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Content and Molecular Weight of Extractable β-Glucan in American and Swedish Oat Samples

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β-(1→3),(1→4)-D-Glucan (β-glucan) was extracted from 93 Swedish and 41 American oat samples using hot water containing $CaCl_2$ and thermostable α-amylase. The samples showed a large variation in both the content of extractable β-glucan (0.76−3.68%) and the average molecular weight ((1.25−1.78) × 10^6 g mol^{-1}). An analysis of the variance of β-glucan content and the molecular weight of Swedish oat samples grown in 2000 and 2001 was done with cultivar and harvest year as factors. It showed that the extractable β-glucan content was a heritable trait whereas molecular weight depended more on environmental factors. The American oat samples had a higher average content of extractable β-glucan (2.24%) and a somewhat higher average molecular weight (1.58 × 10^6 g mol^{-1}) than the Swedish oat samples (1.43% and 1.49 × 10^6 g mol^{-1}).

KEYWORDS: extractable β-glucan; molecular weight; oats

INTRODUCTION

Mixed-linkage β-(1→3),(1→4)-D-glucan (hereafter referred to as β-glucan) is the main constituent of the endosperm cell walls of oats. The (1→4) links occur most often randomly in groups of two (cellotriosyl) or three (cellotetraosyl), separated by isolated (1→3) linkages (*1*). Important physiological effects of oat β-glucan (i.e., improvement of glucose and insulin regulation and lowering of the serum cholesterol level) are attributed to the viscosity of the polysaccharide in the human gastrointestinal tract (*2*, *3*). Two factors that influence the solution viscosity are concentration and molecular weight of the polymer, which in turn are dependent on its extractability and solubility.

Several different methods have been reported in the literature for extracting β-glucan from oats: enzymatic, alkaline, or water extractions with varying temperatures and incubation times (*4*−*7*). Mild conditions could generally extract only 30−70% of the total β-glucan. Three consecutive extractions with hot water (90 °C) containing heat-stable α-amylase extracted 60−75%, and when followed by a dimethyl sulfoxide extraction, the yield increased to 75−80% (*8*). Sodium hydroxide extraction led to a nearly total extraction of β-glucan but decreased the molecular weight (*8*, *9*). The lack of inactivation of endogenous β-glucanase under relatively mild conditions led to an increased total amount extracted but also to a reduced molecular weight (*7*). So far, no complete extraction of β-glucan without degradation has been accomplished.

Because at least some of the beneficial effects of oats are attributed to the high molecular weight of extractable β-glucan, it is important to determine the influence of genotype and environment on these parameters to facilitate plant breeding of oat cultivars. Effects of cultivar and genotype on oat β-glucan concentration have been studied before (*10*−*12*) but with inconclusive results, but no study on the variation of the molecular weight of β-glucan due to environment or genotype has been reported yet.

In the present study, hot water with heat-stable α-amylase was used over a period of 6 h for the extraction of β-glucan. Forty-one American and ninety-three Swedish oat grain samples were analyzed. The results were used to study the variation of extractable β-glucan content and molecular weight due to heritability, location, and year.

MATERIALS AND METHODS

Oat Grains. Forty-one cultivars of American oat samples (ACBaton, ACBelmont, ACPreakness, Ajay, Andrew, Belle, Burton, Calibre, Cayuse, Celsia, CDCBoyer, CDCPacer, Dane, Derby Don, Drumlin, Jerry, Jerry(00), Lamont, Marion, Mem, MN861218, Monida, Newdak, Ogle, Otee, Otana, Paul, Penniline6571, Pennuda, Powell, Prairie, ProvenaD7, RioGrande, SD751187, SD770290, Vista, Whitestone, 87Ab502, 91Ab502, 94Ab5818) were provided by the National Small Grains Collection, Aberdeen, Idaho. MN861218 was grown in 1996; Marion, in 1983; and Penniline6571, SD751187, and SD770290, in 1984 in Aberdeen. The rest of the samples were grown in Aberdeen, or Tetonia, Idaho in 1996−2000.

