Determination of Water-Insoluble β-D-Glucan in the Whole-Grain Cereals and Pseudocereals

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Abstract

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Water-insoluble β-(1,3)-D-glucan (lichenan) was determined in 43 samples of various cereal (oats, barley, wheat, millet) and pseudocereal (buckwheat, amaranth) cultivars using a modified procedure with fungal α-amylase (Fermizyme P 300). The content of water-insoluble β-glucan varied in the dependence on the cereal species and cultivars. The highest content was registered in covered oat cultivars (Cyril and the new breeding cultivar PS-100) ranging from 26.7 to 28.2 g/100 g dry matter (d.m.) followed by less traditional cereals such as millet (*Panicum miliaceum* L.), amaranth (*Amaranthus* sp. L.), and buckwheat (*Fagopyrum*) – more than 20 g/100 g d.m. A somewhat lower average content of water-insoluble β-glucan was found in wheat – 12.7–16.2 g/100 g d.m., in spelt wheat – 8.5 g/100 g d.m., and in oats – varying between 15.3 and 18.7 g/100 g d.m.

Keywords: β-glucan; determination; cereals; pseudocereals; functional foods

The human organism needs a large amount of various substances for its correct functioning, some of which as, for example essential amino acids, some vitamins and minerals, are inevitable for life and therefore their supply into the human body is necessary. However, besides these essential components, there are many compounds the intake of which is not unavoidable for man but their consumption favourably affects the state of the organism (Andlauer & Fürst 2002; Lukáčová & Karovičová 2003). Such substances include isolated nutrients, nourishing supplements, modified "designer foods", functional foods, plant products, and processed products such as cereals, soups, and juices which are rich in phytochemicals and have the protective effect against some diseases (Charalampopoulos *et al.* 2002).

In recent years, the research has been oriented to cereals because of their potential to enable the development of functional foods. Cereals cover as much as 73% of the total world fertile soil and have a 60% share in the world production of foods providing fibres, proteins, energy, minerals, and vitamins required for the human health. As a food, cereals are relatively cheap and, at the same time, they are also an important source of β-glucans. The most significant cereals are oats (*Avena sativa* L.) (2.2–11%) and barley (*Hordeum vulgare* L.) (1.7–33%), depending on the species and cultivar (Jadhav *et al.* 1998).

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The chemical analysis has confirmed that β-glucans constitute as much as 75% of the endosperm cell wall (Prugar 1999; Grundas 2003; Hareland & Manthey 2003; Lindhauer & Dreisoerner 2003; Mazza & Oomah 2003; Kling & Hayes 2004; Lambo *et al.* 2005). Glucans are characterised by a reduced absorption in the intestine, which leads to increased viscosity and to the subsequent slowing down of the gastric evacuation (Mälkki & Virtanen 2001). This results have the importance for the reduction of LDL cholesterol and subsequently lead to a decreased risk of cardiovascular disease, to a decreased glucose level in blood after meals, as well as to the adequate response of insulin (Cavallero *et al.* 2002).

The occurrence, source, structure, and biological effects of β-glucans were already mentioned in detail in our previous experiment in connection with the evaluation of the higher bioactivity of youghurts prepared with the addition of hydrocolloids of fungal β-glucans (Hozová *et al.* 2004). Since no uniform analytical method for the determination of β-glucans in foods and food products exists so far in Slovakia, we paid attention to the choice and introduction of a suitable method for the isolation and detection of water-insoluble β-glucan. In the foreign literature, the application of enzymatic methods on the use of α-amylase, lichenase, and β-glucoseoxidase (Zhang *et al.* 2002; Megazyme 2004) is often an object for discussion. For the detection are used: FIA-CALCOFLUOR methods (Navarro *et al.* 1995; Marklinder *et al.* 1996), HPLC (Pérez-vendrell *et al.* 1995), capillary electrophoresis (Johansson *et al.* 2004), UV-spectra (Genc *et al.* 2001; Demirbas 2005) or IR-spectra (Virkki *et al.* 2005), or non-enzymatic methods such as, for example, filtration and centrifugation dialysis to quantify the dietary fibre (Lambo *et al.* 2004). The dates are documented in Table 1. However, the authors do not indicate whether the point in question is the determination of water-insoluble, soluble, or total β-D-glucan in the cereals analysed. In our work, forty-three model samples of various cereals and pseudocereals (oats, barley, millet, buckwheat, and amaranth) were used with the aim to verify the suitability of the selected analytical procedure for the determination of water-insoluble β-(1,3)-D-glucan, and to compare our results with the literature data. In addition to our primary methodical aim, we tried to determine the breeding of cultivars, namely the genotypes with a significant content of β-glucans that are perspectively thought as a source for the development and production of functional foods, differing from foods commonly used up to now (cereal breakfast, drinks, sauces, youghurts, etc.).

