

# Evaluation of Enzyme and Chemically Treated Wheat Bran Ingredients in Yeast-Raised Breads

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## ABSTRACT

Cereal Chem. 68(3):295-299

Several wheat-bran-based fiber ingredients that had received chemical or enzyme treatments were tested at 10, 15, or 20% flour replacement levels in a sponge-and-dough formulation (containing 2% added gluten) under commercial baking conditions. Mixing and baking properties were determined. The fiber ingredients ranged in neutral detergent fiber content from 52 to 78% (dry weight basis). A commercial wheat fiber ingredient and white wheat bran were used as controls. The water holding capacity, proximate analysis of the fiber ingredients, and effect of hydrogen peroxide

treatment on the color of the treated bran ingredients were also determined. Ingredients with the highest neutral detergent fiber content were produced using an  $\alpha$ -amylase treatment or a combination of  $\alpha$ -amylase and protease treatments. Protease treatment yielded ingredients with relatively poor baking properties. The breads containing bran treated with either  $\alpha$ -amylase or  $\alpha$ -amylase/calcium oxide had the best crumb grain score of the experimental fiber ingredients evaluated.

The nutritional benefits of bran and dietary fiber in foods has increased the demand for high-fiber food ingredients. The primary difficulties of adding high levels of wheat fiber to foods include poor appearance (ie, reduced loaf volume), poor texture and mouthfeel (ie, poor crumb grain scores), presence of a bitter flavor, and a darker color. Recent inventions describe the production of high-fiber ingredients from wheat bran or whole wheat by the removal of nonfiber fractions using enzymes (Conrad 1981, 1983,) fermentation (Rasco and McBurney 1989), or chemical treatments (Morley and Sharma 1986, Sharma 1986, Holmgren 1988).

Evaluation of modified high-fiber ingredients produced from whole grain or bran and used in breads (Conrad 1983, Rasco et al 1990) and formulated foods (Conrad 1981, Sharma 1986, Fulger and Gum 1987, Rasco 1989, Rasco et al 1989) has received increased attention. A primary objective of the present research was to study whether chemical or enzymatic modification of wheat bran could be used to enhance the functional properties of bran in addition to increasing the total dietary fiber content of the foods to which the bran is added.

## MATERIALS AND METHODS

### Fiber Ingredient Production

**Chemical treatment.** White wheat bran (Fisher Mills, Seattle, WA) was suspended in deionized, distilled water (10% by weight, dwb). The suspension was adjusted to pH 3.5 with either citric acid or hydrochloric acid or to pH 10 with either a suspension of 1M calcium oxide or with 1M sodium hydroxide. For suspensions that were to be treated with ethanol, an aqueous suspension of bran was adjusted to pH 7.0 with sodium hydroxide and then diluted with 95% ethanol such that the final concentration of ethanol in the suspension was 10% (w/v). These samples were incubated at 70°C for 1 hr in a metabolic shaking incubator (60 oscillations per minute).

**Enzyme treatments.** To a 10% (w/v) suspension of white wheat bran in distilled, deionized water, a thermostable  $\alpha$ -amylase (0.05% by weight, Termamyl 120 L, No. 684, AA3015, Novo Laboratories, Inc., Wilton, CT), was added and the suspension was heated with stirring at 90-95°C for 90 min. A solids fraction was recovered by vacuum filtration and transferred to another container. Either distilled, deionized water or 10% ethanol was added, such that the solids content of the second suspension was

approximately 10% by weight. At this point, the pH of the treated suspension was adjusted to either pH 3.5 with 0.1M hydrochloric acid or 1M citric acid or to pH 10 with a suspension of 1M calcium oxide or with 0.1M sodium hydroxide. It was then incubated at 70°C for 1 hr as described above.

For a second enzyme treatment with a protease, a suspension that had received the  $\alpha$ -amylase treatment was filtered, and a solids fraction was recovered by vacuum filtration. The filtrant was rinsed with one volume of water and then suspended in 10 volumes of distilled, deionized water. The pH of this suspension was adjusted to 6.0 with 0.1M sodium hydroxide. Then 0.02% (w/v) of a protease (Neutrase, Novo Laboratories, Inc.) was added, and the suspension was incubated for 1 hr at approximately 40°C in a shaking incubator at 60 oscillations per minute. The solids were recovered by filtration and then rinsed twice with one volume of water each time before being dried.