Sixty Swedish oat samples grown at Landskrona were provided by Svalöf Weibull AB (Svalöv, Sweden). Of these, 33 samples (SWE 1-5, Bikini, BoAslak, Bullion, Expander, Lisbeth, Matilda, Neon, NK96166, NK93008, SegerI, SolII, SWBelinda, SWCilla, SWGunhild, SWFreja, SWKerstin, SWMargareta, SWSang, SWSvala, SWVital, SW00137, SW00327, SW97278, SW97290, SW98195, SW98245, SW991420, Veli) were collected from the harvest of 2000 and 2001, and 27 samples (SWE 6-13, Expo, L. P. Firth, NSFreddy, SerChantilly, SW01135, SW01145, SW01183, SW01188, SW01199, SW01217, SW01218,

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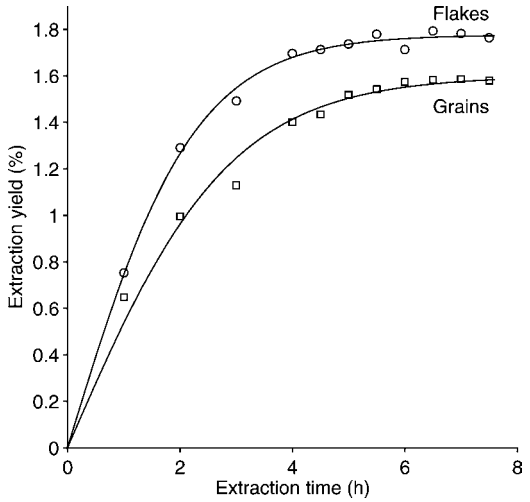

Figure 1. Extraction yield of β-glucan (percentage of dry flour) from oat grains and flakes during extraction with hot water containing $CaCl_2$ and α-amylase over time.

SW01222, SW01243, SW01393, SW01395, SW01398, SW01399, SW01400, SW99305) were collected only in 2001. The average temperature, rainfall, and sunlight received during 2000 and 2001 at Landskrona were normal for both years. The maximum rainfall received in 2000 was distributed in June and July, and that in 2001 was over August and September.

For the development of the extraction method over 6 h, the Swedish cultivar Sang and oat flakes from Cerealia AB (Järna, Sweden) were used. All samples were ground in an ultracentrifugal mill-type ZM 1 with a 0.5-mm ring sieve (Retsch, Hann, Germany).

Extraction of *β*-Glucan. β-Glucan was extracted from oat samples (100 mg) using water (20 mL) containing $CaCl_2$ (0.28 mg/mL of $CaCl_2$) and α-amylase (50 μl, EC 3.2.1.1, 3000 U/ml, Megazyme, Wicklow, Ireland). The extraction was carried out according to Rimsten et al., but samples representing 1 to 7.5 h were taken out at intervals of 1 h up to 4 h and then at intervals of 30 min each to 7.5 h (*13*). After cooling to room temperature, the tubes were centrifuged (1000*g*, 10 min), and the supernatant was filtered (0.45 μm) into vials. Molecular weight distributions and the content of extraction of β-glucan were determined by high-performance size-exclusion chromatography with specific fluorescence detection (HPSEC-FD). The Calcofluor average molecular weight of β-glucan, which is an average taking the whole area for β-glucan over 10^4 g mol^{-1} in molecular weight into account, was determined. Percentiles were also calculated, describing the molecular weight at which 10, 50, and 90% of the distribution fell below that value. Each value is presented as a mean of the duplicate extractions.

Table 1. Calcoflour Average Molecular Weight of Extractable β-Glucan in Oat Grains Sequentially Extracted for 6 Hours

time (h)	molecular weight (10^6 g mol^{-1})
0–1	1.98
1–2	2.02
2–3	1.67
3–4	1.57
4–5	1.57
5–6	1.34

Extraction of American and Swedish Oat Samples. The 41 samples of American oats and 93 samples of Swedish oats were extracted using the above method with the samples being extracted continuously for 6 h, centrifuged, filtered, and analyzed on the HPSEC-FD system.

