

Determination of β -Glucan Molecular Weight Using SEC with Calcofluor Detection in Cereal Extracts

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ABSTRACT

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A high-performance size-exclusion chromatography system (HPSEC) was set up with detection based on the specific binding of Calcofluor to β -glucan for determination of amount and molecular weight of β -glucan in different cereal extracts. To calibrate the HPSEC system, a purified β -glucan was fractionated into narrow molecular weight ranges and the average molecular weight was determined before analysis on the HPSEC system. The detector response was similar for β -glucans from oats and barley and appeared to be independent of molecular weight. Four differ-

ent methods for extraction of β -glucan from different cereal products were tested: two alkaline, one with hot water and added α -amylase, and one with water and added xylanase. Inactivation of endogenous β -glucanase was crucial for the stability of the extracts, even when extracting at high temperature or pH. Yields varied widely between the different extraction methods but average molecular weight and molecular weight distribution were similar. Extraction with sodium hydroxide generally gave a higher yield and molecular weight of β -glucan in the extracts.

Cereal β -glucan is a polysaccharide that consists of linear chains of glucose residues linked through both β -(1 \rightarrow 3) and β -(1 \rightarrow 4)-linkages. The (1 \rightarrow 4)-linked residues occur most often randomly in groups of two (cellotriosyl) or three (cellotetraosyl) separated by isolated (1 \rightarrow 3)-linkages (Wood et al 1994). The physiological properties of β -glucan have, over recent years, gained a lot of interest because it may improve glucose and insulin regulation and lower blood cholesterol levels (Chen et al 1981; Davidsson et al 1991; Sundberg et al 1995). The mechanism is not fully understood but viscosity in the upper gastrointestinal tract is considered to play a central role. Two factors that influence solution viscosity are concentration and molecular weight. Thus, knowledge of these parameters in products is of great importance. There are several methods to determine the content of β -glucan; the most commonly used is the enzymatic method of McCleary and Glennie-Holmes (1985). Another well-known method, one recommended by the European Brewery Convention for analysis of malt and barley, involves the specific binding of Calcofluor to β -glucan (Wood and Fulcher 1978; Wood 1980). This binding results in an increase in fluorescence intensity that is proportional to the concentration of β -glucan in solutions. Development of methods to quantify β -glucan evolved from these findings (Wood and Weisz 1984; Mekis et al 1987; Jørgensen 1988), and has led to the Calcofluor-flow injection analysis (FIA) system. Based on the same principle, a method to determine molecular weight of β -glucan has been developed. In this method, extracts of β -glucan are first separated by size-exclusion chromatography (SEC) and afterwards detected fluorometrically with Calcofluor. It started as an off-line process (Foldager and Jørgensen 1984; Anderson 1990; Manzanares et al 1993) but has now been developed to an online postcolumn detection method (Wood et al 1991a,b; Suortti 1993). The method provides a simple way to determine molecular weights of β -glucan extracts without any prior purification steps.

Two approaches have been used to calibrate molecular weight for the HPSEC-system with Calcofluor detection. One is calibrating with pullulan standards but that has led to overestimation of results (Vårum et al 1991; Wood et al 1991a,b). Another approach is to use purified β -glucan of known average molecular weight for the calibration (Wood et al 1991a,b; Suortti 1993). This is possible if each fraction covers a narrow molecular weight range, while at the same time all fractions together cover the whole range of

molecular weights. So far, there are no β -glucan standards available that fulfill these criteria. The molecular weight of β -glucan is often determined by using the retention time of the peak. This can be misleading if the peak is not normally distributed or if the peak is polymodal and thus cannot be described by a single retention time. It is better to take the whole area under the curve into account to determine molecular weight averages.

Several different methods have been reported in the literature to extract β -glucan from barley and oats, though the most common methods are alkaline or water extractions with varying temperatures and incubation times. Wood et al (1991a,b) compared extractions with water or carbonate buffer at 45, 60, and 80°C. They concluded that the best conditions were carbonate buffer at 60°C with a prior inactivation of endogenous enzymes with 50% EtOH in boiling water for 15 min, although they also saw that an elevated temperature increased the extractability of β -glucan. Different concentrations of NaOH at room temperature have also been tested by Suortti (1993) to extract β -glucan from barley and malted barley. So far, no complete extraction of β -glucan without degradation has been made. The average molecular weight and concentration of β -glucan extracted from oats and barley varied between studies (Wood et al 1991a,b; Suortti 1993; Beer et al 1997). One reason for this is, of course, the different samples used, although another reason could be differences in extractability of β -glucan with the different extraction methods used.

