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Wioletta M. Dynkowska

Plant Breeding and Acclimatization Institute—National Research Institute, Plant Biochemistry and Physiology Department, Radzików, 05-870 Błonie, Poland;
Corresponding author's e-mail: w.dynkowska@ihar.edu.pl;
ORCID ID 0000-0001-8563-7032

RYE (*SECALE CEREALE* L.) ARABINOXYLANS: MOLECULAR STRUCTURE, PHYSICOCHEMICALS PROPERTIES AND THEIR RESULTINGPRO-HEALTH EFFECTS

ABSTRACT

Arabinoxylans are non-starch polysaccharides that are an essential component of dietary fiber, and their health-promoting properties are determined mainly by the content and structural features their biopolymers. Significant amounts of arabinoxylans are contained in cereals, especially rye grain, which is used, inter alia, in the baking industry. Due to the content and chemical structure of these compounds, rye bread is a valuable component of the daily diet. Rye bread is particularly rich in these compounds; their unique features in the context of content and chemical structure of rye arabinoxylans make it a valuable component od daily diet. Long-term studies have shown the positive effect of these compounds in the aspect of prevention of civilization diseases such as type 2 diabetes, obesity, and cardiovascular diseases. Among the description of the physicochemical properties and diversity of arabinoxylans, the article contains a collection of the most important reports regarding the health-promoting effects of these polymers, as well as their metabolism in the human body.

Key words: antioxidant activity, cereal grains, dietary fiber, health potential, short-chain fatty acids, water extract viscosity

INTRODUCTION

Dietary fiber is mainly composed of plant cell wall material that is not digested in the human digestive tract, may affecting the digestion of other dietary components, and causes a lot of metabolic effects, which are beneficial in prevention and treatment the many diet-dependent diseases. The content and composition of dietary fiber in cereal plants depend on the genotype and envi-

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ronmental conditions (Hansen *et al.* 2003), which modify the anatomical structure of the seeds, the thickness of the seed coating and the aleurone layer, as well as the thickness of the endosperm cell walls, the degree of seed coat lignification and the proportion of endosperm size to the size of the embryo. According to the Codex Alimentarius Commission (CAC), the definition of dietary fiber includes carbohydrate polymers with ten or more monomeric units that are not hydrolyzed by endogenous enzymes in the human small intestine (McCleary *et al.* 2011). Depending on the origin, three categories of dietary fiber polymers are distinguished: (1) edible carbohydrate polymers naturally occurring in the food as consumed; (2) carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities; and (3) synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities (Mendis and Simsek, 2014). Chemically, cereal fiber is a complex of many components, among which non-starch polysaccharides, especially arabinoxylans are the dominant factors. Plant-derived dietary fiber may also include lignin fractions and other polysaccharide related compounds in plant cell walls.

Arabinoxylans, as sugar polymers, had been the subject of research since the first positive effects of dietary fiber on human health were observed. The method of their pro-healthy impact depends on the structure, which affects the degree of water solubility of individual fractions of arabinoxylans (Cummings, 1984; Roehring, 1988; Jenkins, 2004). Because of the irregular distribution of different fractions of arabinoxylans in cereal grains (Nyman *et al.* 1984), the health-promoting properties of bread resulting from the content of arabinoxylans strongly depends on the milling degree of flour used for preparing of dough bread. The appropriate choice between flour from wheat or rye is also a key issue. Among cereals, the highest content of arabinoxylans is found in the rye (*Secale cereale* L.) (Knudsen and Lærke, 2010) (Vinkx and Delcour, 1996; Ragaee *et al.* 2001; Hansen *et al.* 2003). The following chapters of this work are dedicated to the characteristics of these biopolymers and the description of the health effects of rye arabinoxylans on human health.

RYE GRAIN ARABINOXYLANS

Arabinoxylans are heteropolymers of arabinose and xylose, and they are the part of non-starch polysaccharides of cereal grains. They build the structural frameworks of cell walls, conditioning cell responses, acting as receptors, and finally displaying regulatory properties (Selvendran *et al.* 1987). Being a component of human daily diet, these polymers have a significant influence on metabolism (Rosen *et al.* 2011). They also affect on physicochemical characteristics of cereal products (Kim and d'Appolonia, 1977).

