Supercritical Carbon Dioxide Extraction of Fatty and Waxy Material from Rice Bran

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ABSTRACT: Waxy and fatty materials were removed from rice bran by supercritical carbon dioxide at pressures up to 28 MPa and temperatures between 40 and 70°C. The yields of the supercritical extraction were only 16–60% of those obtained by Soxhlet extraction with hexane. The highest yield was reached at the highest pressure and temperature used (28 MPa and 70°C), indicating that supercritical extraction of this lipid-bearing material could probably be improved at more severe extraction conditions. The supercritical extract obtained at operational conditions giving high yield was chromatographically characterized. Compared to the hexane extract, the supercritical extract was lighter in color and richer in wax content and long-chain fatty acids C_{20}–C_{34}. Triacontanol was the most abundant alcohol in both extracts. Tocopherol contents were similar. *JAOCS 73,* 1127–1131 (1996).

KEY WORDS: Carbon dioxide, long-chain acids, long-chain alcohols, rice bran, rice bran oil, rice bran wax, supercritical extraction, triacontanol.

Supercritical extraction is an increasingly important separation technology in which traditional liquid solvents are replaced by supercritical fluids (SCF). The main advantages of SCF over liquid solvents is that their high diffusivity, low viscosity, and low surface tension can speed up mass transfer–limited extractions. In addition, because SCF offer the possibility of modifying product solubilities through alteration of pressure and/or temperature, a single SCF may substitute for a variety of liquid solvents.

Among other SCF, supercritical carbon dioxide (SC-CO_2) has gained special attention in the food and pharmaceutical industries for reasons of economy and safety. Thus, it has been applied commercially to the decaffeination of coffee (1) and extraction of the valuable constituents of hop resins and spices (2). In addition, it has been extensively applied in the vegetable oil industry. For example, Friedrich *et al.* (3), List and Friedrich (4), and García *et al.* (5) compared the composition and stability of vegetable oils obtained by SC-CO_2 extraction or recovered and purified by conventional methods; Stahl and collaborators (6,7) performed extensive studies on the solubilities of soybean oil up to high pressures; Zosel (8) and Zhao *et al.* (9) studied the deacidification of oils, and more recently, the selective extraction of compounds from olive oil has been analyzed (10–12).

The successful vegetable oil extraction reported in the literature led us to consider the possibility of extracting oil from rice bran with SC-CO_2. Rice bran is an important by-product of the rice milling industry with an oil content that varies between 12 and 25%, depending on the quality of bran and the degree of polishing. It also contains other valuable chemicals, such as tocopherols, ceryl and miricyl alcohols, squalene, phospholipids, and oryzanol, which reduces serum cholesterol and is effective against autonomic nervous disorders (13,14). In conventional extraction processes, rice bran oil is extracted with an organic solvent, such as hexane; the oil obtained is separated from the solvent by distillation, and the remainig solid material, the meal, is freed from adhering solvent in a desolventizing stage. The two processes, distillation and desolventizing, may be omitted if SC-CO_2 were used as the solvent.

Here we present preliminary results of the supercritical extraction of oil and waxes from rice bran. The aim of the study was to obtain information on the differences between the yields and lipid compositions of the extracts obtained either with SC-CO_2 or with hexane. Moreover, the effect of pressure and temperature on the dissolution of rice brain lipid constituents in SC-CO_2 was analyzed.

EXPERIMENTAL PROCEDURES

Materials. Liquid CO_2 (purity 99.5%) was supplied by Carburos Metalicos, S.A. (Madrid, Spain). Rice bran samples were provided by the rice mill Los Palacios (Pinar del Rio, Cuba). The samples were maintained at 105°C for 1 h to inhibit enzymatic activity. Next, they were allowed to reach ambient temperature and, finally, sieved to obtain several size fractions (0.038–0.1, 0.1–0.3, 0.3–0.4, 0.4–0.5, 0.5–0.7, and 0.7–1 mm). The final moisture content of the samples was 5% w/w on a dry rice bran basis.