Table 1. Content of cereal β-D-glucans

Cereals	β-glucan yield (%)	Method/detection	Source
Barley	3.70	enzymatic/IR spectra	Virkki *et al.* (2005), Johansson *et al.* (2004)
	3.49–4.10	enzymatic (Megazyme)/FIA, HPLC	Pérez-Vendrell *et al.* (1995)
	2.98–8.62	Megazyme	Zhang *et al.* (2002)
	4.70–5.10	enzymatic	Saulnier *et al.* (1994)
	1.70–7.20	enzymatic/UV spectra	Genc *et al.* (2001)
	3.20–4.60	enzymatic/UV spectra	Demirbas (2005)
	1.90–4.00	enzymatic/FIA, Calcofluor	Marklinder *et al.* (1996)
	30.0–33.3	physicochemical	Lambo *et al.* (2005)
Oats	4.00	enzymatic/IR spectra	Virkki *et al.* (2005), Johansson *et al.* (2004)
	2.20–6.60	enzymatic/UV spectra	Genc *et al.* (2001)
	3.90–5.70	enzymatic	Demirbas (2005)
	10.9–11.0	physicochemical	Lambo *et al.* (2005)
Wheat	0.47–1.40	enzymatic/UV spectra	Genc *et al.* (2001)
	0.50–1.10	enzymatic/UV spectra	Demirbas (2005)
Spelt	0.60–1.20	enzymatic/UV spectra	Demirbas (2005)

MATERIALS AND METHODS

Material. For the experimental detection of β-glucan, 43 samples of 6 cereal varieties were used. This number incorporated 20 samples of oats, 6 samples of winter barley, 7 samples of spring wheat, 5 samples of durum wheat, 1 sample of spelt wheat (*Triticum spelta* L.), 2 samples of buckwheat, 1 sample of millet, and 1 sample of amaranth. The Department of Genetic Plant Sources at the Research Institute of Plant Production at Piešťany, Slovak Republic, supplied forty-two samples. The spelt wheat sample was bought in the trade network (Marianna, Ivánka pri Dunaji, Slovak Republic.) A survey of the cereal cultivars analysed and their origin is summarised in Table 2.

Whole grain was ground with weeds on the laboratory vibrating VM4-386 mill produced in 1994. In this way, the wholemeal samples, from which the same amount (1 g with accuracy of 1 mg) was weighed in three parallel doses (n = 3), were prepared. For the degradation of polysaccharides, the enzyme Fermizyme P 300 (fungal amylase, 7000 FAU/g DSM Bakery Ingredients, production 09/2001, batch 1255016 delivered by the firm ADIVIT, Nitra, Slovak Republic) was used.

IR-spectrophotometer Nicolet, Magna FTIR, Germany, 4000–400 cm^{-1} (Department of Inorganic Chemistry of the Faculty of Chemical and Food Technology, Slovak Republic) was used.

Methods. The samples were prepared under pilot plant conditions according to the modified procedure of Kuniak *et al.* (1992). The technological scheme of β-glucan determination is shown in Figure 1.

The IR-spectrum must have absorption bands at the wavelengths of 580, 890, 1042, 1075, 1155, 1200, 1255, 1315, 1400, 1620, 2120, 2922, and 3420 cm^{-1} in the KBr tablet (5 mg of the isolated glucan spread in 800 mg KBr) and must correspond to the spectrum of the comparative substance. The presence of β-(1,3)-D-glucan (lichenan) is confirmed by the absorption band at 893 nm (Firm Norm 01/01, 2001).