Arabinoxylans are the main components of dietary fiber, and their content is for about 64% of the total amount of polysaccharides of rye dietary fiber. Their

concentration in rye grain varies from 6.5 to 11.5% dm (Saastamoinen *et al.* 1989; Hansen *et al.* 2004), while in the endosperm, their content is from 4.0 to 4.7% dm (Cyran and Cygankiewicz, 2004). Due to the even distribution of soluble dietary fiber in cereal grains (Nyman *et al.* 1984), rye wholemeal (2.1 –2.5% dm) and endosperm flour (2.5–2.8% dm) contain similar amounts of soluble arabinoxylans (Bengtsson *et al.* 1992; Cyran and Cygankiewicz, 2004), in contrast to an insoluble fraction, which is almost four times less in endosperm flour than in wholemeal flour.

Structural features

The heterogeneous structure of arabinoxylans is a result of the great diversity subunits building them and being components of the general population, in terms of polymerization degree (molecular mass distribution), structural characteristic of xylose chain and also different kind of linkages from others cell-wall components, such as cellulose, β-glucan, lignin and proteins. Individual subunits of arabinoxylans can be linked permanently by covalent bonds, determining the structure and cell-walls properties, and unstable by coordination bonds, usually hydrogen bonds or van der Waals' forces, stabilizing the polysaccharide helical conformation. Regardless of the type of cell-wall polysaccharide, the glycosidic linkage is a primary covalent bond between its monomers. The intracellular and intercellular interactions, both covalent and coordinative, between the cell-wall subunit result in a three-dimensional heterocomplex, in which cellulose microfibers are entwined with cross-linked xylans and structural proteins and lignin complete a whole.

The fundamental division of arabinoxylans into two fractions is results from the fact, that water-soluble arabinoxylans in opposite to water-insoluble arabinoxylans, are not covalently linked with cell wall but form a layer onto its surface (Mares and Stone, 1973), which allows them to be water extracted. The remaining insoluble fraction is mostly susceptible to extraction by alkali, during which the ester-bonds are gradually hydrolyzed. The high resistance to alkaline hydrolysis of the fraction remaining after extraction with a strong alkali, with a high degree of lignification, is conditioned by the presence of ether-bond and the hydrophobic nature both lignin and cellulose.

The structure of the chain

Arabinoxylans are biopolymers which are characterizing by irregular and variable branching, where the leading polysaccharide chains are composed of the β-D-xylopyranose units joined by the (1→4)-glycosidic bonds (Bengtsson and Åman, 1990; Ebringerova *et al.* 1990; Bengtsson *et al.* 1992; Vinkx and Delcour, 1996). The α-L-arabinofuranose units substituted at the *O*-3 position or rarely in the *O*-2 position, and also the *O*-2 and the *O*-3 positions form the branching off of the basic chain. The α-L-arabinofuranose residues linked in the *O-3* position of principal xylose chain are the site attachment of phenolic acid residues, in particular ferulic acid substituted in the *O*-5 position of the arabinofuranose ring (Saulnier *et al.* 1999). On the other hand, the uronic acid residues, mainly glucuronic and sporadically galacturonic, are gly-

cosidic bond with the main β-D-xylopyranose chain in the *O-2* position (Fincher and Stone, 1986). Occasionally the acetyl groups are present in the *O*-2 position.

The total fraction of water-soluble arabinoxylans contains 49–56% unsubstituted xylopyranose residues, 35–36% xylopyranose monosubstituted in *O*-3 position and 9–15% xylopyranose disubstituted in *O*-2 and *O*-3 positions (9–15%) (Nilsson *et al.* 1997). Hydrogen bonds that are formed between xylopyranose monomers in the unsubstituted part of the polymer cause the aggregation of arabinoxylans, thus decreasing its solubility in water (Andrewartha *et al.* 1979). The presence of arabinofuranose substituents increases the asymmetry of macromolecules resulting in a decrease in their aggregation. The higher arabinozylation degree, the higher an asymmetry exists. These factors reflect directly into both the solubility in water and the functional properties of arabinoxylans (Izydorczyk and Biliaderis, 1995). It has been shown that the arabinozylation degree decreases with the increasing distance from the outer layers of the grain (Bengtsson and Åman, 1990; Ebringerová *et al.* 1990).