Sequential Extraction of *β*-Glucan over 6 Hours. The extraction of β-glucan was carried out using the method described above, but 400 mg of sample was used and the extraction medium was replaced each hour after centrifugation and analyzed on the HPSEC-FD.

Statistical Analysis. The analysis of variance was carried out with the general linear model, and the Anderson−Darling test for normality was performed using Minitab 14 (Minitab Inc, State College, PA).

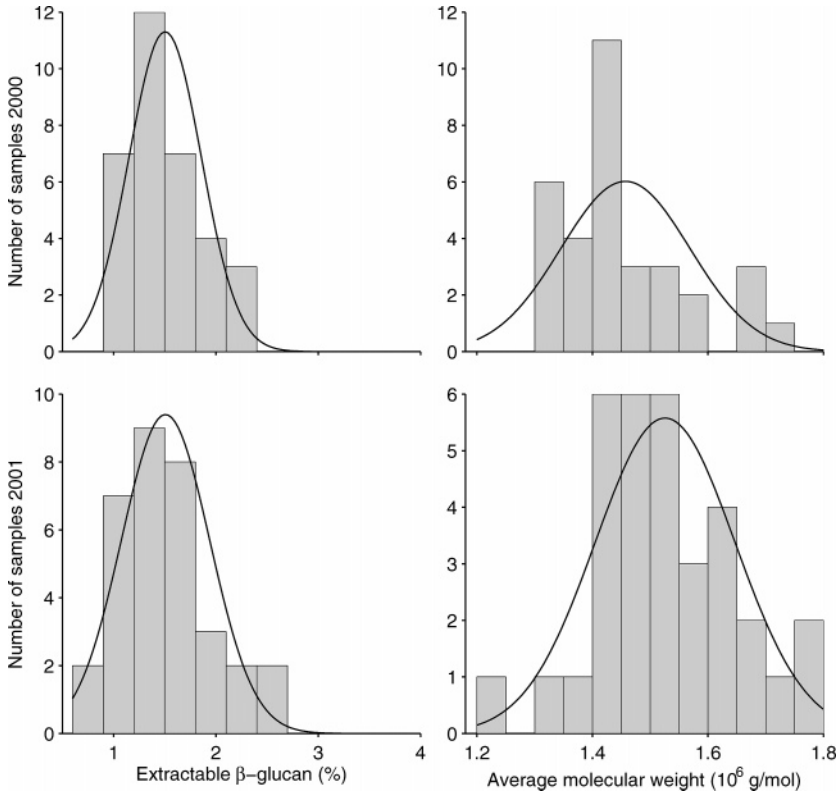

Figure 2. Frequency distribution of extractable β-glucan content (percentage of dry flour) and Calcoflour average molecular weight (10^6 g mol^{-1}) of Swedish oat samples harvested in 2000 and 2001.

Table 2. Summary of Analytical Data from Swedish and American Oat Samples

	Swedish oat samples (harvest year)		oat samples	
	2000	2001	Swedish	American
number of samples	33	33	93	41
extractable β-glucan				
average (% of DM)	1.50	1.51	1.43	2.24
SD	0.35	0.43	0.37	0.55
range	1.09–2.37	0.76–2.65	0.76–2.65	1.34–3.68
molecular weight				
average (10^6 g mol^{-1})	1.46	1.53	1.49	1.58
SD	0.11	0.12	0.11	0.08
range	1.30–1.74	1.25–1.78	1.25–1.78	1.40–1.77
distribution 10%[a]				
average (10^6 g mol^{-1})	0.29	0.34	0.32	0.38
SD	0.02	0.05	0.05	0.05
range	0.25–0.35	0.26–0.46	0.23–0.46	0.28–0.50
distribution 50%[a]				
average (10^6 g mol^{-1})	1.34	1.42	1.38	1.43
SD	0.11	0.13	0.12	0.09
range	1.20–1.60	1.13–1.69	1.13–1.69	1.24–1.65
distribution 90%[a]				
average (10^6 g mol^{-1})	2.63	2.79	2.73	2.93
SD	0.27	0.18	0.22	0.12
range	2.01–3.09	2.37–3.14	2.01–3.14	2.68–3.23

[a] Percentiles describing molecular weight at which 10, 50 and 90% of the distribution fall below that value.

