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PHYTOSTEROL-ENRICHED TRIGLYCERIDE FRACTIONS FROM VEGETABLE OIL DEODORIZER DISTILLATES UTILIZING SUPERCRITICAL FLUID FRACTIONATION TECHNOLOGY

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ABSTRACT

Supercritical fluid fractionation (SFF) technology has been applied as an alternative method to recover phytosterol (St)-enriched triglyceride (TG) fractions from vegetable oil deodorizer distillates (DD). Utilizing a pilot scale high pressure packed column, it was possible to obtain oil fractions with 20 and 31% St, 38 and 30% TG, from rice bran and soybean oil DD, respectively. A two step SFF method was developed further to obtain low free fatty acid and high TG and St content oil fractions from rice bran and soybean oil DD.

Key Words: Deodorizer distillate; Phytosterol; Rice bran; Soybean; Supercritical fluid fractionation

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INTRODUCTION

Deodorizer distillate (DD) is a byproduct of conventional vegetable oil refining process and quite rich in biologically active compounds such as phytosterols, tocopherols, and squalene. Certain phytosterols may be present in vegetable oils in free or esterified form, where they occur as esterified fatty acids (StE) or as ferulic acid esters (FE) (oryzanol). Deodorizer distillate itself, contains mainly free phytosterols.

Phytosterols are used as starting materials in the production of steroids, emulsifiers, and pesticides. Interest in the processing of phytosterol-containing materials has been growing during the last decade due to cited health benefits of such compounds and hence their applications in the nutraceutical market. Fatty acid esters of sterols are preferred for food applications because of their higher solubility in food systems compared to free sterols. However, a relatively new product, which contains a mixture of free phytosterols has been developed as an additive for foods such as yogurt, ice cream, chocolate, and mayonnaise, as well as margarines and salad dressings (1). Animal studies have shown that this product is effective in reducing serum cholesterol levels (1).

Deodorizer distillate has been the major source for preparing sterol compounds of natural origin. Numerous methods have been proposed and patented for the recovery of tocopherols and sterols from DD (2). Conventional sterol concentration or isolation methods involve a number of energy intensive and complex processes such as liquid–liquid extraction, esterification/transesterification, molecular distillation, and crystallization. Furthermore, efficiency and the throughput of these methods are relatively low (3).

Supercritical fluid fractionation (SFF) technology has been studied as an alternative technique for recovering valuable compounds from DD (4–12). The focus of such studies has been the recovery of tocopherols from soybean and palm oil DD. In addition, certain investigators have also studied the phase equilibria and separation behavior of DD mixtures in the above systems (4,5,11,12). The mass transfer efficiency of the counter-current packed columns used to effect the described fractionations have also been analyzed in terms of the height equivalent to a theoretical plate (HETP), as a function of liquid loading, solvent-to-feed, and reflux ratios (6,10).

Rice bran oil (RBO) DD has not been studied to date using a columnar fractionation process or as a potential source of phytosterols for value-added processing. The objective of this study was to develop a SFF process to recover phytosterol-enriched triglyceride (TG) fractions from various vegetable oil DD.

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EXPERIMENTAL

Materials and Methods

Rice bran and soybean oil DD samples were obtained from Riceland Foods Inc. (Stuttgart, AR). Soybean oil DD was used as is without further treatment. Rice bran oil DD experiments were carried out with both original samples and the fraction obtained by removing the wax content in the original DD. The dewaxed fraction of the RBO DD was obtained as follows: the original RBO DD was dissolved in 50/25/25 (v/v/v%) hexane/ethanol/ethyl ether in the ratio of 1:5, DD:solvents, and stored at 4°C for 36 hr. Then the mixture was centrifuged at 3000 rpm for 20 min and the resultant precipitate separated by decanting. The solvent was removed from the supernatant under vacuum, and the lipid fraction (retentate) stored at 4°C for further use.

Supercritical Fluid Chromatography of the Samples

Phytosterol and lipid compositions of the samples were determined by supercritical fluid chromatography (SFC). A SFC unit equipped with a SB-Phenyl-50 capillary column (10 m × 100 mm i.d., 0.5 mm film thickness, Dionex Corp., Salt Lake City, UT) was used for sample analysis. The carrier gas was SFC-grade carbon dioxide (Air Products Inc., Allentown, PA). All oil components were detected and quantified by a flame-ionization detector (FID) held at 350°C. The oven temperature was kept at 100°C. The injector valve sample loop (Valco Inc., Houston, TX) volume and injection time were 200 nL and 1 sec, respectively.

