Aqueous Extraction, Composition, and Functional Properties of Rice Bran Emulsion

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ABSTRACT: The effect of temperature (20–60°C) on the aqueous extraction of emulsified rice bran oil from commercial rice bran was described. The total solids, protein, fat, and carbohydrate contents of the rice bran emulsions extracted at various temperatures were 4.82–6.99, 1.05–1.40, 0.82–1.65, and 2.65–3.36%, respectively. The mean droplet sizes of the rice bran emulsions extracted at 20, 30, 40, 50, and 60°C were 4.35, 2.92, 3.04, 4.40, and 3.73 μm, respectively. The phenolics extracted at various temperatures ranged from 63.28 to 82.51 mg/100 mL emulsion. The antioxidant capacity (oxygen radical absorbance capacity, ORAC) of rice bran emulsions extracted at various temperatures ranged from 2087 to 3505 μmol Trolox® equivalents per 100 mL of emulsion. The relationship between ORAC and the total phenolic content was highly significant ($R^2 = 0.87$). The shear stress and apparent viscosity of rice bran emulsions in response to shear strain were similar to those of homogenized whole milk. Hence, the composition and functional properties show the potential of aqueous rice bran extracts as food-grade emulsions.

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KEY WORDS: Antioxidant capacity, aqueous extraction, droplet size distribution, ORAC, phenolics, proximate analysis, rice bran, rice bran emulsion.

Rough rice and its processed products, brown rice and milled (white) rice, are the major products of rice. Rice bran, a low-valued co-product obtained from rice processing, represents a potential source of edible and healthful 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 also contains several phenolic compounds as well as vitamin E derivatives that have reported health benefits (2).

There are few documented reports of rice bran functional foods (1). Whole grain rice (milled or brown) has previously been used to prepare rice milk without any organoleptic defects (3). Aqueous extracts of defatted rice bran were found to suppress visceral fat accumulation in rats (4). Rice bran milk and rice bran fiber obtained from rice bran aqueous extracts were used to produce acceptable low-fat, high-fiber bakery products (5).

Food utilization of rice bran is limited because of lipid hydrolysis (FFA formation) and subsequent oxidation (off-flavor development) during storage. Lipase must be inactivated or else lipid must be removed before the rice bran can be used as a food ingredient. Many physical and chemical procedures reportedly inhibit lipase activity in rice bran (6–8). Removal of the oil fraction would also improve rice bran stability by removing the substrate for lipase activity. Extraction with either conventional solvents or nonpolar organic solvents is tedious and time-consuming and requires disposal of the solvent (9). However, there have been no reports on the use of water to remove or extract rice bran oil.

We propose an aqueous extraction technique to remove rice bran oil. The rationale of this approach is that lipase activity occurs on the surface of emulsified oil (10). Since lipase activity in rice bran persists over many days, at least part of the rice bran oil should be emulsified. The emulsified oil probably has a hydrophilic surface to allow emulsion stability and thus long-term lipase activity. If such an emulsified oil does have a hydrophilic surface, it should be extracted by aqueous media. Water washing also may simultaneously extract the oil and water-soluble components and nutrients from the rice bran. The goal of this work was to investigate whether the emulsified oil droplets of rice bran can be water-extracted. The objectives of this research were (i) to investigate whether an emulsion can be obtained by water extraction of rice bran emulsified oil, and (ii) to determine the effect of various extraction temperatures on the chemical composition and functional properties of rice bran emulsions.

MATERIALS AND METHODS

Rice bran samples. Commercial rice bran was obtained from Riceland Foods (Stuttgurt, AR). Broken kernels of rice were separated from the bran using a 16-mesh sieve.

Extraction of rice bran emulsion. Rice bran (100 g) was extracted with 400 mL of deionized water for 5 min by using a mechanical stirrer (Arrow 1750; Arrow Engineering Co. Inc., Hillside, NJ) to stir the rice bran and water mixture at 345 rpm. The extraction temperatures were 20, 30, 40, 50, and 60°C. The extracts were separated from bran by filtering through cheesecloth and centrifuged (CRU 500; IEC, Needham Heights, MA) at $2700 \times g$ for 10 min.

Proximate analysis of rice bran emulsions. The total solids content of the rice bran emulsions was determined by the Food Chemical Codex method (11). Total ash was determined by an AOAC method (12) using an isotemp programmable forced-draft furnace (Fisher Scientific, Fairlawn, NJ). The total

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protein content was determined by total nitrogen determination by combustion (13) in a protein analyzer (C.E. Elantech Inc., Lakewood, NJ) and multiplying the nitrogen values by 5.95. Total fat content of the emulsions was determined by Soxhlet extraction (14) with petroleum ether. The total carbohydrate content of the rice bran emulsions was calculated by difference.

