Comparison of Supercritical Fluid and Solvent Extraction Methods in Extracting γ-Oryzanol from Rice Bran

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ABSTRACT: Organic solvents were compared with supercritical CO_2 relative to efficiency for extracting lipid and γ-oryzanol from rice bran. A solvent mixture with 50% hexane and 50% isopropanol (vol/vol) at a temperature of 60°C for 45–60 min produced the highest yield (1.68 mg/g of rice bran) of γ-oryzanol among organic solvents tested. The yield of γ-oryzanol without saponification was approximately two times higher ($P < 0.05$) than that with saponification during solvent extraction. However, the yield (5.39 mg/g of rice bran) of γ-oryzanol in supercritical fluid extraction under a temperature of 50°C, pressure of 68,901 kPa (680 atm), and time of 25 min was approximately four times higher than the highest yield of solvent extraction. Also, a high concentration of γ-oryzanol in extract (50–80%) was obtained by collecting the extract after 15–20 min of extraction under optimized conditions.

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KEY WORDS: Supercritical fluid extraction, rice bran, γ-oryzanol.

γ-Oryzanol is an important fraction, along with tocotrienols and other unsaponifiables, relative to the hypocholesterolemic effects of rice bran oil (1–4). Since γ-oryzanol is readily dissolved in organic solvents, hexane has typically been used in extraction of γ-oryzanol from rice bran (5–7). However, all components of γ-oryzanol contain an alcohol group in the ferulate portion, which gives rise to a relatively high polarity. These components may also be soluble in more polar solvents, such as isopropanol and ethyl acetate, as well as nonpolar solvents, such as hexane or heptane. The polarity of extraction solvent may significantly affect the extractability of γ-oryzanol from rice bran. The effects of various solvents on the yield of γ-oryzanol in extraction have not been reported. Another unclear factor in extraction is the effect of saponification on the efficiency of extraction. In previous studies, saponification was performed prior to the solvent extraction (5–7). Saponification, which is important for reducing interfering lipids and for breaking down the matrix of rice bran for improved recovery of extraction, may have a negative effect on the extraction of γ-oryzanol. It is possible that the ester bond between the ferulate and triterpene components of γ-oryzanol is cleaved under alkali conditions. This could result in the decomposition of γ-oryzanol and decrease the yield of extraction. The effect of saponification on the yield of γ-oryzanol in solvent extraction has not been reported.

Supercritical fluid extraction (SFE) of lipid has received attention as an alternative to organic solvent extraction and has been shown to be an ideal method for extracting certain lipids (8–13). Carbon dioxide is changed to its supercritical fluid state beyond the supercritical point (73 atm, 31°C). Supercritical CO_2 extraction is nontoxic, nonflammable, and simple in operation when compared with traditional extraction using solvents. These advantages may make supercritical carbon dioxide extraction ideal in the food and pharmaceutical industries.

Most studies of the use of SFE in lipid extraction have focused on the yield of extractable material. The supercritical fluid pressure, temperature, and time were optimized to obtain as much extract from sample as possible. In their study Garcia *et al.* (9) reported that the condition giving the highest yield of extract from rice bran was the highest pressure and temperature allowable in their system (28 MPa and 70°C), and the yield was only 16–60% of that obtained by solvent extraction with hexane. In theory, each compound possesses a unique extractability under different conditions of supercritical fluid related to factors such as extraction temperature, pressure, and time. Thus, components in a sample are extracted in an ordered manner from a sample matrix under optimized conditions of SFE. Therefore, the fractionation of the extract becomes possible and convenient. This can reduce the cost and time inherent in traditional solvent extraction for purifying specific components of interest from the extract. The application of supercritical fluid for the purpose of extraction of γ-oryzanol was studied in this project.

EXPERIMENTAL PROCEDURES

Chemical and materials. All solvents were high-performance liquid chromatography (HPLC) grade. Hexane was obtained from Curtis Matheson Scientific Inc. (Houston, TX). Ethyl acetate was obtained from EM Science (Gibbstown, NJ). Methanol, isopropanol, and dichloromethane were obtained from Mallinckrodt Baker Inc. (Paris, KY). Acetonitrile and acetic acid were obtained from Fisher Scientific Inc. (Fair

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Lawn, NJ). Ascorbic acid, sodium hydroxide, and anhydrous sodium sulfate were obtained from Sigma (St. Louis, MO). Carbon dioxide used in SFE was obtained from BOC Gases (Riverton, NJ). Rice bran was obtained from the Riviana Rice Mill (Abbeville, LA).

