

# Enzyme-Assisted Wet Separation of Starch from Other Seed Components of Hull-less Barley

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## ABSTRACT

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A multiple enzyme cocktail containing cellulase, *endo*-(1→3),(1→4)- $\beta$ -D-glucanase and xylanase was used in wet separation of starch, protein,  $\beta$ -glucan, bran, and tailings from four hull-less barleys (HB): SB94794 (0% amylose), CDC Candle (5% amylose), CDC Dawn (24% amylose), and SB550831 (40% amylose). Compared to a conventional procedure, the enzyme-assisted wet extraction reduced slurry viscosity by 50–99%, the amount of water and ethanol used in screening and  $\beta$ -glucan precipi-

tation by 30–60%, and screening time by 20–80%. The enzyme-assisted extraction reduced starch contents and yields of tailings and bran fractions, resulting in a 10% increase in average starch extraction efficiency. However,  $\beta$ -glucan yield was reduced in the enzyme-assisted extraction, particularly in high-viscosity HB. The physicochemical properties of isolated starches were not affected by the enzyme-assisted extraction.

Starch is the most abundant single component of barley, accounting for 60–75% on a dry weight basis (Bhatt 1997). Amylose content of barley starches varies from 0 to 45% (Vasanthan and Bhatt 1995, Bhatt and Rosnagel 1997), thus barley starch has a wide range of potential applications in food and industry. Hoseney et al (1971) reported that barley starch could replace wheat starch in breadmaking. Cationized barley starches, used as wet end additives, resulted in superior paper strength when compared to cationic corn starches (Vasanthan et al 1997). Zheng and Sosulski (*in press*) reported that barley starch pastes were more tolerant to refrigerated storage than other cereal (corn, wheat, rice and oat) and root-tuber (potato and tapioca) starches. Waxy barley starches (0 or 5% amylose) had far less syneresis after repeated freeze-thaw treatments than did waxy corn starch (Bhatt and Rosnagel 1997).

Compared to corn or wheat starches, barley starch isolation by wet extraction presents a few difficulties.  $\beta$ -Glucan, a major cell-wall polysaccharide of barley, produces high viscosities in aqueous solutions and impairs separation of starch by screening and subsequent centrifugation (Petersen and Munck 1993). Dry-milling followed by wet-processing was used to overcome the problem associated with  $\beta$ -glucan. Petersen and Munck (1993) reported that flour from disc-milled barley could be used for wet extraction of starch. Vasanthan and Bhatt (1995) described a procedure for pin-milling and air-classification of hull-less barley (HB) to obtain fractions rich in starch, protein, and  $\beta$ -glucan. The starch-rich fraction, containing 77–78% starch, was subjected to a short wet-processing procedure to obtain pure starch of uniform granule size for cationization. Starch extraction efficiencies from three cultivars of HB were 64–68% in the conventional procedure as compared to 84–88% in the dry and wet extraction of the starch-rich fractions. However, the yields of the starch-rich fractions were only 44–63% (average 51%) of ground barley, thus the final starch yields from this dry- and wet-processing was 29–43% (average 34%), which is similar to that of the conventional wet procedure. A pilot-plant trial using the dry and short wet extraction gave  $\approx$ 30% starch yield from Condor HB (Vasanthan et al 1997).

Enzymes are frequently used to assist starch separation in wet-milling systems. In wheat starch processing, utilization of xylanase reduced slurry viscosity and improved starch-gluten separation and yields when compared to the conventional procedure

(Christophersen et al 1997). Similar results were obtained in pilot-plant experiments with hemicellulases (Weegels et al 1992). Incorporating multiple enzymes in steeping solutions reduced steeping time and enhanced starch separation during wet-milling of maize (Steinke and Johnson 1991). Padmanabhan et al (1993) reported significant improvement of starch recovery from cassava by using pectinase and cellulase. Although barley produces high slurry viscosity, due to its high  $\beta$ -glucan content, it has not been used for starch isolation with enzyme-assisted extraction. The objectives of this study were to evaluate the effect of an enzyme-assisted extraction on starch extraction efficiencies from HB having different starch and  $\beta$ -glucan contents, and on starch separation from other components such as protein,  $\beta$ -glucan, and bran.

