

Distribution of β -Glucan in the Grain of Hull-less Barley

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ABSTRACT

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Nine hull-less barley (HB) containing waxy (0–7% amylose), normal (\approx 25% amylose), or high amylose (\approx 42% amylose) starch with normal or fractured granule make-up and 4–9% (1 \rightarrow 3)(1 \rightarrow 4)- β -D-glucans (β -glucan) were pearled to remove 70% of the original grain weight in 10% intervals. The pearled fractions were analyzed for β -glucan distribution within HB grain. Protein content of the pearled fractions indicated that the three outermost fractions contained pericarp and testa, aleurone, and subaleurone tissues, respectively. For all HB, β -glucan and acid-extract viscosity were very low in the outermost 20% of the kernel. For low β -glucan HB, β -glucan content was the greatest in the subaleurone region

and declined slightly toward inner layers. For high β -glucan HB, however, more than 80% of grain β -glucan was distributed more evenly throughout the endosperm. Acid extract viscosity was significantly ($P < 0.01$) correlated with total ($r = 0.75$) and soluble ($r = 0.87$) β -glucan content throughout the kernel of all HB. Growing conditions, location and year, had significant effects on the concentration of protein, starch and β -glucan. However, protein, starch, and β -glucan distribution patterns were not affected by growing conditions. The difference in β -glucan distribution between low and high β -glucan HB may explain the difference in milling performance of HB with low or high β -glucan.

Quantitative measurement of β -glucan distribution in barley has been difficult because of the difficulty of separating bran from endosperm cells. High affinities of Calcofluor and Congo Red with β -glucan have led to the use of fluorescence microscopy to study cell walls of oats and barley (Wood and Fulcher 1978). Using this technique, Miller and Fulcher (1994) reported that β -glucan in barley was more uniformly distributed throughout the endosperm as compared with oats where high concentration of β -glucan was found in the subaleurone, particularly in low β -glucan oats. However, Oscarsson et al (1997) showed considerable variation in β -glucan distribution among barley genotypes. In their study, cell walls on the ventral side of the kernel and central endosperm in the vicinity of the crease in high β -glucan barley were more heavily stained with Calcofluor than those from low β -glucan barley. Bhatt et al (1997) also showed that the Calcofluor stained cell walls were obvious in the subaleurone region in low β -glucan HB but extended deeply into the endosperm of high β -glucan HB. These results indicated that β -glucan distribution in barley may vary according to β -glucan content. Nevertheless, no quantitative data on β -glucan distribution in HB is available at present.

Pearling, a dry abrasive milling method that removes almost any proportion of outer coverings that can be collected, may be employed for the analysis of component distribution in cereal grains. Gohl et al (1977, 1978) determined the distributions of protein, lipid, ash, and carbohydrates in barley at early or late stages of maturity by collecting layers successively removed by pearling. To our knowledge, no studies have been done on the quantitative distribution of β -glucan and its relationship to viscosity and milling performance of HB. The objective of this study is to quantitate β -glucan distribution in HB with different β -glucan content and starch make-up, and grown in different years and locations.

MATERIALS AND METHODS

HB Samples and Pearling

Two registered HB cultivars, CDC Dawn and CDC Candle, and seven HB genotypes, HB340 (formerly SB94794), SB94022, SB94917, SR93139, SB90354, CDC 92-55-08-31, and CDC 92-

