Supercritical CO_2 Extraction of Rice Bran

M.S. Kuk* and M.K. Dowd

SRRC, ARS, USDA, New Orleans, Louisiana 70179-0687

ABSTRACT: Extraction of rice bran lipids with supercritical carbon dioxide (SC-CO_2) was performed. To investigate the pressure effect on extraction yield, two isobaric conditions, 7000 and 9000 psi, were selected. A Soxhlet extraction with hexane (modified AOCS method Aa 4-38; 4 h at 69°C) was also conducted and used as the comparison basis. Rice bran with a moisture content of 6%, 90% passable through a sieve with 0.297 mm opening, was used for extraction. A maximum rice bran oil (RBO) yield of 20.5%, which represents 99+% lipid recovery, was obtained with hexane. RBO yield with SC-CO_2 ranged between 19.2 and 20.4%. RBO yield increased with temperature at isobaric conditions. At the 80°C isotherm, an increase in RBO yield was obtained with an increase in pressure. The pressure effect may be attributed to the increase in SC-CO_2 density, which is closely related to the value of the Hildebrand solubility parameter. RBO extracted with SC-CO_2 had a far superior color quality when compared with hexane-extracted RBO. The level of sterols in SC-CO_2-extracted RBO increased with pressure and temperature.

JAOCS 75, 623–628 (1998).

KEY WORDS: Carbon dioxide, edible oil extraction, free fatty acid, plant sterol, rice bran oil, supercritical fluid.

As an extraction medium, supercritical fluid (SCF) has versatile solvent properties compared to a liquid extraction agent. Because oilseed lipid extraction is usually conducted at a fixed temperature, slightly below the normal boiling point of the extracting agent, the solubility relationship between solutes and solvent is fixed at the predetermined extraction temperature. The control of lipid solubility and mass transfer properties, such as diffusivity of the extraction medium, is highly desirable in the lipid recovery process. Temperature is the sole variable available for solubility control in lipid extraction with a selected solvent. However, for most commercial oilseed extractions, which are usually conducted with hexane, the control of solubility and mass transfer is severely limited because the solvent's low normal boiling point restricts the window of temperature control.

When a SCF is used in lipid extraction, control of the lipid solubility and diffusivity is expanded to the entire domain of pressure and temperature above its critical point. The control domain of a SCF in selecting lipid solubility and diffusivity is substantially increased, compared to that for a liquid extraction agent.

Recently, rice bran oil (RBO) has received some attention because of its unique health benefits (1) that may be attributed to the sterols present in it. Rice bran contains protein, free fatty acids, glycerides, sterols, and polysaccharides and their degenerated species (2). Extracting RBO aims at recovering triglycerides and sterols and limiting the recovery of free fatty acid (FFA). To achieve good color quality in refined RBO, limiting FFA content in crude oil is essential. This task is difficult to achieve with a liquid extracting agent, such as hexane, because solubility for specific group components of oilseed lipids cannot be controlled.

In this investigation, supercritical CO_2 (SC-CO_2) was used to gain information on whether control of the solubility of such lipid species as FFA and sterols would be feasible in extraction. RBO yield, extract composition, and the resulting oil color, produced by SC-CO_2 at preselected conditions, were examined to gain this information.

MATERIALS AND METHODS

Extraction. Recently harvested and stabilized rice bran, with 6% moisture [AOCS method Aa 3-38 (3)], 90% passable through U.S. sieve #50 (0.297 mm opening), was used for the extraction tests. As a control, rice bran was extracted with hexane for 4 h at 69°C in a Soxhlet extractor, following a modified form of AOCS Method Aa 4-38 (3,10). SC-CO_2 extractions were performed in a commercial extractor (Dionex Model SFE 703, Salt Lake City, UT) at pressures between 7000 and 9000 psi and temperatures between 70 and 100°C. A typical SC-CO_2 extraction was conducted as follows: *ca.* 5 g of rice bran was placed in the extraction cell. The cell was charged with SC-CO_2 at the predetermined temperature and pressure. Ninety minutes was allowed to pass SC-CO_2 through the extraction cell and a restrictor, which controlled the extraction pressure. Extracted rice bran lipid was collected into a cell collector, while CO_2 was vented out to ambient air. Spent rice bran was collected for further lipid extraction with petroleum ether to determine residual lipid content. Extraction oil yields were determined by a gravimetric method on a moisture-free basis.

