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Composition And Molecular-Weight Distribution Of Arabinoxylans Of Winter Rye Grain.

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ABSTRACT

The amounts of carbohydrates were analyzed in six winter rye varieties. Separation and detection monosaccharide's were performed by anion-exchange high-performance liquid chromatography. Varietal differences in the content of polysaccharides were significant. Molecular weight distribution of carbohydrates was evaluated using gel permeation chromatography on Agilent 1260 Infinity chromatograph (Agilent, Germany). Varieties Marusenka, Podarok, Ogonek should be attributed to low-pentosans samples of rye. Arabinoxylans in the grain of these cultivars are not only presented in smaller quantities, but apparently characterized by a lower degree of polymerization. Distinctions in monosaccharide composition of rye grain explained above all to genetic differences in cultivars.

Keywords: winter rye, arabinoxylans, water extract, carbohydrates, variety

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INTRODUCTION

Winter rye (*Secale cereale L.)* is the most freezing tolerant small grain cereal and is well suited for production areas where severe winters occur [1]. Since rye is more tolerant than most other cereals to stress environmental conditions, like drought, salt or aluminum pollution [2]. Owing to its high degree of abiotic stress tolerance, rye is a valued crop in production areas where most small grain cereals are not profitable [3]. The high level of frost tolerance allows winter rye cultivation in northern and continental cropping areas of the temperate zones. Hansen et al [4] reported that rye is a hardy crop, and the production of rye is environmentally friendly. Compared to wheat, rye can advantageously be grown in organic farming, due to reduced requirements of fertilizer and pesticides.

In terms of total production rye is a minor cereal [5]. The world harvest of rye is about 13 million tons. Ninety five percent of world rye production is grown in the northern part of the region from the Nordic Sea to the Ural Mountains. A substantial part of the world rye production (more 3 million tons) is used for bread baking, especially in central, northern, and eastern European countries. Thus, rye bread plays an important role in the nutrition of populations with high rye consumption.

Starch, dietary fiber polysaccharides and protein are the principal chemical constituents of the rye grain [6]. Wide variation in chemical composition has been found, depending on both cultivar and growing conditions. Because cereals are rich in starch, these polysaccharides are usually also referred to as “nonstarch polysaccharides” (NSP). They occur in various tissues of cereal grains and are the predominant matrix polysaccharides in cereal grain cell walls. In this way carbohydrates found in plants can be divided into starch and NSP, also called structural carbohydrates, which are responsible for forming the outer covering of seeds, protecting them against damage developing inside the endosperm [7]. Major NSP are arabinoxylans with about 8% of dm. They primarily consist of the two sugars L-arabinose and D-xylose [8] and represent about 55% of total NSP in rye grains [9, 10].

The first time AX were identified by Hoffman and Gortner in 1927 and described as a viscous gum present in wheat flour [11]. AX were often referred to as pentose-containing carbohydrate polymers called “pentosans,” since they are composed of the pentose’s xylose (Xyl) and arabinose (Ara) [12, 13]. They consist of a linear backbone chain of (b1/4)-linked D-xylopyranosyl residues to which a-L-arabinofuranosyl residues are linked.

AX have high technological importance. As dietary fiber, they have an impact on the nutritional quality of cereal-based foods for human and animal feed. Also AX affect the physicochemical properties and processing behavior of cereal grains in milling, brewing, and baking due to their high viscosity and water retention properties [14]. These properties are also important concerning AX application as food-thickening and stabilizing agents. Tokar et al. [15] fund that, the highest influence on ability to bind water by the pentosans has their molecular weight. Increase of the pentosans molecular weight causes increasing in water absorption. It is probably caused due to differences in molecular weight of pentosans. The substitution pattern (arabinose/xylose) differs for different milling fractions and the functional characteristics of arabinoxylans differ between fractions, e.g., the water-solubility is about 70 percent in the endosperm but almost zero in the aleurone and pericarp/testa.