Solvent extraction. One gram of rice bran was suspended in 5 mL of distilled water in a 25-mL test tube. Ascorbic acid (0.2 g) was added to the test tube. Saponification was performed by adding 0.1 mL of 80% (wt/vol) sodium hydroxide to the test tube. The mixtures with and without sodium hydroxide were vortexed and incubated in a 60°C water bath for 30 min. Five milliliters of test solvent was added, vortexed for 30 s, and centrifuged at 200 g for 15 min. The composition of the solvents in extraction is listed in Figure 1. The organic layer was collected in a separatory funnel. The residue was mixed with 5 mL of the solvent and centrifuged again. The organic layer was combined with the previous collection. Distilled water was added to the funnel to rinse the organic layer. After the funnel had been allowed to stand for 10 min, the distilled water was drained. The rinse step was repeated twice. Then, the organic layer was transferred to a conical tube. The extract was obtained by evaporating the solvent under nitrogen flow in a 40°C water bath.

SFE. SFE was performed using a Dionex SFE-703 supercritical fluid extractor (Dionex Corporation, Sunnyvale, CA). Carbon dioxide (Grade 5.5) was used as a supercritical fluid. The pressure of CO_2 was maintained at 68,901 kPa (680 atm) during extraction. The maximum flow rate of the restrictor was 250 mL/min. The extraction temperatures examined were 30, 40, 45, 50, 55, 60, and 75°C. Seven grams of rice bran was weighed and packed in the extraction cell of the supercritical fluid extractor. Five milliliters of hexane was added to a collection vial, and the vial holder was set at 4°C. After collection, the hexane was evaporated at 40°C under nitrogen flow to obtain the extract.

Quantification for γ-oryzanol. γ-Oryzanol was quantified using a reversed-phase HPLC method (14). A C18 column (25 cm × 4.6 mm) (Rainin Instrument Company, Woburn, MA), a Waters 510 pump (Milford, MA), a Dyntech autosampler (Baton Rouge, LA), and a diode-array ultraviolet-visible detector (Hewlett-Packard, San Fernando, CA) were employed in the HPLC system. The mobile phase consisted of methanol, acetonitrile, dichloromethane, and acetic acid (50:44:3:3 by vol). Flow rate was controlled at 1.4 mL/min. Analytes were detected at 330 nm. A Maxima chromatography workstation (Waters, Milford, MA) was used as the signal recorder and to calculate the concentrations of individual components of γ-oryzanol. The concentration of γ-oryzanol was obtained by summing all individual components.

Statistical analysis. Replication was three times for each solvent extraction and six times for each SFE. The General Linear Model procedure of the Statistical Analysis System (SAS Institute, Cary, NC) was used to evaluate the experiment data. Significant difference between means was considered at $P < 0.05$.

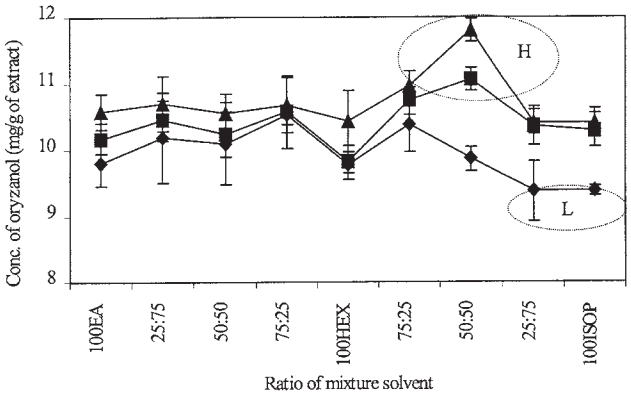

FIG. 1. Concentration of γ-oryzanol in extract using different solvent ratios at 30 (◆), 45 (■), and 60°C (▲) for 60 min. H, significantly higher concentration group; L, significantly lower concentration group; ethyl acetate; HEX, hexane; ISOP, isopropanol.