For certain  $\alpha$ -amylase treatments, the recovered solids were rinsed either once or twice with one volume of water each time. The recovered solids were dried in either a laboratory convection oven at 120°C for 2 hr or with an atmospheric drum dryer (model ALC-4, 6 × 8 in., Blaw-Knox Food and Chemical Equipment Division, Buffalo, NY) at 275-301 kPa (40.4-44.3 psi). Only drum-dried materials were used in the baking tests.

### Bleaching Experiments

Following chemical or enzyme treatment, 50% hydrogen peroxide (Fisher Scientific Co., Pittsburgh, PA, H341-500), 10% by weight, was added to the filter cakes. These treated materials were then dried for 2 hr in a convection oven at 120°C.

### Chemical Analyses

Protein nitrogen (N × 5.7), ash, moisture, and crude lipid were measured using AOAC methods (AOAC 1984) as previously described (Rasco et al 1987). The dietary fiber content (as neutral detergent fiber [NDF]) of each fiber ingredient was measured using the procedure of Dong and Rasco (1987). Bulk density was measured in duplicate for the fiber ingredients by transferring about 5.00 g of each dried product to a 50-ml graduated cylinder, gently tapping the base of the cylinder against a hard surface for 15 sec, and measuring the volume. Water holding capacity was measured by adding about 5.00 g of dried material to a 50-ml conical centrifuge tube, adding 25 ml of distilled, deionized water, and incubating the suspension on a metabolic shaking incubator (90 oscillations per minute) for 30 min at 25°C. The suspension was centrifuged at 3,000 × g for 10 min, and the volume of the liquid imbibed by the solid material was measured.

### Color Measurements

Duplicate analyses for two samples were taken for each fiber ingredient and for bread samples. For the breads, readings were taken from center slices. A HunterLab D25M-9 tristimulus

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colorimeter was used (Hunter Associates Laboratories, Fairfax, VA.) for instrumental color measurements. The unit was standardized with a white tile (C2-11178,  $L = 94.3$ ,  $a = -1.2$ ,  $b = 2.9$ ). Visual lightness and luminous reflectance were measured.

### Commercial Prototype Baking Trials

Bread flour, 60 g (All-Montana flour, Luks Co., Seattle, WA; 12% protein, 14% moisture basis), 10 g of fiber ingredient, 2 g of gluten, 2.5 g of compressed yeast, and 48 g of water were formed into a sponge by mixing for 1 min (model 100-200 A, 100-200 g mixer, National Mfg. Co., Lincoln, NE). These sponges were fermented for 3 hr at 80°F (26.7°C) in a controlled temperature cabinet (Despatch Industries, Inc., Mpls, MN). After fermentation, the following ingredients were added to the sponge: 30 g of flour, 6 g of granulated sugar, 2 g of salt, 2 g of nonfat dry milk, 3 g of hydrogenated vegetable shortening, 40 ppm potassium bromate, 100 ppm ascorbate as ascorbic acid, and water as required for optimal dough formation. These doughs were mixed until optimal development was reached. Dough temperatures after mixing were 77-80°F (25-26.7°C). The doughs were then treated as follows: 10 min of scale time at room temperature (73.4°F, 23°C), 10 min of pan time at room temperature, and a 55- to 58-min proofing period at 112°F (44.4°C) in a humidified cabinet. Breads were baked in a rotary oven at 425°F (218°C) for 17 min. Breads containing higher fiber substitution levels (10, 15, or 20%, w/w, replacement for flour) were prepared in a similar fashion. When fibers replaced flour, the replacement was made on the sponge side of the procedure, and appropriate water changes were also made.

A commercial high-fiber ingredient, Cerelife, used as a control in this study was produced from whole red wheat (Cereteck International, Inc., Bellevue, WA).

Water absorption values were determined by farinograph (AACC Method 54-21, constant flour weight, variable dough weight method) (AACC 1983). Baking absorptions were derived from experimental baking data. Loaf volumes were measured by rapeseed displacement tests. Crumb grain was rated using a

three point scale: S = satisfactory, Q = questionable, and U = unsatisfactory.