The following pressure program was used for the SFC analysis. The initial pressure was held at 10.1 MPa for 5 min and then increased to 15.2 MPa at the rate of 0.51 MPa/min. The ramp rate at this point was changed to 0.2 MPa/min until a pressure of 18.2 MPa was reached. At this point, the pressure-programming rate was changed to 0.51 MPa/min until a pressure of 28.4 MPa was reached. This was followed by rapid inverse pressure-program from 28.4 to 10.1 MPa at a rate of −10.1 MPa/min to reestablish the initial pressure conditions. Each sample was injected at least twice and the average of the two analyses was reported.

Deodorizer distillate samples were dissolved in hexane (about 10 mg/mL). HPLC-grade solvents were purchased from Fisher Scientific (Fairlawn, NJ). Standards of oryzanol (CTC Organics, Atlanta, GA), stigmastanol (TCI, Tokyo Kasei), cholesteryl stearate (Nu-Check-Prep Inc., Elysian, MN), β-sitosterol, and campesterol (Sigma, St. Louis, MO), α- and δ-tocopherol (Aldrich Chemical Co., Milwaukee, WI), and β- and γ-tocopherol (Matreya Inc., Pleasant Gap, PA)

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were used in this study. A mixture of TG, diglyceride (DG), and a FFA mixture consisting of C16:0, C18:0, C18:2 (Sigma, St. Louis, MO) were used for peak identification.

Supercritical Fluid Extraction Experiments

Two sets of SFE experiments were carried out using RBO DD as a model system to establish optimal separation conditions. In one set of SFE experiments, an Isco Model 3560 (Isco Inc., Lincoln, NE) SFE unit equipped with two 100 DX pumps was utilized. In these experiments the effect of temperature (40–80°C), pressure (10.2–34.0 MPa), and ethanol as a co-solvent in supercritical carbon dioxide, SC-CO_2 (5–20%, v/v%) on the TG and phytosterol separation were examined. Two grams of DD mixed with glass beads were placed between two glass wool beds in the extraction cell (10 mL). The samples were extracted with neat or ethanol-modified SC-CO_2. Four extract fractions each were collected from each sample in separate vials using pressurized collection at 0°C at 15 min intervals. The pump liquid CO_2 flow rate was 0.5 mL/min, and the SFE unit's restrictor was set at 80°C.

A second set of extraction experiments was carried out using a larger custom built SFE unit (13). Three grams of DD sample (dewaxed) was mixed with glass beads and placed on a glass wool bed at the bottom of an extractor cell. The extraction cell consisted of a 316 SS tube, pressure rated to 76 MPa at room temperature, with dimensions of 1.75 cm i.d × 55.9 cm. The remainder of the extractor volume was packed with protruded stainless steel packing (0.16 inch Pro-Pak, Scientific Development Company, State College, PA). The extractor cell was placed vertically in an oven for temperature control.

An air-driven booster compressor (Model AG-30-C, Haskel Engineering Corp., Burbank, CA) was used to feed CO_2 from the bottom into the extraction cell. To facilitate this, the CO_2 passed through a 3 m long coiled tube, placed in the oven to ensure thermal equilibrium of the gas before it reached the extractor. The pressure of the system was set and maintained at the desired value by adjusting the air intake valve of the booster compressor. The stream leaving the top of the extractor passed through a high pressure metering valve, which was used to adjust the CO_2 flow rate to the desired value. The extract was separated from the CO_2 in a receiver held at atmospheric pressure. The receiver assembly consisted of a modified vacuum joint and a round-bottom flask. The exit line from the micrometering valve was connected to the vacuum joint through a teflon adaptor to prevent any leakage of the extract-laden gas. The micrometering was electrically heated to prevent freezing to overcome the attendant Joule–Thomson effect caused by rapid expansion of the dense CO_2 through the valve. The volume of the solute-free CO_2 was recorded using a dry gas meter (American Meter Co.,

Horsham, PA) held at ambient temperature (~24°C) and pressure (0.1 MPa) before venting it to the atmosphere. The average CO_2 flow rate was approximately 0.3 L/min, and an extraction time of 3 hr was used for these experiments.

