

# Protein- and $\beta$ -Glucan Enriched Fractions from High-Protein, High $\beta$ -Glucan Barleys by Sieving and Air Classification

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## ABSTRACT

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A commercial, dehulled barley (Portage), a high-protein hulless barley (CI 4362), and a high-protein, high  $\beta$ -glucan hulless barley (Prowashonupana) were ground, sieved, and air-classified to yield fractions with enriched protein and  $\beta$ -glucan contents. Sieving of Prowashonupana flour gave low protein shift but good  $\beta$ -glucan shift values. Air classification of Portage flour resulted in good protein shift and good  $\beta$ -glucan shift values, whereas air classification of CI 4362 and Prowashonupana flours

yielded low protein shift but high  $\beta$ -glucan shift values. The combined high  $\beta$ -glucan air-classified fractions (3, 4, and 5) had a yield of 62% of the defatted Prowashonupana flour and a  $\beta$ -glucan content of 31%. The combined high-protein air-classified fractions (1 and exhaust bag) had a yield of 28% of the defatted Prowashonupana flour and a protein content of 31%. Grinding and air classification of barley flour can result in fractions with enriched  $\beta$ -glucan and protein contents in good yields.

Multiple Risk Factor Intervention Trial showed that there was a strong positive correlation between the risk of heart disease and blood cholesterol levels (American Heart Association 1986). The National Institutes of Health (1985) concluded that the average American would benefit from a reduction in blood cholesterol concentration, and encouraged food industry to develop and market foods for individuals to accomplish that reduction. Consumption of soluble dietary fiber can lower serum cholesterol in humans (Kritchevsky et al 1984; Anderson and Chen 1979; Judd and Truswell 1985; Chen et al 1981, 1984; Van Horn et al 1986).

Barley contains a higher concentration of the nonstarch polysaccharide (1 $\rightarrow$ 3),(1 $\rightarrow$ 4) mixed linked  $\beta$ -D-glucan ( $\beta$ -glucan) than do other cereal grains, and a large fraction of the  $\beta$ -glucan from barley was soluble (Bhatty 1992). Bhatty (1993) extracted  $\beta$ -glucan from barley bran using a number of aqueous solvents and recovered fractions with enriched  $\beta$ -glucan contents by precipitation.

Bhatty (1992) determined  $\beta$ -glucan content and viscosities of barleys and their dry-milled flour and bran products. Knuckles et al (1992) obtained  $\beta$ -glucan-enriched fractions by dry milling and sieving of barley. Wu and Stringfellow (1986) showed that size separation of corn distillers' products produced fractions with reduced or enhanced insoluble or crude fiber. Tyler et al (1981) air-classified eight grain legumes into starch and protein concentrates. Tyler and Panchuk (1982) studied the effects of seed moisture content on the air classification of field peas and faba beans. Han and Khan (1990) reported air classification of dry edible bean fractions. So far, no reports used air classification to obtain enriched  $\beta$ -glucan fractions. This article describes sieving and air-classification procedures that give enriched  $\beta$ -glucan and protein fractions from a commercial dehulled barley, a hulless high-protein barley, and a hulless high  $\beta$ -glucan, high-protein barley.

## MATERIALS AND METHODS

Three different barleys representing a wide range of protein and  $\beta$ -glucan contents as well as hulled and hulless varieties were used in this study. Prowashonupana, a hulless high-protein, high

$\beta$ -glucan barley was received from ConAgra Grain Processing Companies, Omaha, NE. A hulless high-protein barley (CI 4362) was from Montana State University, Bozeman, MT, and dehulled Portage barley was acquired from Quaker Oats Company, Cedar Rapids, IA.

### Sieving, Defatting

Prowashonupana barley kernel was cracked in an Abbe mill, ground in an Alpine pin mill, and then through a Wiley mill equipped with a 0.5-mm screen to obtain flour. Prowashonupana barley flour (500 g) was screened 5 min in a box sifter with 33 cm<sup>2</sup> silk sieves at 25°C and 30% rh until very little material came through. The sieves used were 50GG, 8XX, 10XX, 13XX, and 17XX with openings of 390, 183, 132, 97, and 64  $\mu$ m, respectively. The <64  $\mu$ m fraction was sieved 20 min with a 20.3-cm diameter screen with openings of 43  $\mu$ m. Prowashonupana flour has 5.7% fat and was difficult to sieve. Therefore, some Prowashonupana flour was defatted twice with hexane with a hexane to flour ratio of 6:1 at room temperature. The mixture was stirred 5 min, three times, with 5-min rest between each stirring. After 15 min of standing, the supernatant was siphoned off, and the defatted flour was air-dried overnight at room temperature. Defatted Prowashonupana flour (0.8% fat) sieved easily. Some small suspended particles were not collected during the siphoning. However, 93.1% defatted flour was recovered on dry basis. When the 4.9% fat removed was added to the defatted flour weight, 98% of the weight was accounted for. When the solid that was lost on container wall and that lost on transfer were added to 98%, the weight loss of small particles is minimal. CI 4362 (1.9% fat) and Portage barleys (0.8% fat) were not defatted, because their fat contents were naturally low.

