Thermal Gradient Deacidification of Crude Rice Bran Oil Utilizing Supercritical Carbon Dioxide

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ABSTRACT: The effect of isothermal and temperature gradient operation of a supercritical fluid fractionation column on the composition of rice bran oil (RBO) fractions has been studied. Application of a temperature gradient along the column was found to be beneficial in reducing the triacylglycerol (TAG) lost in the extract fraction. Utilization of higher temperature in the stripping section improved free fatty acid (FFA) removal from crude RBO. FFA acid content of the extract increased, and TAG content decreased with respect to time during the fractionation runs. Increasing the CO_2 flow rate from 1.2 to 2 L/min did not affect the extract composition significantly. By using the above approach, it is possible to obtain RBO fractions with similar total sterol ester content [~23 high-performance liquid chromatographic area (HPLC) %, ferulic plus fatty acid esters] to that of the commercially available sterol ester-enriched (*ca*. 21 HPLC area % fatty acid esters) margarines/spreads.

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KEY WORDS: Deacidification, phytosterols, rice bran oil, supercritical fluid fractionation, temperature gradient.

Rice bran oil (RBO) has been popular in India and Japan mainly as an edible oil source; however, worldwide consumption of RBO is expected to increase due to its potential as a nutraceutical/functional food (1). RBO is rich in phytosterols, tocopherol and tocotrienols, and research studies have shown that RBO has diverse health benefits such as lowering plasma cholesterol levels (2,3), decreasing early atherosclerosis (4), and inhibiting platelet aggregation (5).

The free fatty acid (FFA) content of rice bran may be as high as 30–40% (w/w) if the bran is not processed properly prior to extraction of the oil (6). This is due to the lipolysis caused by the native lipase enzymes present in the bran.

Conventional chemical and physical vegetable-oil processing techniques have several disadvantages, including using large quantities of water and chemicals, generating large quantities of waste, as well as being energy intensive (7). Furthermore, a significant portion of the nutritive RBO components is lost during the conventional refining processes. According to Orthoefer (8), 50% of the RBO phytosterols is lost during the refining process.

Alternative deacidification processes such as methanol extraction of FFA followed by a membrane processing (7) and reesterification of rice bran FFA with monoglycerides (6) have been reported. A supercritical fluid fractionation (SFF) technique has been reported for deacidification of vegetable oils. Refining of lampante oil (9) and deacidification of roasted peanut (10) and olive oil (11) with supercritical carbon dioxide (SC-CO_2) have been studied. Recently, Dunford and King (12) used an SFF tower approach to deacidify crude RBO and determined the optimal conditions for FFA removal, while minimizing phytosterol and triacylglycerol (TAG) losses during the process. The fractionation tower utilized for this study (12) was operated under isothermal conditions. However by applying a temperature gradient along the SFF tower, one may improve the purity of the extract by causing an internal reflux in the column (13). Therefore, the objective of the present study was to compare the effects of an isothermal or thermal gradient SFF column operation on the crude RBO deacidification efficiency. The effect of CO_2 flow rate and fractionation time on the extract and raffinate compositions was also examined.

EXPERIMENTAL PROCEDURES

Methods. Crude RBO, commercially extracted with hexane (Riceland Foods Inc., Stuttgart, AR), was used as a feed material for all the experiments. The crude oil was centrifuged at 3000 rpm for 20 min prior to conducting the fractionation experiments, and the supernatant was used as feed material for the SFF column.

