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Analysis of mixed-linked (1→3), (1→4)-β-D-glucans in cereal grains from Turkey

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Abstract

In this study, (1→3), (1→4)-β-D-glucan content of some culture type cereal grains grown in Turkey, such as barley, wheat, oats, rye, corn, beans, peas and chick peas; and barley, wheat (white, shain, lanser), rye, orabyehis sativa (adi yonca in Turkish) and medicaga satien (adi korunga in Turkish) grown in the plant research institute of the faculty of Atatürk University in Erzurum (Turkey) were determined quantitatively by enzymatic methods. By using pure β-D-glucanase and β-D-glucosidase in the experiments, (1→3), (1→4)-glycosidic bonds of linear polysaccharides found in cell-wall endosperm of plant seeds were hydrolyzed and the resulting β-D-glucans were determined by glucose oxidase/peroxidase solution and measuring the absorbances at 510 nm in a UV-spectrophotometer. The β-D-glucan levels of cultured cereal grains were compared to those obtained from other countries. Mean whole grain β-glucan levels (dry weight basis) for each grain type analyzed in this study were barley, 3.6%, oats, 4.1%, rye, 1.4% and wheat, 0.8%, corns (I, II), 1.4%, 0.5%, beans (I, II) 2.9%, 2.8%, peas (I, II), 0.9% and 1.4% and chickpeas, 0.9%. β-D-Glucan levels of each grain type grown in the Plant Research Institute of Ataturk University analyzed in this study were: barley, 2.8%, rye, 0.9%, wheats (shain, white, lanser), 5.4%, 0.5%, 0.6%, *Orabyehis sativa*, 1.4%, and *Medicaga satien*, 1.1%.

1. Introduction

Recently β-D-glucans extracted from barley, oats, rye and some fungi were used to decrease total blood cholesterol (Özdemir & Genç, 2001). More importantly β-D-glucans also increase the "good" high density lipoprotein (HDL) cholesterol and at the same time decrease the level of the "bad" low density lipoprotein (LDL) cholesterol (Inglett, 1990, 1993). β-glucan is a homopolysaccharide composed of glucose units linked together to form a long polymer chain (Inglett, 1990). Until recently, the β-glucan most studied by cereal scientists was cellulose, the water-insoluble, β-linked D-glucose-polymer. Today, however, many investigators are focusing their attention on the noncellulosic, water-soluble β-glucans of cereal and are working to define their potential role in nutrition and health (Klopfenstein, 1988). In other studies, β-glucan fractions of oats, barley, wheat and sorghum could be freeze-dried and successfully incorporated into white pan bread. Experimental breads containing 7% (of flour weight) oats, barley, wheat and sorghum β-glucan fractions were then prepared. Rats fed with β-glucan-rich breads had lower serum cholesterol levels (Klopfenstein; Klopfenstein & Hoseney, 1987). Both total serum cholesterol and liver cholesterol concentrations were generally lower in animals fed glucan-containing breads than in animals fed the control diet (Klopfenstein; Klopfenstein & Hoseney).

In addition to their cholesterol-lowering and potential cancer-protecting properties, β-glucans maybe useful in controlling blood glucose levels (Klopfenstein, 1988). (1→3)-β-D-glucose polymers of certain plants, including the common yeast, *Saccharomyces cerevisiae*, with which bakers and brewers are well acquainted, have been shown to have anti-tumor and anti-bacterial activity, when injected, as well as cholesterol-lowering activity, when ingested, by experimental animals (Henry, 1987; Klopfenstein; Lehtonen & Aikasalo, 1987; Mekis & Bendek, 1987; Wood, 1984).

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In 1963 De Groot and co-workers (1963) did a series of experiments to determine the cholesterol-lowering properties of barley, wheat, rice and oats and reported that only oat diets significantly lowered rat serum cholesterol (Klopfenstein, 1988). High-serum cholesterol levels decrease the risk (Aktaş, Keha, Yılmaz & Demirbaş, 1999). β-Glucans form viscous solutions and this can cause problems, for instance in the brewing industry (Lehtonen & Aikasalo, 1987). β-Glucans occur in all cereals but their concentration is highest in oats and barley, with values ranging from 2 to 6% (Klopfenstein). Both of these cereals have been shown to have cholesterol-lowering properties (Generald, 1983; Klopfenstein). β-Glucans in cereal-based food products have been determined and, for several different food products, soluble glucan content ranged from 0.49 to 3.90%, whereas total β-glucan content ranged from 0.58 to 8.86% (Carr, Glatter & Jeracl, 1989).