Crude oil analysis. Crude RBO samples were analyzed to determine FFA and phosphorus contents, color, and composition. Crude oil colors were determined with a colorimeter

*To whom correspondence should be addressed at SRRC, ARS, USDA, P.O. Box 19687, New Orleans, LA 70179-0687.
E-mail: mskuk@nola.srrc.usda.gov.

(Colorscan, Tintometer, Salisbury, United Kingdom). Phosphorus contents were determined by an ICP method (induction coupled plasma, Leeman Lab, Lowell, MA) after micro-Kjeldahl digestion, and FFA by AOCS method Ca 5a-40. The composition of crude oil samples was analyzed, after derivatizing the crude oil samples, in a Hewlett-Packard capillary gas chromatograph (GC) (Model 5890; Avondale, PA), equipped with a flame-ionization detector and a split injector (1:50 ratio). A 15 m × 0.25 mm fused-silica column DB-5 (J&W Scientific, Folsom, CA) with 0.1-mm film thickness was used to separate the derivatized compounds. Sterol components were identified with a Hewlett-Packard 5988A quadrapole mass spectrometer, interfaced with a Hewlett-Packard 5890 Series II GC. Derivatization details were as follows: 40 mg of each oil sample was mixed with 500 μL pyridine, 500 μL hexamethyldisilane, and 50 μL trifluoroacetic acid (Pierce Chemical, Rockford, IL), heated at 60°C for 45 min, and periodically shaken to yield a single liquid phase that contained the trimethylsilyl (TMS) ether derivatives. Cholesterol (Sigma Chemical, St. Louis, MO) was used as an internal standard (IS). TMS derivatives of IS were prepared in a similar manner. Commercial triglycerides and sterols (Sigma Chemical) were used to make GC standards.

RESULTS AND DISCUSSION

Crude oil extraction yields with SC-CO_2 ranged between 19.0 and 20.4% and are summarized in Table 1; the maximum was 20.5% with hexane. Extraction of spent rice bran with petroleum ether indicated that greater than 99% lipid recovery was achieved with hexane and with SC-CO_2 at 100°C and 9000 psi. Along with this yield information, the molar volume and density of the SC-CO_2 at the test conditions are listed in Table 1. Crude oil color, FFA, and phosphorus contents are presented in Table 2. These crude oil yields with hexane and SC-CO_2 were similar to soy oil extraction yield data reported by Friedrich *et al*. (4) and by List and Friedrich (5). These investigators obtained extraction yields that ranged from 19 to 20% with hexane, and from 18.3 to 19.9% with SC-CO_2 at temperatures between 50 and 70°C and pressures between 5000 and 8000 psi. These investigators reported that nearly exhaustive extraction of lipids was achieved with SC-CO_2. Recently, Ramsey *et al*. (6) used SC-CO_2 for rice bran extraction at a relatively low temperature and pressure (35°C and 4350 psi) and reported 20.2% crude oil yield with hexane and *ca*. 18% with SC-CO_2. It appears that the low oil extraction yield with CO_2 reported by Ramsey *et al*. (6) can be attributed to the low extraction temperature. It is apparent that SC-CO_2 at 35°C and 4350 psi does not have enough liquid solvent power to extract rice bran lipids, especially for those glyceride components that have melting points far above 35°C. It is true that SC-CO_2 dissolves solid-state materials, such as naphthalene (8); however, the solubilities below the melting point (around 35°C) were low but increased substantially with temperatures in the region near its melting point (80°C). Friedrich *et al*. (4) and List and Friedrich (5) reported that near-exhaustive extraction of lipid was achieved from oil-bearing seeds by using SC-CO_2 at 70 to 80°C and pressures up to 10,000 psi. Considering these properties of SC-CO_2, three temperatures—70, 80, and 100°C—were selected for the present investigation. The selected extraction pressures were 7000 and 9000 psi. The pressure effect on the RBO extraction yield at the 80°C isotherm is shown in Table 1. Although the yield increment is small with the increase in pressure, it indicates that the density increase of SC-CO_2 on the 80°C isotherm caused the increase in the RBO extraction yield. The density difference of SC-CO_2 between the two pressures on the 80°C isotherm was about 9%, based on SC-CO_2 at 7000 psi.