## RESULTS AND DISCUSSION

The various chemical treatments used in these experiments resulted in high-fiber materials with differing fiber contents (Table I). The resultant products with the highest fiber contents were those that had received an  $\alpha$ -amylase treatment. Chemical or enzyme treatments in addition to an  $\alpha$ -amylase treatment did not increase the fiber content of the experimental ingredients dramatically over those treated with  $\alpha$ -amylase alone. Of the chemical treatments (without enzyme), incubation with ethanol alone or suspension in citric acid, calcium oxide, or sodium hydroxide followed by incubation in 10% ethanol yielded products with NDF contents higher than those of other chemical treatments.

Removing the soluble and/or suspended solids from the materials treated with  $\alpha$ -amylase by a simple rinsing treatment with distilled, deionized water was as effective at increasing the dietary fiber content of the treated wheat bran ingredients as either an additional chemical treatment or treatment with a proteolytic enzyme.

All of the fiber materials produced in this study had bulk densities lower than that of the white wheat bran from which they were made. The materials that had received ethanol or alkali treatment in addition to  $\alpha$ -amylase treatment had a higher bulk density than those that had received  $\alpha$ -amylase treatment only.

Bleaching the fiber ingredients with hydrogen peroxide before drying led to a significant increase in product lightness ( $L$  value), reduced redness (reduced  $a$  value), and increased yellowness (increased  $b$  value) (Table II). Bleaching treatment had less lightening effect on materials treated with calcium oxide or sodium hydroxide before bleaching. The use of benzoyl peroxide had little effect on lightening these materials (data not given). All bleached fiber ingredients had a definite tan or tannish yellow coloration. In addition, the bleached materials had a noticeable "chemical" off-flavor, which we predicted would reduce their suitability as a food ingredient (data not given). For this reason, these ingredients were not used in the baking experiments.

The proximate analyses for fiber ingredients used in the baking experiments are given in Table III. These products had NDF contents ranging from 62 to 78% (dwb). These particular ingredients were chosen for evaluation because of their high dietary fiber content relative to those of the other experimental fiber ingredients produced for this study.

TABLE I  
Fiber Content of Experimental Fiber Ingredients from Wheat Bran\*

Treatment	Percent NDF <sup>b</sup> (dwb)	Average Yield <sup>c</sup> (%)	Average Bulk Density <sup>c,d</sup> (g/ml)
<b>Chemical</b>			
Distilled water	55.5 ± 4.9	66.7	0.16
Citric acid	55.9 ± 1.0	87.5	0.17
Hydrochloric acid	52.0 ± 2.1	79.4	0.19
Calcium oxide	45.6 ± 1.8	79.2	0.19
Sodium hydroxide	46.4 ± 0.9	79.9	0.16
Ethanol	65.6 ± 9.0	86.7	0.15
Plus citric acid	58.2 ± 3.9	88.4	0.17
Plus calcium oxide	60.0 ± 1.0	81.4	0.18
Plus sodium hydroxide	59.3 ± 3.9	86.9	0.18
<b>Enzyme</b>			
<b>Amylase</b>			
With no rinse	62.5 ± 7.2	56.7	0.12
With one rinse	67.9 ± 2.8	54.6	0.13
With two rinses	78.4 ± 4.1	52.0	0.12
<b>Amylase</b>			
Plus HCl	69.6 ± 4.0	54.8	0.14
Plus citric acid	75.1 ± 7.2	65.1	0.15
Plus calcium oxide	69.6 ± 4.3	61.8	0.18
Plus sodium hydroxide	75.2 ± 3.9	54.4	0.14
Plus ethanol	73.9 ± 1.4	66.5	0.20
Amylase plus protease	74.7 ± 2.0	53.5	0.20
Wheat bran	47.4 ± 2.1	NA <sup>e</sup>	0.22

\*Mean and standard deviation for triplicate analyses for at least three samples of each material.

<sup>b</sup>Neutral detergent fiber (NDF) analyzed by method of Dong and Rasco (1987).

<sup>c</sup>Duplicate samples from two treatments ( $n = 2$ ).

<sup>d</sup>For materials dried in a forced air convection oven for 2 hr at 120°C.

<sup>e</sup>Not applicable.