RESULTS AND DISCUSSION

Extraction of β-Glucan. The extraction and subsequent analysis of β-glucan by HPSEC-FD used in this study was developed for barley β-glucan, and it has been used routinely for analyzing barley and barley products (*13*, *14*). Because the rate of extraction appeared to be slower for oats than for barley, the appropriate time of the extraction for oats had to be determined. The increase in the amount of β-glucan extracted after 4.5 h was small compared to the amount extracted during the first 4 h. (**Figure 1**). Extending the extraction time beyond 6 h did not increase the extraction amount significantly. Hence, 6 h was fixed for all extractions carried out in this study. A limited lowering of the Calcofluor average molecular weight of extracted β-glucan ((2.1 to 1.7) × 10^6 g mol^{-1}) over this extraction time was detected, as previously observed by Beer et al. (*8*). To determine whether this lowering was caused by degradation during extraction or by a low-molecular-weight fraction of β-glucan being extracted, a sequential extraction of oats was conducted over 6 h. The β-glucan fractions extracted during the first 2 h were of the highest molecular weight, whereas β-glucan fractions of lower molecular weight were extracted during subsequent hours (**Table 1**). This explained the lower average molecular weight obtained during the accumulated extraction over 6 h, but the same yield of extractable β-glucan was reached in both extractions.

Extraction of Swedish and American Oats. Thirty three Swedish oat samples harvested in 2000 and 2001 were analyzed for their extractable β-glucan content and Calcofluor average molecular weight (**Table 2**). Both years showed a normal distribution for extractable β-glucan content, ranging from 1.1 to 2.4% in 2000 and from 0.8 to 2.7% in 2001 (**Figure 2**). The analysis of variance of extractable β-glucan content with cultivar and harvest year as factors showed that cultivar was the only statistically significant factor ($P < 0.001$). This result suggests that extractable β-glucan content is partially controlled genetically and not only by environmental factors. This is in agreement with earlier reports that the heritability of β-glucan concentration