55-06-48, all 2-rowed, were grown in 1997 at the Kernen Crop Research Farm, University of Saskatchewan, Saskatoon, SK. CDC Dawn from the 1995 crop and CDC Candle from the 1996 crop, both grown at Kernen Crop Research Farm, and CDC Candle, CDC 92-55-08-64 and SR93139 from the 1997 crop grown at two different locations (Lashburn and Melfort, Saskatchewan) were used to study the effect of growing season and location. HB grain (200.0 g, as-is moisture basis) was pearled at low speed using a testing mill (model TM05, Satake, Tokyo, Japan) fitted with 36P abrasive roller and 1.0-mm screen. Pearled fines were collected at 10% intervals by successively abrading HB kernels to 90, 80, 70, 60, 50, 40, and 30% of their original weight. For simplicity, the pearled fines were designated as fractions I–VII and the residual kernels were designated as fraction VIII. The time required to achieve the desired percentage of abrasion was recorded for each HB. Fractions I–VII were directly used for chemical analysis without further processing, whereas fraction VIII and whole grain HB were ground in a cyclone mill (Udy Corp., Fort Collins, CO) to pass a 0.5-mm screen before chemical analysis.

Analyses

Acid-extract viscosity (AEV) was measured at a sample to solvent ratio of 1:20 according to Bhatt et al (1991). Aliquots of the acid extract was used for the determination of acid-soluble β -glucan by Calcofluor flow injection (Astrup and Jorgensen 1988). The mixed-linkage β -glucan assay kit (Megazyme Intl., County Wicklow, Ireland) was used for the determination of total β -glucan (Approved Method 32-23, AACC 1995) in wholemeal and pearled fractions. Amylose concentration was determined according to Gibson et al (1997) by using the Megazyme amylose-amylopectin assay kit. Starch was determined by the procedure described by Holm et al (1986). Moisture and protein ($N \times 6.25$) were determined by Approved Methods 44-15A and 46-13 (AACC 1995), respectively. The color of pearled fractions was measured with HunterLab MiniScan XE (model 45/0-L, Hunter Associates Laboratory, Inc., Reston, VA), using HunterLab color scales with D65 daylight illuminant and the 1931 2° standard observer. All analyses were performed in duplicate and data were subjected to the analysis of variance using Minitab statistical software (Minitab, Inc., State College, PA).

RESULTS AND DISCUSSION

HB Grains and Their Pearling Characteristics

The chemical composition and AEV of the HB wholemeal samples are shown in Table I. Starch content varied from 58 to 67% and protein from 14 to 18% on dry weight basis. A negative correlation ($r = -0.71$, $P < 0.05$) was detected between starch and

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TABLE I
Chemical Composition (% dwb) and Acid-Extract Viscosity (AEV, cps) of Wholemeal and Pearled Fractions of Hull-less Barley