### Dry Milling, Air Classification

Dehulled Portage barley was ground in an Alpine model 160Z pin mill and air-classified in a Pillsbury laboratory model classifier (Fig. 1). CI 4362 and defatted Prowashonupana flours were each pin-milled once at 9,000 rpm and three times at 14,000 rpm and air classified with 15, 18, 24, and 30  $\mu$ m settings. (Fraction 1 was collected instead of fractions 1A, 1B, and 1C.) Some fine particles are usually occluded in the coarse fraction, so the coarse fraction is passed through the air classifier again with the same setting until the occluded fine particles are practically removed (a change of less than 20 g between successive passes from an initial weight of 2,000 g). Because of time-consuming nature of air classification, no replication was performed.

### Analyses

Protein was calculated using Kjeldahl N  $\times$  6.25 (method 46-13); fat was determined by petroleum ether extraction (method

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30-26); starch was obtained from an enzymatic method using glucoamylase and glucose oxidase (method 76-11);  $\beta$ -glucan was determined from an enzymatic procedure employing lichenase,  $\beta$ -glucosidase, and glucose oxidase-peroxidase (method 32-22); moisture was obtained gravimetrically after drying at 135°C for 2 hr (method 44-19) (AACC 1983). Nitrogen was determined in triplicate and moisture in duplicate. Not all determinations were made in duplicate due to shortage of some samples. The General Linear Models procedure was used for statistical analysis.

## RESULTS AND DISCUSSION

### Sieving

Table I shows the yield and compositions of nondefatted Prowashonupana barley flour after sieving. The <64- $\mu$ m fraction had higher protein, higher starch, and lower  $\beta$ -glucan contents than the starting flour did, and the remaining fractions had lower protein, lower starch and higher  $\beta$ -glucan contents. In general, the larger particle size fraction had lower fat content than did the smallest particle size fraction (Table I).

The yield and compositions of defatted Prowashonupana barley flour are listed in Table II. Defatted Prowashonupana flour with 0.8% fat content sieved easily, and it was possible to separate the <64  $\mu$ m fraction into 43–64  $\mu$ m and <43  $\mu$ m fractions. The <43  $\mu$ m fraction had higher protein, higher starch and lower  $\beta$ -glucan contents than did the starting flour, while the remaining fractions had lower protein, generally lower starch, and higher  $\beta$ -glucan contents. The starch content of Prowashonupana flour was unusually low compared with that of other barley cultivars, because Prowashonupana flour had unusually high protein and  $\beta$ -glucan contents compared with that of normal barley. Because Knuckles et al (1992) already reported the sieving result of CI 4362 and some other common barley flours, Portage and CI 4362 flours sieving were not done here.

### Air Classification

Table III gives the yield and compositions of defatted Prowashonupana barley flour after air classification. The ultrafine fraction collected in the air exhaust bag had higher protein and fat, but lower  $\beta$ -glucan and starch contents than that of the starting flour. Fraction 1 (<15  $\mu$ m) had higher protein, fat, and starch, but lower  $\beta$ -glucan contents than that of the starting flour. Apparently, protein and starch are enriched in the smaller particles (exhaust bag and fraction 1 for protein, fractions 2 and 3 for starch), whereas  $\beta$ -glucan is enriched in larger particles (fractions 3, 4, 5). The combined high  $\beta$ -glucan fractions (3, 4, and 5) had a yield of 62% of the defatted flour and a  $\beta$ -glucan content of

31%. The combined high-protein fractions (exhaust bag and fraction 1) had a yield of 28% and a protein content of 31%.

Yield and compositions of air-classified fractions from dehulled Portage barley with normal  $\beta$ -glucan and protein contents are shown in Table IV. Exhaust bag, and fractions 1A, 1B, 1C, and 5 had higher protein, higher fat, but lower starch contents than that of the dehulled barley.  $\beta$ -Glucan contents of air-classified fractions increased with increasing particle size from exhaust bag to coarse residue. The difference in protein,  $\beta$ -glucan, and starch contents of fractions 1A, 1B, and 1C are not considered significant enough to justify the extra steps involved for air classification of Portage barley flour. Therefore, Prowashonupana flour and CI 4362 barley were each pin-milled once at 9,000 and three times at 14,000 rpm and air-classified to obtain fraction 1 instead of fractions 1A, 1B, and 1C (Fig. 1).

Table V lists the yield and compositions of CI 4362 barley flour after air classification. Fractions 1 and 2 had higher protein and fat than that of the barley.  $\beta$ -Glucan contents of the air-classified fractions 1–5 increased with increasing particle size.