TABLE II  
Effect of Hydrogen Peroxide on the Tristimulus Color (Lab) Values of Enzyme-Treated or Chemically Treated White Wheat Bran Ingredients\*

Treatment	$L$		$a$		$b$	
	Without H <sub>2</sub> O <sub>2</sub>	With H <sub>2</sub> O <sub>2</sub>	Without H <sub>2</sub> O <sub>2</sub>	With H <sub>2</sub> O <sub>2</sub>	Without H <sub>2</sub> O <sub>2</sub>	With H <sub>2</sub> O <sub>2</sub>
	Distilled water	42	64	10.0	2.5	16.7
Citric acid	45	71	9.7	6.8	17.1	23.0
Hydrochloric acid	42	66	10.5	2.5	15.8	24.2
Calcium oxide	39	57	11.7	2.7	15.3	19.7
Sodium hydroxide	43	64	10.1	1.6	19.0	21.7
Ethanol	42	66	10.3	-0.5	16.5	20.0
Plus citric acid	45	66	10.1	-0.6	16.7	21.1
Plus calcium oxide	45	64	11.1	-0.3	15.6	19.5
Plus sodium hydroxide	44	71	12.3	-2.3	16.0	19.7
Amylase	49	71	11.5	-2.1	19.0	20.8
Plus citric acid	47	56	12.0	6.0	18.2	21.0
Plus hydrochloric acid	51	57	11.5	2.7	18.7	22.7
Plus calcium oxide	48	52	9.7	6.7	14.5	17.7
Plus sodium hydroxide	49	71	12.1	1.0	19.0	23.2
Plus ethanol	50	77	10.7	1.2	17.5	20.9
White wheat bran	67	...	6.5	...	18.5	...

\*Average of duplicate values from samples from two experiments ( $n = 2$ ). Fiber ingredients were dried in a forced air convection oven for 2 hr at 120°C. For other details of sample preparation or analyses, refer to text.

The water holding capacity of the fiber ingredients used in the baking experiments is also given in Table III. The water holding capacity for all of the experimental high-fiber bran ingredients was significantly higher than that for either the commercial wheat fiber ingredient or the white wheat bran, which were used as controls.

The water absorbance by farinograph and by baking tests, the farinograph development times, and the optimal mix times for doughs containing 10–20% treated bran ingredients are given in Table IV. The farinograph values underestimated baking water absorbance by 11–32%. Hydration of bran fiber is slow. During sponge formation, the bran ingredients had 3 hr to become hydrated. This may have been the primary reason for the differ-

ences observed between baking and farinograph absorption values. The optimal mix times for the doughs containing the commercial wheat fiber product or the white wheat bran were shorter than for the treated wheat bran fiber products at the same flour replacement level (Table IV).

Results of the baking tests using 10–20% w/w substitution of the fiber ingredients into a 2% gluten dough are given in Table V. The high-fiber breads with the best crumb grain scores were those containing the commercial wheat fiber ingredient. Although this ingredient was comparable to the others in diluting gluten, its contribution of insoluble dietary fiber to the dough was less than for the treated bran ingredients. The commercial product had approximately 41% NDF (dwb), whereas the treated wheat

**TABLE III**  
Chemical Composition and Water Holding Capacity of Fiber Ingredients (dwb)

Treatment	Composition <sup>a</sup>				Water Holding Capacity <sup>b</sup> (g H <sub>2</sub> O/g material)
	Protein (N × 5.7)	Ash (%)	Lipid (%)	Neutral Detergent Fiber (%)	
Amylase					
With no rinse	17.6 ± 0.2	4.17 ± 0.3	1.89 ± 0.1	62.5 ± 7.2	4.75 ± 0.5
With two rinses	16.0 ± 0.1	3.79 ± 0.2	1.77 ± 0.1	78.4 ± 4.1	5.25 ± 0.5
Plus citric acid	15.5 ± 0.2	3.69 ± 0.5	1.77 ± 0.1	75.1 ± 7.2	4.88 ± 0.7
Plus CaO	16.1 ± 0.2	4.32 ± 0.5	1.52 ± 0.2	69.6 ± 4.3	5.25 ± 0.6
Plus ethanol	16.5 ± 0.3	1.54 ± 0.2	1.85 ± 0.1	73.9 ± 1.4	4.38 ± 0.6
Plus protease	12.6 ± 0.2	4.01 ± 0.1	2.48 ± 0.3	74.7 ± 2.0	4.21 ± 0.4
Commercial product <sup>c</sup>	23.5 ± 0.3	2.50 ± 0.6	1.30 ± 0.1	41.3 ± 1.3	2.10 ± 0.2
White wheat bran	13.9 ± 0.3	6.70 ± 0.01	3.00 ± 0.1	47.4 ± 2.1	3.00 ± 0.1

<sup>a</sup>Data are mean and standard deviation for triplicate analyses of at least three samples from separate lots of product. For preparation or source of fiber ingredients, refer to text.