RESULTS AND DISCUSSION

Solvent extraction of γ-oryzanol using hexane with and without saponification. The concentration of γ-oryzanol in extracted oil using hexane with saponification was significantly lower than without saponification (Table 1). The concentration without saponification (9.8 mg/g) was about two times higher than that with saponification (4.6 mg/g). The structure of γ-oryzanol may decompose during saponification because the ester bond between ferulic acid and the triterpene component of γ-oryzanol could be hydrolyzed under alkali conditions. The advantage of saponification in many lipid extractions is to assist in the release of compounds of interest from the sample matrix and reduce interferences in chromatographic analysis. Rogers *et al.* (6) reported that 0.115 to 0.787 mg γ-oryzanol/g was extracted from refined rice bran oil that was treated using alkaline solutions and other oil refining procedures. The lower concentrations indicated that a large amount of γ-oryzanol could be lost in the oil refining treatments.

Solvent extraction of γ-oryzanol using different solvent ratios at different temperatures. The weights of extracted oil obtained using different solvent mixtures were very close and approximately 0.13 to 0.15 g/g rice bran. However, there were significant differences in concentrations of γ-oryzanol in these extracted oils. Figure 1 shows the concentrations of γ-oryzanol in extracted oils obtained using different solvent ratios. Higher concentrations of γ-oryzanol could be extracted

TABLE 1
Yield of Extract and Concentration of γ-Oryzanol in the Extracts from 1 g Rice Bran Using Hexane With and Without Saponification

	Weight of extract (g)	Conc. of γ-oryzanol in extract (mg/g)
With saponification	0.14 ± 0.01	4.6 ± 0.5
Without saponification	0.15 ± 0.01	9.8 ± 0.2

from rice bran with solvent mixtures of hexane/isopropanol (50:50, vol/vol) at 45°C, hexane/isopropanol (75:25, vol/vol) at 60°C, and hexane/isopropanol (50:50, vol/vol) at 60°C. Lower concentrations of γ-oryzanol were extracted with hexane/isopropanol (25:75, vol/vol) at 30°C and hexane/isopropanol (0:100, vol/vol) at 30°C. The other solvent mixtures at different temperatures extracted intermediate concentrations of γ-oryzanol. Figure 1 also shows a trend toward a positive relationship between concentration of γ-oryzanol in extracted oil and extraction temperature. This may be related to the physical properties of the matrix of the sample, which would be more penetrable at higher temperatures and cause γ-oryzanol to be released from the matrix. Temperature of extraction was an especially important factor affecting concentration of γ-oryzanol when isopropanol was used. Both the highest and lowest concentrations of γ-oryzanol in extracted oil among the solvents tested were obtained when using solvents containing isopropanol at different temperatures. For solvents containing ethyl acetate, the effect of temperature on concentration of γ-oryzanol was not significant. The solvents containing ethyl acetate or only hexane readily penetrated the rice bran matrix to extract γ-oryzanol regardless of the physical property of the sample matrix as affected by temperature. This may be due to the fact that these solvents had lower polarity and viscosity and preferentially extracted lipids. A lower affinity for nonpolar lipid and lower penetration capability of isopropanol compared to hexane or ethyl acetate may be due to its higher polarity and viscosity. Therefore, extraction using solvents containing a high percentage of isopropanol depended on high temperatures to assist in loosening the structure of the sample matrix and increasing the mobility of isopropanol. When the solvent contained isopropanol and hexane and had greater access to lipids, there was greater capacity to extract γ-oryzanol than solvents containing only ethyl acetate or hexane. However, temperatures above 60°C in solvent extraction have disadvantages in practice, such as evaporation of extraction solvent.

Concentration of γ-oryzanol in extracted oil at different extraction times using hexane/isopropanol (50:50, vol/vol) at 60°C. Concentrations after 30, 45, and 60 min of extraction were 11.0, 11.6, and 11.7 mg γ-oryzanol/g extracted oil, respectively when using hexane/isopropanol (50:50, vol/vol) at 60°C. No significant differences in the concentration occurred after 45 min. Therefore, the extraction time could be shortened to 45 min without significantly affecting the concentration of γ-oryzanol in extracted oil.