The determination was done according to STN ISO 712 (1993). This international standard defines the practical reference method for the determination of water in cereals and cereal products.

Statistical evaluation. Means ($\bar{x}$), standard deviations (SD), s_r (%), $R_{max-min}$, and the test of coincidence of arithmetic means by the Lord u-test (criterion u) on the significance level $P \leq 0.05$ (Chatfield 1995).

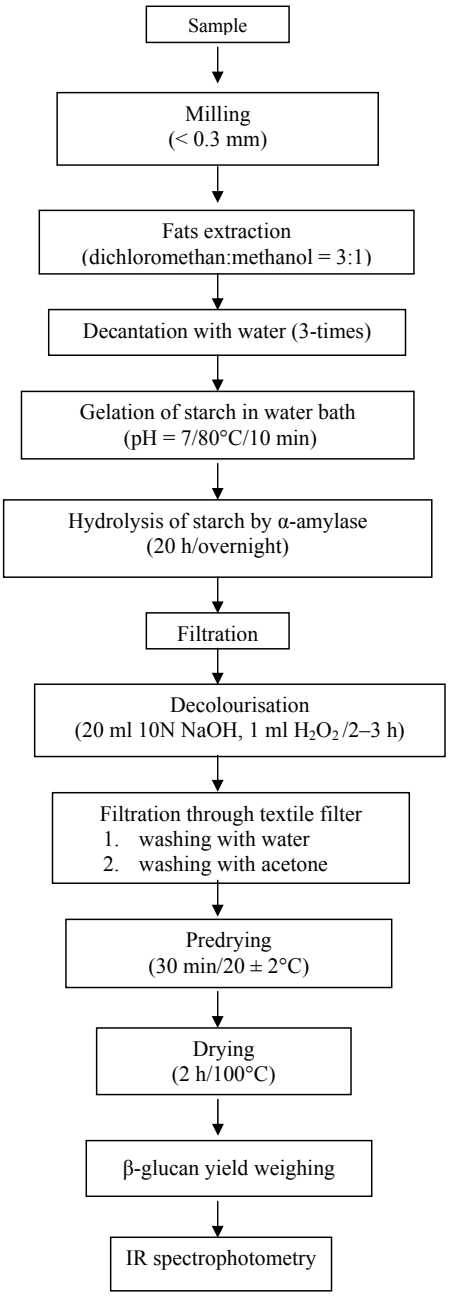

Figure 1. Technological scheme of the water-insoluble β-(1,3)-D-glucan determination

RESULTS AND DISCUSSION

Verification of the appropriateness of the method used

Before the determination of water-insoluble β-glucan in cereal raw materials, i.e. at the first stage of our experiment, it was necessary to verify the appropriateness of the method used (accuracy and reproducibility) by means of the basic statisti-