The temperature effect on the yield at two isobarics at 7000 and 9000 psi was also shown. It is apparent that the lipid extraction yield increased with an increase in temperature of SC-CO_2 under isobaric conditions. The temperature effect was seen more significantly at 9000 psi than at 7000 psi. The raise in temperature was 10°C at 7000 psi and 20°C at 9000 psi. Although the temperature effect at 7000 psi was small, the trend was definite. The temperature effect may be strongly related to the increase in the diffusivity of SC-CO_2. The increase in diffusivity between the two temperatures of SC-CO_2 at the isobarics was estimated to be about 10% [by using the Maxwell-Sutherland equation (9)]. The temperature and pressure effect on the yield can be shown by comparing the yield value with SC-CO_2 at 7000 psi/70°C to that at 9000 psi/100°C. At these two conditions, the density, molar volume of SC-CO_2, and the values for Hildebrand's solubility parameter of SC-CO_2 were estimated to be about equal (11), indicating that the difference in the solubility characteristics of SC-CO_2 was insignificant. As suggested by Giddings *et al*. (12), the value of Hildebrand's solubility parameter for SC-CO_2 is directly propor-

TABLE 1
RBO Extraction Yield, Density, and Molar Volume of Extraction Medium

Extraction medium	T-P conditions (°C)	T-P conditions (psi)	Yield[a] (%)	Density (g/cc)	Molar vol. (cc/g·mol)
SC-CO_2	70	7000	19.0	0.90	48.9
SC-CO_2	80	7000	19.2	0.87	50.7
SC-CO_2	80	9000	19.5	0.93	47.3
SC-CO_2	100	9000	20.4	0.896	49.1
Hexane	69	14.7	20.5	0.65	131.8

[a]Averaged from three measurements (SD < 0.12). RBO, rice bran oil; T-P, temperature-pressure; SC-CO_2, supercritical carbon dioxide.

TABLE 2
RBO Color, Free Fatty Acid (FFA), and Phosphorus Content[a]

Extraction medium (°C/psi)	AOCS color	FFA (%)	Phosphorus (ppm)
SC-CO_2 70/7000	0.4 R/0.6 Y	1.5	250
SC-CO_2 80/7000	0.6 R/1.2 Y	2.4	410
SC-CO_2 80/9000	0.8 R/1.8 Y	2.8	460
SC-CO_2 100/9000	1.0 R/1.8 Y	3.0	—
Hexane	11.0 R/45.0 Y	3.5	1010

[a]For abbreviation see Table 1.

TABLE 3
EI Spectra for TMS Ether Derivatives of RBO Oryzanols

TMS ether derivatives	*m/z*
Campesterol	M + 472,382,367,343,255,145,129,75
Stigmasterol	M + 484,394,357,255,213,159,145,129
β-Sitosterol	M + 486,396,357,255,213,159,129,107,83
Cycloartenol	M + 483,408,393,365,175,159,95,69
24-Methylene-cycloartanol	M + 422,407,379,253,175,159,95,73

[a]EI, electron impact; TMS, trimethylsilyl. For other abbreviation see Table 1.

tional to the density of SC-CO_2. It is therefore rational to consider that the increase in the lipid yield between the two SC-CO_2 points may be due to the increase in diffusivity, which is the dominant physical property in a mass transfer operation such as SC-CO_2 extraction. These two yield points showed that diffusivity of the extracting medium plays a key role in controlling the overall lipid yield. One advantage of SCF extraction is that diffusivity is controllable by selection of pressure and temperature. In general, the diffusion coefficient of an SCF is higher than a liquid extraction agent by two to three orders of magnitude (8).

Color of a refined vegetable oil is closely related to that of crude oil. As shown in Table 3, red values of crude oil produced by SC-CO_2 were far less than that by hexane, regardless of the conditions of SC-CO_2 extraction. The difference in the values for red color was about one order of magnitude. Physical observation indicated that SC-CO_2-extracted crude may not require further refining as far as oil color is concerned. FFA contents are known to affect oil quality. Although the source rice bran before extraction contained FFA, it was clear that there was an appreciable difference in FFA contents between the hexane- and SC-CO_2-extracted crude oil. The amount of FFA content in RBO appeared to follow the pattern of overall extraction yield, which was closely related to the pressure-temperature condition of SC-CO_2. The same trend was observed for phosphorus contents in the RBO crude. In general, SC-CO_2 oil contained far less extracted phosphorus than did hexane-extracted oil. This was also reported in other oilseed extractions with SC-CO_2 (4,5,10).