The diversity of structural subunits included in a general fraction of arabinoxylans was illustrated by their sequential fractionation with using solutions with the increasing concentration of ethanol or ammonium sulfate (Izydorczyk and Biliaderis, 1995; Vinkx and Delcour, 1996; Cyran *et al.* 2003; Cyran and Saulnier, 2005). It has been shown that the increasing concentration of ethanol or ammonium sulfate caused the precipitation of arabinoxylans among a growing degree of arabinozylation (Cyran, 2015). The predominant subunit of water-soluble arabinoxylans in rye wholemeal and endosperm flour, signed as AX-I, constituted 60–70% of the general population of these polymers and contains only xylose units which are monosubstituted of α-L-arabinofuranose residue in *O*-3 position and its arabinozylation degree, expressed by the arabinose-to-xylose ratio 0.47–0.51 (Cyran *et al.* 2003). The content of AX-II subunit, with the higher degree of arabinozylation (0.72–0.9), comprised 20–24% of arabinoxylans population and its chain was built of both unsubstituted xylopyranose units as well as mono- and disubstituted residues with the substituents in *O*-3 position and also *O*-2 and *O*-3 positions, respectively. The highest degree of arabinozylation (0.98–1.28) was characteristic of AX-III subunit, which contained almost exclusively disubstituted xylopyranose residues. The AX-IV subunit with the lowest degree of arabinozylation (0.30–0.31) was isolated from the rye bran, which constituted 8–11% of the total population in whole grain and contains mono- and disubstituted xylopyranose residues in the chain (Cyran and Saulnier, 2005).

The total fraction of water-insoluble arabinoxylans in rye grain is more diverse in terms of the number of subunits with a different degree of arabinozylation, compared to those observed in water-soluble fraction. Using a sequential extraction with barium hydroxide, water, 1M, and 4M sodium hydroxide, only the 90% of the material recovered after extraction was solubilized, the residual 10% of precipitate was an alkali non-extracted fraction, which contains the arabinoxylan with the highest degree of arabinozylation 1.20–1.22. The dominant subunit of arabinoxylans (22–26%) with a degree of arabinozylation 0.38, corresponding with the water-soluble fraction AX-I,

consisted of xylopyranose residues which are unsubstituted and monosubstituted in the *O*-3 position. This part of arabinoxylans was almost totally extractable with barium hydroxide. The subunits with a higher degree of arabinozylation 0.73–0.90 (16–20%) and 0.95–1.23 (11–13%), sequentially extracted with subsequent alkali, contained both mono- and disubstituted xylopyranose residues. However, the lowest degree of arabinozylation 0.07 was observed in the unit precipitated after extraction of 1M sodium hydroxide (16–18%) (Cyran and Saulnier, 2007).

Ferulic acid dehydrodimers (DiFA) play a significant role in the structure of arabinoxylans because of their ability for cross-linking of arabinoxylans (Bunzel *et al.* 2008; Bunzel *et al.* 2005; Dobberstein and Bunzel, 2010; Quideau *et al.* 2011; Quideau and Ralph, 1997; Ralph *et al.* 1998; Ralph *et al.* 1994; Vinkx and Delcour, 1996; Waldron *et al.* 1996). Arabinoxylans cross-linking increases the stiffness of cell walls, furthermore it is a nucleation site of lignin and forms a specific linkage between arabinoxylans and lignin. Binding of these polymers decreases the digestibility of monocotyledons by both digestive enzymes of ruminants, as well as by enzymatic mixtures of β-xylanases and β-xylosidases, likewise contributes to limiting the cell walls expansion and enhances their protection against pathogens (de Buanafina, 2009). Water-soluble arabinoxylans have about 30-fold lower degree of cross-linking (DiFATot = 83 $\mu g \times g^{-1}$ of soluble dietary fiber; degree of cross-linking DiFA/Xyl = 0.2) than the water-insoluble arabinoxylans (DiFATot = 3647 $\mu g \times g^{-1}$ of insoluble dietary fiber; DiFA/Xyl = 6.2), with the 8-8’ dehydrodimer as a dominant cross-linking agent in water-soluble part of arabinoxylans, while the 8-5’ dehydrodimer is the main structure which is present in water-insoluble arabinoxylans (Bunzel *et al.* 2001).