Extract and raffinate fractions were analyzed for FFA, TAG, and phytosterol contents according to methods described in detail by Dunford and King (12). TAG, FFA, and sterol fatty acid ester (StE) contents of the samples were analyzed by high-performance liquid chromatography (HPLC) according to Moreau *et al.* (14). Lipid and sterol components in the oil samples were separated on a LiChrosorb Diol, 5 μm, 100 × 3 mm column (Chrompack Inc., Raritan, NJ). The mobile phase gradient consisted of solvent A, hexane/acetic acid, 1000:1, vol/vol; and solvent B, hexane/2-propanol, 100:1, vol/vol. The linear gradient timetable was: at 0 min, 100:0; at 5 min 100:0; at 12 min 75:25; at 40 min 75:25; at 41 min 100:0; at 60 min 100:0 (% A and % B, respectively). Eluent flow rate was constant at 0.5 mL/min. The evaporative light-scattering detector was operated at 40°C with nitrogen as a nebulizing gas at a flow rate of 1.60 L (standard temperature

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and pressure)/min. The column heater temperature was set at 40°C. Oil samples were dissolved in hexane (about 20 mg/mL), and a 10 μL injection volume was used.

Free sterol and oryzanol compositions of the samples were determined by a supercritical fluid chromatography (SFC), with an SB-phenyl-50 capillary column (10 m × 100 μm i.d., 0.5 μm film thickness; Dionex Corp., Salt Lake City, UT). The carrier gas was SFC-grade CO_2 (Air Products, Inc., Allentown, PA). All oil components were detected and quantified by a flame-ionization detector 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 s, respectively. The following pressure program was used for the SFC analysis: the initial pressure was held at 100 atm for 5 min and then increased to 150 atm at the rate of 5 atm/min. The ramp rate at this point was changed to 2 atm/min until a pressure of 180 atm was reached. Here, the pressure-programming rate was changed to 5 atm/min until a pressure of 280 atm was reached. This was followed by rapid inverse pressure-program from 280 to 100 atm at a rate of –100 atm/min to reestablish the initial pressure conditions. TAG and FFA compositions of the samples were reported as HPLC area percentages, whereas oryzanol, StE, and free sterols were expressed as weight percentages unless otherwise stated. Each sample was injected at least twice, and the average of the two analyses was reported.

Column fractionation. 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 King *et al.* (15). The fractionation experiments were performed isobarically in the pressure range of 13.6–20.5 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. The column was filled with CO_2 before the feed was introduced. For each run, 30 mL of crude oil 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 stroke controller. Then the column was pressurized and allowed to equilibrate until the set temperatures were reached. The deacidification process was carried out in a semibatch mode of operation. CO_2 (The BOC Group, Murray Hill, NE) was in the continuous, and oil was in the batch mode. Extract and raffinate samples were collected from the top and bottom of the column, respectively. The CO_2 flow rate was 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 at 34.0 MPa and 90°C with flowing CO_2 for over 6 h.

Statistical analysis. All fractionation runs and analyses of each extract and raffinate sample were carried out in duplicate and in randomized order with the means being reported. Analysis of variance of the results was performed using General Linear Model procedure of Statistix software (Version 4.1; Analytical Software, Tallahassee, FL). Multiple comparisons of the various means were carried out by least significant difference test at $P = 0.05$.

RESULTS AND DISCUSSION

Crude RBO, which is used as feed material for the deacidification experiments, contained ~7% FFA (Table 1). In a previous study we showed that the SFF process could be an alternative technique for crude RBO oil deacidification (12). The latter study focused on the isothermal operations of the fractionation column to understand the column dynamics and crude oil component interactions during the SFF fractionation process. However, SFF of a mixture can also be achieved by a multiplate approach, which uses a fractionation column over which a temperature and/or pressure gradient (and hence density) is imposed along its height.

In the present study, CO_2, which is supplied to the bottom of the column continuously, moves upward and strips the more soluble components from the feed, which is operating in the batch mode at the bottom of the column. For the described temperature gradient, the higher temperature zones at the top of the column reduce the CO_2 density, condensing the less soluble or volatile compounds to undergo internal refluxing. Therefore, the fluid CO_2 moving up the column contacts this condensate countercurrently, and concentration of extract fraction is achieved according to solubility and volatility in the fluid stream. Clifford (13) describes the lower section of the column, which holds the feed material, as the "stripping section" and the upper part (temperature gradient imposed section) as the "enrichment section." This same terminology will be used throughout this manuscript.