In Figures 1 through 3, compositional analyses of rice bran crude oils, which render useful information on the solubility characteristics of individual components or groups of lipids in SC-CO_2, are presented. Figure 1 represents the chromatogram of crude oil extracted with hexane, and Figures 2 and 3 those with SC-CO_2. Figure 4 shows gas chromatography–mass spectrometry (GC–MS) spectra of TMS-ethers of γ-oryzanols; β-sitosterol, stigmasterol, campesterol, cycloartenol, and 24-methylene-cycloartanol. In all of these chromatograms (Figs. 1–3), the usual glycerides of vegetable oil, as well as sterol esters and saccharides, were identified. The composition analysis from these chromatograms indicated that most of the RBO from either hexane or SC-CO_2 extraction was made up of glycerides, ranging from 70 to 80%, FFA up to 5%, sterols 1 to 3%, trace amounts of glyco- and phospholipids, and the rest was unidentified wax. The sterols extracted by hexane amounted to *ca.* 1.5%, and about the same amount was obtained by SC-CO_2 at 7000 psi and 80°C (Figs. 2 and 3). However, sterol extraction by SC-CO_2 at 9000 psi and 100°C substantially increased to *ca.* 3%. Area counts of GC peaks were integrated, normalized, and compared with IS.

These composition values for SC-CO_2-extracted RBO were generally in agreement with compositions reported in the literature (7). The wax components are believed to be composed of saturated fatty alcohols and alkanes, with carbon numbers between 24 and 32, as reported by Yoon and Rhee (13). Some of these wax materials are shown in Figure 3 as a group of peaks between 24-methylene-cycloartanol and diglycerides. The lipid components at this location are believed to have carbon numbers between 24 and 32. No attempt was made to identify these individual wax components, which should be investigated further.

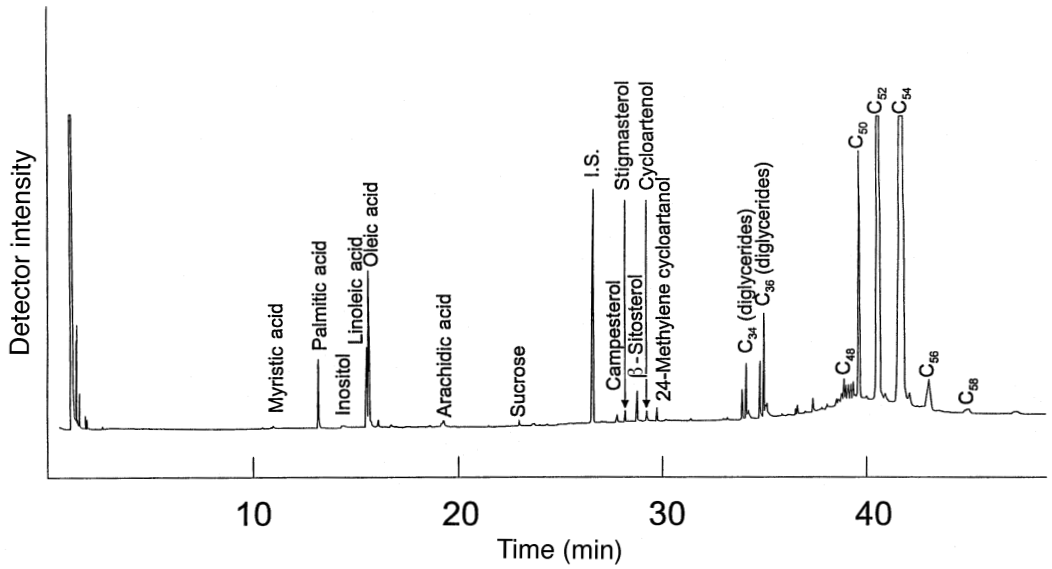

FIG. 1. Gas chromatogram of hexane-extracted rice bran oil (RBO). I.S., internal standard (cholesterol).