## MATERIALS AND METHODS

### Materials

Hull-less barleys, CDC Dawn, CDC Candle, SB 94794, and SB 550831, all from the 1996 crop, were grown at the Kernen Crop Research Farm, University of Saskatchewan, Saskatoon, SK. The barleys were ground into meals in a cyclone sample mill (Udy Corp., Fort Collins, CO) to pass a 0.5-mm screen. The multienzyme cocktail (Roxazyme-G) derived from *Trichoderma viride*, primarily containing cellulase, *endo*-(1→3),(1→4)- $\beta$ -D-glucanase, and xylanase, was obtained from Hoffmann-La Roche Ltd. (Basle, Switzerland). The enzyme complex had a bulk density of 0.6 g/mL and a carboxymethyl cellulose-degrading activity of 8,000 U/g.

### Enzyme-Assisted Wet Separation of Starch and Other Components

A preliminary experiment showed that Roxazyme-G, at 20 mg/100 g of HB meal, was sufficient to reduce slurry viscosity for separation of coarse materials by screening through a 270-mesh (53- $\mu$ m) sieve. Barley meals (100 g, dwb) were suspended in 300 mL of distilled water containing 0 (conventional) or 20 mg of Roxazyme-G (enzyme) and homogenized in a commercial Waring blender for 2 min at high speed. The suspension was agitated at room temperature for an additional 30 min with an overhead stirrer, and its viscosity was determined with a viscometer (model LHV, Brookfield). The slurry was then screened through a 270-mesh (53- $\mu$ m) metal sieve in a rotating-vibratory sieve shaker. Distilled water was added during sieving, and total volume of water use was recorded for each sample. The coarse or bran fraction after sieving was collected and freeze-dried. The extract (pH 5.8–6.1) containing starch, protein, and  $\beta$ -glucan was adjusted to pH 11.5 with 1M NaOH, stirred at room temperature for 15 min, and then centrifuged at 3,000  $\times$  g for 5 min. After pouring the supernatant, the tailing layer on top of the starch sediment was collected, the starch layer was resuspended in water and recentri-

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fused as described above, with the supernatant and tailing layers collected again. This procedure was repeated one more time. The tailings fraction collected after each centrifugation was freeze-dried for calculation of its percent yield and chemical analysis. The pooled starch was suspended in 95% ethanol, filtered through Whatman #114 filter paper, and dried overnight at room temperature. The supernatants after each centrifugation were combined, and protein was precipitated at pH 4.5 (Vasanthan and Bhatti 1995), centrifuged at  $3,000 \times g$  for 10 min and freeze-dried.  $\beta$ -Glucan was precipitated with 50% ethanol from the supernatant after protein removal and recovered by freeze-drying. The amount of ethanol (95%) used for  $\beta$ -glucan precipitation was recorded. Extraction efficiency for starch, protein, and  $\beta$ -glucan was calculated as:

$$\% \text{ Extraction efficiency} = \% \text{ yield} \times \% \text{ component} / \% \text{ component in meal} \times 100$$

### Chemical Analyses

For chemical analysis, the bran and tailings fractions were ground in a commercial coffee mill and passed through a 100-mesh (150- $\mu$ m) sieve. Approved Methods were applied for deter-

minations of moisture (Method 44-15A), ash (Method 08-12), lipids (Method 30-25), and total nitrogen (Method 46-11A) (AACC 1995). Starch was determined according to the procedure described by Holm et al (1986). The mixed-linkage  $\beta$ -glucan assay kit (Megazyme Intl., County Wicklow, Ireland) was used for  $\beta$ -glucan determination (Method 32-23). Amylose content of the starches was determined by the method of Gibson et al (1997). Acid extract viscosity was measured using a digital viscometer at 20°C according to Bhatti et al (1991).

All extractions were repeated twice for each HB and chemical determinations were performed in duplicate for each sample. Data were subjected to analysis of variance using Minitab statistical software (Minitab, Inc., State College, PA).

## RESULTS AND DISCUSSION

### Barleys

On dry basis, HB contained 58–69% starch, 14–15% protein (N  $\times$  6.25), 4–8%  $\beta$ -glucan, 3–4% lipids, and 2% ash (Table I). Amylose content was 0% for SB94794, 4.8% for CDC Candle, 24.4% for CDC Dawn, and 40.3% for SB550831. Lines SB94794 and SB550831 had higher  $\beta$ -glucan and lipids than did CDC Candle and CDC Dawn. Acid-extract viscosity (AEV) was high for CDC Candle and SB94794 as compared to CDC Dawn and SB550831. SB550831 showed low AEV, although it had the highest  $\beta$ -glucan (Table I), suggesting a large variability in acid buffer soluble  $\beta$ -glucan in HB.