Molecular mass distribution and macromolecular parameters

The molecular mass of rye water-soluble arabinoxylans as well as their shape and conformation, defined by the radius of gyration, strongly influences both the water extract viscosity and intrinsic viscosity values, determining the degree of contribution of dissolved substances in the viscosity of the solution. The value of intrinsic viscosity also depends on the hydrodynamic volume of the particles, which, in turn, may be the main parameter ensuring stiffness of arabinoxylans gels. It was found that the rye arabinoxylans are characterized by the largest radius of gyration (Dervilly-Pinel *et al.* 2001); thus, the viscosity of the water extract of rye flour and the intrinsic viscosity is higher than for other cereals. This differentiation occurs within rye varieties as well; the water-soluble fraction of rye arabinoxylans responsible for the high water extract viscosity was a collection of high-molecular-weight macromolecules, showing higher intrinsic viscosity, a longer radius of gyration, larger hydrodynamic radius and a lower degree of branching in comparison with arabinoxylans responsible for small and medium values of water-extract viscosity (Cyran *et al.* 2003; Ragaee *et al.* 2001). Previous studies were shown, that when the average molecular weight was $4.2 \cdot 10^5$ $g \times mol^{-1}$, the water extract viscosity was 11.9 mPa and intrinsic viscosity was 376 $ml \times g^{-1}$. In contrast, a slight, almost 5% increase in average molecular weight ($4.4 \cdot 10^5$ $g \times mol^{-1}$) causes nearly two-fold increase

in water extract viscosity value (21.8 mPa) and about 40% increase in intrinsic viscosity (539 ml × g^{-1}) (Cyran and Saulnier, 2007). However, since the last parameter, described by Mark-Houwink equation, is mostly dependent on the geometry and size of the polymer particles as well as the degree of aggregation of molecules, the determination of molecular masses of arabinoxylans to achieve the desired viscosity parameters is difficult.

The polydispersity (DP) of arabinoxylans, meaning as the degree of their structural homogeneity and described as the relationship of the weight-average molecular weight to the number-average molecular weight, is an essential parameter. With the increase in the DP value, the distribution of the molecular weight of arabinoxylans increases. The range of variability is included in the values 1.00 – 2.20, depending from the rye variety (Cyran *et al.* 2012; Cyran and Saulnier, 2005). By using the values of polydispersity coefficients calculated for both flour and bread, the information about the degree of degradation of arabinoxylans during the dought production and the thermal process can be obtained (Rakha *et al.* 2010); thus the parameter can be used to assess the bakery sustainability of rye variety.

The average molecular weight of rye arabinoxylans available in the literature varies significantly depending on the determination technique used. Molecular sieve separation and the calibration based on pullulan standards resulted in obtainment the particles with molecular weights from 2.75×10^5 g × mol^{-1} to 7.70×10^5 g × mol^{-1} (Girhammar and Nair, 1992). Application the modern chromatographic columns with better separation parameters in high-pressure size exclusion chromatography system with the laser light detection (HP-SEC-MALLS), estimated the absolute molecular weights of polymers showed a greater diversity of results, from 3.5×10^4 g × mol^{-1} to 2.02×10^6 g × mol^{-1}, where the molecular weights of arabinoxylans were different depending on the isolation agent used (water, buffer, alkali), the fractionation method (ethanol, ammonium sulfate) as well as a limit of detection (Andersson *et al.* 2009; Cyran *et al.* 2004; Cyran *et al.* 2003; Cyran, 2010; Cyran and Saulnier, 2007; Cyran and Saulnier, 2005; Izydorczyk and Biliaderis, 1995).