An enzymatic method for analysis of total and insoluble mixed-linked (1→3), (1→4)-β-D-glucans in barley and oats was developed (Klopfenstein, 1988). The method includes complete removal of starch (Aman & Graham, 1987). Another method for analysis of total mixed-linked β-glucans in cereal grains has been developed (Aman & Hesselman, 1985). β-glucan contents of five different winter ryes were studied at three different locations in Finland for 2 years. The values were compared to the values obtained from other countries for rye samples (Saastamoinen, Plaami & Kumpulainen, 1989). A simple and quantitative method for the determination of (1→3), (1→4)-β-D-glucan in barley flours and malt has been described (McCleary & Glennie-Holmes, 1985). The method allows direct analysis of β-glucan in flours and malt slurries. The described assay procedure can readily be applied to the analysis of β-glucan in other cereal grains.

2. Materials and methods

Cereal cultivates were obtained from southern, central and northern parts of Turkey in 1995–1996. In addition, barley, wheat (white, shain, lanser), rye, orabyehis sativa and medicaga satien were received from the plant research institute of the faculty of Atatürk University (in Turkey). The air-dried cereal samples were ground with aATecator Cyclotec with a 0.5 mm screen and then dried in an oven at 80°C for 20 h. The β-D-glucanase (EC.3.2.1.73) from *Bacillus subtilis* was obtained from Fluka (Swiss), β-D-glucosidase (EC.3.2.1.21) from Fluka Biochemika as lyophilized salt-free powder, 6 U/mg, and glucose oxidase/peroxidase (A02466) solution from Glucose Enzymatique Color.

There are several methods for the determination of (1→3),(1→4)-β-D-glucan content of cultured cereal grains. We chose to use the one developed by McCleary and Glennie-Holmes (1985) which is most appropriate and most accurate with the available equipment in our laboratory. In the procedure, highly purified enzymes were employed. The method should be easy to reproduce in any laboratory. Duplicate samples (0.5 g) of flour were weighed and β-D-glucan depolymerized with purified (1→3), (1→4)-β-D-glucanase to oligosaccharides and then hydrolyzed to glucose with specific purified β-glucosidase. Glucose was determined by glucose oxidase/peroxidase solution as D-glucose. β-glucan content was calculated using glucose quantity found in the equation:

$$\text{Glucan }(\%\text{w/w}) = \Delta\text{E}.\frac{F}{\text{mg}}.27$$

where ΔE, the absorbance difference after β-D-glucosidase treatment-blank absorbance; mg, weight of sample; and *F*, a factor for conversion of absorbance values to micrograms of glucose.

3. Results and discussion

The method developed by McCleary and Glennie-Homes (1985) is quite simple and very suitable for the determination of β-glucan in all cereals. The significant components of cereal grains are mixed-linked (1-3) (1-4) β-D-glucan (β-glucan) derived from endosperm cell walls. All cereals contain β-glucan but some cereal grains have different β-glucan contents. Variations in the β-glucan content of cereal grains grown under different environmental conditions have been observed (McCleary & Glennie-Holmes, 1985). The wide diversity in β-glucan content could be significant in the commercial utilization of cereals. In human food, a high content of β-glucan (soluble fibre) may be desirable whereas, in animal food, lower β-glucan contents may be preferable (McCleary & Glennie-Holmes). The β-glucan contents of several cereal grains were studied for 18 varieties during 2 years in Turkey (See Table 1). Significant differences were found in β-glucan contents between different experiments (Table 2). The values were compared to values obtained from the cereals samples imported from Australia, Sweden, USA, England, Finland, Canada, Holland, Germany, Hungary and Russia (Table 3).

Various cereal grains were grown under different environmental conditions but the effects of environmental factors on β-glucan content were not significant (Saastamoinen, Plaami & Kumpulainen, 1989). The increase in β-glucan content due to poor environmental conditions could not be attributed to similar growing times and mean temperatures because, in the entire sample material, there was no correlation between β-glucan content and grain yield (Table 3).

Most of the β-glucans are located in endosperm cell walls, but aleurore cell walls are also rich in β-glucans. Barley β-glucan content ranges from 2 to 10%. β-glucans form viscous solutions and they can cause problems, for instance in the brewing industry, causing very slow filtration or hazerformation (Lehtonen & Aikasalo, 1987). Among the β-glucan contents of barley, that grown in the USA ranked the highest (7.2%) and in Canada the lowest (1.7%).

Table 1
Location of cereal grains in Turkey

Grain and variety	Abbreviation	Situation	Year
Barley	BA	Ankara	1995
Oat	OA	Ankara	1995
Wheat	WH	Kırşehir	1995
Rye	RY	Gümüşhane	1995
Corn I	CO_1	Trabzon	1995
Corn II	CO_2	Trabzon	1995
Beans I	BE_1	Trabzon	1996
Beans II	BE_2	Trabzon	1996
Peas I	PE_1	Trabzon	1996
Peas II	PE_2	Trabzon	1996
Chick Peas	CP	Çorum	1996
Wheat[a]			1996
Shain[a]	SH[a]	Erzurum	1996
White[a]	WI[a]	Erzurum	1996
Lanser[a]	LA[a]	Erzurum	1996
Barley[a]	BA[a]	Erzurum	1996
Rye[a]	RY[a]	Erzurum	1996
Medicage satien[a]	MS[a]	Erzurum	1996
Orobyhis sativa[a]	OS[a]	Erzurum	1996

[a] These were obtained in the Plant Research Institute of Atatürk University (Erzurum in Turkey).