Temperature effect. The effect of isothermal and thermal gradient column operations on extract and raffinate compositions is shown in Table 2. The fractionation experiments were carried out isobarically at a relatively lower pressure (20.5 MPa) since our previous study showed that TAG and phytosterol losses increased with increasing pressure during SFF of crude RBO (12). TAG concentration of the extract fraction was significantly lower than that of the raffinate fraction for all the experimental conditions studied (Table 2). This is due to the low solubility of TAG in SC-CO_2 at this low solvent density. When a thermal gradient is applied along the column, the TAG concentration of the extract was lower and FFA concentration higher, relative to results achieved under isothermal operation. Such a result was expected, since imposing a

TABLE 1
Crude Rice Bran Oil Composition[a]

Triacylglycerols (HPLC area %)	70 ± 2
Free fatty acids (HPLC area %)	7.0 ± 0.5
Oryzanol (wt%)	1.3 ± 0.1
Free sterols (wt%)	0.33 ± 0.03
Sterol fatty acid esters (wt%)	3.6 ± 0.3

[a]HPLC, high-performance liquid chromatography.

TABLE 2
Effect of Isothermal and Thermal Gradient Column Operations on the SFF Fractions[a]

	Column temperature (°C)	TAG[b]	FFA[b]	Oryzanol[c]	Free sterol[c]	StE[c]
Extract	Isothermal at 45°C	52.5[d]	26.9[b]	0.24[a]	0.76[b,c]	1.65[a]
	45/55/65/75[d]	45.7[c]	29.8[c]	0.23[a]	0.90[c]	2.35[b,c]
	Isothermal at 60°C	45.6[c]	31.6[c]	0.24[a]	0.83[b,c]	2.28[b]
	60/70/80/90[d]	24.2[a]	52.0[e]	0.17[a]	1.27[d]	1.60[a]
	Isothermal at 80°C	36.5[b]	36.9[d]	0.45[b]	0.70[b]	2.79[c,d]
	80/85/90/95[d]	24.1[a]	49.3[e]	0.26[a]	1.36[d]	1.72[a]
Raffinate	Isothermal at 45°C	73.2[e]	4.9[a]	1.35[d,e]	0.28[a]	3.55[e,f,g]
	45/55/65/75[d]	71.8[e]	4.4[a]	1.24[c,d]	0.29[a]	3.43[e,f]
	Isothermal at 60°C	71.5[e]	4.8[a]	1.41[e]	0.35[a]	3.84[f,g]
	60/70/80/90[d]	71.6[e]	5.2[a]	1.25[c,d]	0.28[a]	3.58[e,f,g]
	Isothermal at 80°C	72.4[e]	5.1[a]	1.29[c,d]	0.29[a]	4.00[g]
	80/85/90/95[d]	70.7[e]	5.4[a]	1.21[c]	0.29[a]	3.09[d,e]

[a]The numbers in the same column with the same letter are not significantly different ($P > 0.05$). Fractionation experiments were carried out at 20.5 MPa for 180 min.
[b]As HPLC area %
[c]As w/w% (StE = sterol fatty acid esters).
[d]Temperature gradient in ascending order. SFF, supercritical fluid fractionation; TAG, triacylglycerols; FFA, free fatty acids. See Table 1 for other abbreviations.

thermal gradient on the column causes condensation of larger molecules such as TAG, when they encounter the lower CO_2 density present in the higher temperature region under isobaric conditions. Such a condition creates an internal reflux, decreasing TAG and increasing the FFA content of the extract fraction.

TAG concentration of the extract fraction decreased and FFA increased significantly with increasing temperature of the stripping section of the fractionation column (Table 2). TAG loss in the extract fraction can be further reduced by decreasing the pressure of the system. For example, TAG and FFA concentrations of the extract fraction were <10 and >75%, respectively, during a 3-h fractionation run at 12.2 MPa and 45/55/65/75°C. The implication of this finding is important for advocating the feasibility of the deacidification process using the SFF technique, since application of a thermal gradient along the column will increase the efficiency of FFA removal from the feed material and decrease the TG loss in the extract fraction.