Table 2. Survey of cereal and pseudocereal cultivars studied

Cereals	Cultivar	Country of origin
Oats	Zvolen (*Avena sativa* L.)	Slovak Republic
	PS-100	Slovak Republic
	PS-90	Slovak Republic
	Cyril (*Avena sativa* L)	Czech Republic
	Neklan (*Avena sativa* L. var. *aurea* Koern.)	Czech Republic
	PS-106	Slovak Republic
	Detvan (*Avena sativa* L.)	Slovak Republic
	Expander (*Avena sativa* L.)	Austria
	Adam (*Avena sativa* L.)	Czechoslovakia
	Azur (*Avena sativa* L.)	Czech Republic
	Izak (*Avena sativa* L.)	Czech Republic
	Ardo (*Avena sativa* L.)	Czechoslovakia
	Dalimil (*Avena sativa* L.)	Czech Republic
	Abel (*Avena sativa* L.)	Czechoslovakia
	Auron (*Avena sativa* L. var. *aurea* Koern.)	Czechoslovakia
	Flamingsstern (*Avena sativa* L. var. *aurea* Koern.)	Germany
	Euro (*Avena sativa* L).	Austria
	Jakub (*Avena sativa* L.)	Czech Republic
	Roxton (*Avena sativa* L. var. *aurea* Koern.)	Canada
	SV-5	Slovak Republic
Wheat	Astella (*Triticum aestivum* L. var. *lutescens* (Alef.) Mansf.)	Czechoslovakia
	Kris (*Triticum aestivum* L. var. *lutescens* (Alef.) Mansf.)	Germany
	Ekspromt (*Triticum aestivum* L. var. *aestivum*)	Ukraine
	Niagara (*Triticum aestivum* L. var. *lutescens* (Alef.) Mansf.)	Czech Republic
	Banquet (*Triticum aestivum* L. var. *lutescens* (Alef.) Mansf.)	Czech Republic
	Altar (*Triticum durum* DESF. var. *leucurum* (Alef.) Koern.)	Mexico
	Aconchi (*Triticum durum* DESF. var. *leucomelan* (Alef.) Koern.)	Mexico
	Kucuk (*Triticum durum* DESF. var. *leucomelan* (Alef.) Koern.)	Mexico
	Olinto (*Triticum durum* DESF. var. *leucurum* (Alef.) Koern.)	Italy
	Yavaros (*Triticum durum* DESF. var. *leucomelan* (Alef.) Koern.)	Mexico
	Leguan (*Triticum aestivum* L. var. *aestivum*)	Czech Republic
	Aranka (*Triticum aestivum* L. var. *aestivum*)	Czech Republic
	Spelt (*Triticum spelta* L.)	Slovak Republic
Barley	Regina (*Hordeum vulgare* L. subsp. *distichon* (L.) Koern. var. *nutans* Schuebl)	France
	Okal (*Hordeum vulgare* L. subsp. *vulgare* var. *hybernum* VIL.)	Czechoslovakia
	Luxor *(Hordeum vulgare* L. subsp. *vulgare* var. *hybernum* VIL.)	Czechoslovakia
	Barolo (*Hordeum vulgare* L. subsp. *distichon* (L.) Koern. var. *nutans* Schuebl)	France
	Virac (*Hordeum vulgare* L. subsp. *distichon* (L.) Koern. var. *nutans* Schuebl)	Germany
	Oriflame (*Hordeum vulgare* L. subsp. *distichon* (L.) Koern. var. *nutans* Schuebl)	France
Buckwheat	Spacinska 1 (A) (*Fagopyrum esculentum* Monech.)	Slovak Republic
	Spacinska 1 (B) (*Fagopyrum esculentum* Monech.)	Slovak Republic
Amaranth	(*Amaranthus* sp. L.)	Slovak Republic
Millet	Unikum (*Panicum miliaceum* L.)	Czechoslovakia

Table 3. Results of statistical analysis of water-insoluble β-glucan determination from oats cv. Roxton

Yield (%)	
A	B
23.97	23.51
23.98	23.63
23.99	23.66
24.64	24.09
25.06	24.11
25.05	24.31
25.03	24.62
25.21	24.81
25.28	25.01
25.90	25.21
$(\bar{x}_A) = 24.811$	$(\bar{x}_B) = 24.296$
$R_A = 1.93$	$R_B = 1.70$
$\pm s = 0.653$	$\pm s = 0.602$
s_r (%) = 2.6	s_r (%) = 2.5
$u = 0.1419$	

$\bar{x}$ = mean value, n = 10; $R_{A,B}$ = max–min value between series A, B; $\pm s$ = standard deviation; s_r (%): relative standard deviation; u = criterion ($P \leq 0.05$)

cal indices ($\bar{x}$, s, s_r) as well as by means of the test of coincidence of arithmetic means (criterion u). The water-insoluble β-glucan was isolated from 10 parallel weighed doses (with accuracy of 1 mg) of the oat cultivar Roxton and evaluated after comparing the results of two independent series of experiments A and B (between two laboratories). The results achieved by the statistical analysis are summarised in Table 3. The applied Lord test of coincidence of arithmetic means showed that the difference between the acquired means (n = 10) of the water-insoluble β-glucan content in the oat cultivar Roxton (calculation of g/100 g flour) was insignificant. The u value for the significance level $P \leq 0.05$ fulfills the condition $u \geq u_{k(n,P)}$. and therefore the zero hypothesis can be accepted for this test.

