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Extraction of Soluble Dietary Fibers from Defatted Rice Bran

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ABSTRACT

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The influence of extraction reagents on the properties of soluble dietary fiber was examined in defatted rice bran. The effect of soluble fiber preparations on hypocholesterolemic activity in cholesterol-fed rats was also investigated. The extraction reagents examined were: 1) pH 14 sodium hydroxide, 2) pH 12 calcium hydroxide, 3) pH 11 sodium carbonate, 4) pH 3 acetic acid, and 5) pH 0.5 hydrochloric acid solutions. The starch in the rice bran was previously digested with glucoamylase. The yields of soluble fiber extracted from 100 g of starch-free rice bran with extraction reagents 1-5 were: 8, 5, 2, 2, and 4 g, respectively. Composition, protein contamination, and coloration of the soluble fibers varied considerably with the extraction reagents. Soluble fibers extracted with alkaline solutions had the same arabinose-to-xylose ratio, but those extracted with acidic solutions were different. In the isolation of extract 2, trichloroacetic acid treatment could be omitted because the protein content was quite similar between treated and untreated fibers. Soluble fiber extracted with solution 2 had the least coloration, whereas that extracted with solution 1 had the most. Male Sprague-Dawley rats, aged four weeks, were fed a hypercholesterolemic diet containing 2% of each extracted soluble fiber for nine days. The soluble fibers extracted with solutions 1 and 2 suppressed the elevation of serum cholesterol levels. These results indicate that calcium hydroxide is appropriate for the extraction of soluble fiber from defatted rice bran because it produces the least color, gives a desirable composition and yield, and retains the hypocholesterolemic activity.

Rice, one of the major cereals, is used almost exclusively as a food for humans. Rice contains significant amounts of dietary fiber (Normand et al 1987). Rice bran can be also used as a dietary fiber source when prepared by the stabilization treatment (Randall et al 1985). The hypocholesterolemic effect of rice bran was suggested by several studies in which cholesterol-lowering activity was demonstrated by a number of components in rice bran (Saunders 1990). One of the active components was a water-soluble polysaccharide fraction (Vijayagopalan and Kurup 1972). Mod et al (1978, 1979) isolated and chemically characterized the water- and alkali-soluble hemicelluloses from rice bran. Additionally, we reported that a hemicellulose B preparation isolated from defatted rice bran prevented the elevation of serum cholesterol levels in cholesterol-fed rats (Aoe et al 1989).

The term *hemicellulose* was used to describe the complex mixture of polysaccharides that can be extracted from most plant cell walls with dilute alkali (Aspinall 1959). The mixture was fractionated into hemicellulose A, which precipitated from the extract on neutralization, and hemicellulose B, which precipitated on the addition of ethanol to the neutralized solution. Originally in this procedure, a 4% sodium hydroxide solution was used (Southgate 1976). However, extraction in this solution causes strong browning. Dark or brown color is less advantageous for food uses. Bleaching of dietary fiber products from cereals was also conducted, but it is doubtful that bleached dietary fiber can give the same health benefits as an original dietary fiber (Ranum and DeStefanis 1989). Therefore, it is important to develop another extraction reagent that produces less browning.

This study was designed to examine the effect of five extraction reagents on yield, composition, protein contamination, and coloration of soluble fiber in defatted rice bran for use on a large

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TABLE I
Composition of Soluble Fiber Components Extracted with Five Different Reagents

Reagent	Noncellulosic Polysaccharide (wt %)	Composition[a] (wt %)						
		Ara	Xyl	Man	Gal	Glc	Protein[b]	Uronic Acid
NaOH								
Treated with TCA	66.8	22.0	21.9	nd[c]	3.5	10.2	14.6	9.2
Untreated	47.7	16.6	15.9	nd	2.8	5.8	34.2	6.6
$Ca(OH)_2$								
Treated with TCA	67.0	27.0	26.0	nd	3.3	1.5	17.7	9.2
Untreated	66.6	26.7	25.3	nd	3.1	2.1	21.5	9.4
Na_2CO_3	64.3	23.5	21.1	nd	5.2	2.4	13.6	12.1
CH_3COOH	9.9	1.4	1.5	nd	nd	4.3	56.7	2.7
HCl	58.3	2.5	23.2	1.8	3.8	6.1	16.5	20.9

[a] Ara = arabinose, Xyl = xylose, Man = mannose, Gal = galactose, Glc = glucose.
[b] Calculated as N × 5.95.
[c] Not detected.

TABLE II
Hunter Color Characteristics of 1% Soluble Dietary Fiber Solutions

Extractant	Hunter Color[a]			
	L	$-a$	*b*	Chroma
NaOH	35.99	−1.91	5.33	5.66
$Ca(OH)_2$	23.71	−1.38	1.68	2.18
Na_2CO_3	33.66	−2.73	3.14	4.16
CH_3COOH	31.59	−2.08	−1.86	2.79
HCl	30.77	−2.70	2.27	3.53

[a] *L* values for lightness, $-a$, greenness, *b*, yellowness, and chroma values for the degree of coloration.

commercial scale. The effect of the soluble fiber preparations on serum cholesterol levels in rats was also investigated.