The molecular weight of rye arabinoxylans varies depending on analytical technique and the raw material (whole grain or bran). Rye AX can be water soluble or insoluble. Water-extractable arabinoxylan and soluble β-glucan are responsible for the viscous properties of soluble dietary fiber in rye [16], which may contribute to the technological functionalities and the various health effects of rye. Unsubstituted xylans are nearly water insoluble due to high hydrogen bonding occurring intra- and intermolecular. With the increase of Ara residues as side chains, the polysaccharides become more water soluble due to the restriction of intermolecular hydrogen bonding. Moreover, AX can also improve the viscosity, texture, sensory characteristics, and shelf-life of food products [17]. Also AX-derived oligosaccharides have aroused scientific and commercial interest mostly because of their prebiotic character [18].

The objective of this research was to evaluate amount, composition and molecular weight arabinoxylans of whole meal grain, extracted by water, from new winter rye varieties of Russian breeding.

MATERIAL AND METHODS

Plant materials and field design

Grain samples of the winter rye varieties were collected from field trials of Tatar Scientific Research Institute of Agriculture located in the Predkam zone of Tatarstan Republic (the forest-steppe area of the Volga region). Six Russian rye cultivars (Tatarskaya 1, Radon, Ogonek, Podarok, Marusenka, Pamyati Kunakbaeva) were used for this study. Field trials were planted in plots of 20 m2 size with a four-replication randomized block design. The experiments were carried out during 2016-2017 season under uniform agronomic management (fertilizer, herbicides etc.) for winter rye production. The present study was conducted on the grey forest soil with the humus content of 3.1%.

Extraction and Analysis of monosaccharide composition of carbohydrates

The rye grain was milled to whole meal flour with Laboratory Mill 3100 (Perten Instruments) with 0.5 mm sieve. The extract samples for measurement were prepared according to method as detailed below. 2 g of rye whole meal flour were suspended in 20 ml of deionized water and shaken for 1 h at 20 °C. The suspension was centrifuged at 10000 × g for 10 min at 25°C. For subsequent analyses, water extracts were diluted in a ratio of 1: 10.

Separation and detection monosaccharide's were performed by anion-exchange high-performance liquid chromatography on a CarboPac PA-1 column (4 × 250 mm, Dionex, USA) using a pulse amperometric detector (Dionex) [19]. The rate of elution was 1 ml min–1with a column temperature of 30 °C. A gradient elution was conducted with buffer A (100 mM NaOH in 1 m NaOAc) and buffer B (15 mM NaOH) according to the following scheme: 0–20 min in 100 % B; 20–21 min in 90 % B and 10 % A; 22–31 min in 70 % B and 30 % A; after that, washing and equilibrating the column in the 100% B -10 minutes, 100% A -30 minutes. The results were analyzed using PeakNet software according to the calibrations that were obtained for monosaccharide standards that were treated in advance with 2 m TFA at 120 °C for 1 h.

Molecular weight distribution of carbohydrates was evaluated using gel permeation chromatography on Agilent 1260 Infinity chromatograph (Agilent, Germany) on series-connected columns (Shodex, Japan): OHpak SB-G (6.0 × 50 mm, pre-column), OHpak SB-806M HQ (8.0 × 300 mm) and OHpak SB-804 HQ (8.0 × 300 mm) in 0.1 M NaOAc buffer with 0.02% NaN3, at pH 5.5, flow rates 0.5 ml/min, column temperature 40°C. Detection was carried out using a refractometric detector at a cell temperature of 35°C. Dextran 2000 kDa (Sigma, USA) and pullulans were used as standards 380, 186, 100, 48, 23.7, 12.2, 5.8 kDa (Showa Denko, Japan) with low polydispersity index (1.09–1.19) and d (+)-glucose (Merck, Germany). To assess the contribution of water-soluble protein for each of the samples, an additional analysis of the molecular weight distribution of polymers after ten-minute heating to 100°C in a thermoshaker was carried out, followed by centrifugation for the deposition of denatured protein.