### Processing Efficiency and Fraction Yield

CDC Candle and SB94794 produced extremely high slurry viscosities at room temperature in conventional wet extraction (Table II). Compared to CDC Candle and SB94794, the high amylose line SB550831 and CDC Dawn had very low slurry viscosities. Slurry viscosities were significantly ( $R^2 = 0.996$ ) correlated with AEV, suggesting that acid-soluble  $\beta$ -glucan was largely responsible for slurry viscosity. In the conventional wet extraction, 8–18 volumes of water were used for screening, and 770–1,900 mL of ethanol were needed to precipitate  $\beta$ -glucan (Table II). Adding 20 mg of the enzyme cocktail to 100 g of HB meals reduced slurry viscosity by 99% in CDC Candle, 98% in SB94794, 75% in SB550831, and 50% in CDC Dawn (Table II). The volume of water and ethanol use was reduced by 30% (SB550831, CDC Dawn) to 60% (CDC Candle) in the enzyme-assisted extraction when compared to conventional extraction. As a result of reduced slurry viscosity and water use, screening time was reduced by 82% for CDC Candle, 60% for SB94794, 50% for CDC Dawn, and 20% for SB550831 in the enzyme-assisted extraction when compared to the conventional extraction (Table II). Reduced water use and processing time may lead to increased extraction capacity, resulting in improvement of processing efficiency in wet starch extraction (Christophersen et al 1997).

TABLE I

Composition and Acid Extraction Viscosity (AEV) of Hull-less Barleys

Barley	Composition (% dwb)						AEV(cps)
	Starch	Amylose	Protein	$\beta$ -Glucan	Lipids	Ash	
CDC Candle	68.5	4.8	14.4	6.67	3.16	2.31	634
CDC Dawn	67.9	24.4	15.0	4.37	2.76	1.96	8
SB94794	63.6	0.0	15.3	7.26	3.19	2.10	487
SB550831	58.0	40.3	14.7	8.28	3.80	2.28	28
LSD <sup>a</sup>	4.6	0.8	0.1	0.33	0.08	0.07	10
LSD <sup>b</sup>	7.6	1.2	0.2	0.55	0.13	0.12	17

<sup>a</sup> Least significant difference ( $P < 0.05$ ).

<sup>b</sup> Least significant difference ( $P < 0.01$ ).

TABLE II

Slurry Viscosity, Screening Time, and Water and Ethanol Use in Conventional (C) and Enzyme-Assisted (EA) Wet Extraction

Barley	Treatment	Slurry Viscosity (cps)	Screening Time (min)	Water Use (mL)	Ethanol Use (mL)
CDC Candle	C	44,500	17	1,800	1,900
CDC Candle	EA	440	3	750	770
CDC Dawn	C	470	4	750	770
CDC Dawn	EA	250	2	550	560
SB94794	C	37,000	15	1,700	1,830
SB94794	EA	680	6	780	810
SB550831	C	3,520	10	1,100	1,170
SB550831	EA	880	8	800	830
LSD <sup>a</sup>		136	1.6	20	22

<sup>a</sup> Least significant difference ( $P < 0.01$ ).

TABLE III

Fraction Yield and Extraction Efficiency from Ground Barleys by Conventional (C) and Enzyme-Assisted (EA) Wet Extraction

Barley	Treatment	Fraction Yield (% dwb)						Extraction Efficiency (%)		
		Starch	Protein	$\beta$ -Glucan	Bran	Tailings	Total	Starch	Protein	$\beta$ -Glucan
CDC Candle	C	43.3	11.2	4.6	16.9	16.8	92.8	62.0	58.6	46.3
CDC Candle	EA	52.6	10.8	3.4	14.0	11.9	92.7	75.0	57.8	37.6
CDC Dawn	C	53.7	8.4	3.7	19.1	8.2	93.1	77.7	45.3	56.7
CDC Dawn	EA	53.9	8.2	4.2	18.9	8.6	93.8	78.2	44.2	64.6
SB94794	C	43.7	11.3	5.7	16.7	14.7	92.1	67.3	55.8	48.6
SB94794	EA	48.7	11.8	2.8	15.1	13.5	91.9	75.1	57.4	25.9
SB550831	C	42.1	10.6	4.7	23.3	12.1	92.8	70.8	54.3	38.0
SB550831	EA	44.1	11.0	5.1	23.2	11.2	94.6	74.4	54.3	40.6
LSD <sup>a</sup>		1.6	0.8	0.7	1.4	1.0	1.8	1.7	1.5	2.6
LSD <sup>b</sup>		2.3	1.3	1.1	2.2	1.6	2.9	2.7	2.4	4.2

<sup>a</sup> Least significant difference ( $P < 0.05$ ).