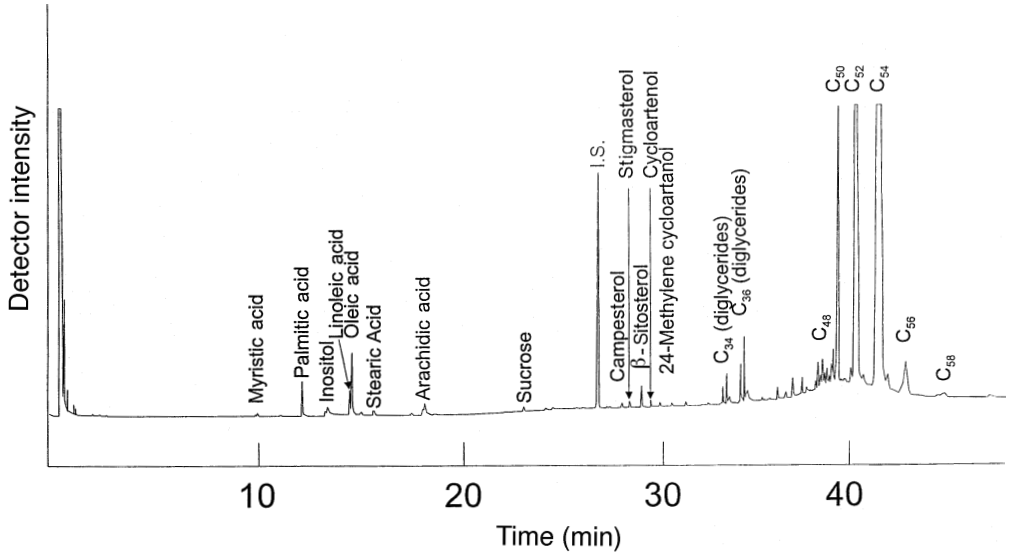

FIG. 2. Gas chromatogram of supercritical carbon dioxide (SC-CO_2)-extracted RBO [trimethylsilyl (TMS)-derivatized]; extraction conditions: 7000 psi and 80°C. For abbreviation see Figure 1.

The amount of individual FFA (palmitic, oleic, and linoleic) extracted by hexane was much greater than those obtained by SC-CO_2 at 7000 psi and 80°C, indicating that hexane has higher affinity to FFA than SC-CO_2 (Figs. 1 and 2).

In Table 3, EI mass spectra for the TMS-ether derivatives of γ-oryzanol extracted by SC-CO_2 are given. The *m/z* values are well supported by the values reported in the literature (14). The chromatogram in Figure 3 and mass spectra in Table 3 indicate that maximum recovery of γ-oryzanol was feasible with SC-CO_2.

With the excellent color quality and the high content of useful γ-oryzanols in SC-CO_2-extracted RBO, one can envision the potential of SCF in producing top-quality RBO, which may have commercial applications in the health-conscious food processing industry.

ACKNOWLEDGMENTS

The authors thank E. Champagne and C. Grimm for providing rice bran and GC–MS analysis, respectively.

REFERENCES

1. Nicolosi, R.J., L.M. Ausman, and D.M. Hegsted, Rice Bran Oil Lowers Serum Total Cholesterol in Non-Human Primates, *Atherosclerosis 88*:133–142 (1991).
2. Kao, G., and B.S. Luh, Chapter 13, *Rice Oil in Rice Utilization,* edited by B.S. Luh, AVI, Westport, 1980, pp. 259–312.
3. *Official Methods and Recommended Practices of the American*