Apparatus and extraction procedure. A schematic diagram of the extraction apparatus is shown in Figure 1. Liquid CO_2 was provided from a steel cylinder (SC). After cooling (LC) and filtering (F), the CO_2 was compressed (P) by a positive

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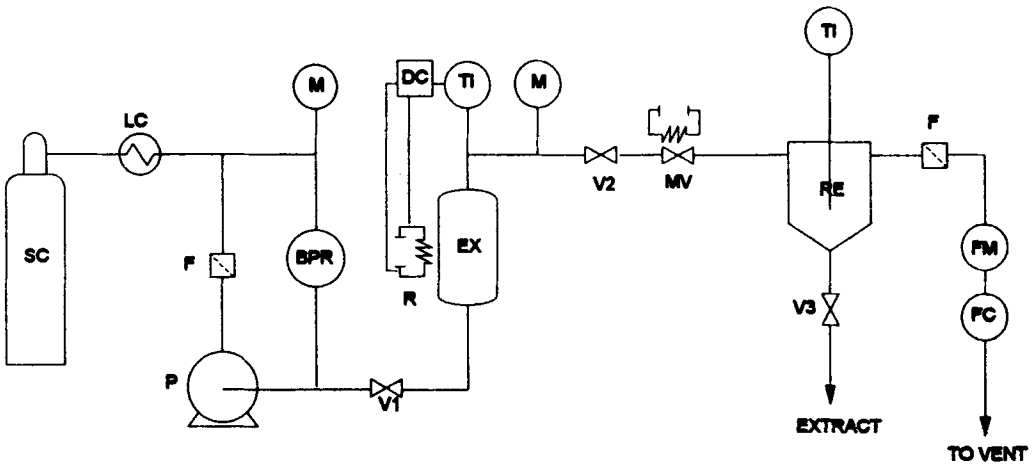

FIG. 1. Flow schematic of the supercritical extraction system. SC, CO_2 steel cylinder; LC, liquid cooler; F, liquid filter; P, pump; BPR, back pressure-regulating valve; M, manometer; V1, V2, and V3, shut-off valves; DC, temperature digital controller; R, resistor; EX, extractor; MV, metering valve; FM, turbine flow meter; FC, flow computer.

displacement high-performance liquid chromatography (HPLC) type pump (miniPump Simplex 396-74; LDC Analytical, Riviera Beach, FL) up to a pressure of 300 MPa. The pressure was regulated (BPR) by a back-pressure regulator (Model 27741; Haskel Inc., Burbank, CA) and checked (M) by a manometer (Duragauge; Autoclave Engineers Inc., Erie, PA). The compressed fluid was passed through a vertically mounted extractor (EX) from the bottom. The extractor was a cylinder (17.48 mm i.d. × 304.8 mm) made of stainless steel (AISI 316) with a working pressure of 590 MPa at 100°C. To keep the extractor temperature at the desired value, a digital controller (Model 808; Eurotherm Corporation, Reston, VA) regulated the electric current through a resistor (R) that surrounded the extractor cylinder. The oil-laden gas from the extractor was passed through a heated metering valve (MV) in which SC-CO_2 was depressurized, and the separated oil was collected in a receiver (RE). The gas flow through the extractor was measured (FM) by a turbine flow meter (Model FT0; EG&E Flow Technology, Inc., Phoenix, AZ) and calculated (FC) by a flow computer (Model FC 70A; EG&E Flow Technology, Inc.). In all experiments, the CO_2 flow rate was mantained at 1 N L/min, and the extraction was run on 20-g rice bran samples.

To study the effects of pressure, temperature, and rice bran particle diameter, several size fractions of rice bran (0.038–0.1, 0.3–0.4, 0.5–0.7, and 0.038–1 mm) were extracted with SC-CO_2 at different pressures and temperatures. The extraction conditions of all experiments are presented in Table 1.

Rice bran oil was also extracted in a Soxhlet apparatus with commercial hexane (Panreac; Montplet & Esteban, S.A., Barcelona, Spain) for 4 h to compare the quality of this oil with that extracted with SC-CO_2. Hexane extraction was completed to the point where residual oil in the rice bran was almost unextractable at 70°C, the highest temperature used in the SC-CO_2 method. After evaporation of the solvent, the oil content was determined gravimetrically.

GC analysis of fatty acids. The methyl esters were prepared by transesterification of the extracts with MeONa/MeOH 1% solution at 85–90°C. The ester fraction was analyzed with a PU-4600 Philips gas chromatograph (Cambridge, United Kingdom), equipped with a SP-2380 column, 30 m length, 53 mm i.d., 0.30 μm film thickness (Supelco, St. Germain-en-Laye, France). Operative conditions were injector and flame-ionization detector (FID) at 250°C; carrier gas, He at 1.5 mL/min and split ratio 1:20; oven temperature from 160 to 174°C at 2°C/min with an initial isotherm of 10 min, then at 4°C/min up to 194°C, and a final isotherm of 13 min.