HYDROLYTIC DECOMPOSITION BY NATIVE ENZYMES OF RYE GRAIN

Arabinoxylans undergo hydrolytic degradation (Fig. 1) under the influence of the action both of endogenous and grain-associated hydrolytic enzymes: xylanases (Dornez *et al.* 2009), xylosidases (Rasmussen *et al.* 2001) and arabinofuranosidases (Saha, 2000) as well as cinnamic acid esterases, especially feruloyl esterases (Kroon *et al.* 1999). The activities of xylanases, xylosidases, and arabinofuranosidases are extensive (0.01–33.7 nkat × g^{-1} dm; 0.29–0.91 nkat × g^{-1} dm; 0.22 – 0.99 nkat·g^{-1} dm; respectively), whereas feruloyl esterase activity is almost the same (0.18 – 0.22 nkat × g^{-1} dm) (Dynkowska *et al.* 2015; Hansen *et al.* 2002). The degradation of arabinoxylans is mostly dependent on their structure, especially on the distribution of arabinofuranoside substituents (Vinkx and Delcour, 1996; Wong *et al.* 1988).

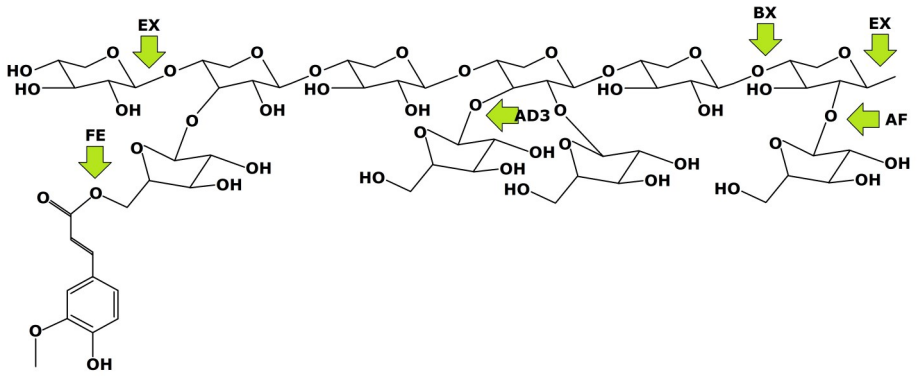

Fig. 1. The structure of arabinoxylan and its susceptibility to hydrolytic enzymes: EX – endo-β-D-xylanase; BX – β-xylosidase; AF – α-L-arabinofuranosidase; FE – feruloyl esterase; AD3 – arabinoxylan arabinofuranohydrolase-D3

Endo-1,4-β-xylanases

Native (endogenous) and microbial (exogenous) xylanases are the main enzymes degrading the arabinoxylan chain. Native xylanases are synthesized during the germination, and the endo-1,4-β-xylanase inhibitors do not inhibit their activity. In contrast, microbial xylanases are present in the outer layer of grain and showing sensitivity to the activity of the inhibitors (Vidmantiene and Juodeikiene, 2010). So far, almost 300 xylanases were identified, which have been classified in six different families of glycoside hydrolases (Collins *et al.* 2005; Dornez *et al.* 2009; Mendis *et al.* 2016). The classification of these enzymes takes into account their origin as well as the heterogeneity and complexity of structures of arabinoxylans (Wong *et al.* 1988).

The action of endo-1,4-β-D-xylanases (EC 3.2.1.8) is based on the hydrolysis of internal (1→4)-glycosidic bond between the β-D-xylopyranose residues in the xylan backbone in a random manner. The degree of arabinosylation and arabinofuranoside substituents distribution in xylose chain influence in enzymatic activity. The low arabinozylation (Ara/Xyl ≈ 0.4) results in the facile hydrolysis of arabinoxylan molecule, whereas if Ara/Xyl ≈ 0.9, their degradation is limited. The end-products of the xylanases activity are short-chain oligoarabinoxylans, mostly with a polymerization degree DP ≥ 6, longer chains are very rare (Szwajgier and Targoński, 2006). Hydrolytic action of endo-1,4-β-D-xylanases is blocked by xylanases inhibitors (SCXI; *Secale Cereale Xylanase Inhibitor*) (Debyser *et al.* 1999; Goesaert *et al.* 2002; Fierens *et al.* 2007).