Total phenolics and antioxidant capacity of rice bran emulsions. Total phenolic content of the rice bran emulsion was measured by the Folin–Ciocalteau method (15) with results expressed as mg of gallic acid equivalents per 100 mL of emulsion. In this method the Folin–Ciocalteau reagent (FCR) reacts with phenolic compounds of the rice bran emulsion to form a colored compound that is measured with a spectrophotometer at 725 nm.

The antioxidant capacity of rice bran emulsions was determined by an automated oxygen radical absorbance capacity assay (ORAC) (16), with results expressed as μmol of Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents per 100 mL of emulsion on a Perkin-Elmer HTSoft 7000 Plus Bio-Assay reader (Norwalk, CT). Fluorescein (400 μL) was added to the samples (40 μL) as a target of free radical attack, 2,2-azobis-(2-amidinopropane)dihydrochloride (10 μL) was used as a free radical generator, and Trolox was used as a control standard. The fluorescence reading was taken every 2 min for 70 min, and all fluorescence measurements were expressed relative to the initial reading. The antioxidant capacities were calculated by using the differences of areas under the fluorescein decay curves between the blank and a sample.

Droplet size distribution of rice bran emulsions. The droplet size distribution of the emulsion was measured using a laser light-scattering instrument (LS230; Coulter Corporation, Miami, FL). Before analyzing the sample, the instrument was blanked by measuring the scattering profile from the continuous phase (de-ionized water) in the absence of emulsion droplets. This scattering pattern was subtracted from that of the emulsion to eliminate scattering from background sources other than the droplets. A refractive index ratio (refractive index of oil/refractive index of aqueous phase) of 1.08 was used in the calculations of particle size. The data were presented as a graph of volume distribution vs. particle size (μm).

Color values of rice bran emulsions. Hunter color values of rice bran emulsions extracted at various temperatures were measured by a Minolta Model DP 301 colorimeter (Minolta, Osaka, Japan). Before each measurement the colorimeter was standardized with the reference white plates provided with the equipment.

Rheology of rice bran emulsions. The interfacial rheology of the rice bran aqueous extracts was determined by a Haake VT 550 Rheometer (Haake MessTechnik Gmbh Co., Karlsruhe, Germany) equipped with an MV-DIN sensor. Samples (40 mL) were placed in the cylindrical cup and the experiment was performed at 23°C. A computer-controlled program (Rheowin; Haake) in a rotational mode was used to shear samples. Shear rate and shear stress data were gathered as rheograms. Rheology curves were generated to show shear rate vs. shear stress and shear rate vs. viscosity.

Statistical analysis. Preparation of rice bran emulsions and all the experiments were carried out in triplicate. A one-way ANOVA was conducted on the data using the PROC GLM procedure of SAS (SAS version 8.1, SAS Institute Inc., Cary, NC, USA). Student's *t*-test was used to differentiate mean values, and significance was defined at $P < 0.05$. A linear regression model was developed with total phenolics and ORAC values to establish the relationship between them (correlation coefficient, R^2).

RESULTS AND DISCUSSION

Rice bran emulsions. Emulsions were obtained from rice bran by water extraction at various temperatures. Visual inspection at room temperature showed a stable milky liquid.

Proximate analysis of rice bran emulsions. The mean and SD of total solids, ash, protein, fat, and carbohydrate of rice bran emulsions obtained by water extraction at various temperatures are presented in Table 1. The total solids content of the rice bran emulsion extracted at 60°C was 6.99% compared to 4.82% at 20°C. At higher temperatures more solids were extracted relative to lower temperatures. This trend was similar for all the components extracted by water at various temperatures. The differences in total solids content of rice bran emulsions extracted at 40°C and higher were not significant. The protein, fat, and carbohydrate contents of the rice bran emulsions extracted at various temperatures were 1.05–1.40, 0.82–1.65, and 2.65–3.36%, respectively. The protein, fat, and carbohydrate contents of the emulsions increased by 33 (1.05 to 1.40%), 101

TABLE 1
Proximate Analysis of Rice Bran Emulsions Obtained by Water Extraction at Various Temperatures[a]

