

The content of water-soluble and water-insoluble β -D-glucans in selected oats and barley varieties

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Abstract

In this paper, data on the inter-variety variability in chemical composition of oats and barley are summarized. The obtained results show that the content of soluble glucans decreases in the following order: barley (3.75 ± 0.14 – 7.96 ± 0.09 g/100 g of dry mass) \geq naked oats (3.91 ± 0.15 – 7.47 ± 0.06 g/100 g of dry mass) $>$ hulled oats (1.97 ± 0.08 – 4.09 ± 0.19 g/100 g of dry mass), whereas the content of insoluble glucans decreases in the order: hulled oats (33.73 ± 1.55 – 13.79 ± 0.51 g/100 g of dry mass) $>$ barley (10.89 ± 0.60 – 21.70 ± 0.73 g/100 g of dry mass) $>$ naked oats (5.15 ± 0.06 – 10.80 ± 0.54 g/100 g of dry mass). When comparing the content of insoluble β -glucan in whole flour, bran and flour it was found that the content decreases from the outer coat to the endosperm. These results were confirmed for both cereals mentioned.

In this work, the influence of warehousing duration on the change in quantity of soluble β -glucans when stored at room temperature (25 ± 2 °C) and in a refrigerator (8 ± 2 °C) was monitored. From the results obtained, it can be concluded that the content of soluble β -glucans decreases with time. Slower alteration in soluble β -glucans content was detected in samples stored in refrigeration; however, lower temperatures did not halt the decrease of soluble β -glucans content in ground samples. The results show the need for stating the duration of warehousing from the time the sample was processed.

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1. Introduction

Oats and barley belong to the group of crops with high energy and nutritional value arising from a high content of biologically valuable proteins, high portion of lipids compared to other cereals, favourable saccharide composition as well as from significant levels of dietary fiber, vitamins and mineral substances (Demirbas, 2005; Jakubecová,

2004). β -D-Glucans have a positive impact on human health in terms of lowering cholesterol and blood glucose levels (Maier, Turner, & Lupton, 2000), increasing immunity against infection and the utilization of β -D-glucans in reducing diets (Minárik, 2004). Reduction of absorption in the bowel, typical for glucans, results in increased viscosity followed by deceleration of gastric excretion. This results in the reduction of LDL cholesterol level (Kerckhoffs, Hornstra, & Mensink, 2003) as well as glucose level and consequently in the prevention of cardiovascular diseases (Liu et al., 2005; Mäkki & Virtanen, 2001). Glucans have a beneficial effect for constipation, haemorrhoids as

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well as probiotic activity. In addition, glucans reduce gall bladder problems and the symptoms of Crohn's disease (Zadák, 2003), lower the risk of absorbing various substances from the bowel and thereby minimize the intake of undesirable substances. Glucans also act as immuno-stimulants (Dongowski, Huth, & Gebhardt, 2003).

Within the context of health protection, nutritionists today focus mainly on one component of fiber, (1 → 3)(1 → 4)- β -D-glucans. Glucan structure consists of cellotriosyll (58–72 %) and cellotetraosyll (20–34 %) units bonded by the β -(1 → 3) linkages; in small amounts, parts bonded by (1 → 4) linkages also occur (Holtekjølén, Uhlen, Bråthen, Sahlstrøm, & Knutsen, 2006; Lazaridou, Biliaderis, Mcha-Screttas, & Steele, 2004; Roubroeks, Anderson, Mastromauro, Christensen, & Aman, 2001). β -Glucans found in yeasts, fungi (Sugawara, Takahashi, Osumi, & Ohno, 2004), algae and bacteria have the (1 → 3) and (1 → 6) linkages in their molecules. β -Glucans constitute a dominant component of the cell wall in cereals (Demirbas, 2005; Trogh et al., 2004; Virkki, Johansson, Ylinen, Maunu, & Ekholm, 2005). The molecular weight of β -glucans ranges from 13,900 to 15,000 Da (McCarthy, Hanniffy, Lalor, Savage, & Tuohy, 2005; Roubroeks, Skjak-Braek, Ryan, & Christensen, 2000).