### Protein Shift and $\beta$ -Glucan Shift

Protein shift is equal to the sum of the protein shifted into the high-protein fractions and out of the low-protein fractions as a percentage of the total protein present in the starting flour (Gracia 1959). Likewise, we can define  $\beta$ -glucan shift as the sum of the  $\beta$ -glucan shifted into the high  $\beta$ -glucan fractions and out of the low  $\beta$ -glucan fractions as a percentage of the total  $\beta$ -glucan present in the starting flour. Sieving Prowashonupana flour gave low protein shift and good  $\beta$ -glucan shift values; defatting did not make large changes in protein shift and  $\beta$ -glucan shift values (Table VI). Air classification of defatted Prowashonupana flour gave improved protein shift and  $\beta$ -glucan shift values over those from sieving. Air classification of CI 4362 flour showed low protein shift but good  $\beta$ -glucan shift. Dehulled Portage flour produced good protein shift and good  $\beta$ -glucan shift after air classification.

TABLE I  
Yield and Compositions of Prowashonupana Barley Flour After Sieving

Fraction, $\mu$ m	% Dry Basis				
	Yield	$\beta$ -Glucan	Protein <sup>a</sup>	Fat	Starch
Flour	...	17.4	23.3 b <sup>b</sup>	5.7 c	24.3 b
390–500	5	24.6	20.5 d	4.2 f	23.8 b
183–390	20	23.4 (0.5) <sup>c</sup>	20.6 d	5.0 e	20.7 c
132–183	6	21.2	21.2 c	6.0 b	17.8 d
97–132	11	23.8	20.9 cd	6.0 b	18.9 d
64–97	14	23.5	19.7 e	5.5 d	20.9 c
<64	44	10.7 (0.2)	26.6 a	6.3 a	28.9 a
<43	0	...	...	...	...

<sup>a</sup> N  $\times$  6.25.

<sup>b</sup> Values sharing the same letter in a column are not significantly different ( $P > 0.05$ ).

<sup>c</sup> Values in parenthesis are standard error.

TABLE II  
Yield and Compositions of Defatted Prowashonupana Barley Flour After Sieving

Fraction, $\mu$ m	% Dry Basis				
	Yield	$\beta$ -Glucan	Protein <sup>a</sup>	Fat	Starch
Defatted Flour	...	19.6 (0.6) <sup>b</sup>	22.4 b <sup>c</sup>	0.8	24.8 bc
390–500	5	28.3	19.5 de	0.7	25.6 b
183–390	15	27.1	20.1 c	0.8	21.0 d
97–183	4	26.0	20.0 c	0.9	19.9 d
64–97	88	24.1	19.9 cd	0.9	16.0 e
43–64	53	27.1	19.4 e	0.8	20.2 d
<43	15	7.3 (0.0)	29.1 a	0.8	28.8 a

<sup>a</sup> N  $\times$  6.25.

<sup>b</sup> Values in parenthesis are standard error.

<sup>c</sup> Values sharing the same letter in a column are not significantly different ( $P > 0.05$ ). The only significant difference for fat is between 390–500  $\mu$ m and 97–183  $\mu$ m and between 390–500  $\mu$ m and 64–97  $\mu$ m fractions.

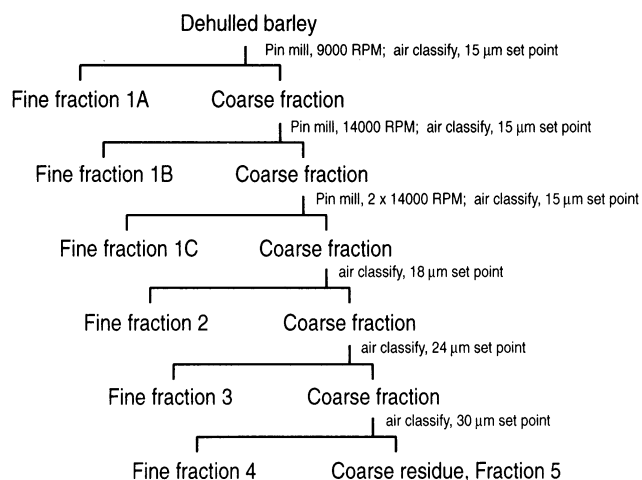


Fig. 1. Flow sheet showing the stepwise air-classification procedure of dehulled Portage barley. CI 4362 and defatted Prowashonupana flours were each pin milled once at 9,000 rpm and three times at 14,000 rpm and air-classified with 15- $\mu$ m setting to collect fraction 1 instead of fractions 1A, 1B, and 1C.