In this study, a high-performance size-exclusion chromatography system with fluorescence detection (HPSEC-FD) was calibrated by using β -glucan fractions of narrow molecular weight ranges. The system was used to compare yields of common and new extraction methods from different cereal products and to study how the different yields affect the molecular weight of β -glucan.

MATERIALS

Samples of common Swedish cereal products were selected, SW 8775 naked barley (Svalöf Weibull AB, Svalöv, Sweden), barley malt (Slottskällans Brewery, Uppsala, Sweden), Soisson wheat (INRA Clermont-Ferrand, France), Kungsörnen wheat bran (Cerealia, Järna, Sweden), Sang-Sigill oats (Odal, Norrköping, Sweden), Kungsörnen oat bran (Cerealia, Järna, Sweden), Olof rye (Svalöf Weibull AB, Svalöv, Sweden), and rye bran. The rye bran was a finely milled bran that was obtained from the pilot plant at Cerealia R&D (Järna, Sweden). Four food products based on oats were also used: soft bread (Fazerbröd AB, Solna, Sweden), crispbread (Wasa Havre, Wasabröd AB, Solna, Sweden), pancake batter (Oatly, Ceba Foods AB, Malmö, Sweden), and oat cereal (Havrefras, Quaker, Chicago, IL).

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METHODS

General Analysis

Before analysis, all samples were ground in a cyclone sample mill (Foss Tecator AB, Höganäs, Sweden) to pass a 0.5-mm screen. The total β -glucan content was determined enzymatically according to the method by Åman and Graham (1987). All analyses were run in duplicate and the results are reported on a dry matter basis, determined by drying samples for 6 hr at 105°C.

Equipment

The chromatographic system was set up according to Wood et al (1991a,b) with some modifications. Our system consisted of two pumps (LC-10AD, Shimadzu, Miniato, Japan) coupled to a degasser (SDU 2006, Prolab, Reinach, Switzerland) one delivering the eluent (0.1M NaNO₃ with 0.02% NaN₃) at a flow rate of 0.5 mL/min and the other one delivering Calcofluor solution (0.05% fluorescent brightener 28 [Sigma] in 0.1M tris(hydroxymethyl)aminomethane [Tris] adjusted to pH 8) at a flow rate of 0.5 mL/min through a pulse reducer. An injector (Midas type 830, Spark, Emmen, Holland) was coupled to the system before a guard column (OHpak SB-G, Shodex, Showa Denko KK, Kawasaki, Japan) and two columns in series (OHpak SB-806HQ and SB-804HQ, Shodex, Showa Denko KK, Kawasaki, Japan). Calcofluor was delivered postcolumn followed by a mixing loop placed together with the columns in an oven maintained at 60°C. For detection, a fluorescent detector (1100 series G1321A, Agilent Technologies, Waldbrook, Germany) was used with the wavelengths $\lambda_{\text{ex}} = 415$ nm and $\lambda_{\text{em}} = 445$ nm according to Suortti (1993) at a gain setting of 8.

Detector Response

Stock solutions from two purified β -glucans from barley (MW 137,000 and 250,000, Megazyme, Wicklow, Ireland) and one from oats (MW 200,000, Megazyme, Wicklow, Ireland) were prepared by weighing 50 mg of sample and adding ≈ 30 mL of deionized water. The β -glucans were heated in a boiling water bath during magnetic stirring until dissolved. After cooling, the solutions were transferred to volumetric flasks and diluted to 50 mL (final conc. 1 mg/mL). The stock solutions were then diluted to 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL, and all solutions were injected (10 μ L) three times on the HPSEC-FD system. A mean of the areas from the three was calculated and plotted against the amount of β -glucan in each sample.