Structure has an impact on the water solubility of β -glucans. Extensive research has been done on the structure and properties of water-soluble β -glucans in contrast to water-insoluble β -glucans (Johansson et al., 2000; Ren, Ellis, Ross-Murphy, Wang, & Wood, 2003). The content of β -glucans in various cereals, according to the literature references is shown in Table 3. As indicated by the study carried out by Johansson et al. (Genc, Mustafa, & Demirbas, 2001; Johansson, Tuomainen, Ylinen, Ekholm, & Virkki, 2004), which examined the structural differences between soluble and insoluble β -glucans in oats and barley, the molecular weights of soluble β -glucans are greater than those of the insoluble glucans, however, this ratio does not differ between oats and barley. Izydorczyk, Marci, and MacGregor (1998) studied the structural properties of non-starch polysaccharides with the result that, in contrast to the insoluble β -glucans, the soluble β -glucans have a greater ratio of β -(1 → 4) linkages as well as of cellotriosyll units. The more (1 → 4) linkages present in the molecule, the lower is the solubility found in the polymers. The most soluble polymers comprise approximately 30% of (1 → 3) linkages and 70% of (1 → 4) linkages (Lambo, Öste, & Nyman, 2005). The solubility in cereals decreases in the following order: oats > barley > wheat. The structure of β -glucan is of significant importance in activation of the immune system, with chains emerging laterally from the main chain, playing a crucial role in this process. Multipath branching of glucan, as well as its higher molecular weight, activates the immune system more intensely.

Cereals present potentially, the least expensive source of glucans and can thereby satisfy the first and determining condition for their application as nutritive supplements and food ingredients to functional foods accessible to the

general public (Hozová & Šturdík, 2005). The addition of cereal β -glucans to food increases the nutritional value of food and improves the quality parameters, in particular, the stability during warehousing (Liu et al., 2005). Cereals offer many possibilities for preparing functional foods. Suggestions for increasing nutritional and sensory value can be found in the literature which recommends the exploitation of cereals in the form of oat flakes, oat milk, pudding, yoghurt (Hozová, Kuniak, & Kelemenová, 2004), biscuits (Sudha, Vetrimani, & Leelavathi, in press), cereal gruel as a dessert, cereal gravy (Müller, Bohatiel, Blortz, & Frank, 1995), fruit juices and drinks (Kovacs, 1998) as well as cereal soups (Gormley & Morrissey, 1999). It is also appropriate to produce various types of bread, store-bread and noodles using oat flour instead of wheat flour (Plaami & Kumpulainen, 1994).

In this paper, the inter-variety variability of oats and barley based on the content of water-soluble and water-insoluble β -glucans is compared. The aim of this study was to select the most suitable representatives for further exploitation based on the detection of the evaluated parameters. Cereals, as the least expensive source of glucans, can be utilized as nutritional supplements and food ingredients. The understanding of inter-variety variability of β -glucans content is of great importance also for exploiting this knowledge in the agricultural breeding and growing of oats and barley.

2. Experimental

2.1. Materials

Seed samples of 33 oat varieties (4 naked, 29 hulled) and 10 barley varieties have been used in this study. All samples were obtained from the Gene Bank of the Slovak Republic from the Research Institute of Plant Production – Slovak Agricultural Research Centre in Piešťany.

The samples were prepared in two ways according to their next use. When observing the β -glucans content in whole grains, the whole flour with the granularity <0.30 mm was prepared. Whole grains with hulls were milled in laboratory conditions using Laboratory Vibrating Mill (VM4-386, OPS Přerov, Czech Republic). For the estimation of the β -glucans content in single fractions, fractions of white flour and bran were obtained using vibrating mill and set of sieves (size of holes 0.30 mm, wire thickness 0.20 mm). The ratio of flour and bran was in oats 1:1 and in barley 1:2.

2.2. Methods

2.2.1. Insoluble β -glucan

The content of insoluble β -glucan was determined by using the modified method of advance isolation and adjustment of fungal β -glucan of oyster mushroom (*Pleurotus ostreatus*) following the patent of KUNIAK and co-workers (Kuniak, Augustín, & Karacsonyi, 1992).

The first step of β -glucan extraction was a single-stage contra flow extraction of presented fats. The 5 ml solution of dichloride methane and methanol was used. The rate of the solution was 3:1. The sample was quantitative removed from a plastic still to Erlenmeyer flask and suspended in a small quantity of distilled water. The mixture was settled and than 3-times decanted. Into the rest, 2 ml of phosphate solution buffer (pH 7) and water (10 ml) were added and warmed in water bath (80 °C during minimal 10 min) for running starch gelatinization. After cooling of mixture, degradation of starch by α -amylase (approximately 20 h) was followed. The mixture was consequently filtrated through textile filter and washed by some distilled water. Filter cake was thereafter suspended in 20 ml of 10 N fusions of NaOH and 1 ml H₂O₂, too. After 2–3 h waiting at laboratory temperature and consistent filtration through textile filter, stripped β -glucan was washed by water and acetone. β -Glucan prepared in this way, was pre-dried (30 min, 20 °C) and than finally dried (2 h, 100 °C). After measuring the weight, the presence of β -glucan was detected spectrophotometrically in IR-area (Spectrophotometer NICOLET, Magna FTIR, Germany, 4000–400 cm⁻¹).

