Chromatography of Barley β-Glucans*

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Abstract

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Size exclusion chromatography with a set of two columns Separon Hema Bio 1000 and Separon Hema Bio 100 was found to give efficiency for the determination of molecular mass of β-glucans isolated from barley. The set was calibrated with dextrans of known molecular mass. β-glucans from the barley cultivars ranged in molecular mass from 1.2×10^6 g/mol to 1.5×10^6 g/mol in the case of soluble β-glucans and from 0.9×10^6 g/mol to 1.4×10^6 g/mol in the case of total β-glucans. The new Czech varieties had higher values of molecular mass in comparison to parental genotypes. The differences between molecular mass of parental genotypes of high glucan barley and new varieties were statistically significant.

Key words: β-glucans; barley; molecular mass; size-exclusion chromatography; biological value

Cereal grains contain various levels of polysaccharides hereafter referred to as β-glucans (CARR *et al.* 1990; HENRY 1987; WOOD 1984). Barley β-glucans have been studied extensively within the brewing industry and in animal feeding studies. In the brewing industry, barley β-glucans are responsible for retardation of malting, poor woort separation, difficulties in beer filtration, and formation of undesirable beer precipitates (BAMFORTH 1982).

The role of cereal β-glucans in human nutrition and health has found a great importance recently. Hull-less cultivars of barley are a potentially useful grain for enrichment of human nutrition with β-glucans (NEWMAN *et al.* 1989; NEWMAN & NEWMAN 1991; MÄLKI *et al.* 1992; BHATTY 1995). β-glucans, a part of dietary fibre, is believed to be the active cholesterol-lowering component of barley bran or flour. Hull-less or naked barley (HB) has been rediscovered in North America.

The Czech Republic tries to cultivate new spring barley materials by crossing with high glucan barley from the USA. The main aim of research is to obtain the high grain yield, the resistance to mildew and lodging. The cultivars differ not only in the content of β-glucans but also in the molecular mass of this biopolymer. The physicochemical properties, together with the molecular mass distribution of biopolymers, affect both their biological and technological activity (SNOEREN *et al.* 1975; WOOD *et al.* 1991; DIETRICH *et al.* 1992; KEY-WHANG 1998). It was confirmed (ANDERSON 1990; IDORCZYK *et al.* 1998; KNUCKLES & MEI-CHEN-MCHIU 1999) that gel chromatography or size exclusion chromatography are a valuable analytical tool for characterization of polysaccharides and other important biopolymers. The size-exclusion chromatography was also used for monitoring changes in molecular mass of β-glucan through the digestive tract of the rat (WOOD *et al.* 1991).

Size exclusion chromatography on the set of two columns Separon Hema Bio 1000 and Separon Hema Bio 100 was used for the determination of molecular mass distribution of β-glucans in new hull-less cultivars of the Czech barley. Enzymatic assay allowed to determine the content of β-glucans in samples.

MATERIAL AND METHODS

Sample preparation and extraction: Before analysis grains were ground in a Retsch ZM 1000 sample mill at 10 000 rpm to pass 0.5 mm screen and stored at 4°C until use. Dry matter was determined for 2 h at 130°C.

Samples (4 g) were extracted (CARR *et al.* 1990) twice with refluxing 80% (v/v) ethanol (100 ml) for 0.5 h each.

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The extracted residues were recovered quantitatively after cooling and dried at room temperature. The aqueous ethanol extracts were discarded.

Water-soluble β-glucan was extracted from the ethanol treated residues with water (100 ml) in a shaking water bath at 100°C for 1 h. The extracts were centrifuged to remove insoluble material and the supernatants were collected. The residues were washed with an addition 10 ml of water and the combined supernatants were then pooled and adjusted to a volume 250 ml before aliquoting.