Genotype and Fraction	Starch	Protein	β-Glucan		AEV	Genotype and Fraction	Starch	Protein	β-Glucan		AEV
			Total	Acid-Soluble					Total	Acid-Soluble	
HB340^a						SR93139 (continued)					
Wholemeal	62.1	14.9	7.03	3.18	23.0	Fraction V	69.9	14.3	10.26	6.76	771.5
Fraction I	10.0	22.2	2.00	1.40	7.7	Fraction VI	71.4	12.0	10.30	7.21	773.5
Fraction II	35.4	26.2	5.78	4.04	25.6	Fraction VII	74.6	10.0	9.70	6.48	260.0
Fraction III	54.3	21.8	7.69	5.38	68.8	Fraction VIII	73.6	6.8	9.33	3.41	32.5
Fraction IV	64.0	17.8	8.59	6.01	155.0	LSD	2.5	0.4	0.31	0.41	6.9
Fraction V	73.2	13.5	8.95	6.27	127.5	CDC Dawn					
Fraction VI	76.6	9.4	8.38	6.39	77.4	Wholemeal	67.4	14.1	4.31	2.14	12.3
Fraction VII	78.6	7.3	7.93	6.38	65.6	Fraction I	10.5	21.2	2.41	0.97	13.9
Fraction VIII	76.9	6.0	7.00	3.85	16.5	Fraction II	36.9	22.9	4.41	2.62	17.9
LSD ^b	2.5	0.3	0.34	0.43	5.8	Fraction III	52.7	18.9	5.04	2.68	29.6
SB94022						Fraction IV	60.8	15.8	5.01	3.78	33.3
Wholemeal	58.4	17.8	8.10	3.81	64.0	Fraction V	70.2	14.2	4.92	3.83	38.2
Fraction I	8.8	23.9	2.86	2.00	9.2	Fraction VI	76.4	10.2	4.66	3.87	29.7
Fraction II	26.5	28.8	6.45	4.51	35.2	Fraction VII	79.1	8.3	4.38	3.66	37.9
Fraction III	44.9	25.0	8.51	5.95	108.9	Fraction VIII	81.6	5.6	4.11	1.83	12.0
Fraction IV	54.2	21.2	8.82	6.17	159.0	LSD	1.9	0.3	0.25	0.40	3.5
Fraction V	61.4	16.9	9.16	6.42	188.0	SB90354					
Fraction VI	68.1	13.2	9.49	6.64	229.5	Wholemeal	62.6	15.3	6.51	3.40	25.5
Fraction VII	70.6	10.8	9.44	6.60	155.5	Fraction I	10.1	23.5	3.95	2.28	12.3
Fraction VIII	74.0	7.6	8.78	5.26	38.0	Fraction II	34.7	26.0	7.29	4.55	42.5
LSD	1.9	0.3	0.28	0.51	6.3	Fraction III	51.5	21.7	8.05	5.52	113.0
CDC Candle						Fraction IV	63.6	18.3	8.16	6.45	151.0
Wholemeal	61.4	14.9	7.45	3.69	28.7	Fraction V	70.1	13.6	7.79	5.42	149.5
Fraction I	7.9	22.2	2.27	1.59	6.2	Fraction VI	72.5	10.5	7.21	5.14	107.0
Fraction II	33.8	24.8	5.47	3.98	20.5	Fraction VII	76.6	8.7	6.96	5.66	70.2
Fraction III	51.0	21.2	6.87	5.69	56.5	Fraction VIII	77.6	5.7	5.23	2.14	21.5
Fraction IV	67.6	17.9	7.64	5.72	114.0	LSD	2.2	0.3	0.34	0.47	4.8
Fraction V	73.9	13.4	8.01	6.14	132.5	92-55-08-31					
Fraction VI	75.0	10.5	7.96	6.08	103.1	Wholemeal	58.3	18.1	7.75	1.96	14.3
Fraction VII	78.2	8.3	7.94	5.42	72.7	Fraction I	7.3	22.1	2.56	1.17	10.2
Fraction VIII	78.5	5.9	7.62	3.81	30.0	Fraction II	23.2	26.6	5.96	2.60	12.8
LSD	2.1	0.4	0.30	0.45	3.9	Fraction III	40.0	24.4	7.94	3.39	31.3
SB94917						Fraction IV	57.8	22.0	9.23	5.29	46.1
Wholemeal	61.6	15.4	8.08	2.20	13.8	Fraction V	66.6	18.3	9.56	4.12	57.5
Fraction I	6.3	21.2	2.76	1.37	12.8	Fraction VI	72.7	14.9	9.16	3.73	36.7
Fraction II	24.6	25.4	7.43	3.66	23.6	Fraction VII	74.3	11.9	8.99	3.58	35.2
Fraction III	48.5	22.5	9.45	6.04	79.8	Fraction VIII	77.7	8.2	8.01	2.06	12.3
Fraction IV	62.6	18.7	9.96	6.57	85.8	LSD	2.1	0.2	0.23	0.38	3.3
Fraction V	69.1	13.8	10.18	6.22	84.8	92-55-06-48					
Fraction VI	71.5	10.8	9.73	5.37	52.8	Wholemeal	60.8	13.7	8.85	3.32	28.2
Fraction VII	75.0	9.2	9.04	5.10	29.2	Fraction I	9.8	18.8	2.35	1.31	11.3
Fraction VIII	77.8	6.3	7.39	1.57	10.5	Fraction II	32.0	22.1	6.21	3.60	21.0
LSD	2.2	0.3	0.29	0.46	3.3	Fraction III	51.7	19.4	7.79	4.97	84.3
SR93139						Fraction IV	64.8	14.9	8.66	4.68	179.0
Wholemeal	60.3	15.5	8.91	3.88	71.8	Fraction V	71.2	13.1	9.73	6.07	298.0
Fraction I	10.2	22.3	3.41	2.30	11.8	Fraction VI	74.4	9.4	10.26	6.08	295.0
Fraction II	37.6	23.2	7.74	5.11	96.2	Fraction VII	75.4	8.1	10.57	6.10	236.0
Fraction III	56.4	21.1	9.43	6.30	371.0	Fraction VIII	72.2	6.3	10.64	5.00	40.0
Fraction IV	65.1	18.4	10.05	6.49	639.5	LSD	2.5	0.3	0.33	0.54	5.9