Extraction temperatures (°C)	Total solids (%)	Total ash (%)	Total protein (%)	Total fat (%)	Total carbohydrate (%)
20	4.82 ± 0.82^{b}	0.29 ± 0.04^{d}	1.05 ± 0.10^{c}	0.82 ± 0.34^{b}	2.65 ± 0.33^{b}
30	$5.57 \pm 0.97^{a,b}$	$0.33 \pm 0.07^{c,d}$	$1.26 \pm 0.14^{b,c}$	1.07 ± 0.38^{b}	$2.91 \pm 0.40^{a,b}$
40	5.99 ± 0.90^{a}	0.38 ± 0.06^{c}	1.34 ± 0.10^{b}	$1.28 \pm 0.28^{a,b}$	$2.97 \pm 0.46^{a,b}$
50	6.74 ± 0.46^{a}	0.48 ± 0.01^{b}	1.47 ± 0.05^{a}	1.47 ± 0.20^{a}	3.32 ± 0.31^{a}
60	6.99 ± 0.62^{a}	0.57 ± 0.09^{a}	1.40 ± 0.01^{b}	1.65 ± 0.29^{a}	3.36 ± 0.33^{a}

[a]Values (mean ± SD; $n = 3$) with different superscript letters in each column are significantly ($P < 0.05$) different.

(0.82 to 1.65%), and 26% (2.65 to 3.36%), respectively, when the temperature of water extraction was increased from 20 to 60°C. This may be due to the greater extractability and solubility of the components at higher temperatures.

Total phenolics and antioxidant capacity of rice bran emulsions. The mean and SD for measurements of total phenolics and antioxidant capacity of rice bran emulsions obtained by water extraction at various temperatures are presented in Table 2. At higher temperatures more phenolics were extracted from rice bran relative to lower temperatures. The phenolics extracted at temperatures from 20 to 60°C ranged from 63.28 to 82.51 mg/100 mL emulsion, respectively. The corresponding antioxidant capacity (ORAC) varied from 2087 to 3505 μmol Trolox equivalents (TE) per 100 mL of rice bran emulsion, respectively. The relationship between ORAC and total phenolic content was highly significant ($R^2 = 0.87$). The antioxidant capacity, expressed as ORAC equivalents per 100 mL rice bran emulsion, was similar to that obtained from two to five servings of fruits and vegetables (17). Prior and Cao (18) suggested that an increase of 1000 to 2000 ORAC equivalents per day to the average diet would be necessary to realize the beneficial health effects of fruit and vegetable consumption. Hence, rice bran emulsion has potential as an alternative source of phenolics and antioxidants in the daily diet.

Droplet size distribution of rice bran emulsions. The droplet size distribution and the mean droplet size of the rice bran emulsions obtained by water extraction at various temperatures are presented in Figures 1 and 2, respectively. The size of oil droplets in rice bran emulsions extracted at various temperatures was within the range of 0.10 to 20.0 μm (Fig. 1). The differences in mean droplet size of the rice bran emulsion extracted at various temperatures were not statistically significant. The size of the fat globules in native milk emulsion range from less than 0.2 μm to 20 μm (19). Rice bran emulsion extracted at 30 and 40ºC had a narrower range of droplet sizes than rice bran emulsion extracted at 50 and 60°C, probably reflecting an increase in oil droplet coalescence at higher temperatures. In general, rice bran emulsion extracted at higher temperatures had a wider range of droplet sizes than at lower temperatures. The relatively large average particle size (4.35 μm) of emulsion extracted at 20°C may be due to the aggregation of fat globules at lower temperatures.

TABLE 2
Total Phenolics and the Oxygen Radical Absorbance Capacity (ORAC) of Rice Bran Emulsions Obtained by Water Extraction at Various Temperatures

Extraction temperature (°C)	Total phenolics (mg/100 mL emulsion)	ORAC (μM TE/100 mL emulsion)
20	63.28 ± 2.93[c]	2087 ± 94[c]
30	71.06 ± 6.40[b,c]	2898 ± 27[b]
40	73.01 ± 4.03[b]	3318 ± 70[a]
50	79.20 ± 6.70[a,b]	3616 ± 65[a]
60	82.51 ± 2.08[a]	3505 ± 110[a]

[a]Values (mean ± SD; $n = 3$) with different superscripts in each column are significantly ($P < 0.05$) different. TE, Trolox® equivalents.