<sup>b</sup> Least significant difference ( $P < 0.01$ ).

Starch yield in the conventional extraction varied from 42% (SB550831) to 54% (CDC Dawn), giving 62% (CDC Candle) to 78% (CDC Dawn) extraction efficiencies (Table III). Low starch yield of SB550831 was due to its low initial starch content (Table I). High slurry viscosities of CDC Candle and SB94794 made screening very difficult, resulting in low yields and starch extraction efficiencies. Compared to the conventional extraction, starch yield in the enzyme-assisted extraction was 21, 11, and 5% higher in CDC Candle, SB94794, and SB550831, respectively. Enzyme-assisted extraction increased starch extraction efficiency by 22 and 11% in CDC Candle and SB94794, respectively (Table III). For CDC Dawn, neither starch yield nor starch extraction efficiency showed significant differences between conventional and enzyme-assisted extractions. Average starch extraction efficiency from the HB excluding CDC Dawn, increased from 67% in the conventional extraction to 75% in the enzyme-assisted extraction.

$\beta$ -Glucan yield in the conventional extraction was 4.6–5.7%, giving 38–57% extraction efficiency (Table III). Low  $\beta$ -glucan yield from CDC Dawn was probably due to its low initial  $\beta$ -glucan content (Table I). For CDC Candle and SB94794, the enzyme-assisted extraction reduced  $\beta$ -glucan yield and extraction efficiency by 26–51% and 9–23%, respectively. For SB550831 and CDC Dawn,  $\beta$ -glucan yield and extraction efficiency increased in the enzyme-assisted extraction by 9–56% and 3–8%, respectively.

Protein yield was only 8% for CDC Dawn, with an extraction efficiency of  $\approx$ 44% as compared to  $\approx$ 11% yield and 54–59% extraction efficiency for other barleys (Table III). Extraction method seemed to have little effect on protein extraction.

In the conventional extraction, the yield of bran fraction varied from 17% in CDC Candle and SB94794 to 23% in SB550831 (Table III). Extraction method did not significantly affect bran fraction yield in CDC Dawn and SB550831. However, the enzyme-assisted extraction reduced the yield of bran fraction by 10 and 17% in SB94794 and CDC Candle, respectively. The enzyme-assisted extraction reduced the yield of tailings fraction by 29, 8, and 7% for CDC Candle, SB94794, and SB550831, respectively (Table III). The reduction in the yields of bran and tailings fractions by the enzyme-assisted extraction was mainly due to reduced

starch contamination in these fractions (Table IV), which in turn resulted in improvements in starch yield and extraction efficiencies (Table III).

### Composition of the Fractions

The starch fraction contained 98% starch and <0.6% protein (Table IV), which approached the composition generally reported for commercially produced corn starch. Enzyme-assisted extraction tended to have lower protein content than the conventional extraction. Compared to the normal and high-amylose barleys, starch extracted from CDC Candle and SB94794 had higher  $\beta$ -glucan. The starch fraction was very low in free lipids (0–0.01%).

The protein fraction contained 73–81% protein ( $N \times 6.25$ ), qualifying as a protein concentrate (Lusas and Rhee 1995). This fraction contained  $\approx$ 4–8% starch, a level which was reduced in the enzyme-assisted extraction (Table IV). The protein fraction was rich in lipids, accounting for 40–52% of the total lipid in HB. During wet extraction, the lipids formed a layer on the surface after centrifuging the alkaline slurry. This layer was apparently concentrated into the protein fraction after acid precipitation and centrifugation. Lipid contents in protein fractions isolated by the enzyme-assisted extraction were somewhat higher than in those prepared by the conventional extraction.

The  $\beta$ -glucan fraction contained 62–74%  $\beta$ -glucan, 5–12% starch, 6–10% protein, and 0.1–0.3% lipids (Table IV). Although extraction method had little effect on the chemical composition of the fraction,  $\beta$ -glucan obtained by the enzyme-assisted extraction gave low viscosity, indicating its breakdown.