^a Formerly SB94794.

^b Least significant difference ($P < 0.05$).

TABLE II
Cumulative Pearling Time (min) for the Removal of 70% Original Kernel Weight of Hull-less Barley

Barley	HB340	SB90422	CDC Candle	SB94917	SR93139	CDC Dawn	SB90354	CDC 92550831	CDC 92550648
Time	50.2	52.8	56.3	102.3	61.2	43.8	59.5	57.2	87.3

protein concentrations. This was in agreement with previous findings where a significant negative relationship between starch and protein was reported in a variety of HB genotypes (Bhatty 1999) or for Condor HB grown at different locations (Edney et al 1992). Amylose concentration (dry starch basis) was 0% for HB340, 1.0% for SB94022, 4.5% for CDC Candle, 5.0% for SB94917, 7.0% for SR93139, 25.4% for CDC Dawn, 24.6% for SB90354, 36.4% for CDC 92-55-08-31, and 41.7% for CDC 92-55-06-48. In agreement with previous reports (Bhatty 1997, 1999), waxy and high amylose HB contained more β-glucan than regular starch HB. AEV was high for two waxy HB (SB94022 and SR93139), but low for CDC Dawn and SB94917. Log-transformed AEV was

positively correlated ($r = 0.90$, $P < 0.001$) with acid-soluble β-glucan concentration. This was in agreement with Bhatty et al (1991).

The cumulative pearling time required to remove 70% of original kernel weight varied from 44 to 102 min (Table II). SB94917, a waxy genotype with fractured starch granules, required the longest time, while CDC Dawn required the shortest time to achieve the same degree of pearling. Pearling time was reported to correlate with HB grain hardness (Bhatty and Rosnagel 1998).

Whiteness (L value) of HB wholemeal had a range of 85–89 (Fig. 1). L value of pearled fractions increased progressively from fractions I to III; accordingly, the variability of L value decreased. The trend in L value suggested that the color of HB was largely

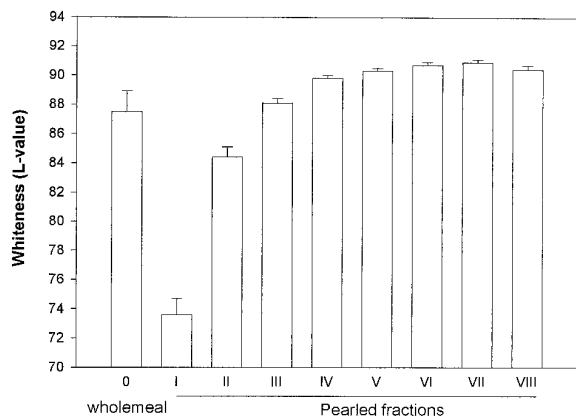


Fig. 1. Whiteness of wholemeal and pearled fractions of hull-less barley samples. Average *L* values and standard deviations were obtained from nine hull-less barley genotypes. Vertical bars indicate standard variations.

determined by the outermost 30% of the kernel. Fractions IV–VII were of similar whiteness; the somewhat lower *L* value of fraction VIII was probably a reflection of the coarser particles resulting from the cyclone mill as compared with pearling. Bhatti (1997) determined, in a microscopy study of sequentially pearled Condor HB, that the outermost 30% of the kernel constituted the true bran of HB (pericarp and testa, aleurone, and subaleurone layers). These tissues are known to contain phenolic compounds (Cochrane and Duffus 1983) which would have contributed to the lower *L* values of the outermost fractions.