One barley and one oat extract were made by weighing 400 mg of sample and extracting with thermostable α -amylase in boiling water as described below. The concentration of glucose residues as a measure of β -glucan content in the ethanol precipitate was determined using the method by Andersson et al (1999), which is a modified method of Theander et al (1995). Four dilutions were

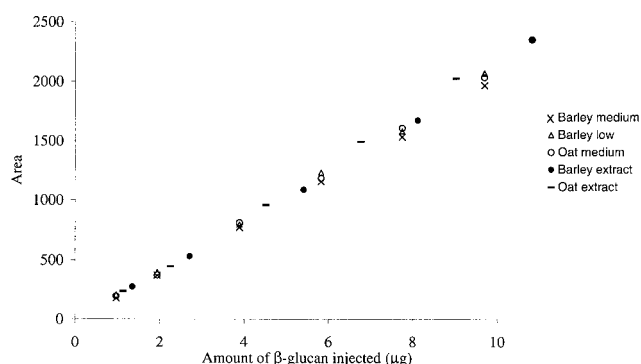


Fig. 1. Response curve for purified β -glucan standards from barley with low viscosity and medium viscosity, oat with medium viscosity, and for hot water extracts from barley and oats.

made from each extract and injected three times on the HPSEC-FD. A mean of the areas from the three injections was calculated and plotted against the amount of β -glucan in each sample.

Molecular Weight Calibration

A solution was made (5 mg/mL) of a purified β -glucan from germinated barley by heating in a boiling water bath during constant stirring. After filtering (0.45 μ m), the sample was injected (100 μ L) on a high-performance size-exclusion system combined with a multiple angle laser scattering and refractive index detector (HPSEC-MALLS-RI) described by Roubroeks et al (2000). An average molecular weight was calculated for each minute interval of the peak. The same sample was injected a second time and separated on the HPSEC-MALLS-RI system by three serially connected columns (OHpak SB-806M HQ, OHpak SB-804 HQ, and OHpak SB-803 HQ, Shodex, Showa Denko KK, Miniato, Japan) controlled at 35°C. The flow of the eluent (0.1M NaNO₃ with 0.02% NaN₃) was 0.5 mL/min. Peak fractions were collected this time from the sample at the same minute interval as previously calculated. The isolated fractions were thereafter injected (50 μ L) on the HPSEC-FD system. A calibration curve was set up by plotting the retention time from each peak from the HPSEC-FD system against log molecular weight obtained from the HPSEC-MALLS-RI system. By using the regression line of the calibration curve, a Calcofluor average molecular weight (\bar{M}_{cf}) could be calculated. The \bar{M}_{cf} over the distribution divided into n slices was defined as

$$\bar{M}_{cf} = \frac{\sum_{i=1}^n (w_i c_i)}{\sum_{i=1}^n c_i} \quad (1)$$

where w_i is the molecular weight at slice i given by the calibration and c_i is the corresponding concentration expressed as Calcofluor response. This average only includes β -glucan molecules large enough to be detected by Calcofluor. Percentiles were also calculated, describing the molecular weight at which 10, 50, and 90% of the distribution fall below that value.

Extraction Methods

Four methods were tested to extract β -glucan, two alkaline extractions with 20 mM Na₂CO₃ (2 hr, 60°C) according to Wood et al (1991a,b) and with 50 mM NaOH for 17 hr in room temperature according to Suortti (1993). The third method tested was hot-water extraction with thermostable α -amylase (EC 3.2.1.1, 3,000 U/mL, Megazyme, Wicklow, Ireland), where 20 mL of deionized water with CaCl₂ (0.28 mg/mL of H₂O) and 50 μ L of thermostable α -amylase was added to 200 mg of sample in test tubes that were placed directly in a boiling water bath for 90 min with occasional stirring. After cooling, the tubes were centrifuged (1,500 \times g for 15 min) and the supernatant was filtered (0.45 μ m) and analyzed on the HPSEC-FD system. The fourth method tested was extraction in water with different xylanases added. The xylanases used were Bio-Feed Wheat L (0.03 and 30%) supplied by Novoenzymes A/S (Bagsvaerd, Denmark), Grindamyl H640 (0.02 and 0.18 mg/mL, Danisco, Copenhagen, Denmark), and endoxylanase M1 from *Trichoderma viride* (1 and 5%, 896 U/mL, Megazyme, Wicklow, Ireland). With all xylanases, 200 mg of sample was mixed with 20 mL of water containing different amounts of a xylanase. Samples were incubated for 2 hr at 40°C with magnetic stirring. After centrifugation (1,500 \times g for 15 min), the supernatants were boiled for 15 min and filtered (0.45 μ m) before injection. To each extraction with xylanase, control samples were included with solutions of purified β -glucan from oat mixed with the xylanase used to test whether β -glucanase was present in the enzyme. All samples were analyzed at least in duplicate.