The 1,4-β-xylosidase (EC 3.2.1.37) is a supplement of endo-1,4-β-xylanase action. It catalyzed the hydrolysis of non-reducing unsubstituted xylose residues of xylooligosaccharides (Jordan, 2008). The activity of 1,4-β-xylosidase correlates with the activity of other enzymes present in cereal grain, mainly with the esterases of ferulic acid and p-coumaric acid (Borneman *et al.* 1990).

Arabinofuranosidases

α-L-Arabinofuranosidases (α-1-arabinofuranosidase arabinofuranohydrolase, EC 3.2.1.55, arabinofuranosidase) are exogenous enzymes hydrolyzing terminal non-reducing α-arabinofuranoses from arabinoxylans (Saha, 2000). The substrate specificity determines their division into the AXH-m type, hydrolyzing glycosidic bonds between arabinofuranose and monosubstituted xylose residue in the *O*-2 or the *O*-3 position (McCleary *et al.* 2015) and the AXH-d3 type, capable of releasing arabinofuranose residue from disubstituted xylose residues (Lagaert *et al.* 2010; McCleary *et al.* 2015). However, the lack of α-arabinofuranosidases activity has been demonstrated in the case of arabinofuranoside residues esterified with ferulic acid (Biely *et al.* 2016). The successive hydrolysis of arabinofuranose residues is carried when arabinofuranose chain are present as substituents of arabinoxylans because the steric hindrance does not allow to hydrolyze the internal glycosidic bonds (Saha, 2000). Seven families of glycoside hydrolases were identified, and the specificity of the action, including substrate selectivity, was the selection factor (Lombard *et al.* 2014).

Ferulic acid esterases

Ferulic acid esterases (EC 3.1.1.73) are a group of carboxylesterases hydrolyzing the ester bond not only between the C-5 arabinose carbon atom and ferulic acid carboxylic group (Biely *et al.* 2016) but also between the two residues of ferulic acid which formed diferulic bridges linked two polymer chains of cell wall polysaccharides. The researches showed their hydrolytic ability concerning the three kinds of dehydrodiferulic bridges present in plant cell walls: 5-5', 8-O-4', and 8-5' benzofuran form (Garcia-Conesa *et al.* 1999). These enzymes also participate in the degradation of bonds between the various components of cell walls, including arabinoxylans with lignin (Fazary and Ju, 2007).

Depending on the place of their activity, the two types of esterases have been found; thus, these enzymes can be one of the analytical tools allowing us to determine the degree of dimerization. Type A esterases hydrolyze the bonds in regions with more hydrophobicity and larger substituents attached to the aromatic ring. In contrast, type B esterases preferred the substrates located at the hydrophilic regions with the smaller substituents on the benzene ring. The presence of ferulic acid dehydrodimers increases the hydrophobicity of arabinoxylans compared to places where the monomeric ferulic acid residues are attached. The enzyme activity is spatially limited, thereby the selectivity of its action implicated (Kroon *et al.* 1999; Kroon and Williamson, 1999).

Microbial esterases significantly contribute to increasing the degradation of cell wall polysaccharide chains mainly by the removal substituents of arabinoxylan chains, especially ferulic acid residues and its dehydrodimers, which protect the enzyme access to the xylose backbone. Also, the synergistic effects of esterases with endo-1,4-β-xylanase has been demonstrated (Szwajgier and Targoński, 2006).