Protein and Starch Concentration in Pearled Fractions

For all HB, protein concentration was high in fractions I–III, with the highest levels being detected in fraction II. Protein then declined almost linearly toward the center of HB grain (Table I). In contrast, starch concentration was low for the first three fractions, averaging 9% for fraction I, 32% for fraction II, and 50% for fraction III, respectively (Table I). Average starch concentration increased to 62% in fraction IV and 70–78% in fractions V–VIII. A significant negative correlation ($r = -0.81$, $P < 0.01$) was detected between starch and protein in fractions I–VIII. In cereals, including barley, bran is characterized by high protein and low starch concentrations. Among bran tissues, the aleurone layer is known to be high in protein. The high protein concentration of fraction II suggested that it contained the aleurone layer, whereas fractions I and III contained pericarp and testa and subaleurone, respectively. This was in agreement with Bhatti (1997), who reported that pearling HB to 90, 80, and 70% of its original weight removed pericarp and testa, aleurone, and subaleurone layers, respectively. The relatively high starch concentration in fractions III and IV was indicative of endosperm contamination due to pearling. The protein concentration in pearled fractions, after complete removal of the bran, showed a progressive linear decrease toward the kernel center for all HB, indicating that protein in the endosperm of HB was not evenly distributed but rather decreased from outer layers toward central core of the kernel. Similar protein distribution patterns were reported in feed barley harvested at early or late stages of maturity (Gohl et al 1977, 1978) and in fluorescence microscopy studies of hulled and hull-less barley (Bhatti 1997, Oscarsson et al 1997). Although pearling is not concentric and the seed-ends tend to be removed more quickly than the body, protein and starch distribution data suggest that sequential pearling is useful for the study of component distribution within the kernel of HB.

β -Glucan Distribution and AEV

β -Glucan concentration was only 2–4% in fraction I for all HB (Table I), indicative of low β -glucan in pericarp and testa of HB.

Fraction II contained, on average, twofold β -glucan compared with fraction I, which reflected a higher β -glucan concentration in the aleurone layer of HB than in the pericarp and testa. This agrees with microscopy studies (Bhatti et al 1991, Oscarsson et al 1997) where thicker cell walls stained with Calcofluor were observed in the aleurone layer. Bacic and Stone (1981a,b) also reported β -glucan being the second, after arabinoxylan, most abundant carbohydrate in aleurone cell walls of mature barley grain. β -Glucan distribution in fractions III–VIII showed different patterns according to grain β -glucan. The normal starch and low β -glucan HB, CDC Dawn and SB90354, showed the highest β -glucan concentration in fractions III–IV followed by a gradual decrease in subsequent fractions.

In contrast, high β -glucan HB including both waxy and high amylose starch HB exhibited the highest β -glucan concentration in fractions V–VI followed by a slight decrease (HB340, SB94022, SB94917, SR93139, CDC Candle, and CDC 92-55-08-31) or increase (CDC 92-55-06-48) in subsequent fractions (Table I).

Although β -glucan in barley has been extensively studied from both breeding and nutritional perspectives, its distribution within the grain remains largely unknown. The present study is probably the first to report the quantitative distribution of β -glucan within HB. Miller and Fulcher (1994), studying five barley cultivars containing 2.8–11% β -glucan by microspectrofluorometric method, reported that the distribution of β -glucan in barley was more uniform than that in oats where a high concentration of β -glucan was detected in the subaleurone layer.

