LETTER TO THE EDITOR

Tocopherol, Tocotrienol, and Oryzanol Content of Rice Bran Aqueous Extracts

Sir:

Rice bran, a low-valued co-product obtained from rice processing, represents a potential source of value-added healthy products. Commercial rice bran contains from 11.5 to 17.2% protein, 12.8 to 22.6% fat, 6.2 to 14.4% total fiber, and 8.0 to 17.7% ash depending on the processing conditions (1). Rice bran oil also contains several phenolic compounds as well as vitamin E derivatives that have reported health benefits (2). In our recent paper (3), we reported an aqueous extraction procedure for oil from rice bran that is apparently emulsified. The total solids, protein, fat, and carbohydrate contents of the rice bran extracts obtained at various temperatures were 4.82–6.99, 1.05–1.40, 0.82–1.65, and 2.65–3.36% (w/w, wet basis), respectively. The phenolic content of extracts obtained at various temperatures (20 to 60°C) ranged from 63.28 to 82.51 mg/100 mL aqueous extracts with corresponding antioxidant capacities (Oxygen Radical Absorbance Capacity, ORAC) of 2087 to 3505 μmol Trolox equivalents per 100 mL of rice bran extracts. The relationship between ORAC and total phenolic content was highly significant ($R^2 = 0.87$). The antioxidant capacity of the rice bran aqueous extracts expressed as ORAC equivalents per 100 mL rice bran extracts was similar to that obtained from 2 to 5 servings of fruits and vegetables (4). The composition and functional properties of the extracts showed potential as a food-grade milk analog. Therefore, it was of interest to determine the tocopherol, tocotrienol, and oryzanol contents of the rice bran aqueous extracts, since these are the major antioxidants in rice bran.

Rice bran (100 g) was extracted using 400 mL of deionized water by the method of Monsoor *et al.* (3). The extraction temperatures were 20, 30, 40, 50, and 60°C, respectively. The extracts were separated from bran by filtering through a cheesecloth and followed by centrifugation (CRU 500; IEC, Needham Heights, MA) at 2700 × *g* for 10 min. The rice bran aqueous extracts were lyophilized in a freeze-dry system (Model 77530; Labconco Corporation, Kansas City, MO) for antioxidant analysis.

Total lipid was extracted from 2.0 g freeze-dried rice bran extracts (FDE) using 8.0 mL hexane. The hexane and FDE mixture were vortexed for 4 min, then centrifuged for 10 min at 2700 × *g*. The supernatants were collected and evaporated to dryness under nitrogen. The lipid fraction obtained from FDE was redissolved in 4 mL of acetonitrile and methanol (3:1), then centrifuged, filtered (0.45 μm), and transferred to HPLC autosampler vials.

The lipid fraction of FDE was analyzed for tocopherol, tocotrienol, and oryzanol contents by reversed-phase HPLC by the method of Rogers *et al*. (5). A Waters 2690 HPLC (Alliance, Milford, MA) system equipped with 250 × 4.6 mm C18 column connected to both fluorescence and UV detectors, allowing simultaneous measurements of tocotrienols, tocopherols, and oryzanols, was used. The mobile phase was acetonitrile, methanol, and water (60:35:5), which changed in 1 min to 0% water, 40% methanol, and 60% acetonitrile. The mobile phase then changed linearly to a ratio of 20:80:0 for acetonitrile, methanol, and water, for the next 14 min and was held for 5 min before returning to initial conditions. Total tocotrienols, tocopherols, and oryzanols were quantified by comparing their peak areas with those of standards. Total tocopherol levels in FDE were calculated by the summation of α- and γ-tocopherols. Total tocotrienol levels in FDE were calculated by the summation of their isomers, α, δ, and γ.

Total lipids, tocopherol, tocotrienol, and oryzanol contents of the 100-mL rice bran aqueous extracts at various temperatures are presented in Table 1. The lipid contents of the rice bran aqueous extracts extracted at various temperatures ranged between 0.81 and 1.20% (w/w, wet basis). More lipids were extracted at higher temperatures relative to lower temperatures. This may be due to the greater extractability and solubility of emulsified oils at higher temperatures. Higher temperatures also extracted more antioxidants relative to lower temperatures. The amount of antioxidant extracted appeared to increase with the amount of lipid extracted. More oryzanols (5.66 to 7.59 mg/100 mL extracts) were extracted than tocopherols (0.22 to 0.35 mg/100 mL extracts) and tocotrienols (1.19 to 1.65 mg/100 mL extracts). The oryzanol, tocopherol, and tocotrienol contents of the hexane extracts of 25 g rice bran (used to prepare 100-mL extracts) were 72.50, 2.05, and 6.20 mg, respectively. Extraction temperatures used in this study had no detrimental effects on the antioxidant composition of rice bran emulsified oil. The range of lipid extracted at various temperatures was 22–32%. The extractability (calculated from the ratio of actual amount in the bran and the amount in the aqueous extracts) for tocotrienol (19–27%) was greater than the extractability of oryzanol (8–9%) and tocopherol (10–17%) by aqueous media. This indicates that the solubility of tocotrienol in emulsified oil probably is much greater than oryzanol and tocopherol, since the presence of these lipid-soluble phytochemicals in the extracts depends on the extent of aqueous extraction of emulsified oil from rice bran.

Paper no. J11035 in *JAOCS 82*, 463–464 (June 2005).

TABLE 1

Oryzanol, Tocopherol, and Tocotrienol Contents[a] of Rice Bran Aqueous Extracts (100 mL) at Various Temperatures

Extraction temperatures (°C)	Total lipids (%)	Extractability (%)	Oryzanol (mg/100 mL)	Extractability (%)	Tocopherol (mg/100 mL)	Extractability (%)	Tocotrienol (mg/100 mL)	Extractability (%)
20	0.81 ± 0.09^{c}	22.0	5.66 ± 0.16^{d}	7.8	0.22 ± 0.01^{c}	10.8	1.19 ± 0.02^{d}	19.2
30	0.92 ± 0.04^{c}	24.9	6.22 ± 0.24^{c}	8.6	0.27 ± 0.01^{b}	13.2	1.34 ± 0.04^{c}	21.7
40	0.93 ± 0.12^{b,c}	25.2	6.70 ± 0.21^{b}	9.2	0.30 ± 0.02^{a,b}	14.6	1.49 ± 0.07^{b}	24.0
50	1.09 ± 0.10^{a,b}	29.5	7.38 ± 0.35^{a}	10.2	0.34 ± 0.01^{a}	16.6	1.59 ± 0.03^{a}	25.6
60	1.20 ± 0.06^{a}	32.5	7.59 ± 0.70^{a}	10.5	0.35 ± 0.01^{a}	17.1	1.65 ± 0.07^{a}	26.6
Rice bran (25 g)	3.69 ± 0.16		72.50 ± 1.82		2.05 ± 0.08		6.20 ± 0.42	

[a]Results are expressed as the mean ± SD of the three measurements. Values with different superscripts in each column are significantly ($P < 0.05$) different.

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[Received January 20, 2005; accepted April 27, 2005]

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