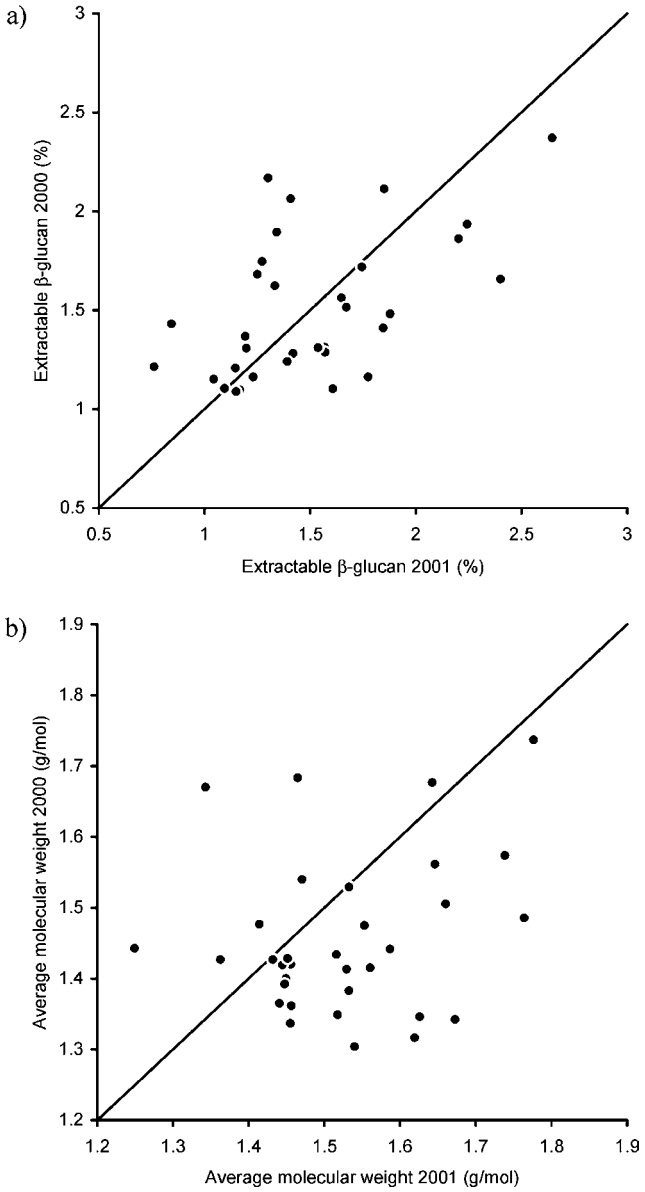

Figure 3. Scatter plots showing (**a**) the variation in extractable β-glucan content and (**b**) the variation in average molecular weight of Swedish oat cultivars harvested in 2000 and 2001.

is controlled by gene action (*15*−*17*). The content of extractable β-glucan for each individual cultivar is represented as a dot in the scatter plot showing the values for each year on the *x* and *y* axes (**Figure 3a**). The consistency of the cultivars during the 2 years is represented by their proximity to the unity line.

The Calcofluor average molecular weight showed normal distribution curves in both years and was spread over a range of (1.3−1.7) × 10^6 and (1.3−1.8) × 10^6 g mol^{-1} in 2000 and 2001, respectively (**Figure 2**). The analysis of variance for the Calcofluor average molecular weight with the cultivar and year as factors showed that the year was the only statistically significant factor ($P = 0.011$). Thus, molecular weight appears to be controlled more by environmental factors, which differ in rainfall distribution over the years, than by genetic factors. This information could be of much practical importance in an oat variety development program. The variation in molecular weight and its inconsistency in oat cultivars across the 2 years are illustrated by the scatter plot (**Figure 3b**), confirming the absence of significant cultivar effects in the ANOVA.

Forty-one American oat samples were analyzed for extractable

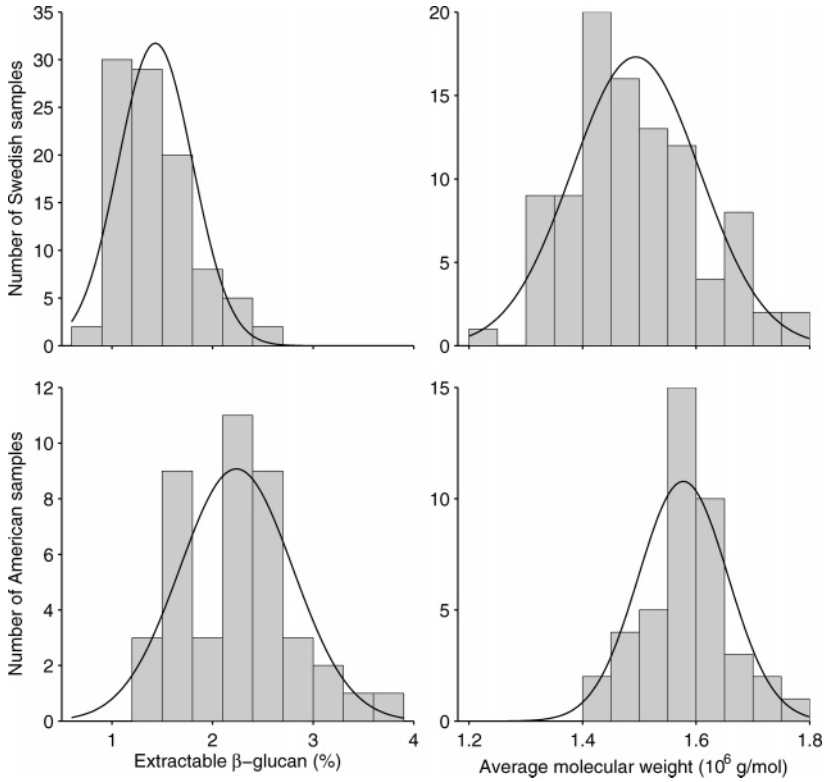

Figure 4. Frequency distribution of extractable β-glucan content (percentage of dry flour) and average molecular weight (10^6 g mol^{-1}) of Swedish and American oat samples.