## CONCLUSION

It is possible to enrich protein and  $\beta$ -glucan contents of sieved and air-classified fractions from barley flour. Protein shift was higher for normal-protein dehulled barley than for hullless high-protein or high-protein, high  $\beta$ -glucan barley after air classification.  $\beta$ -Glucan shift was good for high-protein, high  $\beta$ -glucan

barley after sieving;  $\beta$ -glucan shift was even better after air classification. Enriched  $\beta$ -glucan and enriched protein fractions in good yield can be obtained from air classification of high  $\beta$ -glucan, high-protein barley.

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**TABLE III**  
Yield and Compositions of Defatted Prowashonupana Barley Flour After Air Classification

Fraction	Size, $\mu\text{m}$	% Dry Basis				
		Yield	$\beta$ -Glucan	Protein <sup>a</sup>	Fat	Starch
Defatted flour	...	...	19.6 (0.6) <sup>b</sup>	22.4 d <sup>c</sup>	0.8 c	24.8 (0.3)
Exhaust bag	...	6	2.5	47.8 a	3.0 a	19.0
1	<15	22	4.8	26.8 b	1.0 b	42.5
2	15-18	10	9.5	18.1 e	0.8 c	45.1
3	18-24	26	28.5	23.4 c	0.8 c	16.7
4	24-30	5	37.9 (0.2)	17.8 e	0.6 d	12.6
5, coarse	> 30 $\mu\text{m}$	31	31.3 (1.0)	18.6 e	1.0 b	15.7

<sup>a</sup> N  $\times$  6.25.

<sup>b</sup> Values in parenthesis are standard error.

<sup>c</sup> Values sharing the same letter in a column are not significantly different ( $P > 0.05$ ).

**TABLE IV**  
Yield and Compositions of Dehulled Portage Barley After Air Classification

Fraction	Size, $\mu\text{m}$	% Dry Basis				
		Yield	$\beta$ -Glucan	Protein <sup>a</sup>	Fat	Starch
Defatted barley	...	...	5.8	10.9 f <sup>b</sup>	0.48 d	66.2
Exhaust bag	...	1.2	1.5	36.2 a	1.15 b	48.8
1A	<15	4.7	1.8	28.4 b	1.23 b	60.5
1B	<15	8.0	2.2 (0.1) <sup>c</sup>	25.3 c	1.27 ab	58.7
1C	<15	7.0	3.0	23.1 d	1.41 a	57.3
2	15-18	14.3	3.0	9.8 f	0.54 d	72.3
3	18-24	31.4	3.3	4.6 h	0.29 e	74.0
4	24-30	19.3	9.1	6.1 g	0.42 de	68.0
5, coarse residue	>30 $\mu\text{m}$	13.2	14.7 (0.3)	13.4 e	1.00 c	41.0

<sup>a</sup> N  $\times$  6.25.

<sup>b</sup> Values sharing the same letter in a column are not significantly different ( $P > 0.05$ ).

<sup>c</sup> Values in parenthesis are standard error.

**TABLE V**  
Yield and Compositions of CI 4362 Barley Flour After Air Classification

Fraction	Size, $\mu\text{m}$	% Dry Basis				
		Yield	$\beta$ -Glucan	Protein <sup>a</sup>	Fat	Starch
Barley	...	...	8.0	18.6 c <sup>b</sup>	1.9 cd	54.5
1	<15	9.4	1.1	28.5 a	4.0 a	52.6
2	15-18	15.0	1.9	22.8 b	2.9 b	58.1
3	18-24	34.0	5.9	14.4 e	1.5 d	64.6
4	24-30	14.3	8.1	16.1 d	1.7 cd	58.0
5, coarse residue	>30 $\mu\text{m}$	27.3	14.6 (0.6) <sup>c</sup>	18.8 c	2.1 c	63.3

<sup>a</sup> N  $\times$  6.25.

<sup>b</sup> Values sharing the same letter in a column are not significantly different ( $P > 0.05$ ).

<sup>c</sup> Values in parenthesis are standard error.

**TABLE VI**  
Summary of Protein Shift and  $\beta$ -Glucan Shift After Air Classification or Sieving

Barley	% Dry Basis		Air Classification		Sieving	
	Protein	$\beta$ -Glucan	Protein Shift, %	$\beta$ -Glucan Shift, %	Protein Shift, %	$\beta$ -Glucan Shift, %
Portage, dehulled	10.9 (0.1) <sup>a</sup>	5.8	61	64	nd <sup>b</sup>	nd
CI 4362	18.6 (0.1)	8.0	18	51	nd	nd
Prowashonupana, defatted	22.4 (0.1)	19.6 (0.6)	20	58	9	41
Prowashonupana	23.3 (0.1)	17.4	nd	nd	13	36

<sup>a</sup> Values in parenthesis are standard error.

<sup>b</sup> Not determined. High fat for Prowashonupana flour interferes with air classification. Sieving results of CI 4362 and some other common barley flours were already published by Knuckles et al (1992).

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