RESULTS AND DISCUSSION

Detector Response

The detector response for all β -glucans had similar linear relationship over increasing concentration (Fig. 1). Because no difference in response between β -glucan from oats or barley could be seen, structural differences like the ratio between cellotriosyl and cellotetraosyl units do not seem to influence the binding of Calcofluor. Also, the crude extracts of β -glucan from barley and oats with molecular weights 10 \times that of the purified β -glucans had the same response. Therefore, it could also be concluded that there is no difference in response between the β -glucans of low average molecular weight compared with those of high average molecular weight in the range tested (0.1–1 mg/mL) or between purified β -glucans and β -glucans in a crude extract. Thus, the response curve from the purified β -glucans can be used to determine the concentration of β -glucan in extracts. A regression line was made including all data points from the purified oats and the two barley β -glucans. The regression line had an R value of 0.998 and was used for the determination of the β -glucan content in extracts.

Molecular Weight Calibration

Peak fractions of a germinated barley β -glucan sample were collected from the HPSEC-MALLS-RI system. The collected fractions were directly run on the HPSEC-FD system. The same mobile phase and column types were used for both systems, and the collected fractions had a concentration of β -glucan that was $\approx 10\times$ lower than the concentration before separation, which resulted in a final concentration of ≈ 0.5 mg/mL that suited the detection limits of the HPSEC-FD system. The peaks from each fraction were symmetrically distributed and covered a narrow molecular weight range (Fig. 2). The molecular weight calibration

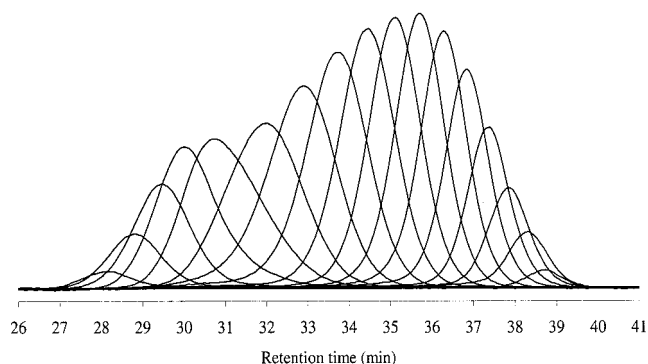


Fig. 2. HPSEC-FD chromatograms of the isolated β -glucan fractions of germinated barley.

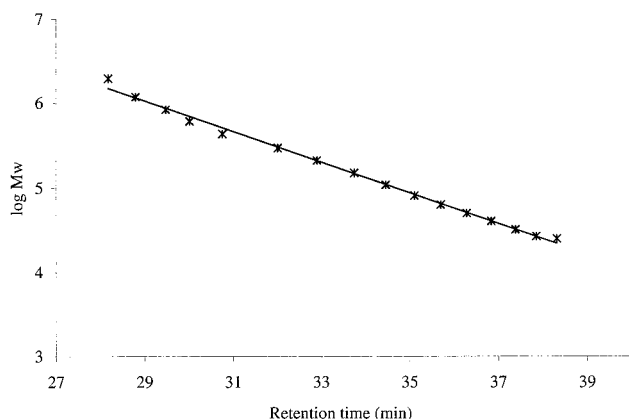


Fig. 3. Plot of log molecular weight (M_w) from HPSEC-MALLS-RI against retention time for fractions from HPSEC-FD of germinated barley.

curve showed a linear ($r^2 = 0.995$) relationship between log average molecular weight calculated from HPSEC-MALLS-RI and retention time from HPSEC-FD (Fig. 3).

If β -glucan from different cereals have differences in conformation, this would influence their retention in the size-exclusion columns. Purified β -glucans from oats and barley were injected on the HPSEC-MALLS-RI to test whether the calibration is applicable for all β -glucan extractions regardless of the source. The barley and oat samples showed the same ratio between retention time and molecular weight determined by light scattering. The molar ratio of cellotriosyl to cellotetraosyl units was different in barley compared with oats (Wood et al 1991a,b). It should be possible, therefore, to use the same calibration for all cereal samples.