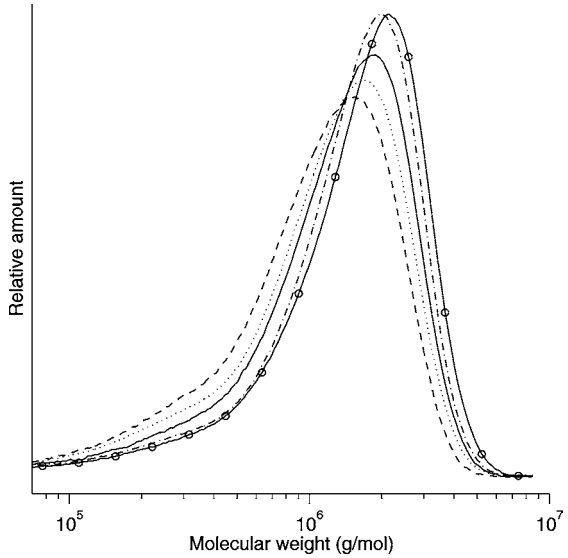

Figure 5. Molecular weight distribution of extractable β-glucan from Matilda (- - -), NK93008 (•••), Expander (–), Bikini (-•-), and Lisbeth (–◦–).

β-glucan content and molecular weight. They were compared with 93 Swedish oat samples, including the 66 samples discussed above and 27 other cultivars grown in 2001. The analysis of variance of extractable β-glucan content showed that the American samples had significantly higher values (2.2%) compared to the Swedish samples (1.4%) ($P < 0.001$) (**Table 2**). The majority of the American oat samples had a content higher than 2%, whereas most Swedish samples had a content below that value (**Figure 4**). The Swedish samples covered a somewhat wider range ((1.3−1.8) × 10^6 g mol^{-1}) of molecular weights than the American oat samples ((1.4−1.8) × 10^6 g mol^{-1}) (**Figure 4**). The analysis of variance of average molecular weight showed that the American oats grown in Aberdeen, or Tetonia, Idaho, had a significantly higher average molecular weight ($P < 0.001$) and could thus be more suitable candidates for the production of functional foods with oat β-glucan (*18*).

Table 3. Extractable β-Glucan Content (Percentage of Dry Flour), Average Molecular Weight, and Percentiles of Each Peak at 10, 50, and 90% for Five Oat Samples Equally Spaced along the Average Molecular Size Scale

sample	extractable β-glucan content	average molecular weight (10^6 g mol^{-1})	distribution (10^6 g mol^{-1})[a] 10%	50%	90%
Matilda	1.39	1.25	0.26	1.13	2.37
NK93008	1.48	1.38	0.27	1.27	2.58
Expander	1.65	1.51	0.37	1.41	2.75
Bikini	2.20	1.65	0.40	1.57	2.91
Lisbeth	1.67	1.78	0.46	1.69	3.13

[a] Percentiles describing molecular weight at which 10, 50, and 90% of the distribution fall below that value.

Five samples covering the total range of average molecular weight (equally spaced along the average molecular size scale) were chosen from the total of 134 samples of oats analyzed, and their molecular weight distribution of extractable β-glucan is shown in **Figure 5**. The oat samples that had a low average molecular weight were deficient in β-glucan chains of high molecular weight (above 4.5 × 10^6 g mol^{-1}). This may be of technological and nutritional importance because the longest chains have the greatest impact on solution viscosity. The oat samples of high average molecular weight had lower levels of extractable β-glucan with molecular weight below 10^6 g mol^{-1}. This is also evident from a comparison of their 10 and 90% values (**Table 3**), which could possibly serve as a criterion for the choice of oats enriched in long-chain β-glucans.

Information on heritability is vital for the development of cultivars. The results from this study indicate that the extractable

β-glucan content is a heritable trait whereas molecular weight depends on environmental factors. The American oat samples had a higher average β-glucan content and molecular weight than the Swedish oat samples. These results are of interest because the demand for high-quality oats for the production of functional foods is increasing.

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