Column Fractionation Experiments

The SFF experiments were carried out on a pilot-scale (1.70 m height and 1.43 cm i.d.) packed column. Details of the column design and controls were previously described by Dunford and King (14) and King et al. (15), which utilized 0.16-inch protruded stainless steel packing, available from Scientific Development Company, State College, PA. The fractionation experiments were performed at a constant pressure over the pressure range of 13.6–27.2 MPa. The SFF column consisted of a preheater and four separately heated zones. Each zone was heated to the desired temperature (40–90°C) using heating mantels (Glas Col Inc., Terre Haute, IN). The temperature of the preheater was set at the same temperature as the first heated zone, and the column was filled with CO_2 before the feed was introduced.

The feed sample, which was very viscous at room temperature, was heated to 40–45°C just before pumping it into the column. Likewise, the associated feed lines were also heated to avoid sample solidification. For each run, 10 mL of DD (wax-removed fraction) was pumped into the column above the first heated zone by a liquid metering pump (Model MS-188, Haskel Inc., Burbank, CA) connected to a pump stroke controller. Then the column was pressurized and allowed to equilibrate until the set temperatures for each individual thermostated zone were reached.

Fractionation experiments were carried out in a semi-batch mode of operation, in which CO_2 (BOC Group, Murray Hill, NJ) was introduced continuously into the column to fractionate a fixed amount of DD in the column. Extract and raffinate samples were collected over the duration of the experiment from the top and bottom of the column, respectively. The carbon dioxide flow rate was set at 1.2 L/min, as measured at room temperature and pressure. Fractionation run times were 180 min unless otherwise stated. The column was depressurized and residual oil was drained at the end of each run. Then the column was cleaned between runs at 34.0 MPa and 90°C by flowing CO_2 in the column for 6 hr.

Statistical Analysis

All fractionation runs and analysis of each extract and raffinate sample were carried out in duplicate and in randomized order with the means being

reported. Analysis of variance (ANOVA) of the results was performed using General Linear Model procedure of Statistix software software (Version 4.1, Analytical Software, Tallahassee, FL). Multiple comparison of the various means were carried out by LSD (Least Significant Difference) test at $p = 0.05$.

RESULTS AND DISCUSSION

Phytosterol and overall lipid composition of both RBO and soybean oil DD are quite similar (Table 1). FFA are the main lipid components (30–40%) in the DD and the majority of the phytosterols are present in the free form (10–20%). Although a significant portion of the oryzanol present in the crude RBO was lost during the conventional oil refining process (14), we were not able to detect any oryzanol in the RBO DD. The oryzanol content of a RBO soap stock sample from the same company, which provided the DD samples was ~2%.

Supercritical Fluid Extraction

As noted before, an Isco 3560 automated SFE unit was used to screen for optimum processing conditions to separate phytosterols and TG from the DD mixture. Table 2 lists the processing parameters which were utilized for optimizing separation efficiency. As shown in Table 3, it was not possible to achieve a significant separation of DD components using any of the conditions listed in Table 2. These results showed that extract and residual oil compositions were not significantly different ($p > 0.05$), compared to the feed material.

The effect of solvent residence time and contact area between DD and $SC\text{-}CO_2$ on the separation efficiency of the lipid components was examined using the larger extraction cell in the custom built SFE unit. In an effort to simulate a fractionation column, the extraction cell was filled with Pro-Pak packing material

Table 1. Phytosterol and Lipid Composition of RBO and Soybean Oil DD (Area Percentage from SFC Analysis)

	RBO DD	Soybean Oil DD
FFA	32 ± 2	38 ± 3
TG	12 ± 1	14 ± 2
Free sterols	13.0 ± 0.8	18 ± 1
Sterol esters	2.6 ± 0.8	3.2 ± 0.9

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Table 2. Typical RBO DD Extraction Parameters for Isco Model 3560 Experiments

Feed	Pressure (Mpa)	Temperature (°C)	Solvent
Original DD	10.2	40	CO_2
Original DD	12.2	40	CO_2
Original DD	13.6	40	CO_2
Original DD	20.4	40	CO_2
Original DD	27.2	40	CO_2
Original DD	34.0	40	CO_2
Original DD	12.2	60	CO_2
Original DD	12.2	80	CO_2
Original DD	12.2	40	CO_2/20% EtOH
Wax-removed fraction	12.2	40	CO_2
Wax-removed fraction	12.2	40	CO_2/5% EtOH
Wax-removed fraction	12.2	40	CO_2/10% EtOH
Wax-removed fraction	12.2	40	CO_2/20% EtOH

to increase contact area between the DD and SC-CO_2. In this case, it was possible to achieve a significant separation of DD components. For example, the FFA concentration of the extract fraction was found to be significantly higher than that of the feed and raffinate samples (Fig. 1). Also, during the extraction process, the TG and free sterols were concentrated in the raffinate fraction, similar to the trends reported by Brunner et al. (6). These results indicate that it is possible to recover sterol-enriched TG fractions from DD utilizing the described process. Based on these results, it was decided to further examine the SFF of DD on a larger scale fractionation column.