Proper determination

Figures 2–5 illustrate graphically the average yields (%) of water-insoluble β-glucan (n = 3) in oats, barley, and wheat samples as well as in the less traditional crop-buckwheat, millet, and amaranth. The results are calculated to g/100 g d.m. (the d.m. content of the flour was ranging around 90 ± 1%); the examples of the absorption spectra of the cereal cultivars chosen are indicated in Figure 6.

Oats

As can be seen from the results in Figure 2, the values of the content of the isolated water-insoluble β-glucan in 20 oat samples differ substantially. The highest content was determined in the cultivar Cyril (28.2 g/100 g d.m.), the lowest one in the cultivar

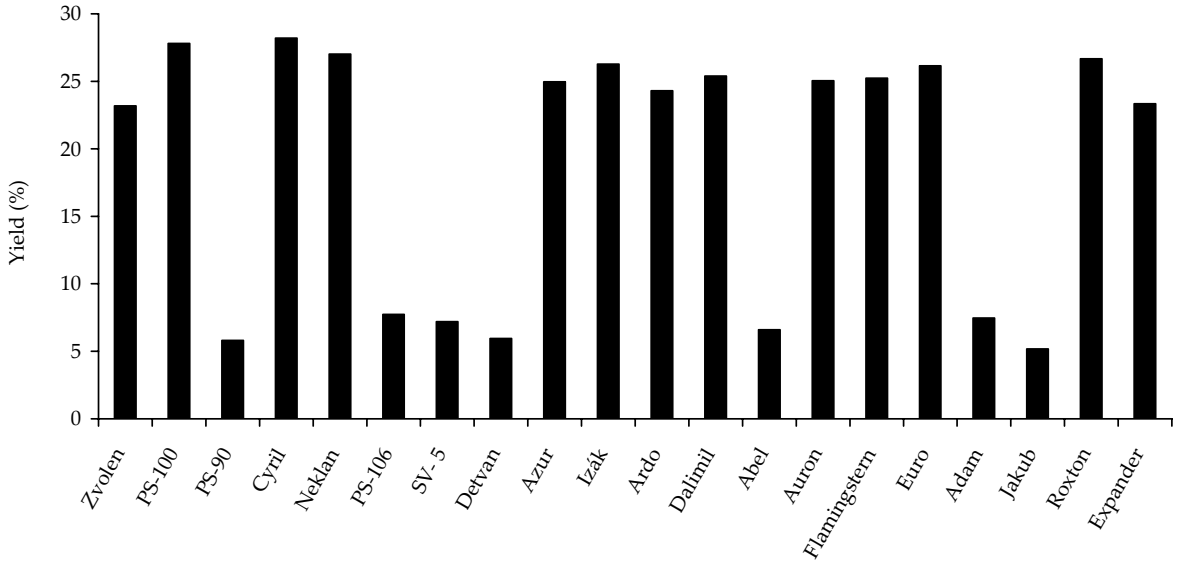

Figure 2. Content of water-insoluble β-glucan (%) in 20 oat samples ($\bar{x}$, n = 3)

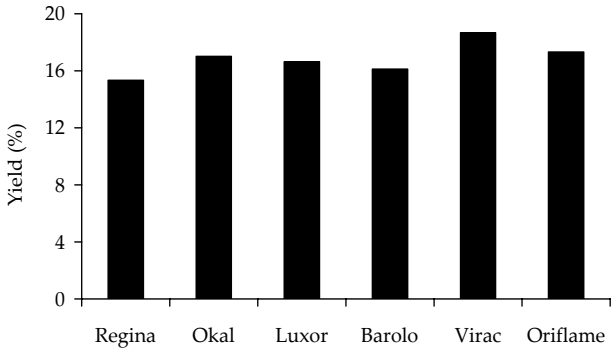

Figure 3. Content of water-insoluble β-glucan (%) in 6 barley samples ($\bar{x}$, n = 3)

Jakub (5.18 g/100 g d.m.). In Slovak oat cultivars, the content of water-insoluble β-glucan ranged from 5.8 g/100 g (cv. PS-90) to 27.81 g/100 g d.m. (cv. PS-100). In the accessible literature data (Table 1), one can learn that the content of β-glucans in oats covers the interval from 2.2 g/100 g d.m. to 11 g/100 g d.m. About 30% of our results are comparable with these values. Similar values were determined by us in the following cultivars: Jakub, Abel, Adam, Detvan, SV-5, PS-106 and PS-90. Other cultivars showed much higher contents of water-insoluble β-glucan.