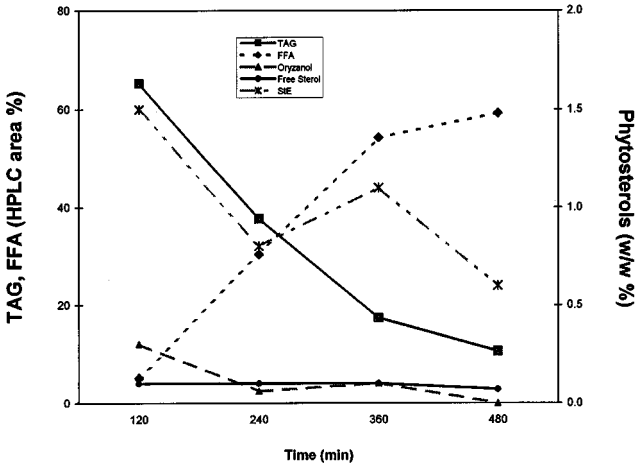

FIG. 1. Effect of fractionation time on the composition of extract fraction. The crude rice bran oil (RBO) fractionation was carried out at 13.6 MPa and 45/60/80/90°C (temperature gradient in ascending order) and 1.2 L/min CO_2 flow rate. TAG, triacylglycerol (■); FFA, free fatty acid (◆); oryzanol (▲); free sterol (●); sterol fatty acid ester (×). HPLC, high-performance liquid chromatography.

Oryzanol and StE contents of the extract fractions were significantly lower than those of the raffinate samples (Table 2). However, free sterol content of the fractions showed an opposite trend, a higher amount of free sterols was lost with the extracts (Table 2). Oryzanol content of the fractions did not show an apparent trend with either isothermal or thermal gradient column operations. However, free sterol content of the extract fractions was significantly higher, and StE content was lower under thermal gradient column operation (Table 2).

Fractionation time. TAG and FFA content of the extract samples changed significantly with the fractionation time (Fig. 1). TAG content of the extract fractions decreased from 65.3 to 10.6% while FFA content of the same sample increased from 5.1 to 59.3% during a 480-min fractionation run. Free sterol composition of the extract fraction was not affected significantly by the fractionation time. Although StE content of the fractions showed a sharp decline during the initial stages of the process (0–240 min), these changes became less pronounced later in the process (360–480 min). A slight decrease was observed in the oryzanol content of the extracts. Changes in extract composition in time are due to the changes in the composition of oil present in the column during the semicontinuous fractionation process.

CO_2 flow rate. The fluid flow rate in the fractionation column is an important operation parameter, which affects the efficiency of the fractionation process (12). Therefore, we examined the effect of increasing the CO_2 flow rate on the FFA-removal efficiency. In this particular study, increasing CO_2 flow rate from 1.2 to 2 L/min did not affect the composition of the extract fraction significantly (Table 3). Hence, operating the column at a higher CO_2 flow rate, 2 L/min, can reduce

TABLE 3
Effect of CO_2 Flow Rate on the Extract and Raffinate Composition[a,b]

	Flow rate (L/min)	TAG[c]	FFA[c]	Oryzanol[d]	Free sterol[d]	StE[d]
Extract	1.2	52.5[a]	26.9[b]	0.24[a]	0.76[b]	1.65[a]
	2.0	49.7[a]	27.6[b]	0.20[a]	0.81[b]	2.07[a]
Raffinate	1.2	73.2[b]	4.9[a]	1.35[b]	0.28[a]	3.55[b]
	2.0	75.0[b]	4.2[a]	1.30[b]	0.27[a]	4.74[c]

[a]Fractionation experiments were carried out at 20.5 MPa and 45°C for 180 min.
[b]The numbers in the same column with the same letter are not significantly different ($P > 0.05$).
[c]As HPLC area %.
[d]As w/w%. See Tables 1 and 2 for abbreviations.

the fractionation time without impairing the FFA-removal efficiency.