<sup>b</sup>Mean and standard deviation for duplicate analyses for two samples of materials from separate lots (n = 2).

<sup>c</sup>Cerelife.

**TABLE IV**  
Mixing Properties of Flour Doughs Containing Chemically and Enzymically Treated Wheat Bran<sup>a</sup>

Product	Substitution Level (%)	Farinograph <sup>b</sup>		Baking Tests <sup>c</sup>	
		Absorption (%)	Development Time (min)	Optimal Mix Time (min)	Absorption (%)
Flour control	NA	60	8	2.1	67
Control + 2% gluten	NA	...	...	2.1	...
Amylase-treated					
With no rinse	10	65	19	4.3	79
	15	...	...	6.5	84
	20	70	39	6.7	87
With two rinses	10	67.5	17	4.3	79
	15	...	...	6.1	87
	20	72	42	7.9	92
Plus citric acid	10	68	13.7	4.2	79
	15	...	...	6.2	89
	20	72	17	7.4	98
Plus CaO	10	66	25	4.4	80
	15	...	...	6.1	88
	20	71	61.5	7.5	94
Plus EtOH	10	65	16	4.0	77
	15	...	...	6.6	83
	20	70	44.5	4.6	90
Plus protease	10	67	15.5	3.4	78
	15	...	...	4.0	84
	20	72	17.5	5.5	91
Commercial product <sup>d</sup>	10	60	12	2.9	73
	15	...	...	3.4	71
	20	61	10.5	5.2	74
White wheat bran	10	62	13	2.5	75
	15	...	...	2.7	77
	20	67	7	3.1	79

<sup>a</sup>Mixing properties are average values.

<sup>b</sup>Average values for two samples.

<sup>c</sup>Averages for flour and flour plus gluten controls (n = 8), for doughs containing 10% of the fiber ingredients (n = 4), and for doughs containing 15 or 20% of the fiber ingredient (n = 2).

<sup>d</sup>Cerelife.

brans ranged from 62–78% NDF (dwb). The treated wheat bran ingredients that exhibited the best baking properties were those receiving  $\alpha$ -amylase treatment alone, or  $\alpha$ -amylase treatment followed by calcium oxide treatment. These breads had crumb grain scores of Q–S at the 10% substitution level and Q or Q–S at the 15% substitution level. All of the breads containing the treated bran ingredients at the 20% substitution level had unsatisfactory crumb grain scores.

Removal of essentially all of the aqueous soluble components from the bran ingredients may have been the primary reason why the baking performance of these ingredients was poorer than expected. In addition, some residual enzyme may have been present in a number of the ingredients tested. Although the enzyme-treated ingredients were dried at temperatures that would inactivate the enzymes, and enzyme carryover was expected to be low, it cannot be ruled out. In addition to the presence of residual enzymes, removal of suspended solids by rinsing and filtration may have been the reason why the loaf volume of the first bran treatment ( $\alpha$ -amylase, no rinse) was higher than that of the second bran treatment ( $\alpha$ -amylase, two rinses) (Table V).  $\alpha$ -Amylase can increase bread softness, enhance loaf volume, and improve crumb structure.

Treatment with an acid following the  $\alpha$ -amylase step appeared to be more detrimental to the functionality of the bran ingredient than treatment with a base, at least with regard to crumb grain scores; loaf volume and proof height appeared to be equally affected. It is possible that addition of acid-treated bran led to a small reduction of the dough pH, which resulted in reduced activity of any residual  $\alpha$ -amylase. However, a more likely explanation is that the additional rinsing steps used on the acid- or base-treated bran ingredients removed substantially all of the aqueous soluble components, including any of the residual

enzyme. Treatment with ethanol may have resulted in the loss of polar lipids and other minor components that are important in enhancing gluten development. Ethanol could also have altered the solubility of residual bran protein(s) or polysaccharide(s), reducing the ingredient's wettability and adversely affecting its incorporation into a dough matrix. The alcohol-treated wheat bran had one of the lower water holding capacities (Table V). The poor performance of the wheat bran ingredient treated with amylase plus protease is most likely due to residual protease activity, which leads to gluten damage during fermentation and proofing.

