Comparison of Isopropanol and Hexane for Extraction of Vitamin E and Oryzanols from Stabilized Rice Bran

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ABSTRACT: The effects of solvent-to-bran ratio (2:1 and 3:1, w/w), extraction temperature (40 and 60°C), and time (5, 10, 15, 20, and 30 min) were studied for hexane and isopropanol extraction. Increasing the solvent-to-bran ratios and extraction temperature increased the amounts of crude oil, vitamin E and oryzanol recovered for both solvents. An extraction time of 15 min was sufficient for optimum crude oil, vitamin E, and oryzanol extraction. Preheated isopropanol (3:1 solvent/bran ratio and 60°C) extracted less crude oil ($P < .05$) but more vitamin E ($P < .05$) and similar amounts of oryzanol ($P > .05$) relative to preheated hexane. The data suggest that isopropanol is a promising alternative solvent to hexane for extraction of oil from stabilized rice bran.

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KEY WORDS: Extraction, hexane, isopropanol, oryzanol, rice bran oil, vitamin E.

Crude rice bran oil consists of 88 to 89% neutral lipids, 3 to 4% waxes, 2 to 4% free fatty acids, and approximately 4% unsaponifiables (1,2). The unsaponifiable fraction in rice bran oil contains a unique complex of naturally occurring antioxidant compounds, such as vitamin E (tocopherols, tocotrienols), and oryzanols (3). Antioxidant compounds in rice bran oil contribute to lowering low density lipoprotein and total serum cholesterol in both animals and humans (4–8).

Hexane is used as a solvent to commercially extract oil from rice bran (9). However, hexane poses potential fire, health, and environmental hazards (10). Short-chain alcohols, especially ethanol and isopropanol, have been proposed as alternative extraction solvents due to their greater safety and reduced probability of regulation (9,11,12). Alcohols tend to extract more nonglyceride materials than hexane, due to their greater polarity (9). Typically, alcohol-extracted oils contain more phosphatide and unsaponifiable compounds (9).

Although numerous studies have been conducted on cottonseed and soybean oil extraction with isopropanol as a solvent (13–19), only a few studies with isopropanol or ethanol as extraction solvent have been conducted on rice bran. Isopropanol has been used to extract rice bran oil rich in B-vitamins (20), and ethanol has been used to extract rice bran oil rich in tocopherols and B-vitamins (21). However, there are no reports in the literature of using isopropanol to extract oil rich in vitamin E and oryzanol from stabilized rice bran. The objective of this study was to compare isopropanol and hexane as extraction solvents for recovery of vitamin E and oryzanol from stabilized rice bran and investigate the effect of solvent-to-bran ratios and temperature.

EXPERIMENTAL PROCEDURES

Bran preparation, storage, and sample analysis. Extrusion-stabilized rice bran was obtained from a commercial source in California. The bran was received, vacuum-packaged, and stored at −20°C until processing. Initial moisture and lipid contents of bran samples ($n = 50$ and 9, respectively) were determined by AOAC methods 7.007 and 27.006 (22), respectively.

Extraction. Hexane (Mallinckrodt, Paris, KY) or isopropanol (Mallinckrodt) at 20°C was mixed with stabilized rice bran (25 g) at 2:1 or 3:1 w/w solvent-to-bran ratio in flasks, capped, and immersed in a constant-temperature water bath at 40 or 60°C. After extraction times of 5, 10, 15, 20, and 30 min, the miscella from each flask was separated from the defatted rice bran by vacuum filtration. Vacuum evaporation (BÜCHI Labortechnik AG, Flawil, Switzerland) of solvent from the miscella yielded crude rice bran oil. The crude oil yield was measured and expressed as g oil/kg dry full-fat rice bran. Two replicates were conducted for each ratio–temperature–time treatment combination.