Simultaneously, the results of our experiment confirm that β-glucans are found mainly on the surface casing of cereal-grain layers. Naked oat cereals showed the lowest average values of the β-glucan content (from 5.18 g/100 g d.m. in the cv. Jakub up to 7.74 g/100 d.m. in the cultivar PS-106), while the values in covered cultivars ranged from 23.18 g/100 g (cv. Zvolen) to 28.2 g/100 g dry matter. The presence of β-(1→3), (1→4)-D-glucan in the concentrate is confirmed by a peak in the region of 890 cm^{-1} which can be clearly visible on all absorption curves. In our case, absorption maxima were recorded at 896 cm^{-1} corresponding to lichenan (cv. Cyril) (Figure 6a).

Barley

In the barley samples, the contents ranged from 15.34 g/100 g d.m. (cv. Regina) to 18.67 g/100 g dry matter (cv. Virac) (Figure 6b). The data obtained from the world-wide accessible sources indicate the range of the β-glucan content in barley from 1.7 g/100 g d.m. to 33.3 g/100 g d.m. (Table 1). It is obvious that the results of our determination correspond more or less to those obtained by other authors' analyses. The IR-absorption curve in Figure 6b proves the presence of β-(1→3), (1→4)-D-glucan in the selected barley sample (cv. Virac). The peak in the region of 897 cm^{-1} is clearly visible.

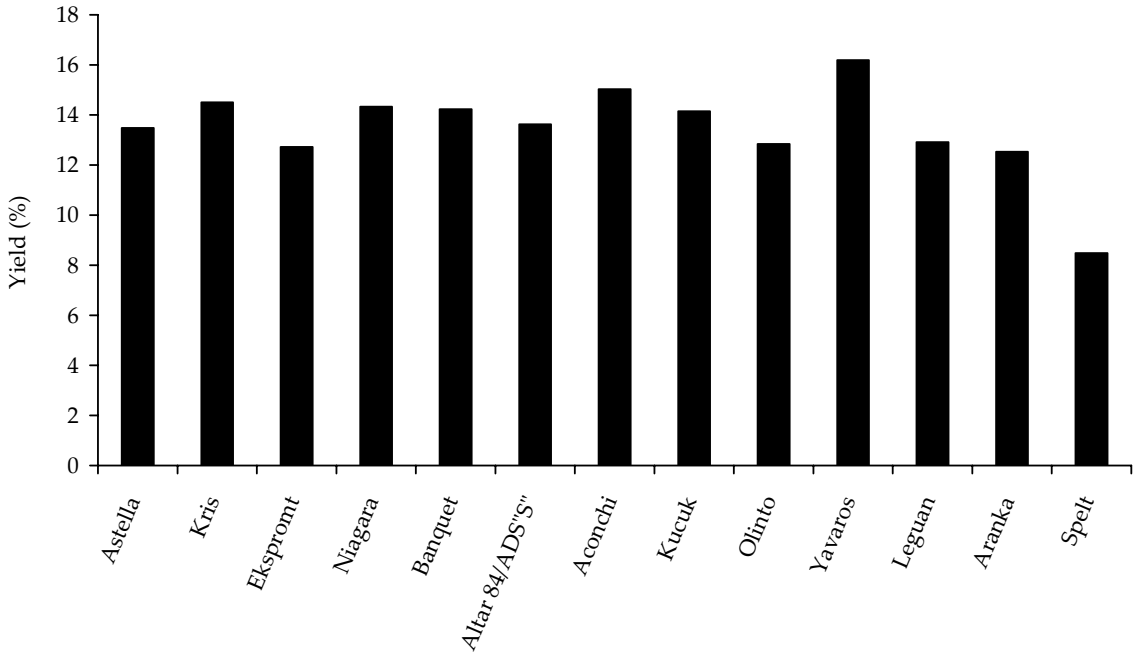

Figure 4. Content of water-insoluble β-glucan (%) in 13 wheat samples ($\bar{x}$, n = 3)