In other studies (Bhatti 1997, Oscarsson et al 1997) however, thicker cell walls were observed in the region of subaleurone and endosperm immediately adjacent to subaleurone of barley kernel. This was particularly evident in low β -glucan genotypes. Microspectrofluorometry (Miller and Fulcher 1994) also showed relatively higher fluorescence intensities in the subaleurone region of low β -glucan compared with high β -glucan barley cultivars. In the present study, fractions III–IV of low β -glucan HB showed higher β -glucan concentration than other fractions, indicating that β -glucan was more concentrated in the subaleurone and the endosperm adjacent to subaleurone in low β -glucan HB.

For high β -glucan HB, however, β -glucan was more uniformly distributed throughout the endosperm, although the central core tended to contain less β -glucan than the mid-layers of endosperm, with the exception of the high amylose genotype CDC 92-55-06-48, which exhibited higher β -glucan concentration in the center of the kernel.

AEV showed virtually similar distribution patterns for all HB (Table I) irrespective of total β -glucan distribution. AEV was very low in fraction I, increased progressively toward fractions V–VI, then declined in fractions VII–VIII. AEV distribution pattern reflected the distribution of acid-soluble β -glucan in pearled fraction from all HB, and a significant positive correlation ($r = 0.89$, $P < 0.001$) was found between log-transformed AEV and acid-soluble β -glucan concentration. This agrees with previous reports (Bhatti et al 1991, Xue et al 1991, Yoon et al 1995). Beer et al (1997) also suggested that β -glucan content was the predominant factor in determining the viscosity of barley extracts because little difference was found in molecular weight distribution between low viscosity nonwaxy and high viscosity waxy barley.

It was noted that the ratio of acid-soluble β -glucan to total β -glucan was lower for wholemeal and fraction VIII than for the pearled fractions for all HB (Table I). The average β -glucan solubility in acid buffer was 62–70% for pearled fractions I–VII, whereas that of wholemeal and fraction VIII was only 54 and 44%, respectively. One possible explanation for the difference in β -glucan solubility was the difference in fineness between the pearled fractions and the cyclone-milled samples. The pearled fractions were finer, therefore β -glucan extraction with the acid buffer was greater than the two cyclone-milled samples for each HB.

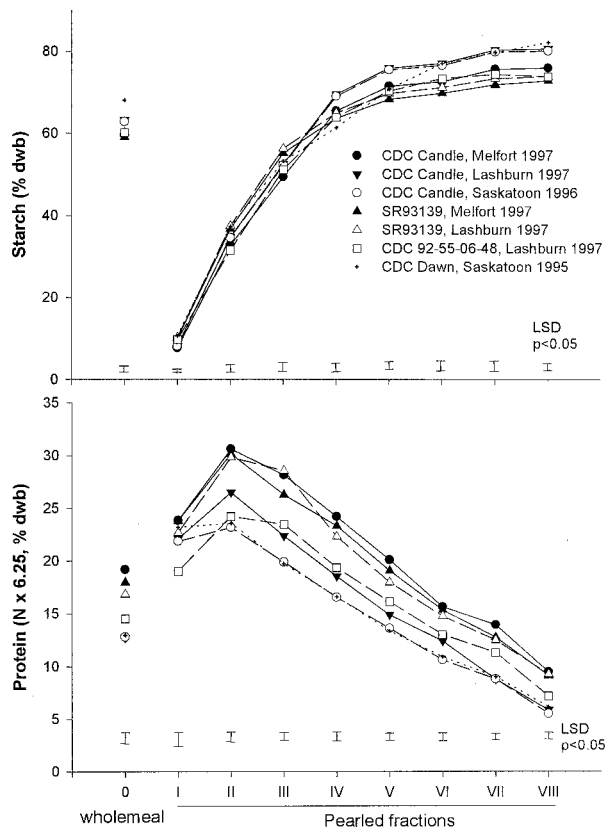


Fig. 2. Starch and protein concentration of wholemeal and pearled fractions of hull-less barley grown at different locations and in different years.