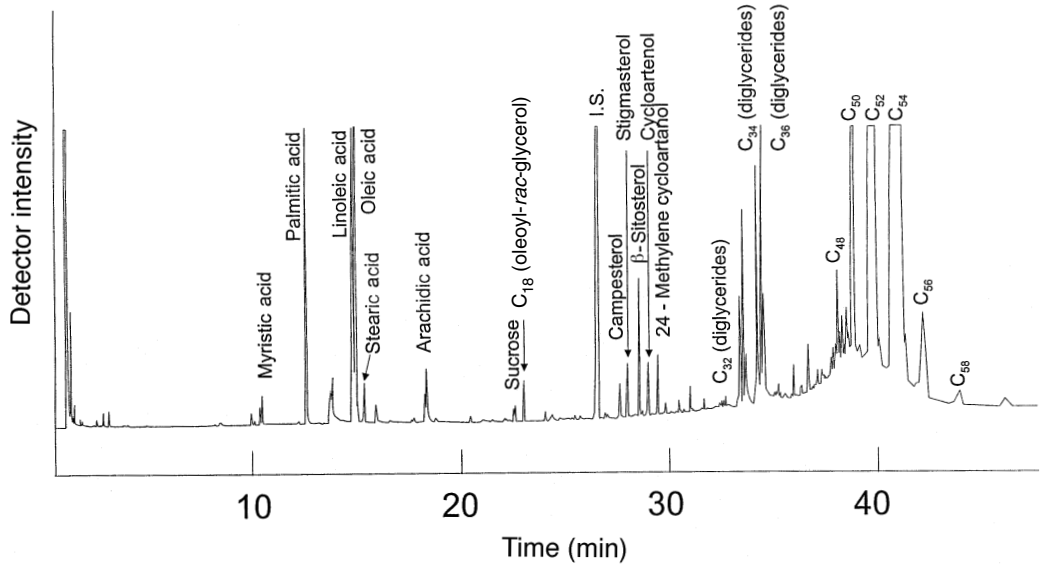

FIG. 3. Gas chromatogram of SC-CO_2-extracted RBO (TMS-derivatized); extraction conditions: 9000 psi and 100°C. For abbreviations see Figures 1 and 2.