GC analysis of long-chain alcohols. The alcohol derivatives were prepared with the silanizing agent *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide (Sigma Aldrich Quimica, S.A., Madrid, Spain). The analyses were performed with a gas chromatograph (Model PU 4600; Philips), equipped with an OV-101 column filled with chromosorb w A/w 100–120 mesh

TABLE 1
Extraction Conditions in the Different Experiments

Experiment number	Extraction solvent	D_p (mm)	T (°C)	P (MPa)	Extraction time (h)
E-1	SC-CO_2	0.038–1.0	70	28.0	0.5
E-2	SC-CO_2	0.038–1.0	70	28.0	1.5
E-3	SC-CO_2	0.038–1.0	70	28.0	2.0
E-4	SC-CO_2	0.038–1.0	70	28.0	4.0
E-5	SC-CO_2	0.038–1.0	70	22.0	2.0
E-6	SC-CO_2	0.038–1.0	70	16.5	2.0
E-7	SC-CO_2	0.038–1.0	70	10.0	2.0
E-8	SC-CO_2	0.038–1.0	60	28.0	2.0
E-9	SC-CO_2	0.038–1.0	50	28.0	2.0
E-10	SC-CO_2	0.038–1.0	40	28.0	2.0
E-11	SC-CO_2	0.038–0.1	70	28.0	2.0
E-12	SC-CO_2	0.3–0.4	70	28.0	2.0
E-13	SC-CO_2	0.5–0.7	70	28.0	2.0
E-14	Hexane	0.038–1.0	70	0.1	4.0

(Philips). Conditions were injection and detection temperature 320°C; oven temperature programmed from 130–145°C at 10°C/min; and the carrier gas, Ar at 30 mL/min.

HPLC analysis of tocopherol. Tocopherol was measured with MeOH-H_2O (96:4, vol/vol) as a mobile phase and a fluorescence detector set at Ex 295 nm, Em 330 nm. The HPLC device (Model 4110; Philips) was equipped with a 5-μm silica gel column 4.6 mm × 25 cm (HYPERSIL H5ODS; Shandon Scientific, Ltd., Astmoor, United Kingdom). The analytical conditions were: temperature 25°C (isocratic); and mobile phase at 1.6 mL/min.

RESULTS AND DISCUSSION

The accuracy of the experimentally determined extraction yields was determined by comparing the results from five independent runs that were carried out under identical conditions (P = 28 MPa, T = 70°C, D_p = 0.038–1 mm). In these experiments, the extraction yields were similar (70.7, 71.9, 70.1, 71.0, and 71.8 mg/g dry rice bran), indicating that reproducibility of the data was good, and that replication of the experiments was not necessary. Nevertheless, to minimize experimental errors, triplicate experiments were performed under all experimental conditions. Thus, the extraction yields reported in this paper are the average of three experimentally measured yields. The standard deviation was between 0.7 and 2.9.

The extract yield recovered from rice bran with SC-CO_2 ranged from 19.0 to 71.1 mg/g dry rice bran, while the hexane extract was 120 mg/g dry rice bran. In other words, the SC-CO_2 extraction yields were only 15.9–59.3% of those obtained by conventional extraction with hexane. This result was not unexpected because, according to the literature (3,6), in the processing of lipid-bearing materials, higher extraction pressures are needed to reach the extraction capability of hexane.

Figure 2 shows the variation of SC-CO_2 extraction yields with time (experiments E-1–E-4 of Table 1). It shows that no significant yield increase occurs after 2 h from the beginning of the experiment. Because similar results were found at all experimental conditions, only the yields reached in 2 h of operation are shown in Figures 3 and 4.

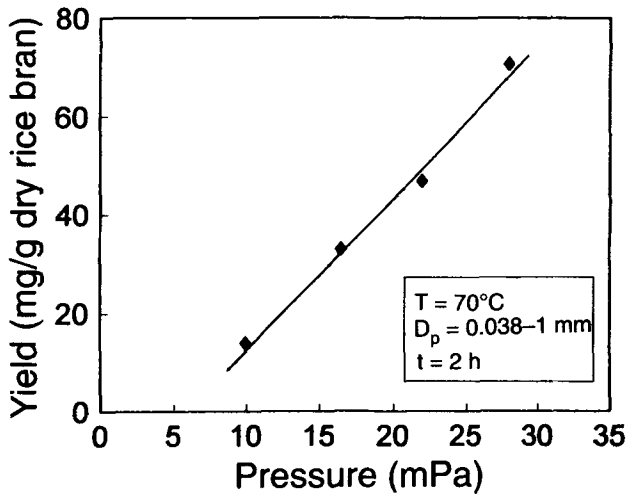

FIG. 3. Supercritical CO_2 extraction of rice bran at 70°C. Yield vs. extraction pressure.