MATERIALS AND METHODS

Samples and Sample Preparation

Rice bran (defatted with *n*-hexane) was supplied by Boso Oil & Fat Co., Chiba, Japan. The defatted rice bran was ground to pass through a 0.59-mm sieve. The components (wt %) of the defatted rice bran were: crude protein (17.5%), fat (3.1%), carbohydrate (56.3%), insoluble fiber (22.7%), soluble fiber (2.8%), and ash (12.3%). The starch in the rice bran was digested with glucoamylase solution (filtered solution of 0.1% enzyme suspension, Nagase Biochemicals Ltd., Osaka, Japan) at pH 4.8 for 24 hr, and the residue was recovered by filtration (milk filter, Azumi Filter Paper Co., Osaka, Japan). The starch-free residue was further washed with water four times and then air-dried.

Extraction of Soluble Fiber

Soluble fibers were extracted by a modification of the procedure of Cartaño and Juliano (1970), and the extraction time and temperature were determined according to Graham et al (1988), who suggested that extraction of soluble fibers with pH 5.0 acetate buffer for 4 hr at 60°C gave significantly higher values than did the other conditions. The starch-free residue (50 g) was blended in a colloid mill with 1 L of reagents and extracted by shaking for 4 hr at 60°C. The reagents were: 2% NaOH (pH 14 at 20°C), 2% $Ca(OH)_2$ (pH 12), 2% Na_2CO_3 (pH 11), 3% CH_3COOH (pH 3), or 4% HCl (pH 0.5). The extract was centrifuged and neutralized with concentrated acetic acid or 5*N* sodium hydroxide. The neutralized supernate was treated with trichloroacetic acid to a final concentration of 7%. After centrifugation, the supernate was dialyzed under running tap water for three days. After dialysis, the extract was stirred while being poured into four volumes of 95% ethanol and stored overnight. The precipitate was collected by centrifugation, redissolved in water, and freeze-dried. White or brown powder was obtained. Another soluble fiber preparation, extracted with NaOH or $Ca(OH)_2$ solution but untreated with trichloroacetic acid, was also prepared to examine the effect of protein contamination in the soluble fiber.

Analysis of Fiber

The polysaccharides were fractionated using the Southgate (1969) procedure, and a noncellulosic polysaccharides fraction was obtained. The hydrolyzed sugars were reduced with sodium borohydride and acetylated with acetic anhydride using the pyridine catalyst method at 100°C for 30 min. The alditol acetates formed were quantified by gas chromatography on a Hitachi model 263-30, fitted with a 1.5% silicone ov-225 column (3 mm × 2 m; nitrogen gas flow 50 ml/min; 200°C). Quantitative estimates of the neutral sugars were made by comparing the peak areas with that of 2-deoxy-D-glucose, an internal standard. The uronic acid content was determined by the carbazole method of Bitter and Muir (1962). D-α-Galacturonic acid (Tokyo Kasei Ind. Co.) was used for reference.

Color Characteristics of Fiber Solutions

Soluble fibers were dissolved in water to a concentration of 1%. The Hunter color characteristics were measured and calculated on a color difference meter (model CD-200, Murakami Color Research Laboratory, Tokyo, Japan). The Hunter color scale indications are: $L = 100$ (white), $L = 0$ (black), $+a$ = red, $-a$ = green, $+b$ = yellow, and $-b$ = blue. The chroma parameter indicates the degree of coloration.

Animal Experiments

Male rats of the Sprague-Dawley strain (four weeks of age) were purchased from Clea Japan Inc, Tokyo, Japan. They were divided randomly into five groups of 10 rats each, placed individually in stainless steel cages and fed commercial chow (CE-2, Clea Japan Inc.) for four days before being fed the test diets. Rats were fed on the cholesterol-enriched test diets for nine days. Diets and tap water were provided ad libitum. Each group was fed one of five test diets similar to the AIN-76 diet (American Institute of Nutrition 1977) except for dietary fiber, cholesterol, and bile acid. Cholesterol (1%), sodium cholate (0.25%), corn oil (1%), and lard (9%) were added to all test diets. A casein protein source was added to adjust N content to 2.7% in all test diets. Sucrose was added to adjust dry matter content. The control diet was supplemented with 4% cellulose. The test diets containing soluble dietary fiber replaced cellulose with 2% of each soluble fiber preparation. The evaluation of the soluble fiber extracted with acetic acid was omitted because the dietary fiber content was quite low. After a 17-hr fast, rats were anesthetized with diethyl ether and blood was drawn from the inferior vena cava. Serum cholesterol levels were determined enzymatically (TC-5, Kyowa Medex Co., Tokyo, Japan).

Statistical Analysis

Group means were compared by one-way analysis of variance. Means were considered to be significantly different ($P < 0.05$) as determined by Dunnett's multiple comparison test (1964).