Endogenous Activity in Samples During and After Extraction

Inhibition of endogenous β -glucanase activity in samples for determination of β -glucan molecular weight is a crucial step in the analysis. Though there are several ways to inhibit β -glucanase activity, previous total inactivation of β -glucanase activity was difficult. For example, a high pH reduced activity of β -glucanase (Bamforth et al 1979; Knuckles and Chiu 1999). Reduction in activity can also be achieved by an elevated temperature in water or in combination with ethanol (Forrest and Wainwright 1979; Wood et al 1991a,b; Knuckles et al 1997; Knuckles and Chiu 1999). Thus, the extraction conditions used here would be expected to give a relatively low activity of β -glucanase. To test whether this was true, the stability of the β -glucan extracts was investigated (with and without the prior inactivation step with 50% EtOH in boiling water for 15 min) by injecting a β -glucan extract from SW 8775 repeatedly during 48 hr. The average molecular weight in extracts from the hot water extraction with α -amylase that was not inactivated before extraction decreased by 20.1% after 48 hr, while the inactivated sample was degraded by 10.6% (Fig. 4). The inactivated sample seemed stable up to 24 hr after extraction. Extracts from NaOH decreased by 44.2% without inactivation, while the inactivated sample decreased by 13.8% after 48 hr. Samples were also stable for up to 24 hr when the inactivation step was included. Extracts from the Na_2CO_3 extraction without inactivation decreased by 33.2%, while the inactivated sample decreased by 19.9%. It was not possible to determine how long a time these extracts were stable because degradation occurred continuously, even for the inactivated sample. This agrees with previous observations in the study by Wood et al (1991a,b). There was a difference between β -glucan in the extracts with and without prior inactivation for the alkaline extractions, even though the high pH (≈ 12) in the extracts was expected to minimize the β -glucanase activity (Bamforth et al 1979; Knuckles et al 1999).

TABLE I
Total Content of β -Glucan (% of dry matter) and Percentage of Extracted β -Glucan (% of total β -glucan in sample) in Cereal Products and Oat-Based Foods by Hot Water Extraction with α -Amylase, NaOH Extraction, or Na_2CO_3 Extraction

	Total Content of β -Glucan	Extracted β -Glucan		
		Hot Water	NaOH	Na_2CO_3
Cereal products				
Barely	3.6	49.6	89.0	57.6
Barley malt	0.5	14.8	31.8	17.2
Rye	2.0	20.3	27.8	14.1
Rye bran	0.9	50.9	77.2	30.9
Wheat	1.0	7.1	19.0	16.2
Wheat bran	0.9	18.0	32.8	18.2
Oat	3.4	35.9	96.1	96.1
Oat bran	8.3	28.3	85.6	97.6
Oat-based products				
Bread	1.1	32.3	52.7	35.8
Crisp bread	2.5	46.8	70.1	64.6
Pancake batter	1.0	75.2	94.7	96.7
Oat cereal	3.2	51.7	81.3	66.6

It could also be seen, for all three methods, that the extraction without a prior inactivation step gave a lower starting value compared with the inactivated sample. One reason for this is most probably endogenous β -glucanase activity during the extractions. Another reason might be, as observed earlier (Knuckles et al 1997), the change in extractability of the β -glucan due to the prior inactivation step. The largest difference in molecular weight was seen for the extraction with NaOH where the sample with prior inactivation had a starting value 1.5 \times higher than the sample without inactivation. This extraction was over a longer period of time than the other two, which would indicate that the largest effect is from endogenous β -glucanase and not the change in extractability.

Yield and Molecular Weight of β -Glucan in Different Extracts

Different extraction methods of β -glucan were compared for some cereal products and oat-based foods (Table I). These cereals (barley, oats, rye, and wheat) were chosen because they are the most common in this country, have varying contents of β -glucan and represent structurally different matrixes. The oat bran had the highest total content of β -glucan (8.3%), while malted barley had the lowest content (0.5%). The oat-based foods were included to see whether processing affected the extractability of β -glucan. The extraction methods studied were two alkaline extractions, one hot water extraction with added thermostable α -amylase, and one extraction with water and different xylanases added.