Preheated solvent extraction. Preheated hexane or isopropanol at 60°C was mixed with rice bran (25 g) at 3:1 w/w solvent-to-bran ratio in flasks, capped, and immersed in a constant-temperature water bath at 60°C. After extraction times of 5, 10, 15, 20, and 30 min, the miscella from each flask was separated from the defatted rice bran by vacuum filtration. Vacuum evaporation of solvent from the miscella yielded crude rice bran oil. The crude oil yield was measured and expressed as g oil/kg dry full-fat rice bran. Two replicates were conducted for both preheated hexane and isopropanol extraction.

Analytical analysis. Vitamin E (tocopherols and tocotrienols) and oryzanol contents in the oil were determined by high-performance liquid chromatography (HPLC) (23)

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and are reported as mg/kg dry full-fat rice bran. All determinations were made in duplicate.

Standards. Tocopherols and tocotrienols were prepared from natural sources (24). Oryzanol was isolated from crude rice bran oil (25).

Sample preparation for HPLC. A rice bran oil sample (100 mg) was placed in a 15-mL test tube with 5 mL ethanol and 0.1 g ascorbic acid. The sample was saponified with 0.15 mL of 80% KOH for 10 min at 80°C. After saponification, the flask was placed in an ice bath, and 5 mL water and 5 mL hexane were added. The mixture was transferred to centrifuge bottles and centrifuged at 120 × *g* for 1 min. The upper layer was transferred to a 125-mL separatory funnel. Extraction of the sample with 5 mL hexane was repeated twice. The pooled hexane layer was washed with 5 mL water three times, filtered through Na_2SO_4, and then evaporated under a stream of nitrogen. The oil sample was diluted with 1 mL isooctane.

HPLC. The HPLC system consisted of a Waters (Milford, MA) 510 HPLC pump, a 715 Ultra WISP injector, a 470 scanning fluorescence detector with excitation at 290 nm and emission at 330 nm. A Supelcosil™ (Supelco, Bellefonte, PA) LC-Si, 5 μm, 15 cm × 4.6 mm i.d. column was used. The mobile phase was isooctane/ethyl acetate/acetic acid/DMP (98.5:0.7:0.7:0.1) with a flow rate of 1.4 mL/min.

Statistical analysis. The experimental plan was a completely randomized design with 5 × 2 × 2 factorial treatment structure. The data were analyzed with the SAS general linear models (GLM) procedure (SAS Institute, Inc., Cary, NC). A contrast procedure was used to test significant differences ($P < .05$) among solvent-to-bran ratio (2:1 and 3:1, w/w), extraction temperature (40 and 60°C), and extraction time (5, 10, 15, 20, and 30 min). Least square means (LSM) and standard errors of LSM for all data with different factor combinations were obtained. Test of significant differences ($P < .05$) between preheated isopropanol and hexane was performed with a GLM procedure with appropriate contrast statement (26).

RESULTS AND DISCUSSION

The stabilized rice bran used in this study contained 9.0 ± 1.0% moisture and 24.9 ± 0.9% lipid (dry weight basis). The lipid contained 608 ± 72 ppm vitamin E (343 ppm tocopherols and 265 ppm tocotrienols) and 13,400 ± 570 ppm oryzanols. These results agree with those reported by Zhao *et al.* (27), who showed that hexane-extracted rice bran oil contained 330 ppm tocopherols and 14,000 ppm oryzanol in the oil. Also, crude oil with similar amounts of oryzanol (12,221 ppm) and tocopherols (262 ppm), and a larger amount of tocotrienols (713 ppm) has been reported by Nicolosi *et al.* (28).

Three-factor factorial analysis of crude oil, vitamin E, and oryzanol showed that they increased with increasing solvent-to-bran ratio and temperature in hexane and isopropanol extraction (Table 1). For hexane extraction, an increase in solvent-to-bran ratio (w/w) from 2:1 to 3:1 extracted 10.8% more crude rice bran oil that contained 13.0% more vitamin E and 12.2% more oryzanol ($P < .05$). Extraction at 60°C produced 3.6% more crude bran oil that contained 7.1% more vitamin E and 3.6% more oryzanol ($P < .05$) than that extracted at 40°C. When rice bran was extracted with hexane, the vitamin E increased twice as much as either the crude oil or oryzanol content as the extraction temperature was increased from 40 to 60°C. This showed that temperature influenced vitamin E solubility in hexane more than that of crude bran oil or oryzanol.