As for barley, oats contain β-glucans which are located mainly in the endosperm. β-glucan is a polysaccaride composed entirely of glucose units linked together to form a long polymer chain. Although structurally related to both starch and cellulose, its glucose units are joined together in a way that confers different properties (Inglett, 1990).

Oats are the major cereal crop in Turkey, and used as an animal feed. The β-glucan contents were compared with oat samples from other countries (Table 3). The β-glucan content of oats grown in the USA was the highest (6.6%) and that of oats grown in Sweden was the lowest (2.2%). Rye is like barley and oats but β-glucan contents of rye samples are much lower than those of barley and oats (Table 3). Wheat grains also contain β-glucan, but in lower concentrations than barley, oat and rye grains (Table 3).

Among these the crop of the plant research institute of Atatürk University ranked the highest with a β-glucan level of 5.4%. Shain species is different in appearance and physical properties from bayez, lanser and all other wheat grains in Turkey.

Table 2
Average β-glucan content and growing time, mean temperature (°C) and grain yield of cereal in Turkey

Abbreviation	Growing time (days)	Mean temperature (°C)	Grain yield (kg/ha)	β-Glucan content[a,b] (%)
BA	248	18.5	1750	3.6±0.1
OA	245	18.5	1500	4.1±0.19
WH	263	19.5	2300	0.8±0.3
RY	240	18	1200	1.4±0.2
CO I	240	16.5	750	1.4±0.1
CO II	240	16.5	750	0.5±0.2
BE I	230	17	500	2.9±0.2
BE II	230	17	600	2.8±0.1
PE I	210	16	300	0.9±0.4
PE II	210	16	200	1.4±0.2
CP	215	18	1650	0.9±0.1
WH[c]				
SH[c]	260	16.5	1800	5.4±0.5
WI[c]	260	16.5	1800	0.5±0.1
LA[c]	260	16.5	1800	0.6±0.2
BA[c]	260	16.5	1650	2.8±0.3
RY[c]	260	16.5	1100	0.9±0.3
MS[c]	220	15	170	1.1±0.2
OS[c]	200	15	140	1.4±0.2

[a]

Duncan-test	Sum of square	d.f.	Mean square	*F*-ratio
Between groups	170.68980	17	10.04	999.999
Within groups	0.01956	72	0.00272	
Total	170.70936	89		
Significance	$P < 0.05$			

[b] Content of dry matter.

[c] These were obtained in the Plant Research Institute of Atatürk University (Erzurum in Turkey).

Table 3
Avarage β-glucan content of cereal sample (%)

Grain and variety	Country of origin	Average β-glucan content		Reference
		Highest	Lowest	
Barley	Australia	4.6	3.8	Henry, 1987
Barley	Sweden	4.5	3.0	Aman and Graham, 1987
Barley	USA	7.2	4.3	Prentice, Babler and Faber, 1980
Barley	England	6.0	4.0	Ahluwalia and Ellis, 1984
Barley	Finland	5.2	2.8	Lehtonen and Aikasalo, 1987
Barley	Canada	2.8	1.7	Wood and Weisz, 1984
Barley	Holland	4.9	–	Carr et al., 1989
Oat	Sweden	3.2	2.2	Aman and Graham, 1987
Oat	USA	6.6	4.8	Prentice et al., 1980
Oat	Canada	4.9	3.2	Wood and Weisz, 1984
Rye	USA	2.9	–	Prentice et al., 1980
Rye	Canada	1.5	–	Wood and Weisz, 1984
Rye	Finland	1.6	–	Saastamoinen et al., 1989
Rye	Germany	1.3	–	Saastamoinen et al., 1989
Rye	Hungary	1.9	–	Saastamoinen et al., 1989
Rye	Russia	2.0	1.8	Saastamoinen et al., 1989
Rye	Sweden	1.3	1.2	Lehtonen and Aikasalo, 1987
Wheat	USA	1.4	–	Prentice et al., 1980
Wheat	Sweden	0.54	0.47	Aman and Hesselman, 1989

Corn I, Corn II and Beans I, Beans II and Peas I, Peas II are different species. Corn is grown for human food and beans are also a good food source for humans. Peas are similar. Chickpeas are consumed in dry form. *Medicage satien* and *Orobyhis sativa* are grown to feed animals in the winter. The values obtained from chickpeas are very similar to *Orobyis sativa* but the values of the other species are significantly different (Table 2).

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