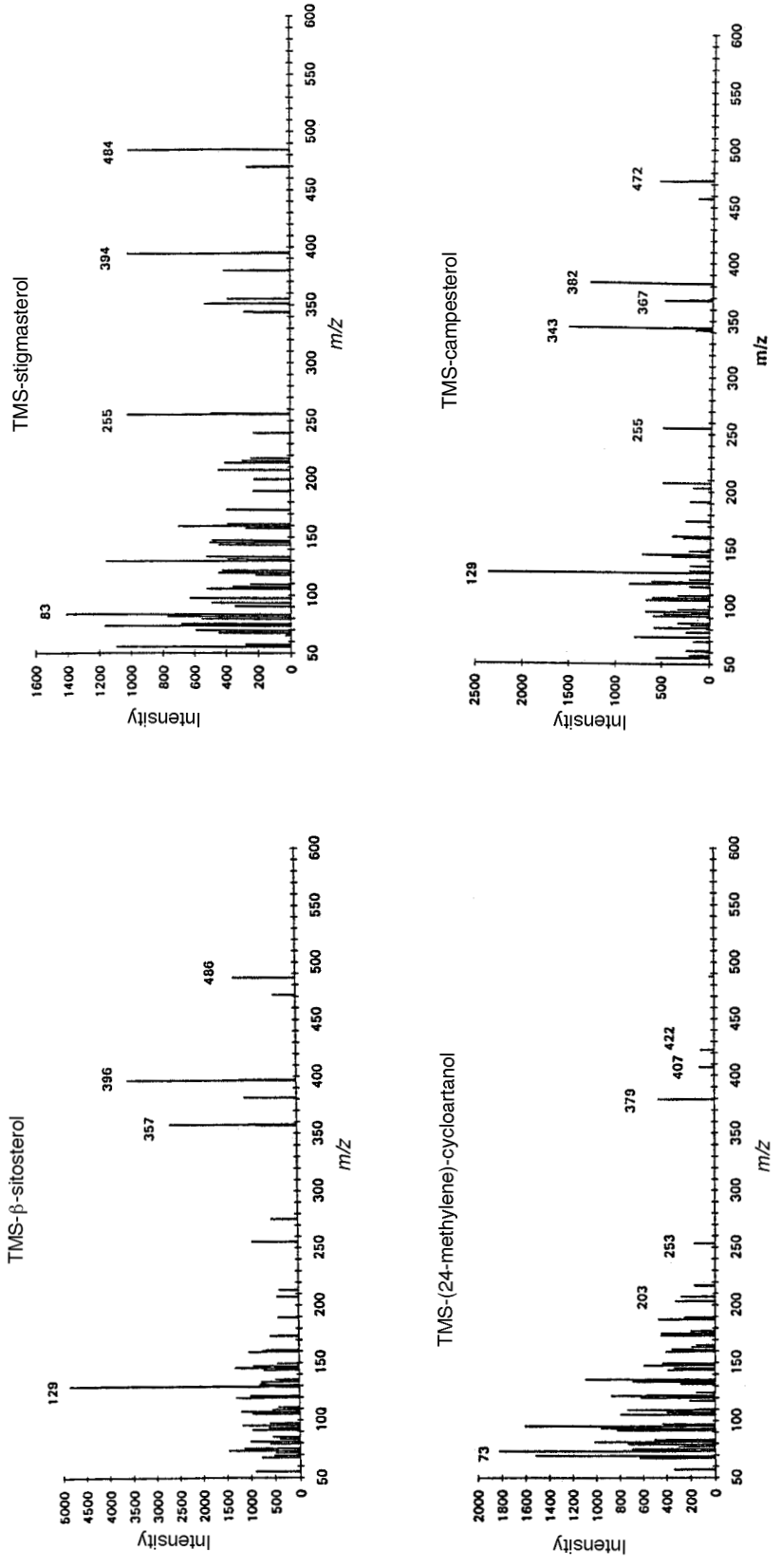

FIG. 4. Electron impact mass spectra for TMS-derivatized RBO oryzanol: TMS ethers of campesterol, stigmasterol, β-sitosterol, cycloartenol, and 24-methylene cycloartenol. For abbreviations see Figures 1 and 2.

Oil Chemists' Society, 4th edn., edited by D. Firestone, American Oil Chemists' Society, Champaign, 1989.
4. Friedrich, J.P., G.R. List, and A.J. Heakin, Petroleum-Free Extraction of Oil from Soybeans with Supercritical Carbon Dioxide, *J. Am. Oil Chem. Soc. 59*:288–292 (1982).
5. List, G.R., and J.P. Friedrich, Processing Characteristics and Oxidative Stability of Soybean Oil Extracted with Supercritical Carbon Dioxide at 50°C and 8000 psi, *Ibid. 65*:82–85 (1985).
6. Ramsey, M.E., J.T. Hsu, R.A. Novak, and W.J. Reightler, Processing Rice Bran by Supercritical Fluid Extraction, *J. Food Technol. 45*:98–104 (1991).
7. Zhao, W., A. Shishikura, K. Fujimoto, K. Ara, and S. Saito, Fractional Extraction of Rice Bran Oil with Supercritical CO_2, *Jpn. Agric. Biol. Chem. 51*:1773–1777 (1987).
8. Paulitis, M.E., M.A. McHugh, and C.P. Chai, Chapter 6, *Solid Solubilities in Supercritical Fluids at Elevated Pressures,* edited by M. Paulitis, J.M.L. Penninger, R.D. Gray, Jr., and P. Davidson, Ann Arbor Science, Ann Arbor, 1983, pp. 139–158.
9. Gilliand, E.R., Diffusion Coefficients in Gaseous Systems, *J. Ind. Eng. Chem. 26*:681–685 (1936).
10. Kuk, M.S., and R.J. Hron, Sr., Supercritical Carbon Dioxide Extraction of Cottonseed with Co-Solvents, *J. Am. Oil Chem. Soc. 71*:1353–1356 (1994).
11. Czubryt, J.J., N.M. Myers, and J.C. Giddings, Solubility Phenomena in Dense Carbon Dioxide Gas in the Range of 290–1900 Atmospheres, *J. Phys. Chem. 74*:4260–4265 (1970).
12. Giddings, J.C., M.N. Myers, L. McLaren, and R.A. Keller, High Pressure Gas Chromatography of Nonvolatile Species, *Science 162*:67–73 (1968).
13. Yoon, H.S., and J.S. Rhee, Composition of Waxes from Crude Rice Bran Oil, *J. Am. Oil Chem. Soc. 59*:561–563 (1982).
14. Rogers, E.J., S.M. Rice, R.J. Nicolosi, D.R. Carpenter, C.A. McClelland, and L.J. Romanczyk, Jr., Identification and Quantification of γ-Oryzanol Components and Simultaneous Assessment of Tocols in Rice Bran Oil, *Ibid. 70*:301–307 (1993).

[Received May 5, 1997; accepted November 13, 1997]