All of the experimental high-fiber breads were significantly darker, redder and yellower than the control (Table VI). Color differences relative to wheat bran at the same substitution levels were more pronounced for the wheat fiber ingredient that had received the protease treatment.

## CONCLUSION

A number of different chemical (acid, base, ethanol) treatments, alone or in combination with enzyme (amylase, protease) treatments, can be used to significantly enhance the dietary fiber content of wheat bran (to 63–78%). However, the mixing and baking properties of these ingredients are adversely affected. Optimal mixing time increased and loaf volume and crumb grain decreased for yeast-raised breads (sponge-and-dough) containing the treated fiber ingredients relative to white wheat bran at the same flour substitution level (10–20% by weight). The treated bran materials with the best baking characteristics were those treated with  $\alpha$ -amylase alone, or with  $\alpha$ -amylase and calcium oxide.

TABLE V  
Baking Parameters of Breads Containing Experimental Fiber Ingredients at 10, 15, or 20% Substitution Levels

Product	Substitution Level (%)	Dough Weight (g)	Loaf Weight (g)	Height at Proof (in.)	Loaf Volume (ml)	Crumb Grain <sup>a</sup>
Flour control <sup>b</sup>	0	176.2 ± 2.2	148.4 ± 2.5	0.96	954 ± 20	S
Control <sup>b</sup> + 2% gluten	0	172.8 ± 6.0	149.7 ± 1.8	0.91	1,003 ± 23	S
Amylase-treated						
With no rinse	10 <sup>c</sup>	188.3	161.4 ± 2.7	0.88	806 ± 51	Q-S
	15 <sup>d</sup>	190.3	163.0	1.2	790	Q
	20 <sup>d</sup>	191.6	162.6	1.0	680	U
With two rinses	10	188.0	160.9 ± 2.5	1.0	843 ± 51	Q-S
	15	193.6	164.6	1.2	775	Q
	20	197.3	171.0	0.81	643	U
Plus citric acid	10	188.8	162.2 ± 1.4	1.1	856 ± 26	Q
	15	194.7	166.7	1.3	828	Q
	20	203.8	174.4	1.3	765	U
Plus CaO	10	189.1	160.6 ± 2.2	1.1	876 ± 13	Q-S
	15	193.6	164.9	1.4	820	Q-S
	20	200.2	171.4	1.1	763	U
Plus EtOH	10	187.5	159.1 ± 3.2	0.75	849 ± 36	Q
	15	190.0	162.5	0.88	773	Q
	20	196.0	167.3	1.1	738	U
Plus protease	10	138.6	160.0 ± 2.1	1.1	885 ± 43	Q
	15	191.5	163.3	1.3	828	Q
	20	196.1	167.6	1.2	708	U
Commercial product <sup>e</sup>	10	183.7	155.3 ± 1.4	1.1	960 ± 48	S
	15	178.5	152.2	1.3	850	S
	20	178.9	152.7	1.3	808	Q-S
White wheat bran	10	184.9	157.6 ± 1.5	1.2	935 ± 13	Q-S
	15	184.2	156.4	1.3	945	Q-S
	20	181.2	157.2	1.3	865	Q

<sup>a</sup>Crumb grain was rated using a three-point scale with S = satisfactory, Q = questionable, and U = unsatisfactory.

<sup>b</sup>Values for flour and flour plus gluten controls are for duplicate values from four baking trials (n = 8).

<sup>c</sup>Values for breads containing the 10% substitution level of fiber ingredients are averages from four loaves from two baking trials (n = 4).

<sup>d</sup>Values for breads containing the 15 or 20% fiber substitution level are for two replicate loaves from one baking trial (n = 2).

<sup>e</sup>Cerelife.