Total (alkali soluble) β-glucan was determined similarly by extracting the ethanol-treated residues with the solution of NaOH (c = 1.0 mol/l) at room temperature for 16 h. The extraction mixtures were neutralized with solution of HCl (c = 1.0 mol/l), centrifuged to remove insoluble material and the supernatants were collected. The residues were washed with an additional 10 ml of water and the combined supernatants were then pooled and adjusted to a volume 250 ml before aliquoting.

β-glucan Assay: β-glucan was determined using highly purified lichenase (endo-1,3(4)-β-glucanase, Fluka, 7.2 U per mg) and β-glucan-D-glucosidase (Fluka, 5.8 U/mg). β-glucan was specifically hydrolyzed by lichenase to oligosaccharides, which were quantitatively cleared to glucose by β-glucosidase. Glucose was measured using glucose-peroxides-buffer mixture.

Sample aliquots were transferred to tubes containing 5 ml sodium-phosphate buffer (0.1 mol/l, pH = 6.5) and 0.5 ml of the lichenase solution (2 U, endo-1-3,1-4-β-glucanase) and incubated at 40°C for 1 h. Then to 0.6 ml aliquots in tubes 0.6 ml of the sodium acetate-HCl buffer (0.2 mol/l, pH = 4.0) containing 0.2 U β-glucosidase was added. Tubes were incubated at 40°C for 15 min.

The glucose released was measured at 520 nm, using glucose oxidase-peroxidase reagent (Glukosa, Lachema CR).

Size-exclusion Chromatography: A set of two columns Separon Hema Bio 1000 and Separon Hema Bio 100 (250 × 8 mm, particle size 10 μm, Tessek CR) and a Laboratorní přístroje (Praha, CR) model HPP5001 pump were used for size exclusion chromatography. Samples were filtered (1,2 μm membrane) before analysis. The columns set, fitted with a Separon Hema Bio 1000 guard column of 30 × 3 mm, was maintained at 20°C and eluted with sodium 2-(N-morpholino) ethane sulfonate (MES) buffer (c = 0.05 mol/l, pH = 6.5), containing sodium azide (c = 5 mmol/l) at 0.8 ml/min. An analytical valve Ecom (Praha, CR) with 200 μl loop was used for injection of samples. Waters R401 detector at sensitivity range 4 was used for refractive index (RI) detection.

The data were processed by a BASIC program of Spectra Physics integrator SP4100.

Dextran standards in range of 2×10^6–15×10^5 g/ml were dissolved by stirring in mobile phase. The regression equation was evaluated from standard data:

$$\log M = -0.33.t_r + 9.86$$

where: M – the molecular mass and t_r is the retention time

The Basic program evaluated the average M_m (average molecular mass), M_n (the number of average molecular mass) and M_m/M_n (polydispersity) in range of molecular mass from 15×10^5 to 2×10^6 g/mol.

RESULTS AND DISCUSSION

Published results indicate that β-glucans are responsible for the biological property of barley which was demonstrated in the diet of chickens namely (NEWMAN & NEWMAN, 1991; MICHNIEWICZ & GASIOROWSKI 1994). The most important effect is the hypocholesterolemic activity. Gel chromatography proved that the most effective barley is the variety with the highest molecular mass of water-soluble β-glucans. The highly branched β-glucan raises viscosity of water extracts. NEWMAN (1989) suggested that the high viscosity of barley extracts due to the degree of β-glucan polymerization is the major factor in the hypocholesterolemic effect of β-glucans.

The presented procedure together with the set of chromatographic columns Separon Hema Bio 1000 and Separon Hema Bio 100 (Fig. 1) allowed to establish the molecular mass of β-glucans in a new generation of barley materials that were developed by crossing the Czech varieties with the high glucan barley from the USA. The basic properties of parental genotypes and new materials are reviewed in Tables 1 and 2.