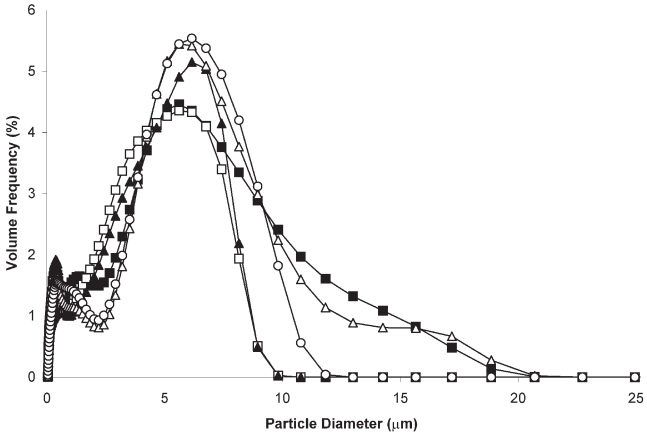

FIG. 1. Droplet size distribution of rice bran emulsions extracted at various temperatures. ■, 20°C; □, 30°C; ▲, 40°C; △, 50°C; ○, 60°C.

The extraction at 30 and 40°C showed relatively smaller particle sizes (2.92 and 3.04 μm, respectively), perhaps because of the lower aggregation and coalescence. At 50 and 60°C, the mean size of the particles was also increased, again probably owing to increased coalescence of the oil droplets. Thus, the mean droplet sizes of rice bran emulsions extracted at various temperatures were comparable to those of raw milk, which range between 2.5 and 4.5 μm (19).

Color values of rice bran emulsions. Mean Hunter color values and their SD for rice bran emulsions extracted at 20, 30, 40, 50, and 60°C are presented in Table 3. There were significant differences in brightness of the rice bran emulsions extracted at various temperatures. Those extracted at higher temperatures were brighter than those at lower temperatures. This may be due to the higher solids contents of the rice bran emulsions at high temperatures. The lower mean Hunter 'a' values show that the rice bran emulsions extracted at higher temperatures were more red. Rice bran emulsions extracted at higher temperatures were more yellow than emulsions extracted at lower temperatures, which is evident by increased Hunter 'b' values. The increase in redness and yellowness in emulsions extracted at higher temperatures was probably due to increased recovery of phenolics (Table 2). The emulsion

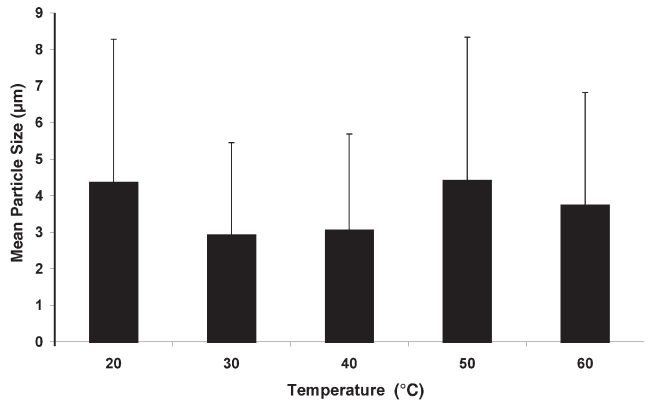

FIG. 2. Mean droplet size of rice bran emulsions extracted at various temperatures. Error bars represent SD. $n = 30$.

TABLE 3
Hunter Color Values (L, a, and b) of the Rice Bran Emulsions Obtained by Water Extraction at Various Temperatures

Extraction temperature (°C)	'L' value	'a' value	'b' value
20	62.13 ± 1.99[c]	–2.47 ± 0.11[a]	5.38 ± 0.18[c]
30	66.03 ± 2.85[b]	–2.37 ± 0.02[a]	7.86 ± 0.56[b]
40	70.86 ± 2.24[a,b]	–1.81 ± 0.03[b]	10.32 ± 0.64[a]
50	73.03 ± 3.93[a]	–1.31 ± 0.17[c]	12.48 ± 1.25[a]
60	74.48 ± 3.88[a]	–1.13 ± 0.26[c]	13.18 ± 2.12[a]

[a]Values with different superscripts in each column are significantly ($P < 0.005$) different ($n = 3$).

with higher brightness, redness, and yellowness would represent more total solids and higher phenolics contents. Emulsions with higher phenolics contents would be more desirable to a consumer.

Rheology of rice bran emulsions. The shear stress and viscosity as a function of shear rate of rice bran emulsions extracted at 20, 30, 40, 50, and 60°C, and of homogenized whole milk are presented in Figures 3 and 4, respectively. All the rice bran emulsions showed very low shear stress (0 to 0.5 Pa) in response to shear rate (Fig. 3). The viscosity of the rice bran emulsions extracted at various temperatures also was very low (0 to 5 cP). Flow behavior of the rice bran emulsions was similar to that of homogenized whole milk (Figs. 3 and 4). The slight differences in rheological properties of rice bran emulsions relative to those of homogenized whole milk may be overcome by homogenizing the rice bran emulsions. This may also improve the stability of the emulsions. The rheology curve showed that temperatures of extraction did not have any effect on the rheology of the rice bran emulsions.