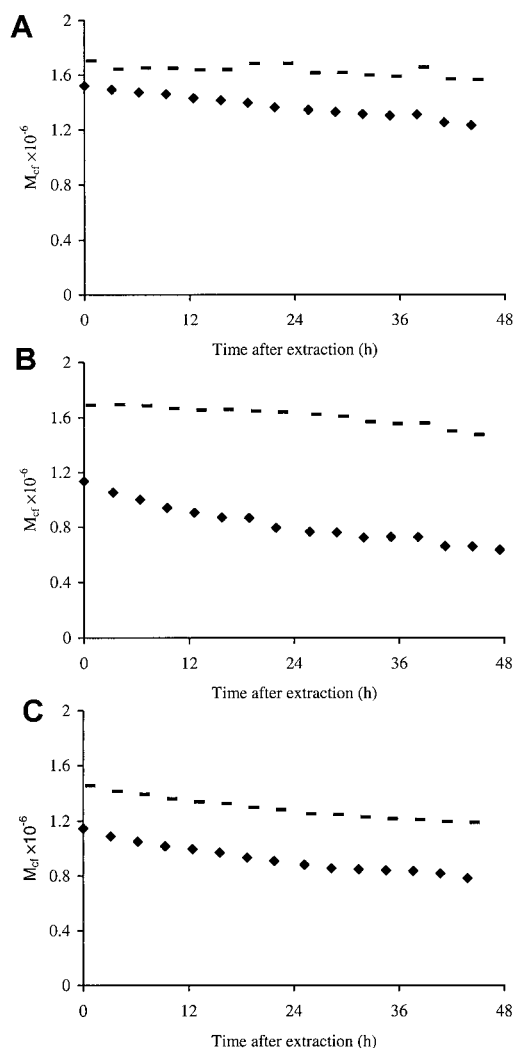


Fig. 4. Change in molecular weight ($\overline{M}_{cf} \times 10^{-6}$) of β -glucan in extracts of samples, with (–) and without (♦) prior inactivation from hot water extraction with α -amylase (A), NaOH extraction (B), or Na_2CO_3 extraction (C) stored at room temperature.

Two of the xylanases tested, Bio-Feed Wheat L and Grindamyl H640, had a contamination with β -glucanase that resulted in a degradation of β -glucan in both samples and in controls that was not acceptable for analysis of molecular weight (data not shown). The contamination with β -glucanase in Xylanase M1 (Megazyme) is claimed to be <0.001 U/mg and the degradation of β -glucan was much lower here (Fig. 5). Still, the effect on the molecular weight profile of β -glucan from the contamination with β -glucanase was evident. This is probably proof of the high sensitivity of the chromatographic method rather than an erroneous value of the contamination with β -glucanase. The extraction with a low concentration of Xylanase M1 was combined with the hot water extraction with α -amylase. This gave a larger yield of β -glucan for the bran samples compared with extraction with only the hot water extraction, but with a lower average molecular weight of β -glucan (data not shown).

The contents of β -glucan in the extracts were determined by using the area of the peak. In all three extraction methods, there was a large variation in yield of β -glucan depending on the sample (Table I). The highest yields of β -glucan were obtained from the NaOH extraction (19–96%), except for oat bran for which the β -glucan yield was highest in Na_2CO_3 extraction. The NaOH extraction yield for barley was a bit lower than in the study by Beer et al (1997), while the extraction yield for oats was in the same range. For the hot water extraction with added α -amylase, the extraction yields varied from 7 to 52%, except for pancake batter, which had an extraction yield of 75%. The extraction yield for barley was in the same range as that found by Beer et al (1997), while the extraction yield for oats was significantly lower. The Na_2CO_3 extraction showed the largest variation in yields of β -glucan (14–98%), with the highest from pancake batter, oats, and oat bran. The β -glucan yield for barley from the Na_2CO_3 extraction was in the same range as in the study by Wood et al (1991a,b), while the extraction yield for malted barley and rye was much lower. The deviation between replicates was generally $<10\%$ for all three extraction methods.