Statistical Analysis

Results of all analysis were expressed as mean ± standard deviation of at least 3 replicates of each sample. The one-way ANOVA (analysis of variance) was calculated for the obtained data, applying the Microsoft Excel for Windows 7.0 and SPSS software package. Fishers least significance tests were used to determine statistical significant differences (p-value was lower or equal to 0.05) between rye varieties. Agilent GPC/SEC software was used for analysis of chromatograms and data interpretation.

EXPERIMENTAL

The ANOVA analysis showed that the studied six varieties did not differ significantly (at $P < 0.05$) in the total amount of carbohydrates obtained as a result of water extraction of rye whole meal (Fig 1). The content ranged from 41 to 53 mg/ml for various cultivars. Suspensions were 4-5% (by weight) solutions of carbohydrates in mono -, oligo- and polysaccharide forms. The ratio of these forms of carbohydrates varied depending on the genotype of the variety. At the same time, we noted that there were significant differences in the content of polysaccharides. For example, the cultivar Marusenka had these carbohydrates 4.3 mg/ml in water extract, and varieties Tatarskaya 1 and Radon - 2 times more (11.1 mg/ml). The content of

oligosaccharides varied from 17.4 to 28.8 and monosaccharide's changed from 12.8 to 21.7 mg/ml of water extract (Fig. 1). Carbohydrates in the monosaccharide form were from 24 to 47% of all water-soluble carbohydrates of rye meal.

Testing extracts of rye meal of different varieties contained 23-40 mg/ml of carbohydrates in oligo- and polymer form, which ranged from 52 to 74% of all extracted carbohydrates (Fig 2). The extracted water composition of oligo- and polymeric carbohydrates included xylose (Xyl), glucose (Glc), arabinose (Ara) and galactose (Gal). It indicates the presence in this fraction of oligomeric fragments and polymers of starch, arabinoxylans and, possibly, mixed-linked glucans. Galactose was a minor sugar of water-soluble carbohydrates (0.9-1.4 mg/ml). It is important to note that the Glc content in the polymer form is comparable to the Xyl content (Fig 2). The glucose content changed from 8.6 to 18.3, while the xylose varied from 8.8 to 15.7 mg/ml, i.e. water extraction of rye meal releases similar in meaning amounts of starch and/or mixed-bound glucans and arabinoxylans. At the same time, we identified that cultivars Ogonek, Podarok and Marusenka had a lower Xyl content (9-11 mg of xylose per ml of water extract), and varieties Tatarskaya 1, Radon and Pamyati Kunakbaeva had higher content (14-16 mg of Xyl per ml of extract).

The ratio of Ara / Xyl, which is usually used to characterize the degree of substitution of arabinoxylans, for polymers of water extract of rye meal was about 0.3, that is, of the 10 residues of Xyl in the skeleton, only 3 can be replaced by Ara.

AX was calculated as the sum of arabinose and xylose all fractions (Fig 3). Our determinations of AX in various rye grain samples showed that their content in three cultivars (Marusenka, Podarok, Ogonek) was significantly lower (13.5; 16.0; 16.9 mg/ml, respectively) than other, although all varieties were grown under the same conditions.

Gel permeation chromatography was carried out on columns with a resolution range from 180 to 40,000,000 Da to assess the degree of polymerization of carbohydrates in water extracts of rye meal. The experiments were performed twice: for centrifuged samples (Fig. 4, left) and for the same samples that were heated before centrifugation to denature the protein in them (Fig. 4, right).

All cultivars were the mix of mono-, oligo- and polysaccharides. We indicated seven peaks, including a monosaccharide. Their set was identical for all varieties, although the ratios of peaks for different varieties were dissimilar somewhat. After boiling, none of the peaks disappeared completely, i.e. the contribution of protein to the observed pattern was relatively small.

Table 1 presents the differences in the ratio of peaks between varieties, the mass and content of carbohydrates in each of them. The peak 1 represented by the most massive carbohydrates, contained polysaccharides with a mass of about 4000 kDa (table 1). Varieties Ogonek, Podarok and Marusenka were characterized by lower carbohydrate content in this peak (0.6–1 mg / ml extract) compared with Tatarskaya 1, Radon and Pamyati Kunakbaeva (1.4–1.6 mg / ml extract). Differences in the amount of high-molecular carbohydrates, thus, may cause varietal difference in the viscosity of water extracts of whole meal.