For isopropanol extraction, an increase in solvent-to-bran ratio (w/w) from 2:1 to 3:1 extracted 9.4% more crude bran oil that contained 10.0% more vitamin E and 8.4% more oryzanol ($P < .05$) (Table 1). Extraction at 60°C produced 6.4% more crude bran oil with 9.4% more vitamin E and 6.8% more oryzanol than oil extracted at 40°C ($P < .05$), indicating that temperature affects vitamin E extraction more than crude oil or oryzanol extraction.

The hexane extraction time did not have a significant effect on the amount of crude oil or vitamin E extracted (Figs.

TABLE 1
Crude Oil, Oryzanol, and Vitamin E Extracted by Hexane or Isopropanol with Different Solvent-to-Bran Ratios and Temperatures

	Hexane extraction			Isopropanol extraction		
	Crude oil[a]	Vitamin E[b]	Oryzanol[b]	Crude oil[a]	Vitamin E[b]	Oryzanol[b]
Ratio (w/w)						
2:1	186.2 ± 2.5[c]	133.4 ± 1.8[c]	2510 ± 41[c]	170.5 ± 1.2[c]	145.9 ± 1.1[c]	2452 ± 20[c]
3:1	206.3 ± 2.5[d]	150.7 ± 1.8[d]	2815 ± 41[d]	186.5 ± 1.2[d]	160.5 ± 1.1[d]	2657 ± 20[d]
Temp. (°C)						
40	192.8 ± 2.5[c]	137.2 ± 1.8[c]	2615 ± 41[c]	173.0 ± 1.2[c]	148.5 ± 1.1[c]	2471 ± 20[c]
60	199.7 ± 2.5[d]	146.9 ± 1.8[d]	2709 ± 41[d]	184.1 ± 1.2[d]	157.9 ± 1.1[d]	2638 ± 20[d]
Preheated						
60°C, 3:1 w/w	211.0 ± 2.7[e]	157.3 ± 2.8[e]	2847 ± 39[e]	201.2 ± 2.7[f]	171.0 ± 2.8[f]	2930 ± 39[f]

[a]The results are expressed as g/kg dried rice bran.

[b]The results are expressed as mg/kg dried rice bran. Significantly different values ($P < .05$) in the same column of each ratio and temperature treatment are indicated by different letters [c,d]. Significantly different values ($P < .05$) of oil, vitamin E, and oryzanol by preheated hexane and preheated isopropanol extraction are indicated by different letters [e,f].

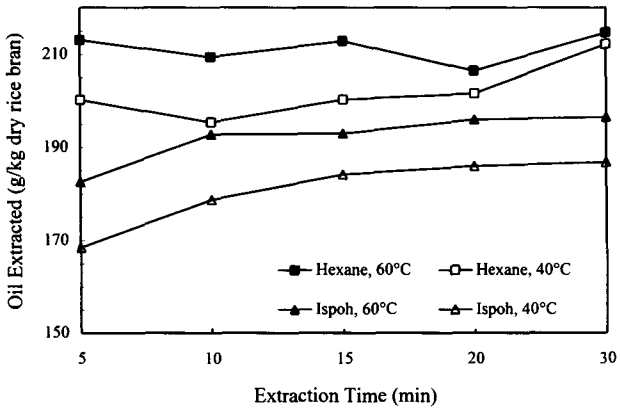

FIG. 1. Extraction curves of crude rice bran oil with hexane and isopropanol (Ispoh) at 3:1 w/w solvent-to-bran ratio and extraction temperatures of 40 and 60°C.