The largest \overline{M}_{cf} of β -glucan for each sample was obtained from the extraction with NaOH (2.07×10^5 to 2.19×10^6), except for oats, for which it was largest for the extraction with hot water (Table II). The \overline{M}_{cf} for the extraction with hot water varied from 1.94×10^5 to 2.25×10^6 , while that for the extraction with Na_2CO_3 varied from 1.94×10^5 to 1.81×10^6 . Even though the molecular weights for some samples varied between extraction methods, the same levels of molecular weight were obtained. The extraction with hot water gave \overline{M}_{cf} values for barley and oats that were in

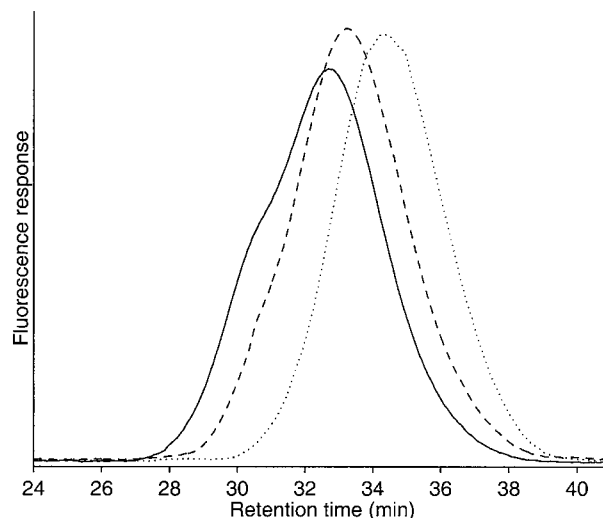


Fig. 5. Molecular weight distribution of purified β -glucan, purified β -glucan incubated with a low concentration of Xylanase M1, and purified β -glucan incubated with a high concentration of Xylanase M1.

the same range as the peak molecular weights in the study by Beer et al (1997) and Knuckles et al (1997). In those studies, lower peak molecular weights were obtained for samples extracted with NaOH than for samples extracted with hot water. In study by Beer et al (1997), they suspected these effects were due to degradation of the β -glucan molecule during extraction with NaOH. This might be an effect of higher concentration of NaOH because, in our study where a lower concentration of NaOH was used (25 \times lower), it was not observed. The \overline{M}_{cf} values for barley, rye, oats and oat bran for the extraction with Na₂CO₃ were lower than the peak molecular weights obtained in the study by Wood et al (1991a,b), while the \overline{M}_{cf} for malt was in the same range. The deviation between replicates for \overline{M}_{cf} was generally <5% for all three extraction methods. The average molecular weight of β -glucan can sometimes be misleading unless the molecular weight distribution is considered as well.

Percentiles describing the molecular weight at which 10, 50, and 90% of the material is smaller is, therefore, a useful complement to \overline{M}_{cf} . Comparing the percentiles for each sample from the different extraction methods showed that the shapes of the β -glucan peaks were similar for all methods. Thus, we can conclude that even if the extraction yields varied, the same populations of β -glucans were extracted.

Comparing the β -glucan profiles of the cereal products and oat-based foods from an extraction with hot water shows that there are differences in shape (Fig. 6), though oats and oat bran have profiles that look similar to that of the barley profile. These samples seem to have only one population of β -glucan, while the other samples have at least two. The p10 percentiles visualize the differences in small-sized molecules between samples while the p90 percentiles show differences in large sized molecules. The p50 percentile gives a value for the β -glucan distribution for a sample where half of the distribution has a lower molecular weight and the other half has a higher molecular weight. For a sample with a symmetrical distribution, the p50 percentile and the \overline{M}_{cf} will be the same; therefore, it may be interesting to compare these two values. No large differences were seen for \overline{M}_{cf} and the p50 percentile for oats, oat bran, and barley, while the two bread samples had the largest difference. These peaks had two populations of β -glucan, which is probably due to degradation that occurs during baking. The β -glucans from pancake batter were of lower molecular weight than all the others. The oat cereal had a profile that was similar to that of oats and, therefore, it can be assumed that the production of oat cereal does not influence the β -glucan molecular weight. The molecular weight distributions of the oat products show how easily changes in molecular weight of β -glucan due to differences in processing can be detected by this method.

CONCLUSIONS

All β -glucans studied showed a linear relationship between detector response and increasing concentration. The same response curve could be used for all samples to determine the amount of β -glucan in extracts because neither different average molecular weights of β -glucan nor sources influenced the response.

Fractionation of a sample with a broad molecular weight range was a reliable way of calibrating HPSEC-FD systems because the fractions gave peaks with narrow molecular weight distribution over a large range.