Figure 3 shows that the extraction yield increases linearly with increasing pressure (experiments E-3, E-5, E-6, and E-7). This result conforms with the general principles of supercritical fluid extraction (7) and other studies on the extraction of lipid-bearing materials (3,6). The studies state that, in general, at constant temperature, the solubility of a substance in an SCF increases with pressure.

The effect of temperature (experiments E-3, E-8, E-9, and E-10) is presented in Figure 4, where extraction yield is plotted against CO_2 density. The extraction yield increases with temperature from 50–70°C, probably because the vapor pressure of the extracted materials plays a major role on the extraction efficiency in this temperature range. The opposite ef-

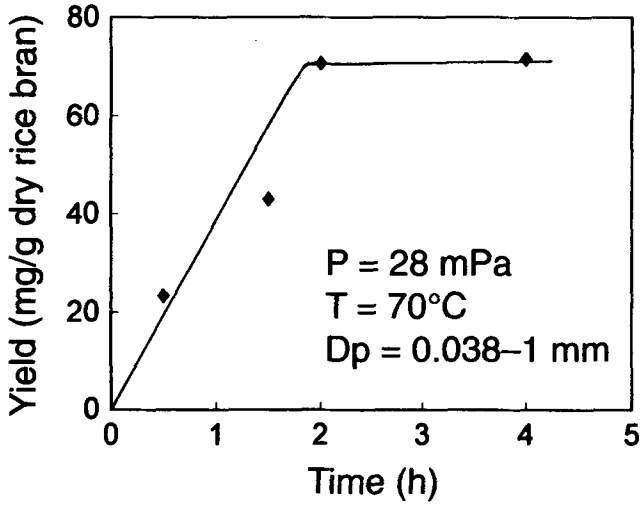

FIG. 2. Supercritical CO_2 extraction of rice bran at 28 MPa and 70°C. Yield vs. extraction time.

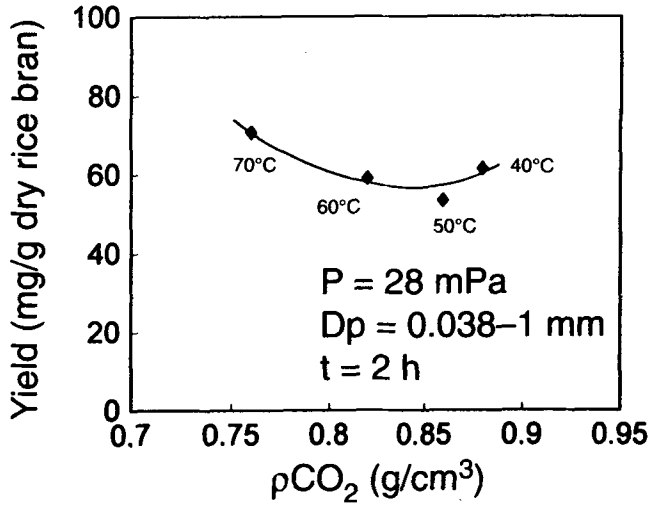

FIG. 4. Supercritical CO_2 extraction of rice bran at 28 Mpa. Yield vs. CO_2 density.

TABLE 2
Fatty Acid and Fatty Alcohol Composition of Hexane and SC-CO_2 Extracts

	SC-CO_2 extract				Hexane extract				
Comp.	Fatty acids[a]		Fatty alcohols[b]		Fatty acids[a]		Fatty acids[a,c]	Fatty alcohols[b]	
$C_{14:0}$	0.02	(0.4)	—	—	0.02	(0.19)	(0.4–1)	—	—
$C_{16:0}$	0.84	(17.0)	—	—	2.65	(25.0)	(12–18)	—	—
$C_{18:0}$	0.01	(1.1)	—	—	0.16	(1.5)	(1–3)	—	—
$C_{18:1}$	0.95	(19.3)	—	—	3.71	(35.0)	(40–50)	—	—
$C_{18:2}$	0.80	(16.6)	—	—	2.76	(26.0)	(29–42)	—	—
$C_{18:3}$	0.01	(0.2)	—	—	0.18	(1.7)	(≤1)	—	—
$C_{20:0}$	0.44	(8.4)	—	—	0.12	(1.1)	(≤1)	—	—
$C_{22:0}$	0.52	(10.6)	0.02	(1.4)	0.21	(2.0)		0.02	(1.50)
$C_{24:0}$	0.94	(19.0)	0.20	(14.0)	0.42	(4.0)		0.11	(14.5)
$C_{26:0}$	0.09	(1.8)	0.19	(13.3)	0.13	(1.2)		0.12	(16.4)
$C_{28:0}$	0.03	(0.6)	0.29	(20.7)	0.06	(0.6)		0.15	(20.1)
$C_{30:0}$	0.15	(3.0)	0.42	(29.6)	0.18	(1.7)		0.73	(30.2)
$C_{32:0}$	0.05	(1.0)	0.28	(20.0)	—	—		0.23	(16.6)
$C_{34:0}$	0.05	(1.0)	0.01	(1.0)	—	—		0.01	(0.7)
Total	4.95	(100)	1.41	(100)	10.6	(100)		1.37	(100)