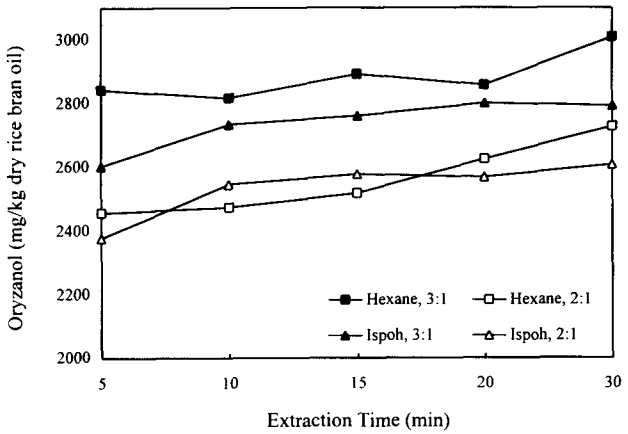

FIG. 3. Oryzanol in rice bran oil extracted by hexane and isopropanol (Ispoh) at extraction temperature of 60°C and solvent-to-bran ratios of 3:1 and 2:1 w/w.

1 and 2). However, the amount of oryzanol extracted after 30 min was greater than that obtained after 5 or 10 min (Fig. 3). Regression analysis of ratio, temperature, and time effects on measured variables showed that, for hexane extraction, a solvent-to-bran ratio of 3:1 and extraction temperature of 60°C for 15 min was sufficient for crude oil and total vitamin E extraction. The results are consistent with those of other investigators (29), who claimed that the extraction time to reach 1% residual oil was 10 min for extrusion-stabilized rice bran extracted at a solvent-to-bran ratio of 1.77:1 and extraction temperature of 60°C. The amount of oryzanol was highest with a 30-min extraction time (Fig. 3).

Crude oil, vitamin E, and oryzanol yields for isopropanol extraction were not statistically different after 10, 15, 20, and 30 min of extraction, although they were elevated relative to a 5-min extraction (Figs. 1–3). A solvent-to-bran ratio of 3:1 w/w and extraction temperature of 60°C for 10 min were sufficient for isopropanol extraction.

Nonpreheated hexane and isopropanol comparisons indicated that average amounts of oil and oryzanol were greater with hexane extraction (Figs. 1 and 3). However, the average amount of vitamin E with isopropanol extraction was greater than that with hexane extraction (Fig. 2).

Preheating hexane to 60°C did not give rise to significant differences in extraction of crude oil, oryzanol, and vitamin E when compared to nonpreheated hexane extraction (Table 1). However, preheated isopropanol significantly increased all measured variables (Table 1) due to longer time at 60°C compared to cold isopropanol that needed to be warmed to 60°C. The amount of oryzanol in crude rice bran oil extracted by preheated isopropanol at a solvent-to-bran ratio of 3:1 and 60°C was not significantly different from that of preheated hexane under the same conditions (Fig. 4).

In summary, the optimal conditions for crude rice bran oil, vitamin E, and oryzanol extraction from stabilized rice bran with hexane are 3:1 solvent-to-bran ratio (w/w), 60°C extraction temperature, and 15 min extraction time. Use of preheated isopropanol (60°C) to extract stabilized rice bran at

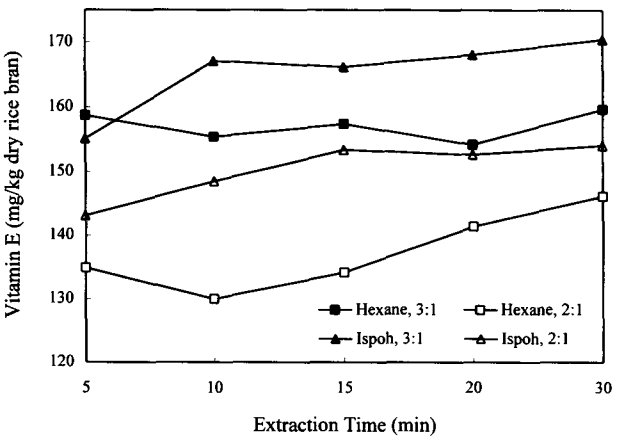

FIG. 2. Vitamin E in rice bran oil extracted by hexane and isopropanol (Ispoh) at extraction temperature of 60°C and solvent-to-bran ratios of 3:1 and 2:1 w/w.