The emulsified oil with high phenolics content and antioxidant capacity was water-extracted from rice bran. The mean diameter of the oil droplets in rice bran emulsions was similar to that of raw milk. Overall rheological properties of the rice bran emulsions were comparable to those of homogenized whole milk. Hence, the composition and functional properties of rice bran aqueous extracts show potential as food-grade emulsions. In addition, the effect of dairy processing techniques such as homogenization and pasteurization on the functional properties of rice bran emulsions should be evaluated.

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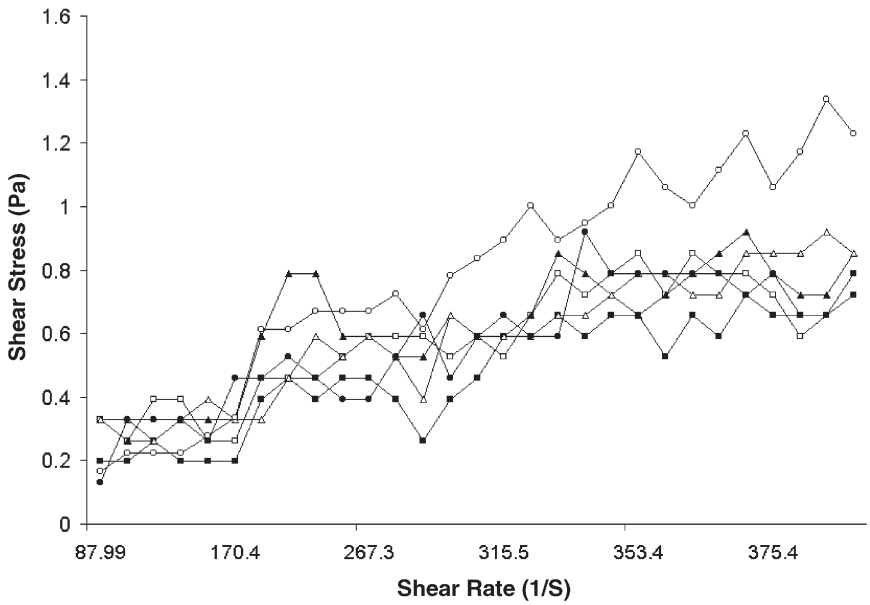

FIG. 3. Changes in shear stress as a function of increasing shear rate (1/S) of homogenized whole milk (○) and of rice bran emulsions extracted at various temperatures (■, 20°C; □, 30°C; ▲, 40°C; △, 50°C; ●, 60°C).

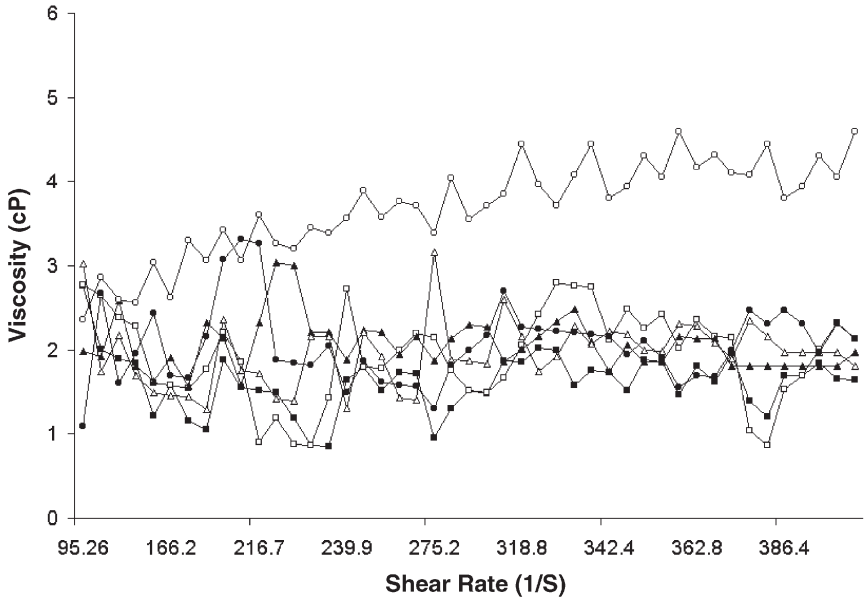

FIG. 4. Changes in viscosity as a function of increasing shear rate (1/S) of homogenized whole milk (○) and rice bran emulsions extracted at various temperatures (■, 20°C; □, 30°C; ▲, 40°C; △, 50°C; ●, 60°C).

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