[a]Grams of fatty acid extracted from 100 g of dry rice bran; percentages of total fatty acids are given in parentheses.
[b]Grams of fatty alcohol extracted from 100 g of dry rice bran; percentages of total fatty alcohols are given in parentheses.
[c]Data reported by Gupta (Ref. 13).

fect is observed at lower temperatures (40–50°C), which indicates that the solvent density (which varies from 0.86 g/cm^3 at 50°C to 0.88 g/cm^3 at 40°C) is the factor that enhances the extraction capability of the SC-CO_2.

The last supercritical extraction experiments (E-11, E-12, and E-13) were performed to check the effect of particle size on the extraction yield. No significant influence of the variable was found in the experimental range analyzed.

Analysis of the SC-CO_2 extracts by thin-layer chromatography showed their fatty acid and alcohol contents to be similar at all extraction conditions; therefore, only the hexane extract and the SC-CO_2 extract obtained at the experimental conditions that produced the highest yield (28 MPa and 70°C) were further analyzed by gas chromatography to compare the properties of the fractions extracted with hexane and SC-CO_2. Results from these analyses are shown in Table 2.

Compared to the extract obtained with hexane, the SC-CO_2 extract was lighter in color and richer in waxy components (Table 3). Concerning fatty acid and alcohol compositions, both extracts contained the same compounds but at different ratios. Hexane dissolved more palmitic, oleic, linoleic, and linolenic acid, while the fraction soluble in SC-CO_2 contained a higher proportion of long-chain fatty acids C_{20}–C_{34}, mainly tetracosanoic and docosanoic acids. The fatty alcohol proportions were similar in both extracts. Triacontanol appeared most abundant as the alcohol in the hexane and CO_2 fractions. Only a slight change was observed in the wax composition. Tocopherol content was slightly lower in the SC-CO_2 extract (280 ppm) than in the hexane-extracted oil (295 ppm), indicating that the oxidative stability of both extracts is similar.

TABLE 3
Comparison Between Hexane and SC-CO_2 Extracts

Solvent	Oil[a]	Waxes[a]	Iodine no. (mg I_2/100 g oil)	Saponification no. (mg KOH/100 g oil)
Hexane (70°C)	86	14	64	173
SC-CO_2 (28 MPa-70°C)	64	36	51	140

[a]g /100 g extract.

The phospholipids, free fatty acids, and glycerides behavior in extractions with hexane and SC-CO_2 was also analyzed. The hexane-extracted oil contained about 1.5% phospholipids (based on the accepted calculation (3): % phospholipids = % phosphorous × 31.7), whereas the SC-CO_2-extracted oil contained about 0.15% on the same basis. Phosphorous content was measured by atomic absorption. The free fatty acid proportions were similar in both extracts (≈18 μmols/g oil). Free fatty acids were determined according to the method given in Reference 15. Finally, values of iodine and saponification numbers (see Table 3) suggest that triglyceride content in both extracts is quite similar.

The poor dissolution of smaller molecular weight (C_{14}–C_{18}) fatty acids in SC-CO_2, compared to their dissolution in hexane, can easily be explained by considering that the fatty acid solubilities in SC-CO_2 are smaller than in hexane at the experimental conditions used in this work (7,12). According to this, SC-CO_2 should also have extracted less long-chain fatty acids (C_{20}–C_{26}) than hexane. Experimentally, however, the opposite was found. This could be due to the existence of a weak interaction between these fatty acids (C_{20}–C_{26}) and the solid matrix containing them, which in turn, somehow, favored their extraction by SC-CO_2.

Table 2 also shows the fatty acid composition of Indian rice bran oil reported by Gupta (13). Data from this author

shows that the fatty acid composition of Indian and Cuban rice bran oils extracted with hexane are slightly different. In Cuban oil, the proportion of palmitic, linolenic, and icosanoic acids is larger, while the myristic, oleic, and linoleic fractions are smaller. To the authors' knowledge, no literature data exist for comparing the fatty alcohol compositions of hexane and SC-CO_2 extracts.

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