Peak 2 contained carbohydrates with average molecular weight of 37-48 kDa (table 1). This peak had a small admixture of protein (see Fig.4, right). We found that there were, respectively, 2 and 3 mg per ml of carbohydrate in water extract at this peak in varieties Marusenka and Ogonek. The concentration of carbohydrates of this molecular weight in cultivars Podarok, Pamyati Kunakbaeva, Tatarskaya 1 and Radon was higher and ranged from 4.4 to 6.3 mg / ml (table 1).

Peak 3 in all varieties was represented by polysaccharides with masses of approximately 11 kDa. Water extracts of meal varieties Podarok and Marusenka contained 1.6 mg of carbohydrates in this peak per ml after heating and precipitation. Cultivars Pamyati Kunakbaeva and Ogonek had in peak (~11 kDa) 2.7 and 2.5 mg / ml and varieties Tatarskaya 1 and Radon - 3.3 and 3.2 mg / ml, respectively.

Peaks 4, 5 and 6 are oligosaccharide fragments with polymerization degrees respectively of ~ 17, 7 and 3 monosaccharide residues, calculated on xylose. Presumably, such fragments cannot have a significant effect on the viscosity properties of the solution. However, they constituted the bulk of the polymerized carbohydrates present in the water extracts of rye meal.

Peak 7 (~ 180 Da) contained mainly monosaccharides, the number of those was varied from 12.8 for Tatarskaya 1 to 21.7 mg/ml for Pamyati Kunakbaeva.

RESULTS AND DISCUSSION

It is known that most of the dietary fiber in rye is constituted by AXs, which are mostly bound to other cell wall components [10]. The most common method to determine arabinoxylans from grains is by water extraction. Water extractions are typically carried out at temperatures in the 25-100 ^{0}C range, allowing the recovery of high molecular weight AX with the highest preservation of their native structure [20]. AXs can form covalently stabilized gels under oxidizing conditions. This leads to a strong increase viscosity in solution, as the molecular weight of the AXs increases. Moreover, structural characteristics of AXs such as molecular weight and branching degree play also a significant role on their functionality [21]. As seen in other investigations high-molecular carbohydrates can effectively participate in the formation of supramolecular associates and gel-like structures [22].

In our study we found a variation in the composition of whole meal water-soluble carbohydrates in various winter rye cultivars. Glucose was the dominant component in all cases. Apparently, this is due to the partial degradation of starch. The presence of arabinose, xylose and galactose in approximately equal proportions indicates partial degradation of arabinoxylans and arabinogalactans (Fig. 2). In addition to arabinogalactans galactose could also be part of the pectin, although in this situation in the samples should be present galacturonic acid, as a typical component of the skeleton of pectin substances. The existence of monosaccharides in the water extract of meal is probably the result of the work of exo-enzymes of rye grain in the course of water extraction. It should be concluded that the greatest activity of these enzymes are characterized by varieties Marusenka and Pamyati Kunakbaeva, and the lowest – Tatarskaya 1. This is confirmed by the results of the analysis of peak 7 which was presented mostly by monosaccharide's.

Varieties Marusenka, Podarok, Ogonek should be attributed to low-pentosans samples of rye. Arabinoxylans in the grain of these cultivars are not only presented in smaller quantities, but apparently characterized by a lower degree of polymerization. In contrast, varieties Tatarskaya 1, Radon and Pamyati Kunakbaeva were characterized by a high content of arabinoxylans in water extract (20.4; 18.7; 18.7 mg/ ml, respectively) (Fig. 3). These polysaccharides are also represented by supermassive molecules (Table 1), presumably involved in the formation of high-viscosity solutions. Our previous research has shown that composition and content of pentosans are indicators to diversify rye grain use [23]. Cultivars with low and high pentosans content were identified in Russian and foreign gene pools. The smallest amounts of water-soluble pentosans in flour and meal were characteristic of the Russian varieties Marusenka and Ogonek. The structural heterogeneity of AX is also observed on the degree of polymerization (DP) of the xylan backbone, as well as on the ratio of Ara to Xyl residues.