2.2.2. Soluble β -glucan

For the determination of the content of soluble β -glucan a method accepted by the AACC (Method 32-23), AOAC (Method 995-16) and ICC (Method 168) (Commercial kit of the company Megazyme, Ireland) was used. The mixture of β -glucans was cleaved to β -gluco-oligosaccharides using lichenase. These, reacting with β -glucosidase, were cleaved to glucose, which was thereafter detected spectrophotometrically ($\lambda = 510$ nm) and retroactively calculated in the content of soluble β -glucan according to the formula:

$$\beta\text{-glucan (\%)} = \Delta E \times (F/W) \times 8.46$$

where ΔE is the absorbance after β -glucosidase treatment minus blank absorbance; F is the factor for conversion of absorbance values to micrograms of glucose; and W is the weight of sample analysed (Megazyme, 1985).

2.2.3. Estimation of the dry matter

The dry matter was estimated according to the norm STN ISO 712, which defines practical reference method for the estimation of the content of water in cereals and cereal-based products, $n = 2$.

2.2.4. Mathematical-statistical evaluation

The standard deviation was calculated according to the formula:

$$s = \pm \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n - 1}}$$

Statistical significance of the variances in the results of analysis from two different samples obtained by a uniform method was tested using the Student's test, in which the number of estimations of the monitored parameters was four (Eckschlager, Horsák, & Kodejš, 1980).

3. Results and discussion

Measured values of dry mass, soluble and insoluble β -D-glucan of oats and barley are given in Tables 1 and 2, in which the hulled oat varieties were divided into yellow, black and white according to the colour of glumes. In the inter-variety comparison of cereals, the highest content of insoluble β -D-glucan was found in the black hulled oat variety G2 Taiko (33.732 g/100 g of dry mass) and barley bear variety with 21.7 ± 0.73 g/100 g of dry mass. The results indicate that the insoluble β -D-glucans content in hulled oat varieties is significantly higher than in the varieties of naked oats. This is opposite the soluble glucans. The content of soluble β -D-glucans was 3.860–7.460 g/100 g of dry mass in naked oats varieties, 2.960–3.880 g/100 g of dry mass in black-hulled, 2.550–3.885 g/100 g of dry mass in yellow-hulled and 1.965–4.095 g/100 g of dry mass in white-hulled oat varieties.

In the majority of varieties, the content of soluble and insoluble β -glucan differs significantly and the levels of soluble glucans are comparable to those obtained by other authors showed in Table 3.

Based on the results indicating that insoluble β -glucan content is significantly higher in hulled varieties, it can be concluded that the insoluble β -glucans are found mainly in the coat parts of the grain, whereas the soluble glucans are located in the inner parts of the grain. Subsequent experiments to confirm this assumption were done. In selected varieties (oats: Cyril, barley: Oriflame), the insoluble β -D-glucan content was determined in whole flour, flour divided from the glumes and in the glumes. The amount of insoluble β -glucans in whole flour (oats: 28.30 ± 0.60 g/100 g of dry mass, barley: 17.34 ± 0.26 g/100 g of dry mass), bran (oats: 40.27 ± 0.51 g/100 g of dry mass, barley: 24.32 ± 0.38 g/100g of dry mass) and in the white flour, that is the flour divided from the glumes (oats: 9.53 ± 0.51 g/100 g of dry mass, barley: 13.19 ± 0.57 g/100 g of dry mass) is shown in the Fig. 1. The results suggest that the oat whole flour contains approx. 3 times more insoluble β -D-glucans than the white flour, with a similar situation observed in barley. Compared to the white flour, approx. 1.3 times and 1.8 times more insoluble β -D-glucans were found in the whole flour and bran than in the white flour, respectively.

