil solubilities in the SC-CO_2 are greatly reduced with the passage of time at all experimental conditions, since fatty acids of high solubility in the soybean sludge are extracted preferentially at the initial stage, while, monoglycerides, tocopherols and diglycerides of low solubility are enriched in the remaining soybean sludge. However, it should be noted that, since fatty acids are the major components in the soybean sludge, the tocopherol content in the remaining mixture can be gradually enhanced up to a certain point by the simple semi-continuous extraction method used in this work. The changes of the tocopherol concentration in the extract are plotted against the percentage of the overall extracted oil in Figure 3. In Figure 3, tocopherol concentrations in the extracts are

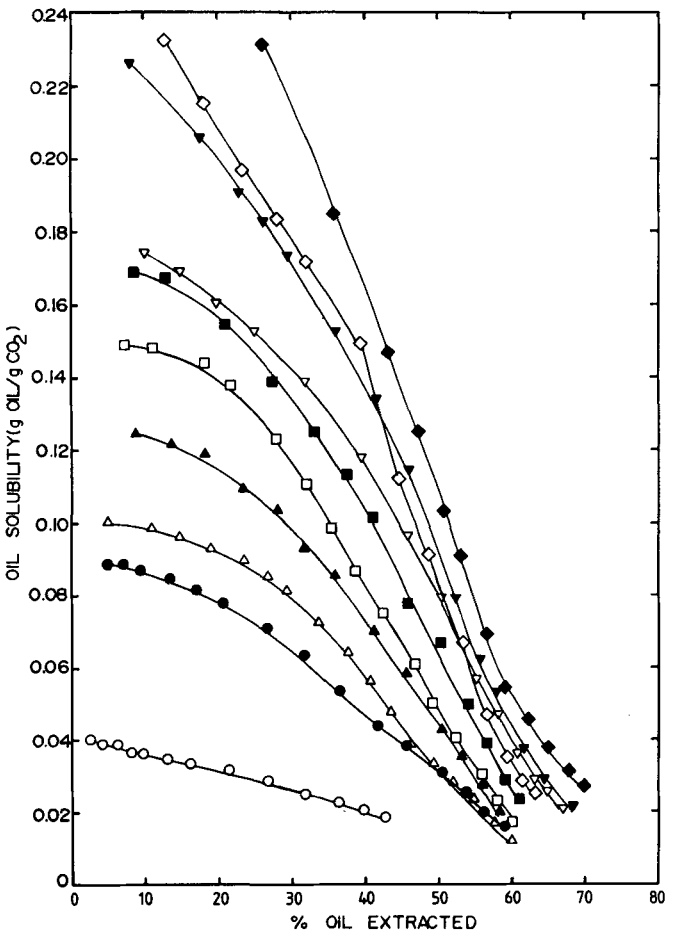

FIG. 2. Extraction curves of esterified soybean sludge at different temperatures and pressures. ○—70°C, 200 bar; ●—70°C, 250 bar; △—45°C, 200 bar; ▲—35°C, 200 bar; □—45°C, 250 bar; ■—35°C, 250 bar; ▽—70°C, 300 bar; ▼—45°C, 300 bar; ◇—35°C, 300 bar; ◇—45°C, 350 bar.

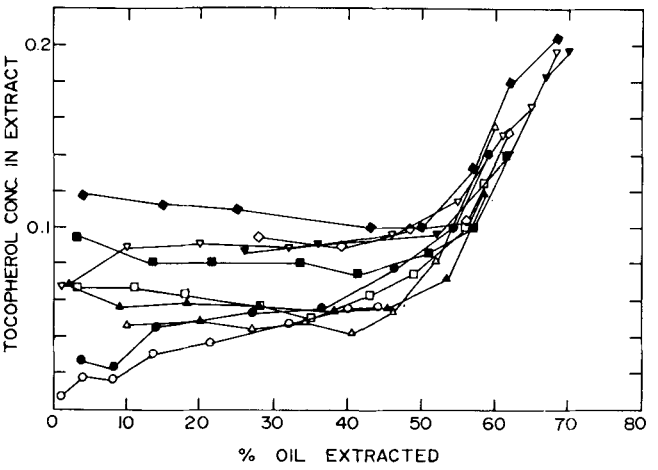

FIG. 3. Tocopherol concentration in the extract at different temperatures and pressures. The symbols are the same as those used in Figure 2.

almost constant up to approximately the 50 wt% extraction rate of the initial feed content with values of 6 wt% (200 bar), 8 wt% (250 bar) and 10 wt% (300 bar), respectively, at 35°C, whereas at higher extraction rates the tocopherol concentration increases rapidly. Similar behavior has been found at other temperatures, as shown in Figure 3. Since the tocopherol concentration of the initial esterified soybean sludge is about 17–18 wt%, the tocopherols in the vessel are gradually enriched together with glycerides by the semi-continuous and one-stage operation used in the present study. However, the enriched tocopherols in the vessel are stripped out above the 50 wt% extraction rate, resulting in a decrease of the tocopherol concentration. By this batch-type method, the tocopherols contained in the esterified soybean sludge could be concentrated up to a maximum of 40 wt%, indicating that a countercurrent multistage separation column will be necessary to obtain a higher tocopherol concentrate when pure SC-CO_2 is used with or without entrainers. The overall results stated above are preliminary and further investigations are needed to better design the SFE process. Studies involving the hydrodynamic and mass transfer characteristics of the SFE column are in progress in our laboratory.

ACKNOWLEDGMENTS

This work was supported by NON-DIRECTED RESEARCH FUND, Korea Research Foundation, 1989 and research grants from Genetic Engineering Center in Korea.

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[Received October 23, 1990; accepted May 22, 1991]