Distinctions in monosaccharide composition of rye grain explained above all to genetic differences in cultivars. Similar findings were also obtained in another study by Boros and Fraś (2015) [24]. Authors confirm the continued slight decrease in soluble NSP in new varieties of rye.

The development of new rye cultivars with enhanced quality, therefore, requires methods to exploit this variation and it is essential to understand and modulate the mechanisms controlling the key events of cell-wall polymer synthesis. This knowledge is essential to understand AX properties and defined possible targets for plant breeding [25].

On the other hand, some studies highlight that arabinoxylans molecules of various molecular sizes have been reported within the water extractable AX and solubilized water unextractable AX fractions of rye grain [12]. The observed high influence of the genotype on the viscous properties of the grain was most likely caused by differences in AX structure, such as the degree of branching and/or ferulic acid cross-linking of AX molecules, as well as the molecular size of the AX. The molecular size of unextractable AX was not measured in this study but might also be important. Similar findings were reported by Scoles et al. [26], who added that it was correlated with the proportion of high molecular weight (>500 kDa) AX in the water unextractable AX.

Gel permeation chromatography (GPC) of water extracts of rye whole meals revealed the presence of a high molecular weight fraction (HMWF), which was found in higher concentration in the ryes than in wheat

[27]. A significant positive correlation (r= 0.84, p< 0.05) was observed between HMWF content (expressed as a proportion of the total carbohydrate in water extracts) and extract viscosity of rye whole meals.

In like manner in our work the GPC method allows computing the ratio Ara/Xyl which indicates the degree of branching in the polymer chain, thus permitting to deduce information about the AX structure of different winter rye varieties. We found that this indicator in the studied new varieties ranged from 0.27 (Radon) to 0.31 (Ogonek). Ara/Xyl ratio, calculated from arabinose (A) and xylose (X) content determined by HPLC/RI after hydrolysis of arabinoxylans, in Polish variety Amilo was 0.7 [27]. Higher ration corresponds to a higher proportion of mono-substituted xylosyl residues and a lower proportion of unsubstituted xylosyl residues. Izydorczyk and Biliaderis [14] observed that the specific arrangement of arabinose residues along the backbone chain could also be an important factor as intermolecular alignment between unsubstituted AX molecules might occur, which in turn would lead to aggregation of AX isolates. The degree of substitution of xylan backbone is relevant for predicting the cereal behavior when subjected to different technological processes.

CONCLUSION

This paper contains new information about quantitative and qualitative composition of the carbohydrate fraction of rye grain modern varieties.

From a technological point of view, the quantification and the assessment of variations in the overall AX content is relevant because AX is generally considered to have a significant effect on rye grain functionality and also to affect suitability for certain applications. It is clear that there are significant varietal differences in the composition and amounts of water-soluble polysaccharides. Rye varieties are rich in AX, have a strong potential for healthy food products that contain not only high dietary fiber content but also increased prebiotic oligosaccharides. At the same time there are new varieties that have arabinoxylans content and structure has changed significantly.