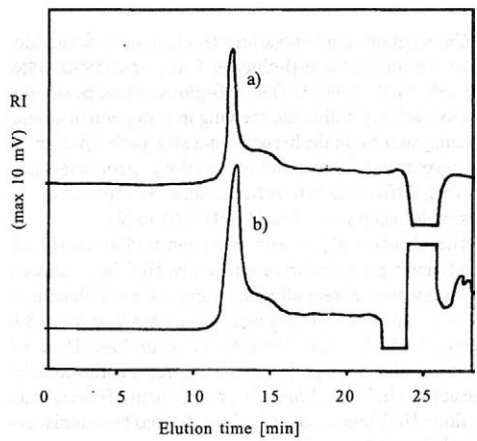

Fig. 1. Elution profile for β-glucan in water (a) and alkali (b) extracts of barley sample 6 (Wanubet)

Table 3 sums up the values of the dry solids and the total and soluble β-glucan content. The values of average molecular mass (M_m), the number of average molecular mass (M_n) and the polydispersity (M_m/M_n) of all sets of barley samples are presented in Table 4.

Before discussion on variety of parental and new barley cultivars there is a necessity to evaluate the standard deviation of all analytical procedures.

Table 1. Characterization of parental genotypes and new barley materials

Sample No	Variety	Characterization	Sample information
1	Wabet	hulled	Samples 1–8 are high glucan barley from the USA
2	Wabet	hulled	(University of Montana)
3	Wapana	hulled	
4	Wapana	hulled	
5	Wanubet	naked	
6	Wanubet	naked	
7	Washonubet	naked	
8	Washonubet	naked	
9	Akcent	hulled	Samples 9–10 are the Czech standard malt variety
10	Akcent	hulled	
11	KM 2037-124/93	naked	Samples 11–14 are the Czech naked spring barley
12	KM 2045-7/93	naked	materials which were tested for grain yield*
13	KM 2048-284/93	naked	
14	KM 2048-286/93	naked	
15**			Samples 15–24 are the Czech naked new spring barley
16	KM 2152-31/94		materials which were produced by crossing
17	KM 2154-80/94n	naked	
18	KM 2115-112/94	naked	Czech varieties KM and Dominique with high barley
19	KM 2109-167/94	naked	1–6 from the USA
20	KM 2152-44/91	hulled	High grain yield, resistance to lodging and mildew
21**		naked	were the main aim of research
22	KM2127-609/94	hulled	
23	KM 2127-415/94	hulled	
24**		naked	

*These new barleys were not developed by crossing with high glucan barley
**This variety was rejected from breeding

Table 2. Yield performance, mildew and lodging resistance of high ß-glucan, standard and new developed spring barley varieties

Sample No	Grain yield [t/ha]	Mildew scale 9–1	Lodging scale 9–1
1–2	5.99	3.0	2.00
3–4	5.43	2.3	3.30
5–6	5.14	3.5	1.33
7–8	4.45	2.5	1.50
9–10	7.17	6.4	6.20
11	7.52	7.3	3.00
12	7.15	5.8	6.33
13	8.40	5.7	4.91
14	7.73	4.8	7.0
16	11.06	8.0	5.0
17	5.12	8.0	4.5
18	7.32	5.5	3.0
19	9.40	7.8	4.0
20	6.42	5.2	4.0
22	9.11	9.0	5.0
23	9.11	7.2	6.0

In the case of water soluble β-glucans, the standard deviation of the method (Table 5) is STD = 34 g/mol and the standard deviation of the molecular mass values in the range of all varieties is STD = 132 g/mol. In the case of alkali soluble β-glucans, the standard deviation of the method is STD = 70 g/mol and the standard deviation of the molecular mass values in the range of all varieties is STD = 151 g/mol.

These results proved that the presented procedure of size exlusion chromatography allows to compare molecular mass of β-glucans of different barley varieties. The conclusion is confirmed by the value of *F*-distribution (Table 5). There is a sufficient sample evidence to warrant rejection of the claim that the standard deviation of procedure STD_2 and the standard deviation of the whole range of varieties STD_1 are equal. It means that differences between measured varieties were greater than differences of the molecular mass determination of a particular sample. The barley Wabet was chosen as a particular sample. The molecular mass determination of Wabet barley was repeated five times by both water extraction and alkali extraction.