Yield of extract using SFE under different conditions. Figure 2 shows the yield of extract using SFE at various times and temperatures, and a pressure of 68,901 kPa (680 atm). The yields of extract in 10 min were significantly higher at 55, 60, and 75°C than at temperatures below 55°C. For temperatures above 55°C, yields were maximum and changed only slightly, after 10 min extraction. For temperatures of 30, 40, and 45°C, yields continued to increase over time. The density of supercritical CO_2 is decreased with increasing temperature under a constant pressure. However, the effect of

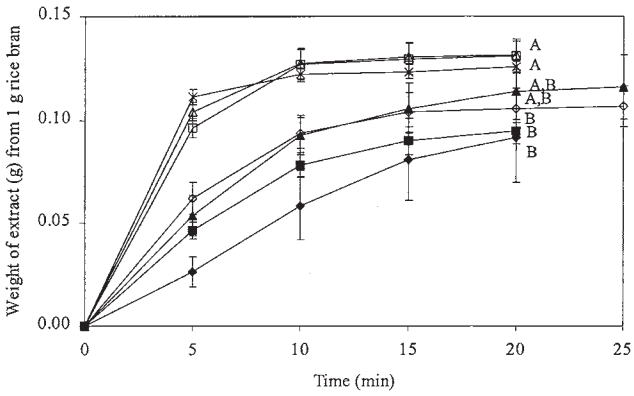

FIG. 2. Weight of extract of supercritical fluid extraction at different extraction times, temperatures, and pressure of 68,901 kPa (680 atm). Significant difference ($P < 0.05$) is expressed by different letters. 30°C (◆); 40 (■); 45 (▲); 50 (◇); 55 (□); 60 (△); 75 (X).

higher temperature in SFE may alter the physical properties of the rice bran matrix and make it more penetrable by extraction fluid. Another effect is related to retrograde behavior in which the solubility of the solutes increases with increasing temperature if pressure is sufficiently greater than the critical pressure owing to increased vapor pressure of the solute. Therefore, a high temperature in SFE resulted in higher yields at shorter extraction times in this study.

The range of yields of extracts in 20 min extraction time was 0.09 g at 30°C to 0.13 g at 60°C from 1 g of rice bran (Fig. 2). The percentage yield of extract from rice bran was 9 to 13%. Yields were lower than that of solvent extraction (approximately 14%). Garcia *et al.* (9) reported that the highest yields of total extract from rice bran for SFE were obtained at a pressure of 28,000 kPa (280 atm) and a temperature of 70°C for 4 h, but the total yield was only 7.1%. Also, they concluded that the yield of extract in rice bran could be improved with more severe extraction conditions. In the present study, however, the yield of extract at higher temperatures (75°C) was not significantly higher than that of 55 and 60°C. Tsuda *et al.* (13) found that the yield of extracts at 40°C was higher than that at 60 and 80°C in the extraction of antioxidative components from tamarind seed coat. This suggests that severe SFE conditions may not lead to increased yield of extract. It is possible that more extract escaped from the vent of the SFE system because it was not condensed in the collection vial owing to high extraction temperature and pressure. Therefore, the yield of extract was not positively associated with extraction temperature in the actual SFE system. However, long extraction times with lower temperatures may not be feasible because they result in higher consumption of supercritical carbon dioxide.

Figure 3 shows the concentrations of γ-oryzanol in extracts prepared using various extraction times and temperatures using SFE. In 10 min of extraction, the concentration of γ-oryzanol in the extract was significantly higher at temperatures of 55, 60, and 75°C than at lower temperatures. After 10 min, however, no further increases in γ-oryzanol concen-