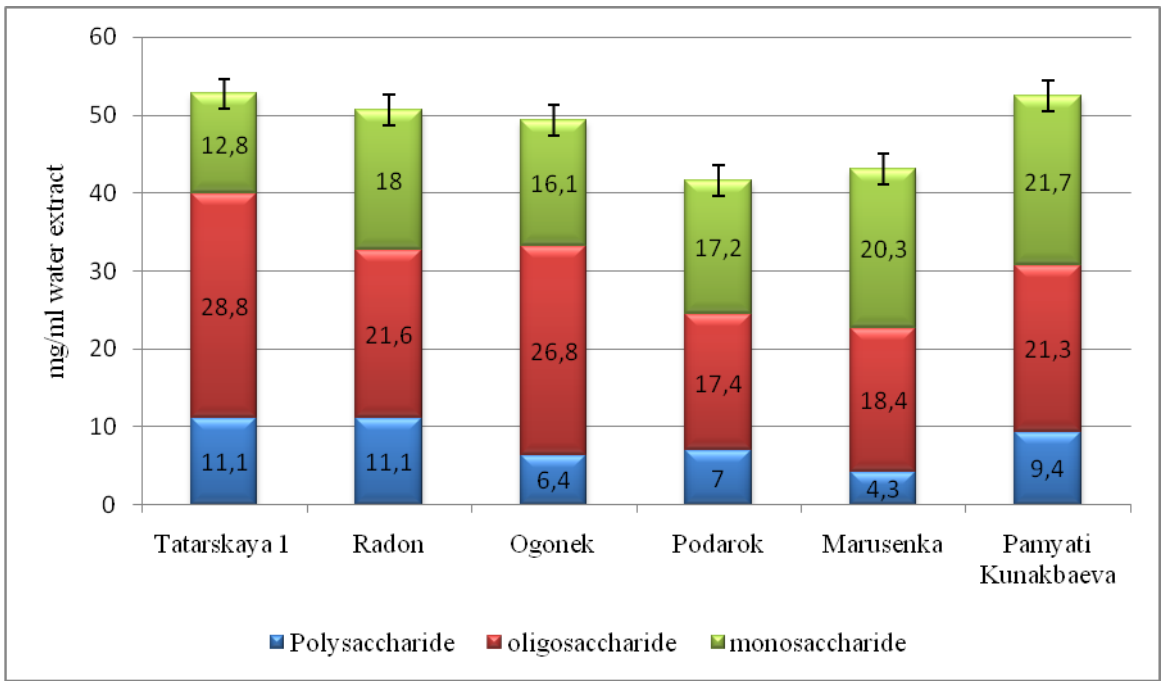

Figure 1. The carbohydrate fraction composition of rye wholemeal water extracts of winter rye varieties

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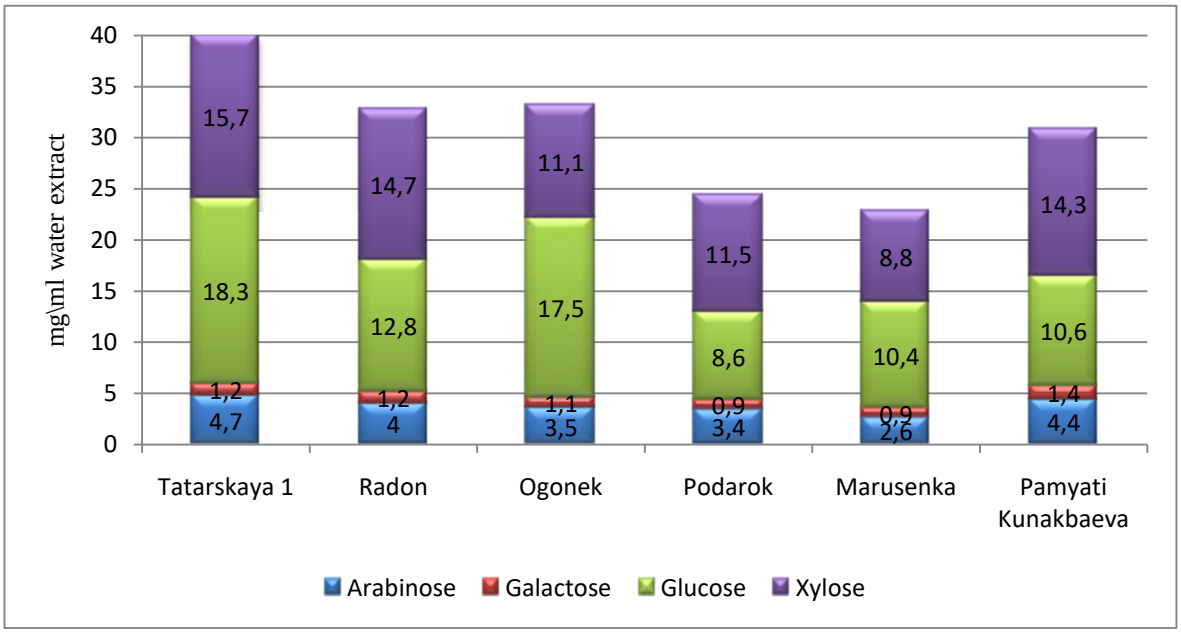