3.1. The impact of warehousing on soluble β -D-glucans content

The values of soluble β -glucans on days 0, 7, 14, 21, 35 and 49 of warehousing of the oat (Kanton) and barley (Oriflame) samples are presented in Figs. 2 and 3. Warehousing

Table 1

The content of dry mass in water-soluble and insoluble β -D-glucan in evaluated oats varieties

Oat varieties		Dry mass content		β -D-Glucan content (g/100 g of dry mass)			
				Insoluble		Soluble	
		Mean value	SD	Mean value	SD	Mean value	SD
Naked	Ábel	91.97	0.04	6.55	0.07	4.08	0.01
	Adam	91.57	0.02	7.54	0.60	7.46	0.06
	Jakub	91.87	0.25	5.15	0.06	5.43	0.03
	Neon	90.45	0.09	10.80	0.54	3.91	0.15
<i>hulled</i>							
Black	Autevil	92.72	0.10	22.77	0.23	3.88	0.03
	G2 Taiko	92.22	0.06	33.73	1.55	2.93	0.13
	Sirene	93.48	0.08	27.93	1.35	3.17	0.16
Yellow	Aveia Peluda	91.32	0.09	32.78	1.23	3.42	0.02
	Borka	92.24	0.09	32.26	1.04	2.55	0.01
	Caracas	92.22	0.09	30.66	1.35	2.63	0.12
	Cyril	90.27	0.60	28.30	0.60	3.71	0.03
	Flamingstern Trend	92.11	0.32	28.63	0.32	3.44	0.03
	G2 Aragon	92.11	0.51	13.79	0.51	3.25	0.11
	G2 Indio	94.72	0.32	24.12	0.99	3.89	0.01
	German	93.45	0.09	24.38	0.75	3.28	0.01
	Jumbo	93.01	0.93	20.81	0.96	3.00	0.14
	Kanton	92.78	0.51	29.75	0.91	3.16	0.06
	Lutz	92.81	0.91	27.80	0.75	3.39	0.06
	Senator	94.25	0.75	28.31	1.07	3.53	0.18
	Šampionka	92.99	0.09	17.93	0.51	2.92	0.14
	Tarra	93.08	0.68	30.02	1.40	3.70	0.21
White	Argentina	92.97	0.75	31.07	0.99	2.98	0.15
	Edit	94.23	0.51	26.19	0.44	2.85	0.03
	Flamingstern Plus	91.93	0.44	26.97	1.09	2.81	0.16
	G2 Seven Antree	93.39	0.60	28.45	0.93	4.09	0.19
	Gambo	92.41	0.32	29.48	1.02	3.57	0.07
	Maris Oberon	92.49	0.51	30.02	0.91	3.26	0.08
	Master	92.37	0.68	28.23	0.37	3.67	0.16
	Orfine	93.03	0.97	20.08	0.37	2.52	0.12
	Pendek	93.04	0.76	22.23	0.68	3.52	0.04
	Revisor	92.05	0.75	21.37	0.97	3.05	0.04
	Sanova AS 181 325	93.10	0.09	17.61	0.76	1.97	0.07
	Stormon Sceptre	91.33	0.51	31.45	0.99	3.73	0.13

Table 2

Dry mass content of water-soluble and water-insoluble β -D-glucan in evaluated barley

Barley varieties	Dry mass content		β -D-Glucan content (g/100 g of dry mass)			
			Insoluble		Soluble	
	Mean value	SD	Mean value	SD	Mean value	SD
Bear USA	87.82	0.68	21.70	0.73	5.47	0.04
CI 5 DEU	90.73	0.09	11.30	0.46	5.57	0.12
CI 15 DEN	90.39	0.14	18.71	0.61	6.51	0.21
Hyproly	89.59	0.06	15.57	0.33	7.96	0.09
KM 1057	88.55	0.75	17.36	0.53	3.75	0.14
KM 1910	87.34	0.75	10.89	0.60	4.54	0.15
KM 2037	87.70	0.10	15.40	0.68	5.13	0.07
Oriflame	88.95	0.45	17.34	0.26	4.18	0.01
Peregrine	89.04	0.51	14.81	0.69	4.66	0.11
Tibet White	87.95	0.23	16.63	0.47	6.71	0.07

of flour and bran was compared at room temperature (25 ± 2 °C) and in refrigeration at a temperature of 8 ± 1 °C. After the first week of warehousing at room temperature, a decrease of 45.5% (1.45 g/100 g of dry mass) in soluble β -glucans content was observed in the samples of

oat flour (Fig. 2), whereas a decrease of only 9.8% (2.4 g/100 g of dry mass) was measured for the refrigeration storage. Forty-nine days after refrigeration warehousing, a decrease of 77.8% (0.59 g/100 g of dry mass) in soluble β -glucans content was observed when compared