Complete inactivation of endogenous β -glucanase in samples prior to extraction is an important step to retain molecular weight during extraction and storage. Including an inactivation step of 15 min in boiling water with 50% ethanol made the extract from hot water with α -amylase or from NaOH extraction sufficiently stable

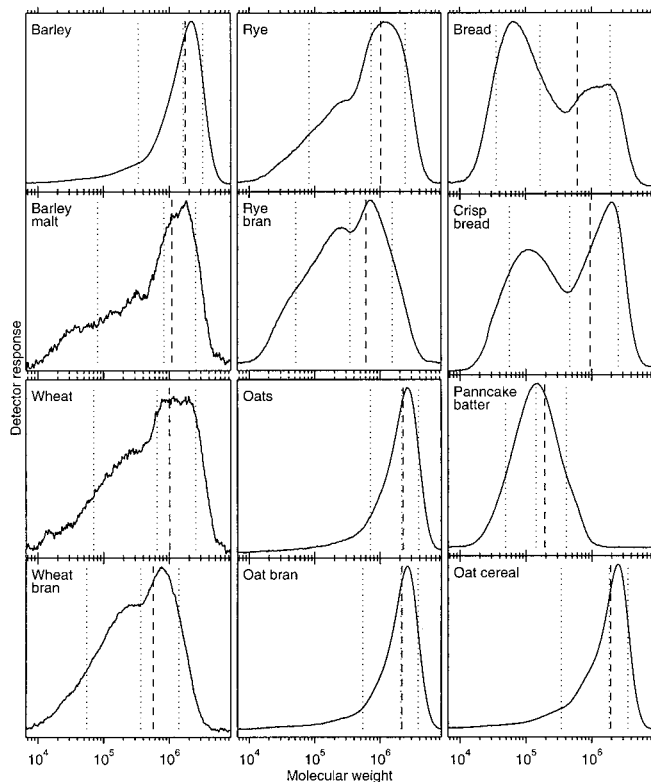


Fig. 6. β -Glucan profiles from extraction with hot water and added α -amylase of cereals products and oat-based foods. Dotted lines are the percentiles 10, 50, and 90%, while dashed line is the average molecular weight (\overline{M}_{cf}) for the sample.

TABLE II
Average Molecular Weight ($\overline{M}_{cf} \times 10^{-4}$) of β -Glucan and Percentiles ($\times 10^{-4}$) of Each Peak at 10, 50, and 90% for Hot Water Extraction with α -Amylase, NaOH Extraction, or Na₂CO₃ Extraction

	Hot Water Extraction				NaOH Extraction				Na ₂ CO ₃ Extraction			
	\overline{M}_{cf}	p10	p50	p90	\overline{M}_{cf}	p10	p50	p90	\overline{M}_{cf}	p10	p50	p90
Cereal products												
Barley	164	28.9	150	310	173	34.9	162	318	162	26.4	150	304
Barley malt	99.4	5.2	76.7	233	113	2.2	80.6	278	108	4.5	87.3	246
Rye	97.6	7.5	69.2	233	104	6.6	75.9	245	96.6	6.1	65.8	237
Rye bran	63.7	4.8	36.5	163	79.3	5.2	50.8	198	75.9	4.1	37.3	209
Wheat	98.2	5.7	61.7	246	103	2.3	63.7	267	91.8	3.6	56.4	234
Wheat bran	58.9	5.9	39.0	147	97.4	5.3	62.2	249	93.2	4.0	52.7	244
Oat	225	71.1	213	387	185	46.0	175	324	181	42.8	172	327
Oat bran	215	54.1	207	379	219	53.8	215	372	170	45.3	161	301
Oat-based foods												
Bread	64.9	3.5	16.7	206	77.2	3.2	21.2	238	59.7	3.7	19.9	178
Crisp bread	94.9	5.6	46.3	253	134	6.8	94.5	320	95.0	5.3	44.8	255
Pancake batter	19.4	4.9	14.2	40.9	20.7	4.7	14.1	42.3	19.4	4.7	13.7	40.5
Oat cereal	193	34.6	186	355	195	44.6	191	339	158	32.4	149	290

up to 24 hr, while the extract from Na₂CO₃ extraction had a continuous degradation over time that was only slowed down by the inactivation. The extraction methods gave different extraction yields for the different products but that did not significantly influence the average molecular weight or distribution of β-glucan.

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