Figure 2. Total oligo-and polysaccharide fragments composition of carbohydrates of water extracts of rye meal, mg/ml water extract.

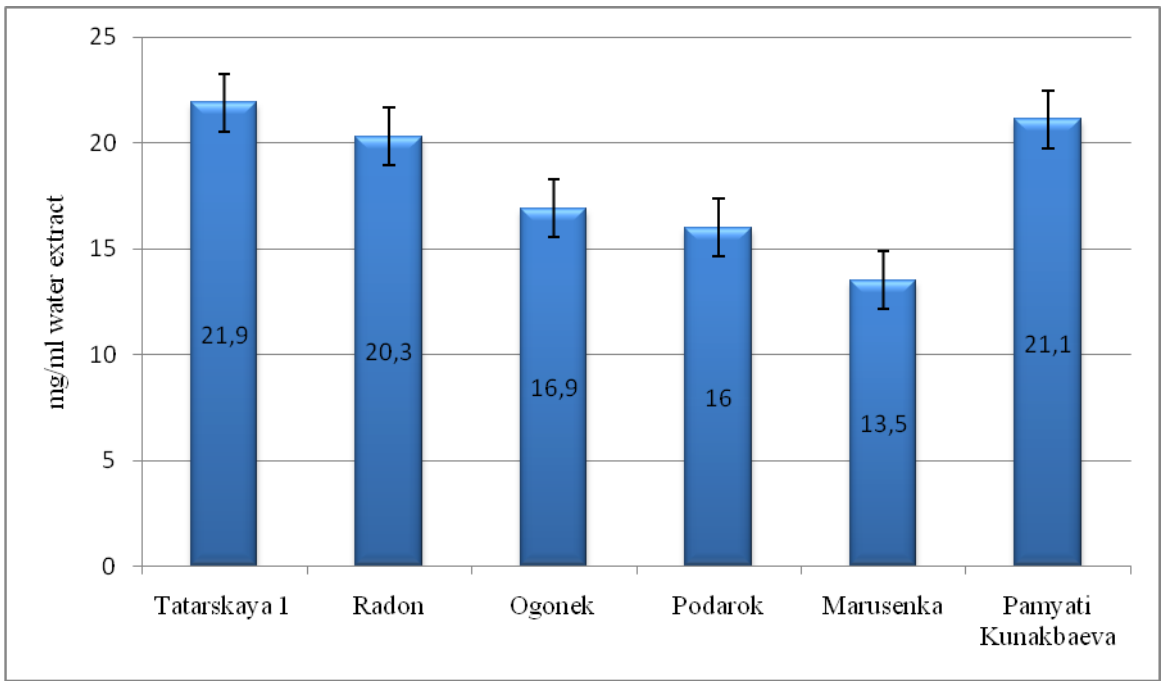

Figure 3. Content AX of water extracts of winter rye varieties (calculated as the sum of arabinose and xylose all fractions)