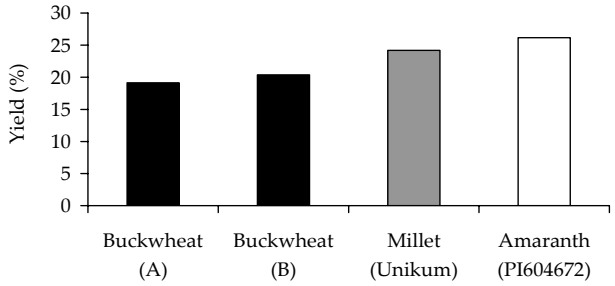

Figure 5. Content of water-insoluble β-glucan (%) in buckwheat, millet, and amaranth samples ($\bar{x}$, n = 3)

Wheat

The average insoluble β-glucan contents in the dry matter of wheat samples ranged from 8.48 g/100 g d.m. to 16.09 g/100 g dry matter. The lowest β-glucan content was estimated in spelt wheat and the highest one in the cultivar Yavaros (Figure 4). The accessible world-wide literature indicates the β-glucan content in wheat in the range of 0.47 g/100 g d.m. and 1.4 g/100 g d.m (Table 1). Our results indicate that they exceed the afore-said values several times. In the wheat cultivars, analysed the yield of insoluble β-glucan was on average twice as high as the above-mentioned values. The absorption curves in Figure 6c refer to the presence of β-(1→3), (1→4)-D-glucan in the concentrate. The peak at the absorption maximum of 894 cm^{-1} (cv. Kucuk) can serve as an evidence.

Buckwheat, millet and amaranth

Figure 5 presents the results obtained by the detection of insoluble β-glucan in less traditional cereals and pseudocereals such as buckwheat (A and B), millet, and amaranth. They show that the content of insoluble β-glucan is influenced also by climatic conditions during the year of cultivation and by the quality of the agricultural soil in which the cereal was grown. In the case of the buckwheat cv. Spacinska (A and B) these two factors reflected in the slight difference of the average β-glucan content of 1.26 g/100 g d.m.

Figure 5 shows that the less common cereals and pseudocereals contain more insoluble β-glucan in comparison with common crops. As for the β-glucan content, broom corn millet and amaranth can be compared to our best results obtained in