Table 3. Composition of Extract and Raffinate Fractions from Isco 3560 SFE Experiments (Area Percentage from SFC Analysis)

	Extract	Raffinate
FFA	29–35	30–33
St	10–12	11–13
TG	9–12	10–11

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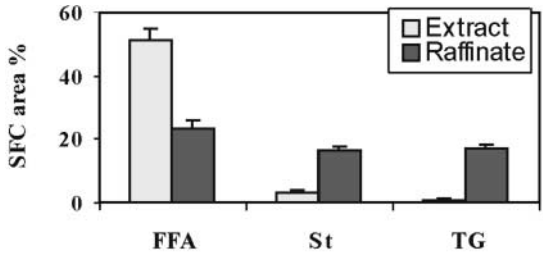

Figure 1. The sterol and lipid compositions of extract and raffinate fractions obtained during the SFE of RBO DD (wax-removed fraction). The experiments were carried out at 10.2 MPa and 40°C. Twenty-two liters of CO_2 were passed through the system at a flow rate of 0.3 L/min during the extraction process.

Supercritical Fluid Fractionation

The effect of pressure and temperature on the fractionation efficiency was examined utilizing the pilot scale fractionation column. As shown in Fig. 2, it is apparent that more FFAs were removed in the extract using a higher fractionation temperature. The triglyceride and St content of the extract fraction was significantly lower than those of the feed and raffinate samples at both temperatures studied. This trend is due to the lower solubility of these compounds in SC-CO_2 at the lower pressure (10.2 MPa). Although the St and TG concentration of the extract fraction was found to be slightly higher at the higher temperature, the observed differences were not significant statistically ($p > 0.05$).

The effect of pressure on the separation efficiency was investigated at three pressure levels 13.6, 20.4, and 27.2 MPa. The FFA content of the extract was

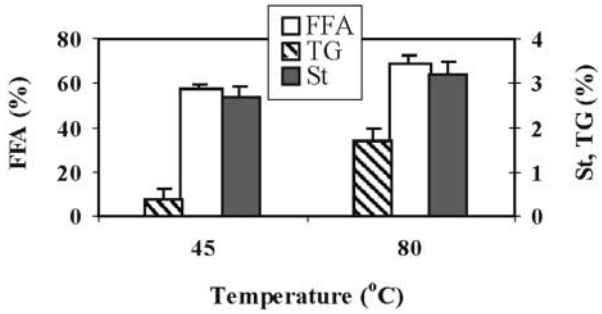

Figure 2. The effect of temperature on the composition of extract fraction collected during the SFF of RBO DD (wax-removed fraction). The experiments were performed at 13.6 MPa.

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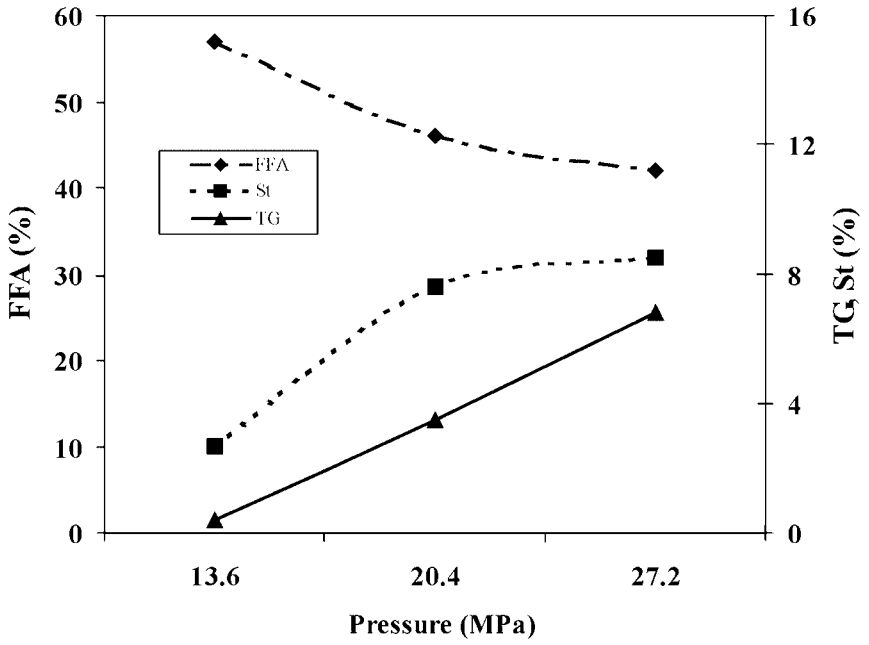