Environmental Effect on β -Glucan Distribution

Wholemeal of CDC Candle, SR93139, and CDC 92-55-08-31 exhibited significant ($P < 0.05$) differences in protein, starch, total and acid-soluble β -glucan concentrations grown at different locations or in different years (Figs. 2 and 3, and in comparison with data in Table I). For the same growing season (1997), samples grown at Melfort were higher in protein but lower in β -glucan and AEV than samples grown at Lashburn or Saskatoon. For grains grown in Saskatoon, CDC Dawn showed less variance in chemical composition than CDC Candle for different years. The influence of environmental factors on chemical composition of HB was not the focus of this study since a great deal of earlier work has dealt with the effect of environment on chemical composition of barley (Fastnaught et al 1996, Perez-Vendrell et al 1996, Oscarsson et al 1997). Regardless of location and growing season, the distribution of protein and starch (Fig. 2), total and soluble β -glucan (Fig. 3) showed similar patterns as compared with the corresponding genotypes shown in Table I. These results indicated that environment did not alter the distribution of β -glucan within HB kernel, although the concentration of β -glucan was significantly affected by growing conditions.

CONCLUSIONS

The results obtained from this study clearly indicated that β -glucan was relatively high in the subaleurone region for low β -glucan HB but not for high β -glucan HB. For HB that contained medium to high β -glucan, the starchy endosperm contained more β -glucan than subaleurone. These results may explain, in part, the difference in milling behavior between high β -glucan HB and low β -glucan HB (Bhatty 1997, 1999). Compared with high β -glucan HB, the endosperm of low β -glucan HB contains less β -glucan (the present study) with thinner cell walls (Bhatty 1997, Oscarsson et al 1997) and therefore is more easily roller-milled, resulting in higher flour yields.

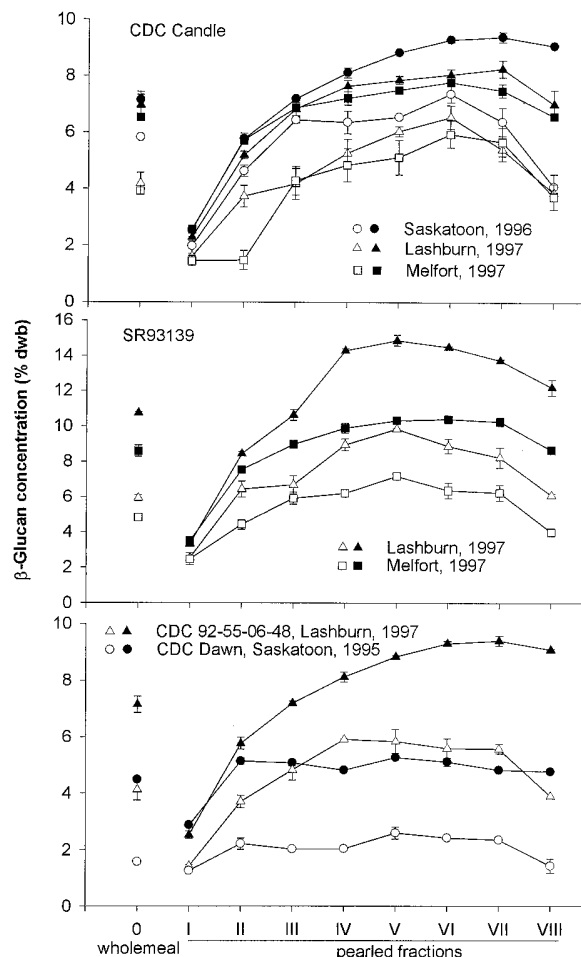


Fig. 3. Total (solid symbols) and acid-soluble β -glucan (open symbols) concentrations of wholemeal and pearled fractions of hull-less barley grown at different locations and in different years. Vertical bars indicate standard errors.

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