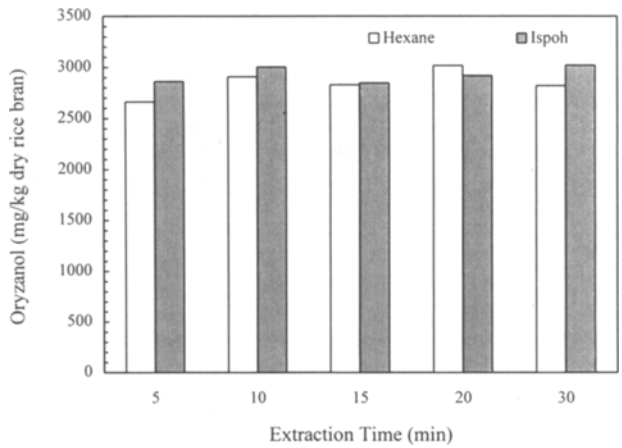

FIG. 4. Oryzanol in rice bran oil extracted by preheated hexane and isopropanol (Ispoh) at 60°C and solvent-to-bran ratio of 3:1 w/w.

3:1 solvent-to-bran ratio (w/w) for 10 min can produce crude rice bran oil with the optimum vitamin E (174.8 mg/kg dry rice bran) and oryzanol (3003 mg/kg dry rice bran) content. Under respective optimal extraction conditions, isopropanol extracted less crude oil ($P < .05$), more vitamin E ($P < .05$), and the same amount of oryzanol ($P > .05$) from rice bran compared with hexane. Isopropanol is a promising alternative solvent to extract oil from stabilized rice bran.