Figure 3. The effect of pressure on the composition of extract fraction collected during the SFF of RBO DD (wax-removed fraction). The experiments were performed at 45°C.

found to decrease significantly with increasing pressure (Fig. 3), while the concentration of TG and St in the extract increased with increasing column pressure. Previous SFF experiments, which were carried out in our labs with vegetable oils have yielded similar results (14). Similar trends were also observed when the solubility of pure sterols was measured in SC-CO_2 (16,17). These results indicate that low pressure and high temperature conditions should yield sterol-enriched TG fractions from the DD, since under these conditions less TG and St are found in the extract fraction.

Based on these findings, a two step SFF fractionation method was developed to obtain low FFA and high St content oil fractions from DD. Initially, the FFA content of the DD was reduced to $<10\%$ at a relatively lower pressure and temperature, 13.6 MPa and 45°C, respectively. The first extraction step was designed to remove the FFA, since these compounds are not desirable in the edible oils due to their negative effects on the oil's stability and the shelf life of the final product.

A sterol ester-enriched TG fraction can be collected at a higher pressure, 20.4 MPa, by utilizing a second extraction step. Figure 4 shows the composition of the final products obtained in this case from both RBO and soybean oil DD.

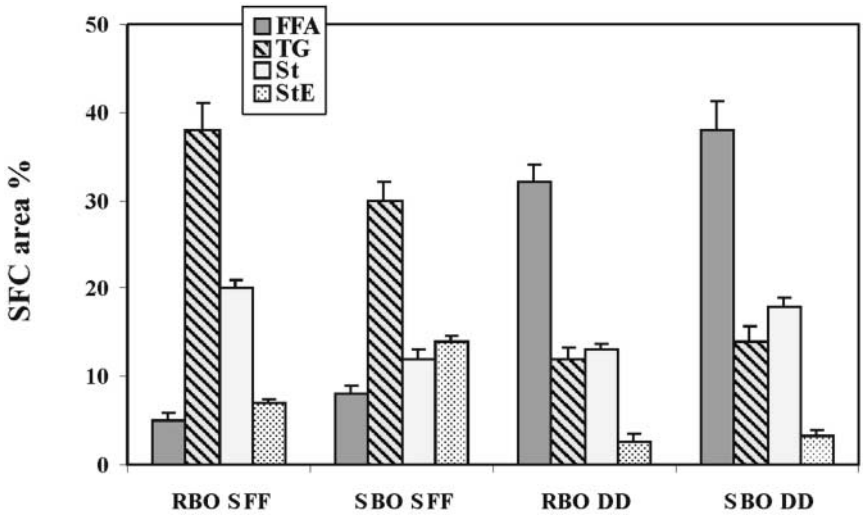

Figure 4. Comparison of sterol and lipid compositions of final products obtained by a two step SFF method (RBO SFF and SBO SFF) with the starting materials (RBO DD and SBO DD). The first extraction was carried out at 13.6 MPa and 45°C. The second extraction was performed at 20.4 MPa and 80°C. The composition of extract fraction from the second extraction is reported. RBO = rice bran oil; SBO = soybean oil; DD = deodorizer distillate; SFF = supercritical fluid fractionation.

Using such an approach, it was possible to obtain oil fractions with ~20 and 30% sterol content from RBO and soybean oil DD, respectively. Note that the FFA content of these products were relatively low; 5 and 8%, respectively. If desired the FFA content of these products can be further reduced by increasing the amount of SC-CO_2 passed through the column during the first extraction step. The second extraction step further improves the product quality because some of the color-producing compounds in the DDs, which remained in the system during the first SC-CO_2 extraction, can be separated from the final product.

CONCLUSIONS

In conclusion, this study demonstrates that a SFF technique can be utilized for a value-added processing scheme by recovering St-enriched TG fractions from a vegetable oil refining by-product. The two step SFF method developed in this study is an advancement over conventional methods which are used to obtain phytosterol-enriched products, since this new method consists of only two extraction steps as opposed to several unit operations involved in existing

processes. Furthermore, the described method does not leave any solvent or chemical residues in the final product, nor generate additional waste streams requiring subsequent disposal.

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