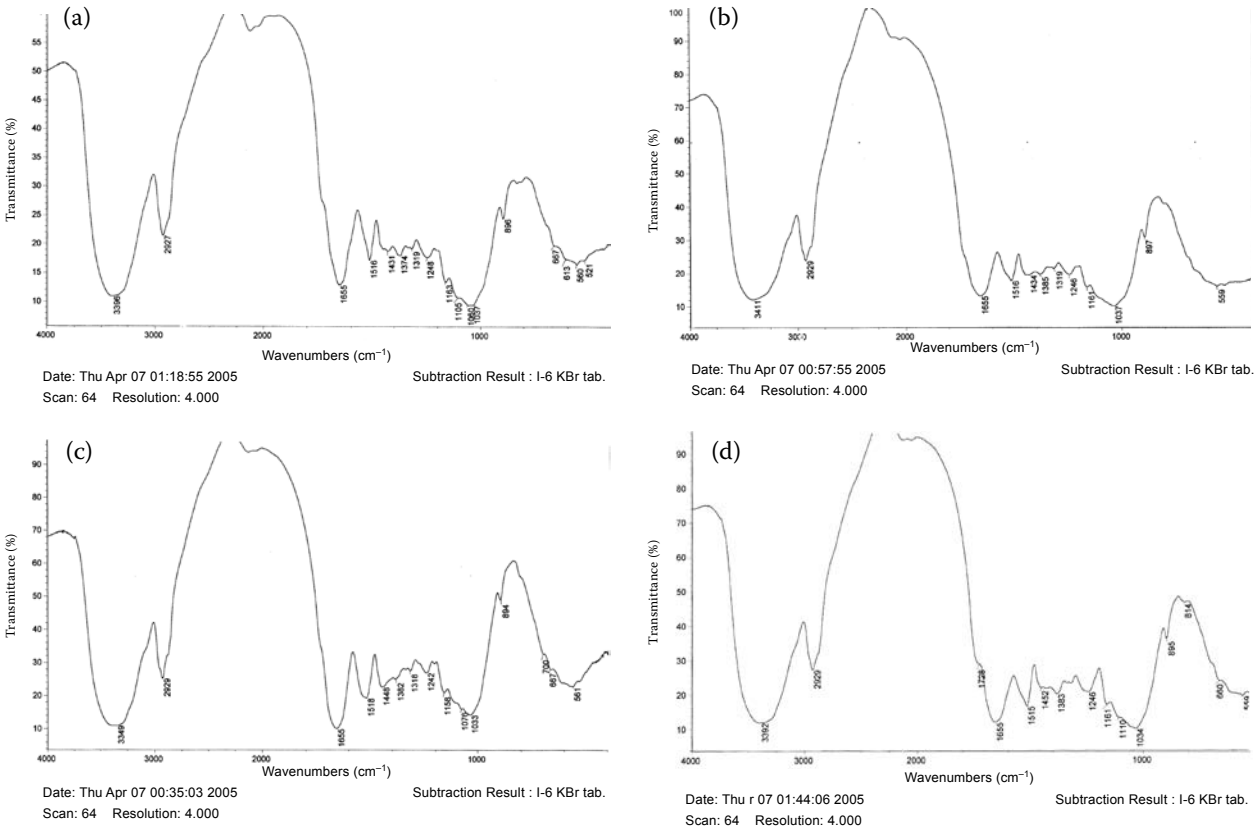

Figure 6. Examples of IR spectra: oats (a), barley (b), wheat (c), buckwheat A (d)

the case of the oat sample (24–26 g/100 g d.m.). Figure 6d illustrates the absorption IR-spectrum of the buckwheat cv. Spacinska 1 (A) (895 cm^{-1}).

CONCLUSIONS

On the basis of the experimental results, it is possible to conclude that some cereal cultivars, mainly oats, are a convenient source for the production of high fiber functional foods due to the high content of insoluble β-glucan. A very important role will be played in the future by the easily accessible newly cultivated Slovak oat cultivars containing a high amount of β-glucans. As an example can be indicated the cultivars of Zvolen and PS-100 (Research Plant Breeding Station, Vígľaš, Slovak Republic) which will certainly find an extensive application as a raw material and an additive to high fiber functional foods in the food practice. Their consumption can considerably contribute to the improvement of the health state of the population.

Among the accessible non-traditional crops, the attention should be directed to buckwheat which has so far not been sufficiently appreciated in the food practice. With regard to its high β-glucan content (about 20%) and well-known nutritional (lysine) and therapeutic (flavonoids) effects, buckwheat can be employed with good prospects as the next important source of functional foods. From the point of view of the total evaluation of the results, it is possible to maintain that our values are higher than those published by other researchers . This fact can be attributed to many factors such as the choice of the cultivar (purposefully cultivated for the enlargement of the monitored parameter), differentiated treatment of the sample designed for the analysis (fractionation), the applied method of determination (water-soluble, water-insoluble, total β-glucan), etc, which the authors mostly do not comment in their works. It is important for these data to be specified because, for example, soluble β-glucan constitutes only about 1/3 to 1/4 of the total glucan value (according to our previous experimental experiences with Megazyme). Besides this, in the word the most frequently applied enzymatic method Megazyme (2004) is designed only for the detection of soluble glucan (mainly in malting barley) performed with the accuracy of up to 4% of the total β-glucan. However, as the decisive factor in the evaluation should be considered the immunostimulative effect of β-glucans present in food and their beneficial influence on the total improvement of the health state of the man.

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