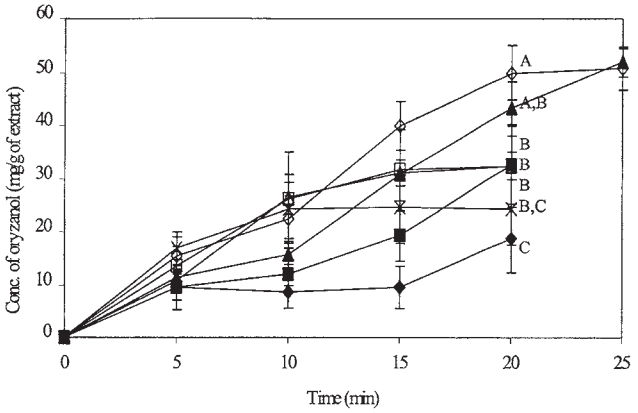

FIG. 3. Concentration of γ-oryzanol in extract using supercritical fluid extraction at different extraction times, temperatures, and pressure of 68,901 kPa (680 atm). Significant difference ($P < 0.05$) is expressed by different letters. 30°C (◆); 40 (■); 45 (▲); 50 (◇); 55 (□); 60 (△); 75 (X).

tration in the extract were attained at these extraction temperatures. Furthermore, the concentration of γ-oryzanol obtained at temperatures of 60 and 75°C for 20 min was significantly lower than that at a temperature of 50°C. In the first 10 min of extraction, a large amount of extractable material in rice bran was already extracted at these higher extraction temperatures (Fig. 2). During 10 to 20 min of extraction, a smaller amount of material in rice bran was extracted at these temperatures. It is possible that γ-oryzanol and other components were more mobile in the SFE system at lower concentrations and higher extraction temperatures. These components may not have condensed completely in the collection vial and may have escaped from the vent. Thus, yields of γ-oryzanol did not increase further.

Table 2 lists yields and concentrations (above 100 mg/g of extract) of γ-oryzanol in extracts at different extraction times. The highest yield of γ-oryzanol in SFE was at 15–20 min at a temperature of 45°C, and the highest concentration in the extract was at 15–20 min at a temperature of 50°C. From the results in Table 2, it is suggested that γ-oryzanol could be concentrated and extracted from the sample matrix under a period of optimal extraction conditions. SFE conditions can be selected based on extraction either for high yield or high concentration of γ-oryzanol. However, limitations of the SFE device, such as fluctuation of flow rate and pressure, caused relatively high standard deviations.

Comparison of yields and concentrations of γ-oryzanol in extracts prepared by SFE or solvent extraction. Table 3 lists the yields of γ-oryzanol from rice bran and concentrations in extracts obtained by solvent extraction and SFE, respectively. The yield by SFE was three times higher than that by solvent extraction. Also, savings of solvent and time associated with traditional solvent extraction could be achieved using the SFE method. Furthermore, a much higher concentration of γ-oryzanol was obtained by SFE when the extract was collected during a specific period (15–20 min) of extraction time.

The results of this study have demonstrated that SFE produces a high yield and concentration of γ-oryzanol without consuming the large amounts of solvent, time, and complex purification processing that usually are associated with solvent extraction.

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TABLE 2
Yields and Concentrations of γ-Oryzanol from 1 g of Rice Bran in Extract at Various Times, Various Temperatures, and a Pressure of 68,901 kPa (680 atm) Using Supercritical Fluid Extraction[a]

Temperature (°C)	Time (min)	Yield (mg)	Concentration (mg/g)
40	15–20	1.33 ± 0.24[a]	332.3 ± 136.8[b,c]
45	15–20	1.69 ± 0.11[a]	227.7 ± 65.4[b,c]
	20–25	1.10 ± 0.53[a]	484.0 ± 88.1[a,b]
50	15–20	1.11 ± 0.53[a]	674.6 ± 148.1[a]
	20–25	0.13 ± 0.10[b]	132.1 ± 81.8[c]

[a]Means within columns with common superscript letters are not different ($P > 0.05$).

TABLE 3
Comparison of Yields from Rice Bran and Concentrations in Extracts of γ-Oryzanol Between SFE and Solvent Extraction at Each Optimum Condition

	Solvent extraction[a]	SFE[b]	SFE[c]
Yield (mg/g of rice bran)	1.68 ± 0.02	5.39 ± 0.43	1.11 ± 0.07
Concentration (mg/g of extract)	11.8 ± 0.2	51.0 ± 5.5	674.6 ± 148.1

[a]Extraction with hexane/isopropanol (50:50) at 60°C for 60 min.
[b]Extraction under 68,901 kPa (680 atm) at 50°C for 25 min.
[c]Extraction under 68,901 kPa (680 atm) at 50°C and collection between 15–20 min.

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