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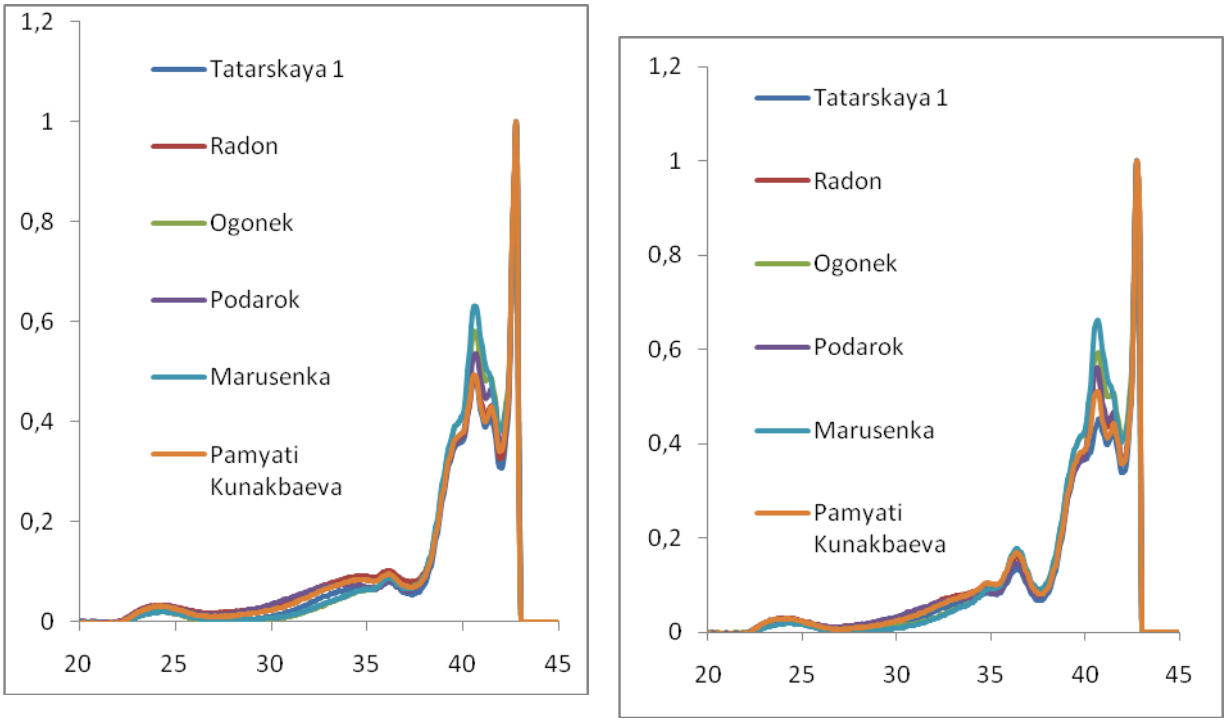

Figure 4. Molecular mass distribution of water-soluble carbohydrates of whole meal winter rye varieties. Chromatogram of supernatants - left, chromatogram of supernatants after heating - right. Horizontal scale – time of elution (minutes). Vertical scale – carbohydrate profiles. Chromatograms are aligned at the height of the monosaccharide peak (peak 7, Mn ~ 180 Da).

Table 1. The amounts and carbohydrates average molecular weight (Mn) of rye water extracts after heating (Da)

Molecular mass distribution	Tatarskaya 1	Radon	Ogonek	Podarok	Marusenka	Pamyati Kunakbaeva
Peak 1	4283890 1.6	4565455 1.6	4285725 0.9	4259977 1.0	3902936 0.6	3847741 1.4
Peak 2	43404 6.2	53313 6.3	38543 3.0	51324 4.4	36597 2.1	45528 5.3
Peak 3	11290 3.3	11775 3.2	12033 2.5	11526 1.6	11244 1.6	11114 2.7
Peak 4	2428 10.8	2345 9.1	2403 10.2	2525 6.0	2515 6.2	2394 8.2
Peak 5	957 11.5	922 7.9	938 10.6	965 7.4	946 8.5	952 8.4
Peak 6	474 6.5	485 4.6	482 6.0	477 4.0	476 3.7	482 4.7
Peak 7	217 12.8	222 18.0	222 16.1	219 17.2	223 20.3	222 21.7

in numerator – average molecular weight (Da), in denominator - the carbohydrate content of various molecular weights according to refractometry data (mg / ml of water extract rye meal). The carbohydrate content is calculated by the ratio of the peak areas in the chromatograms after heating, based on the total number of oligo-and polysaccharides.

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