Relevance to producing sterol ester-enriched RBO. This study demonstrates that phytosterol-enriched oil fractions can be obtained from crude oil by utilizing an SFF column. Based on the findings of this study, we developed a two-step semicontinuous process to demonstrate the application of SFF technology to obtain RBO fractions with similar sterol ester contents to that found in commercially available phytosterol-enriched margarines/spreads. Initially, the FFA content of the crude RBO was reduced to an acceptable level (<0.5%) at a relatively lower pressure and under a thermal gradient column operation (i.e., 13.6 MPa and 40/60/70/80°C). The temperature of the stripping sections was chosen as 40°C for this particular experiment, even though our previous experiments indicated that more FFA was removed at higher temperature. The reasons for this choice were twofold: (i) The deacidification process takes longer to complete at a higher temperature (i.e., 80°C) due to the low density and low solvent power of SC-CO_2 at a low pressure such as 13.6 MPa. Thus, the temperature of the stripping section was set at 40°C to maintain a higher solvent density and to speed up the deacidification process. (ii) As a common practice, temperature gradient is applied to the column in an ascending order, starting from the stripping section. Hence, higher stripping section temperature would require even higher temperature settings for the enrichment section. The use of high temperatures (i.e., >80°C) can cause oxidation and/or degradation of some of the lipid components. Then, sterol ester-enriched oil was extracted from the raffinate fraction at a moderately higher pressure (20.5 MPa).

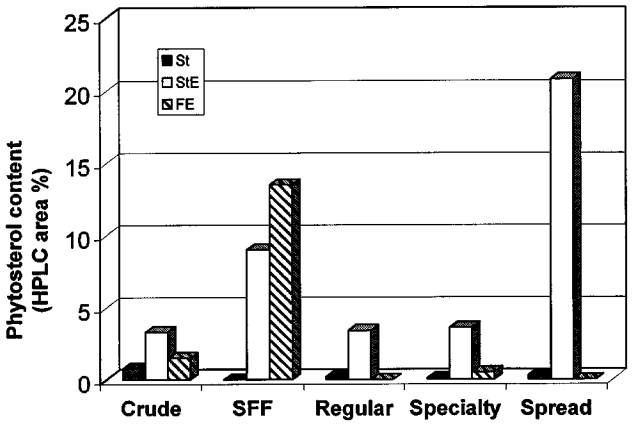

FIG. 2. Comparison of a supercritical fluid fractionation (SFF) product with commercially available RBO samples. SFF product was obtained by a two-step fractionation method. Initially, FFA content of the crude RBO oil was reduced from 7 to 0.5% at 13.6 MPa and 40/60/70/80°C; then raffinate was further extracted at 20.5 MPa and under the same thermal gradient conditions. Flow rate of the CO_2 was 2 L/min for both extraction steps. The figure shows the phytosterol composition of the extract fraction from the second-step fractionation. The abbreviations in the figure are as follows: StE = fatty acid esters of phytosterols, FE = ferulic acid esters of phytosterols (i.e., oryzanol), St = free sterols, crude = crude RBO (Riceland Foods Inc., Stuttgart, AR), regular = commercially refined RBO (Riceland Foods), specialty = commercially produced "high oryzanol" RBO (Riceland Foods), spread = phytosterol enriched-margarine (Benacol) purchased at a local grocery store. All of the samples were analyzed in our labs. See Figure 1 for other abbreviations.

The experimental results presented in Figure 2 illustrate the efficacy of the approach described in this study. We were able to obtain RBO fractions with ~23% (combined StE and oryzanol HPLC area %) total sterol ester content, which is very similar to that of a commercially available sterol ester-enriched margarine/spread, ~21% (HPLC area %StE). Sterol esters in the SFF product were in the form of both sterol esters of fatty acids and ferulic acid (oryzanol), whereas the commercial product contained only the StE. We were not able to detect any oryzanol in the RBO, which was refined using conventional methods. Oryzanol content of the specialty oil, which was marketed as "high oryzanol" oil, was only 0.5%, which was much lower than that of the SFF product, containing 13.5%. According to the manufacturer, a special refining process was used for the production of "high oryzanol" RBO, but details of the process are not revealed due to the proprietary nature of the information. The SFF product had a very similar FFA content (0.1%) to the commercially available RBO products (0.09%).