The set of samples was then divided into four groups (Table 5), the varieties from the USA (sample No 1–8), the

Table 3. The content [%] of the dry solids and the total and soluble β-glucans in barley samples

Sample No	Dry solids	Total β-glucans	Soluble β-glucans	Sample No	Dry solids	Total β-glucans	Soluble β-glucans
1	89.3	11.7	9.9	13	90.3	7.7	2.8
2	89.1	12.6	9.1	14	90.1	6.3	2.7
3	89.2	12.7	8.6	15	90.4	7.6	3.1
4	89.9	12.6	9.0	16	90.7	7.5	3.1
5	89.9	11.4	8.6	17	90.5	6.7	2.8
6	90.3	10.1	7.8	18	90.3	6.2	2.3
7	89.8	14.8	10.8	19	90.5	6.6	3.4
8	89.6	14.2	9.4	20	90.6	6.0	2.6
9	89.3	6.0	2.6	21	90.7	6.1	2.4
10	90.3	5.8	2.0	22	90.6	7.1	2.5
11	90.1	7.3	1.5	23	90.7	6.9	2.8
12	90.1	7.1	1.8	24	90.3	6.1	3.2

standard Czech malt barley (sample No 8–9) and the new Czech cultivars (sample No 11–24). Molecular masses of groups differ from each other and the new Czech cultivars have the highest value $M_m = 1{,}4 \times 10^6$ g/mol The alkali extraction gives the lower molecular mass of estimated β-glucans generally.

The hypothesis of a relationship between the content of β-glucan in barley and its molecular mass was not con-

Table 4. The distribution of the molecular mass of the total and soluble β-glucans in barley samples

Sample No	Total β-glucans			Soluble β-glucans		
	M_m [g/mol $\times 10^{-3}$]	M_n [g/mol $\times 10^{-3}$]	M_m/M_n	M_m [g/mol $\times 10^{-3}$]	M_n [g/mol $\times 10^{-3}$]	M_m/M_n
1	998	928	1.1	1204	1148	1.1
2	1190	1082	1.1	1283	1166	1.1
3	1092	969	1.1	1248	1179	1.1
4	1205	1116	1.1	1249	1176	1.1
5	932	821	1.1	1279	1162	1.1
6	897	821	1.1	1254	1140	1.1
7	1133	933	1.2	1300	1250	1.0
8	1263	1131	1.1	1310	1191	1.1
9	1252	1156	1.1	1276	1184	1.1
10	1054	958	1.1	1169	1063	1.1
11	1198	1089	1.1	1230	1190	1.0
12	1089	990	1.1	1268	1206	1.1
13	1266	1151	1.1	1598	1510	1.1
14	810	745	1.1	1270	1155	1.1
15	1090	991	1.1	1240	1127	1.1
16	1025	948	1.1	1542	1402	1.1
17	1266	1201	1.1	1526	1422	1.1
18	1262	1227	1.0	1238	1207	1.0
19	1167	1112	1.0	1416	1318	1.1
20	1050	1004	1.0	1424	1337	1.1
21	1332	1290	1.0	1582	1454	1.1
22	1400	1321	1.1	1617	1508	1.1
23	1418	1358	1.0	1275	1159	1.1
24	1266	1146	1.1	1400	1273	1.1