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REFERENCES

1. Hemavathy, J., and J.V. Prabhakar, Lipid Composition of Rice (*Oryza sativa* L.) Bran, *J. Am. Oil Chem. Soc. 64*:1016–1019 (1987).
2. Orthoefer, F.T., and R.J. Nicolosi, Rice Bran Oil Composition and Characteristics, presented at the 84th American Oil Chemists' Society Annual Meeting, Anaheim, California, April 25–29, 1993.
3. Wells, J.H., Utilization of Rice Bran and Oil in Human Diets, *Louisiana Agriculture 36*:5–8 (1993).
4. Sharma, R.D., and C. Rukmini, Rice Bran Oil and Hypocholesterolemic in Rats, *Lipids 21*:715–717 (1986) .
5. Rukmini, C., Chemical, Nutritional and Toxicological Studies of Rice Bran Oil, *Food Chem. 30*:257–268 (1988).
6. 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).
7. Kahlon, T.S., R.M. Saunders, R.N. Sayre, F.I. Chow, M.M. Chiu, and A.A. Betschart, Cholesterol-Lowering Effects of Rice Bran and Rice Bran Oil Fractions in Hypercholesterolemic Hamsters, *Cereal Chem. 69*:485–489 (1992).
8. Hegsted, M., and M.M. Windhauser, Reducing Human Heart Disease Risk with Rice Bran, *Louisiana Agriculture 36*:22–24 (1993).
9. Johnson, L.A., and E.W. Lusas, Comparison of Alternative Solvents for Oils Extraction, *J. Am. Oil Chem. Soc. 60*:229–242 (1983).
10. *Health and Safety Guide No. 59: n-Hexane Health and Safety Guide*, World Health Organization, Geneva, 1991.
11. Lusas, E.W., L.R., Watkins, and S.S. Koseoglu, Isopropyl Alcohol To Be Tested as Solvent, *INFORM 2*:970–976 (1991).
12. Watkins, L.R., S.S. Koseoglu, K.C. Rhee, E. Hernandez, M.N. Riaz, W.H. Johnson, Jr., and S.C. Doty, New Isopropanol System Shows Promise, *Ibid. 5*:1245–1253 (1994).
13. Beckel, A.C., P.A. Belter, and A.K. Smith, The Nondistillation Alcohol Extraction Process for Soybean Oil, *J. Am. Oil Chem. Soc.* 25:10–11 (1948).
14. Harris, W.D., F.F. Bishop, C.M. Lyman, and R. Helpert, Isopropanol as a Solvent for Extraction of Cottonseed Oil, I. Preliminary Investigations, *Ibid. 24*:370–375 (1947).
15. Rao, R.K., M.G. Krishna, S.H., Zaheer, and L.K. Arnold, Alcohol Extraction of Vegetable Oils, I. Solubilities of Cottonseed, Peanut, Sesame, and Soybean Oils in Aqueous Ethanol, *Ibid. 32*:420–423 (1955).
16. Rao, R.K., and L.K. Arnold, Alcoholic Extraction of Vegetable Oils. Part I. Solubilities of Vegetable Oils in Aqueous 2-Propanol, *Ibid. 34*:401–404 (1957).
17. Rao, R.K., and L.K. Arnold, Alcoholic Extraction of Vegetable Oils, Pilot Plant Extraction of Cottonseed by Aqueous Ethanol, *Ibid. 35*:277–281 (1958).
18. Baker, E.C., and D.A. Sullivan, Development of a Pilot-Plant Process for the Extraction of Soy Flakes with Aqueous Isopropyl Alcohol, *Ibid. 60*:1271–277 (1983).
19. Hron, R.J., and S.P. Koltun, An Aqueous Ethanol Extraction Process for Cottonseed Oil (Biorenewable Alternative to Hexane), *Ibid. 61*:1457–1460 (1984).
20. Meinke, W.W., B.R. Holland, and W.D. Harris, Solvent Extraction of Rice Bran. Production of B-Vitamin Concentrate and Oil by Isopropanol Extraction, *Ibid. 26*:532–534 (1949).
21. Talwalkar, R.T., N.K. Grag, and C.R. Krishna Murti, Rice Bran —a Source Material for Pharmaceuticals, *J. Food Sci. and Technol. 2*:117–119 (1965).
22. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 14th edn., AOAC, Inc., Arlington, 1984.
23. Shin, T.-S, and J.S. Godber, Improved High-Performance Liquid Chromatography of Vitamin E Vitamers on Normal-Phase Columns, *J. Am. Oil Chem. Soc. 70*:1289–1991 (1993).
24. Shin, T.-S, and J.S. Godber, Isolation of Four Tocopherols and Four Tocotrienols from a Variety of Natural Sources by Semi-Preparative High-Performance Liquid Chromatography, *J. Chromatogr. 678*:49–58 (1994).
25. Kim, I., and Kim, C., Separation of Oryzanol from the Refining By-product of Rice Bran Oil, *Korean J. Food Sci. Technol. 23*:76–80 (1991).
26. *SAS/STAT User's Guide*, Version 6, 4th edn., SAS Institute, Inc., Cary, 1989.
27. Zhao, W., A. Shishikura, K. Fujimoto, K. Arai, and S. Sait, Fractional Extraction of Rice Bran Oil with Supercritical Carbon Dioxide, *Agric. and Biol. Chem. 51*:1773–1777 (1987).
28. Nicolosi, R.J., E.J. Rogers, L.M. Austman, and F.T. Orthoefer, Rice Bran Oil and Its Health Benefits, in *Rice Science and Technology*, edited by W.E. Marshall and J.I. Wadsworth, Marcel Dekker, Inc., New York, 1994, pp. 421–437.
29. Kim, C.J., - S.M. Byun, H.S. Cheigh, and T.W. Kwon, Comparison of Solvent Extraction Characteristics of Rice Bran Pretreated by Hot Air Drying, Steam Cooking and Extrusion, *J. Am. Oil Chem. Soc. 64*:514–516 (1987).

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