Starch, at 29–42% and 54–75%, respectively, was the primary component of the bran and tailings fractions (Table IV). Enzyme-assisted extraction reduced starch content, particularly in the tailings fraction. Christophersen et al (1997), using xylanase, reported significant reductions of slurry viscosity and tailings fraction in wheat starch extraction. As the enzyme-assisted extraction generally reduced starch content and yields of bran and the tailings fractions (Tables III and IV), it resulted in higher starch yields and extraction efficiencies than the conventional extraction (Table III), particularly in high-viscosity HB (CDC Candle and SB94794).

TABLE IV  
Composition (% dwb) of Barley Fractions by Conventional (C) or Enzyme-Assisted (EA) Wet Extraction

Barley Fractions	Starch		Protein		$\beta$ -Glucan		Lipids	
	C	EA	C	EA	C	EA	C	EA
Starch fraction								
CDC Candle	98.1	97.7	0.6	0.6	0.09	0.09	0.01	0.01
CDC Dawn	98.2	98.5	0.4	0.3	0.03	0.02	0.01	0.01
SB94794	98.0	98.1	0.5	0.3 <sup>a</sup>	0.17	0.22	0	0
SB550831	97.5	97.9	0.5	0.4	0.02	0.02	0	0
Protein fraction								
CDC Candle	7.1	6.7	75.3	77.1	2.1	3.0	12.6	13.3
CDC Dawn	4.2	4.0	80.8	80.9	0.3	0.6	13.2	14.7
SB94794	7.7	4.5 <sup>a</sup>	75.5	74.4	1.2	1.8	14.2	13.6
SB550831	5.7	4.1	75.3	72.5	0.5	1.4 <sup>a</sup>	16.5	17.8
$\beta$ -Glucan fraction								
CDC Candle	7.6	5.8	5.7	5.9	67.2	73.8	0.2	0.2
CDC Dawn	6.7	6.3	6.9	7.3	67.0	67.2	0.1	0.1
SB94794	12.3	6.4 <sup>a</sup>	7.4	8.3	61.9	67.2	0.2	0.2
SB550831	5.6	5.4	9.4	9.9	67.0	65.9	0.3	0.3
Bran fraction								
CDC Candle	35.1	30.1 <sup>a</sup>	16.3	16.6	12.9	10.7	4.8	4.4
CDC Dawn	42.2	40.4	17.6	18.0	10.1	8.9	3.1	3.2
SB94794	31.4	29.3	14.7	16.0	17.1	17.6	3.5	4.1
SB550831	33.5	33.2	14.5	14.9	16.9	17.3	3.2	3.3
Tailings fraction								
CDC Candle	68.7	59.2 <sup>a</sup>	9.6	11.1	4.6	9.8 <sup>a</sup>	3.7	3.1
CDC Dawn	75.2	72.1	6.0	4.6	2.7	2.6	1.6	1.8
SB94794	64.3	54.0 <sup>a</sup>	7.3	11.5 <sup>a</sup>	5.6	9.5 <sup>a</sup>	3.8	3.4
SB550831	69.1	66.8	5.9	5.5	3.6	4.1	3.6	2.6

<sup>a</sup> Significantly different from conventional extraction by *t*-test,  $P < 0.05$ . No significant differences were found between conventional and enzyme-assisted extractions for the other determinations.

Protein,  $\beta$ -glucan, and lipid contents of the bran fraction showed little differences between the extraction methods (Table IV). However, the enzyme-assisted extraction increased percent of protein and  $\beta$ -glucan contents in tailings fractions of CDC Candle and SB94794. This was mainly due to their reduced starch contents. Lipid content in tailings fractions was not affected by the extraction method.

The extraction procedure had little effect on physicochemical properties of isolated starches as shown by scanning electron microscopy, particle-size distribution, pasting (amylograph), and gelatinization experiments (data not shown).

### CONCLUSION

Compared to dry and wet starch extractions reported by Vasanthan and Bhatti (1995), the enzyme-assisted wet extraction yielded  $\approx 47\%$  (50 vs. 34% starch yield) more starch. This extraction largely destroyed  $\beta$ -glucan, which reduced slurry viscosity, resulting in significant improvement in processing efficiency for wet separation of starch from other seed components of HB. There may be a need to evaluate the enzyme-assisted extraction for individual barleys. Incorporating the enzyme cocktail at a low enzyme-to-meal ratio without additional heating may benefit industrial starch extraction from barley.

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