TABLE III
Effect of Soluble Fiber Preparations on Body Weight, Food Intake, and Food Efficiency Ratio in Rats[a]

Effect	Control	NaOH	$Ca(OH)_2$	Na_2CO_3	HCl
Initial weight, g	124.8 ± 1.8	124.6 ± 1.4	124.6 ± 1.8	124.8 ± 1.6	124.6 ± 1.8
Final weight, g	191.1 ± 5.2	191.4 ± 3.7	191.0 ± 5.3	184.5 ± 3.1	191.3 ± 5.1
Weight gain, g/9 days	66.3 ± 4.0	66.8 ± 2.5	66.4 ± 4.3	59.8 ± 2.0	66.6 ± 3.6
Food intake, g/9 days	135.5 ± 4.3	146.0 ± 4.2	148.6 ± 6.0	137.9 ± 3.5	146.9 ± 6.4
Food efficiency ratio, %[b]	48.7 ± 1.8	48.7 ± 1.8	44.4 ± 1.5	43.3 ± 0.7[c]	48.7 ± 1.8

[a]Values are mean ± SE ($n = 10$).
[b]Weight gain/food intake × 100.
[c]Significantly different from the control group ($P < 0.05$).

TABLE IV
Effect of Soluble Fiber Preparations on Serum Cholesterol Levels in Rats[a]

Preparation	Ingested Cholesterol (mg)	Serum Cholesterol (mg/dl)
Control	1,355 ± 43	261.4 ± 14.6
NaOH	1,460 ± 42	202.4 ± 12.8[b]
$Ca(OH)_2$	1,486 ± 60	204.6 ± 13.0[b]
Na_2CO_3	1,379 ± 35	226.3 ± 18.3
HCl	1,469 ± 64	260.2 ± 21.5

[a]Values are mean ± SE ($n = 10$).
[b]Significantly different from the control group ($P < 0.05$).

RESULTS AND DISCUSSION

The yields of soluble fiber extracted from 100 g of starch-free residue were: NaOH (8 g), $Ca(OH)_2$ (5 g), Na_2CO_3 (2 g), CH_3COOH (2 g), and HCl (4 g). Strong alkali reagent extraction gave the highest soluble fiber yield, while extraction with pH 11 or pH 3 solution gave the lowest. The composition of soluble fiber components extracted with five different reagents is shown in Table I. The yield of noncellulosic polysaccharides was similar in all fiber except the preparation extracted with acetic acid. Soluble fibers could not be extracted with acetic acid under these conditions. The soluble fibers extracted with the alkali solutions were mainly composed of arabinose and xylose. Their arabinose-to-xylose ratio was 1.0:1.0–1.1. The constant arabinose-to-xylose ratio of alkali-soluble fibers indicates that these polysaccharides are arabino-xylans rather than a mixture of arabinans and xylans (Cartaño and Juliano 1970). However, soluble fibers extracted with hydrochloric acid had a lower arabinose-to-xylose ratio of 0.1:1.0. The lower ratio indicates hydrolysis of arabinose residues during extraction.

In the isolation by sodium hydroxide extraction, the protein and noncellulosic polysaccharides contents were different for trichloroacetic acid treatment and nontreatment. On the other hand, the content of the protein and noncellulosic polysaccharides was quite similar for trichloroacetic acid-treated fibers and untreated fibers extracted with calcium hydroxide. In calcium hydroxide extraction, protein in the bran may be barely extracted, or extracted protein may be precipitated and removed during neutralization and centrifugation. Therefore, trichloroacetic acid treatment can be omitted. This is a great advantage for large-scale commercial usage. The color characteristics of soluble fiber solutions are shown in Table II. The coloration of soluble fiber extracted with calcium hydroxide was the lowest, while that extracted with sodium hydroxide was the highest. Sodium hydroxide caused strong browning. Therefore, this extraction is less of an advantage for food uses, in spite of it having the highest yield. The present study shows that soluble fiber extracted with calcium hydroxide would be more acceptable for food products, especially for beverages, due to the lighter coloration.

There were no significant differences in mean body weight gain and food intake between groups fed test diets (Table III). Food efficiency ratio was significantly lower in rats fed a soluble fiber extracted with Na_2CO_3 than it was in rats fed a control diet ($P < 0.05$). The other soluble fibers did not affect the rat growth.

Mean ingested cholesterol and serum cholesterol levels are given in Table IV. Serum cholesterol levels in rats fed the soluble fiber preparations extracted with alkaline solutions were lower than those in rats fed the control diet. The NaOH and $Ca(OH)_2$ groups were significantly different from the control group ($P < 0.05$), but the Na_2CO_3 group was not. The soluble fiber preparation extracted with NaOH or $Ca(OH)_2$ suppressed the elevation of serum cholesterol levels. The soluble fiber preparation extracted with HCl had no hypocholesterolemic effect. The active component might be degraded or lost during the extraction procedure. The present study shows that the hypocholesterolemic activity in the soluble fiber preparations extracted with NaOH and $Ca(OH)_2$ was maintained during the extraction procedure.

These results indicate that calcium hydroxide is appropriate for the extraction of soluble fiber from defatted rice bran because it produces the least color, gives a desirable composition and yield, and retains the hypocholesterolemic activity. Ongoing studies are developing large-scale preparations of rice-bran hemicellulose.

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