Table 3
Content of β -glucans in the given cereals according to the latest literature references

Cereal	β -D-Glucan content (g/100 g of dry mass)	References
Oats	4.0 ± 0.1^a	Johansson et al. (2004)
	4.1 ± 0.19^a	Genc et al. (2001)
	10.9–11.0 ^b	Izydorczyk et al. (1998)
	23.0–24.0 ^a	Izydorczyk et al. (1998)
	10.37 ^a	Dongowski et al. (2003)
Barley	2.41–8.25 ^a	Holtekjølén et al. (2006)
	3.24–4.62 ^a	Zhang et al. (2002)
	6.0–8.0 ^a	Izydorczyk et al. (1998)
	30.0–33.3 ^b	Izydorczyk et al. (1998)
	3.7 ± 0.1^a	Johansson et al. (2004)
	3.6 ± 0.1^a	Genc et al. (2001)
	3.64–7.96 ^a	Storsley et al. (2003)

^a Poznámka: Water-soluble β -glucan.

^b Poznámka: Total β -glucan.

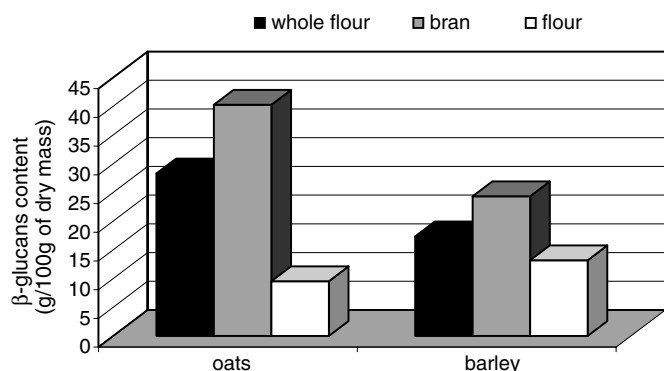


Fig. 1. Comparison of insoluble β -glucans content in whole flour, bran and flour in oats (Cyril) and barley (Oriflame).

with Day 0, which is comparable to the decrease observed in flour stored at room temperature after 7 days (45.5%). In bran, the rate of decrease in β -glucan content was similar in the first days, however, after 35 days of warehousing, the decrease in β -glucan content was approximately similar for the samples stored in refrigeration and at room temperature.

In the barley flour, the decrease in soluble β -glucans content was significantly lower in the samples stored in refrigeration. After the first 2 weeks, a decrease of only 1.3% (3.88 g/100 g of dry mass) was detected. On day 49, a decrease of 34.1% (2.59 g/100 g of dry mass) and 36.6% (2.49 g/100 g of dry mass) in β -glucans content was observed in the samples stored in refrigeration and at room temperature, respectively. In barley bran, a mild decrease was observed within the first 14 days after grain processing. After 49 days of warehousing, β -glucans content decreased by more than one half (50.9%) when stored in refrigeration, compared to as much as a 58.2% decrease in the samples stored at room temperature.

It can be assumed that these changes occur due to enzymes (β -glucanases) naturally present in the grain. During grinding of the grain, destruction of grain structure occurs following by the release of enzymes degrading β -glucans. β -Glucanase cleaves the β -glucans into tri- and tetra-saccharides, not to detectable glucose that could be retroactively counted to the β -D-glucans.

3.2. Interspecies and inter-variety comparison of cereals

For final interspecies and inter-variety comparisons, the values of total β -glucans content were used, that is the sum of soluble and insoluble glucans. The best oat varieties were G2 Taiko (36.66 g/100 g of dry mass), Borka (34.81 g/100 g of dry mass), Aveia Peluda (36.2 g/100 g of dry mass), Caracas (33.29 g/100 g of dry mass), Maris Oberon (33.28 g/100 g of dry mass), Kanton (32.91 g/100 g of dry mass), Cyril (32.01 g/100 g of dry mass), Tarra (33.72 g/100 g of dry mass), G2 Seven Antree (32.54 g/100 g of dry mass) and Sirene (31.10 g/100 g of dry mass). The order for barley varieties was as follows: Bear USA (27.17 g/100 g of dry mass), CI 15 DEN (25.22 g/100 g of dry mass), Hyproly USA (23.53 g/100 g of dry mass), Tibet White DEU (23.34 g/100 g of dry mass), Oriflame (21.52 g/100 g of dry mass), KM 1057 CZE (21.11 g/100 g of dry mass), KM 2037 CZE (20.53 g/100 g of dry mass), Peregrine CAN (19.47 g/100 g of dry mass),