TABLE VI  
Tristimulus Color Values (*L*, *a*, *b*) of High-Fiber Breads Containing 10, 15, or 20% Wheat Bran Fiber Ingredient (w/w) Replacement for Flour

Product	Substitution Level	<i>L</i>	<i>a</i>	<i>b</i>	Color Difference Value vs. Control <sup>a</sup>	Color Differences vs. Wheat Bran <sup>b</sup>		
						Value	Description	
Flour (control)		66.1 ± 1.0	-1.4 ± 0.1	11.3 ± 0.5	...	...	...	
Amylase-treated	With no rinse	10	54.5 ± 2.2	0.5 ± 0.2	14.2 ± 0.7	12.1	2.5	More red, more yellow
		15	49.8 ± 2.7	-2.0 ± 0.2	11.5 ± 0.4	16.3	4.7	Darker, more green, more blue
		20	46.7 ± 3.5	2.3 ± 0.8	14.7 ± 0.9	20.0	5.3	Darker, more red
	With two rinses	10	55.0 ± 1.1	0.2 ± 0.4	13.7 ± 0.3	11.7	1.8	...
		15	48.5 ± 2.2	1.8 ± 0.1	13.3 ± 1.0	18.0	5.7	Darker, more yellow
		20	47.9 ± 2.8	2.0 ± 0.5	14.2 ± 0.6	18.7	4.1	Darker, more red
Plus EtOH	10	55.0 ± 1.0	0.2 ± 0.3	13.3 ± 0.4	11.4	1.7	...	
	15	49.2 ± 1.7	1.6 ± 0.1	13.3 ± 0.6	17.3	5.0	Darker, more yellow	
	20	47.8 ± 1.3	1.9 ± 0.4	14.1 ± 0.1	18.8	4.2	Darker, more red	
Plus CaO	10	52.3 ± 2.1	0.2 ± 0.4	14.0 ± 0.2	14.2	4.4	Darker, more red	
	15	50.0 ± 1.2	1.9 ± 0.3	12.8 ± 0.6	16.5	4.2	Darker, more red	
	20	48.5 ± 1.1	1.8 ± 0.8	14.7 ± 0.5	18.2	3.5	Darker, more red	
Plus citric acid	10	53.6 ± 1.7	0.1 ± 0.2	13.9 ± 0.5	12.9	3.1	More red	
	15	50.0 ± 2.1	1.6 ± 0.1	13.6 ± 0.3	16.6	4.3	Darker, more red	
	20	46.7 ± 2.4	1.7 ± 0.5	14.6 ± 0.6	19.9	5.2	Darker, more yellow	
Plus protease	10	53.9 ± 2.1	0.2 ± 0.2	14.4 ± 0.3	12.7	2.6	More yellow	
	15	48.5 ± 1.0	2.1 ± 0.3	13.7 ± 0.4	18.1	5.8	Darker, more red	
	20	43.9 ± 1.5	2.0 ± 0.4	14.0 ± 0.9	22.6	8.0	Darker, more red	
Commercial product <sup>c</sup>	10	55.3 ± 1.2	0.2 ± 0.1	13.6 ± 0.3	11.1	1.6	...	
	15	49.7 ± 1.7	1.2 ± 0.2	13.2 ± 0.3	16.7	4.5	Darker	
	20	45.9 ± 1.4	2.1 ± 0.3	13.7 ± 0.4	20.6	6.1	Darker, more red, more yellow	
White wheat bran	10	56.5 ± 1.3	-0.8 ± 0.3	13.4 ± 0.6	9.8		...	
	15	54.2 ± 1.2	1.3 ± 0.1	12.9 ± 0.7	12.5		...	
	20	51.8 ± 1.6	0.8 ± 0.4	14.6 ± 0.3	14.8		...	

<sup>a</sup>Color difference values relative to (flour) control that were significantly different ( $P < 0.05$ ) by one-way analysis of variance ( $n = 4$ ).

<sup>b</sup>Color differences relative to white wheat bran at same substitution level that were significantly different ( $P < 0.05$ ) by one-way analysis of variance ( $n = 4$ ).

<sup>c</sup>Cerelife.

#### ACKNOWLEDGMENTS

This research was sponsored in part by the Washington, Oregon, and Idaho Wheat Commissions.

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[Received April 19, 1990. Revision received November 19, 1990. Accepted January 8, 1991.]