Table 5. Statistical evaluation of the molecular mass differences

	Soluble β-glucans				Total β-glucans				
	M_m (average) [g/mol]	STD [g/mol]	F	F_{crit}		M_m (average) [g/mol]	STD [g/mol]	F	F_{crit}
Wabet (Sample No 1)	1 204 000	34			Wabet (Sample No 1)	998 000	70		
Samples No 1–24	1 344 000	132	15	2.8	Samples No 1–24	1 152 000	151	4.6	2.8
Samples No 1–8	1 271 000	32			Samples No 1–8	1 089 000	126		
Samples No 9–10	1 223 000				Samples No 9–10	1 153 000			
Samples No 11–24	1 402 000	143			Samples No 11–24	1 188 000	158		

Table 6. The results of biological tests on laboratory rats

Variety	Sample No	TD [%]	BV	NPU	UP [%]	NB/NP	ED [%]
Akcent	9	78.27	71.79	56.20	5.73	40.24	85.45
Wabet	1	80.75	77.64**	62.71**	6.30**	45.68**	86.73*
Wapana	3	82.22*	70.03*	57.59	6.11	42.05	86.08
Wanubet	5	82.02*	76.26**	62.55**	7.16**	44.89**	92.08**
Washonubet	7	81.55	72.61	59.22*	6.53*	42.32	91.39**

TD – coefficient of true protein digestibility in %
NPU – nett protein utilization (NPU = TD × BV/100)
NB/NP – bilance of N on unit of utilized N
*, **significant at the $P \leq 0.05$, 0.01
BV – protein biological value
UP – usable protein content in %
ED – coefficient of energy digestibility in %

firmed. There is a very low correlation between the content of soluble β-glucans and their molecular mass ($r = 0.33$).

The varieties of sample No 9 (malting barley, the low β-glucans content) and samples No 1, 3, 5, 7 (the high β-glucans content) were tested for their suitability for feeding purposes in biological growth and balance experiments in vivo using laboratory rats. The varietes for biological parameters as TD (coefficient of true protein digestibility in %), BV (protein biological value), NPU (net protein utilization, NPU = TD × BV/100), UP (usable protein content in %, UP = [NPU × content of grain protein – N × 6.25]/100), NB/NP (bilance of N on unit of utilized N) and ED (coefficient of energy digestibility in %) were evaluated (Table 6). The higher content of β-glucans did not affect the biological value of varietes No 1, 3, 5 and 7 negatively, due to coprophagie of the rats, probably.

Conclusion

The described procedure and the set of chromatographic columns Separon Hema Bio 1000 and Separon Hema Bio 100 (Fig. 1) allowed to establish the molecular mass of β-glucans in barley materials. The chromatographic procedure was worked out with an aim to qualify the new Czech generation which was obtained by crossing high β-glucan varieties from the USA and the Czech malt variety. The content of β-glucans with known molecular mass was then determined by enzymatic method. The molecular mass of individual cultivars had statistically different values but the correlation between the content of soluble β-glucans and their molecular mass was not confirmed. The biological experiments with laboratory rats didn't approve the negative effect of the higher content of β-glucans in barley on the digestibility.

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Polysacharidy označované jako β-glukany se nacházejí zejména v ječmenu a ovsu. Odrůdy ječmene pěstované na území českých zemí byly tradičně šlechtěny tak, aby obsahovaly co nejméně těchto polysacharidů. Polysacharidy β-glukany tvoří část rozpustné vlákniny lidské potravy, takže obilniny se zvýšeným obsahem těchto polysacharidů jsou v posledních letech předmětem zájmů šlechtitelů. Práce se zabývá stanovením molárních hmotností β-glukanů izolovaných z odrůd ječmene šlechtěných a testovaných v Zemědělském výzkumném ústavu Kroměříž. Pomocí gelové chromatografie na dvou kolonách zapojených za sebou (Separon Hema Bio 1000 a Separon Hema Bio 100) bylo zjištěno, že β-glukany z nových odrůd mají zvýšenou molární hmotnost ve srovnání s rodičovskými odrůdami. Rozdíly byly statisticky významné.

Klíčová slova: β-glukany; ječmen; molární hmotnost; gelová chromatografie; biologická kontrola

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