To our knowledge, manufacture of commercially available phytosterol-enriched products involves isolation of free sterols from either tall-oil deodorizer distillate or soybean oil. Since free sterols are not soluble in food systems, they are esterified with fatty acids to obtain sterol esters, which have higher solubility in fats, allowing them to be incorporated into the food systems as functional (cholesterol-lowering) ingredients. A number of very complex and energy-intensive unit operations such as liquid/liquid extraction, esterification, mo-

lecular distillation, and crystallization are utilized during the production of such products. Although the costs involved in the high-pressure processing seem to be a disadvantage for the above-proposed SFF method, the simplicity of the process and higher quality of the final product (no solvent and chemical residues) make this process more attractive.

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REFERENCES

1. Brady, J.A., and N.B. Shaikh, Rice Bran Oil and the Subsequent Isolation of Various Micronutrients, *inform 10*:S32 (1999).
2. Nicolosi, R.J., L.M. Ausman, and D.M. Hegsted, Rice Bran Oil Lowers Serum Total and Low Density Lipoprotein Cholesterol and apo B levels in Nonhuman Primates, *Atherosclerosis 88*:133–142 (1991).
3. Rukmini, C., and T.C. Raghuram, Nutritional and Biochemical Aspects of the Hypolipidemic Action of Rice Bran Oil: A Review, *J. Am. Coll. Nutr. 10*:593–601 (1991).
4. Rong, N., L.M. Ausman, and R.J. Nicolosi, Oryzanol Decreases Cholesterol Absorption and Aortic Fatty Streaks in Hamsters, *Lipids 32*:303–309 (1997).
5. Seetharamiah, G.S., T.P. Krishnakantha, and N. Chandrasekhara, Influence of Oryzanol on Platelet Aggregation in Rats, *J. Nutr. Sci. Vitaminol. 36*:291–297 (1990).
6. De, B.K., and D.K. Bhattacharyya, Deacidification of High-Acid Rice Bran Oil by Reesterification with Monoglyceride, *J. Am. Oil Chem. Soc. 76*:1243–1246 (1999).
7. Kale, V., S.P.R. Katikaneni, and M. Cheryan, Deacidifying Rice Bran Oil by Solvent Extraction and Membrane Technology, *Ibid. 76*:723–727 (1999).
8. Orthoefer, F.T., Rice Bran Oil: Healthy Lipid Source, *Food Technol. 50*:62–64 (1996).
9. Bondioli, P., C. Mariani, A. Lanzani, E. Fedeli, A. Mossa, and A. Muller, Lampante Olive Oil Refining with Supercritical Carbon Dioxide, *Ibid. 69*:477–480 (1992).
10. Ziegler, G.R., and Y.J. Liaw, Deodorization and Deacidification of Edible Oils with Dense Carbon Dioxide, *Ibid. 70*:947–953 (1993).
11. Brunetti, L., A. Daghetta, E. Fedeli, I. Kikic, and L. Zanderighi, Deacidification of Olive Oils by Supercritical Carbon Dioxide, *Ibid. 66*:209–217 (1989).
12. Dunford, N.T., and J.W. King, Phytosterol Enrichment of Rice Bran Oil by a Supercritical Carbon Dioxide Fractionation Technique, *J. Food Sci. 65*:1395–1399 (2000).
13. Clifford, T., *Fundamentals of Supercritical Fluids*, Oxford University Press Inc., New York, 1999, pp. 130–144.
14. Moreau, R.A., M.J. Powell, and K.B. Hicks, Extraction and Quantitative Analysis of Oil from Commercial Corn Fiber, *J. Agric. Food Chem. 44*:2149–2154 (1996).
15. King, J.W., E. Sahle-Demessi, F. Temelli, and J.A. Teel, Thermal Gradient Fractionation of Glyceride Mixtures Under Supercritical Fluid Conditions, *J. Supercritical Fluids. 10*:127–137 (1997).

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