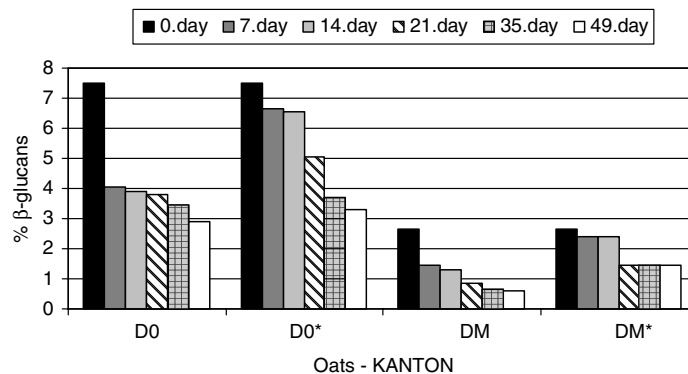


Fig. 2. The effect of temperature on the soluble β -glucans content of oats (Kanton) during warehousing. (C0, oats bran stored at laboratory temperature $25 \pm 2^\circ\text{C}$; C0*, oat bran stored in refrigerator at the temperature of $8 \pm 2^\circ\text{C}$; CM, oats flour stored at laboratory temperature $25 \pm 2^\circ\text{C}$; CM*, oats flour stored in refrigerator at the temperature $8 \pm 2^\circ\text{C}$).

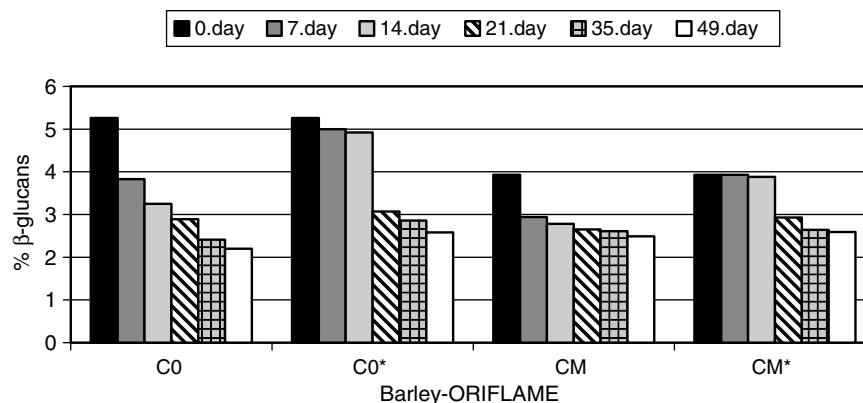


Fig. 3. The effect of temperature on the soluble β -glucans content of barley (Oriflame) during warehousing. (C0, barley bran stored at laboratory temperature $25 \pm 2^\circ\text{C}$; C0*, barley bran stored in refrigerator at the temperature of $8 \pm 2^\circ\text{C}$; CM, barley flour stored at laboratory temperature $25 \pm 2^\circ\text{C}$; CM*, barley flour stored in refrigerator at the temperature of $8 \pm 2^\circ\text{C}$).

CI 5 DEU (16.87 g/100 g of dry mass) and KM 1910 CZE (15.43 g/100 g of dry mass).

Based on these results, it can be concluded that total β -glucans content decreases in the order of, hulled oats (max. value of total β -glucan = 36.66 g/100 g of dry mass) > barley (max. value of total β -glucan = 27.17 g/100 g of dry mass) > naked oats (max. value of total β -glucan = 14.71 g/100 g of dry mass).

4. Conclusions

The aim of this work was to compare the inter-variety variability of oats and barley in terms of soluble and insoluble β -D-glucan content. Thirty-three and 10 varieties of oats and barley, respectively, obtained from the Gene Bank at the Piešťany Research Institute of Plant Production, were evaluated. This information is important for the breeding and growing of oats and barley as well as for the exploitation of these cereals in food production as inexpensive sources of biologically active substances.

The content of soluble β -glucans can normally be found in the available literature. However, the time of warehousing after sample processing is not reported. According to our results these data are of great importance as the content of soluble β -glucans decreases with time during warehousing. It is not possible to halt this process through temperature manipulation; however, the process can be reduced.

Based on the obtained results, it appears necessary to state the duration period of warehousing from the time of sample processing, so that the values measured can be compared with those in the literature.

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