PHYSICOCHEMICAL FEATURES

Ability to elevate extract viscosity

The macromolecular structure of arabinoxylans, capable of forming branched spatial structures, causes that these substances are responsible for the formation of viscous solutions (Ficher and Stone, 1986; Fengler and Marquardt, 1988; Izydorczyk and Biliaderis, 1995). It has been demonstrated that there is a simple relationship between the concentration of water-soluble arabinoxylan and water extract viscosity (Izydorczyk and Biliaderis, 1995; Saulnier *et al.* 1995). The viscosity of water extract is determined by not only the amount of water-soluble arabinoxylans but also its structural features such as the length of xylopyranose chain, arabinozylation degree as well as arabinofuranoside substituents distribution, which imply their hydrodynamic volume and radius of gyration (Courtin and Delcour, 2002; Muralikrishna and Rao, 2007).

The larger radius of gyration of rye arabinoxylans compared to arabinoxylans of other cereals results in higher viscosity values of rye flour water extracts (Dervilly-Pinel *et al.* 2001). Decreasing the viscosity of water extract is correlated with the increased degree of arabinosylation, thus a lower content of ferulic acid residues. The lack of a simple relationship between viscosity values and the arabinoxylan structure, being a result of the discrepancy of the values obtained to the values expected, is suggesting that the specific spatial arrangement of arabinose substituents can be an essential factor affecting the water-extract viscosity (Izydorczyk and Biliaderis, 1995).

Oxidative cross-linking

The presence of ferulic acid linked esterifically with arabinose in the *O*-5 position is the main factor determining the formation of a three-dimensional arabinoxylan network (Moore *et al.* 1990). The oxidative cross-linking mechanism is based on catalytic dehydrogenation of the hydroxyl group at the *C*-4 position of the aromatic ring; thus, highly reactive isomeric phenoxyl radicals are formed. The activity center with the unpaired electron is located at the *C*-5 location in the aromatic ring or the *C*-8 position of the side chain. These radicals can combine each other forming the C-C bonds resulted in the isomeric structures of ferulic acid dehydrodimers are formed (Ralph *et al.* 1994). The catalytic agent is a redox system consisting of hydrogen peroxide and peroxidase (Iiyama *et al.* 1994; Ralph *et al.* 1994) or fungal laccase with the peroxidase linked with the cell wall (Figueroa-Espinoza and Rouau, 1998). Hydrogen peroxide concentration is the factor limiting the cross-linking of arabinoxylans. In contrast, ferulic acid and vanillic acid are cross-linking inhibitors (Moore *et al.* 1990) similar to ascorbic acid and cysteine, which reduce the amount of hydrogen peroxide through competitive reactions (Vinkx *et al.* 1991). The arabinoxylans cross-linking coefficient expressed as the molar ratio of the number of ferulic acid dehydrodimers to the amount of xylose in arabinoxylan fraction is much more representative to describe the cross-linking extent than only ferulic acid or its dehydrodimers content in arabinoxylans (Bunzel *et al.* 2001).

As a result of oxidative cross-linking, ferulic acid dehydrodimers play a crucial role in modifying the mechanical properties of cell walls and limiting their availability, by forming diferulic bridges between two polysaccharide chains (Rybka *et al.* 1993) as well as between polysaccharide and lignin (Hatfield *et al.* 2017; Hatfield *et al.* 1999). The researches indicate the relationship of the amount of ferulic acid dehydrodimers and extractability of arabinoxylans by increasing the molecular weight of arabinoxylans and also possibly arabinoxylan-lignin cross-linking thus including to insoluble part of dietary fiber. Besides, the presence of diferulic bridges and the degree of arabinoxylans cross-linking have been shown to have a significant effect on enzymatic degradation by the inhibition of exogenic enzymes, which describes the physiological role of arabinoxylans in the organism (Bunzel *et al.* 2001).

Hydration

The presence of the hydroxyl group in the structure of arabinoxylans causes the formation of hydrogen bonds. The coordination bonds forming by the free electron pairs of the oxygen atom allow to intramolecular connections between hydroxyl groups of polysaccharides. Surface tension forces cause the water molecules to penetrate the structure of arabinoxylans, thereby allowing the formation of coordination bonds between arabinoxylans hydroxyl groups and water as well. The water molecule can form the bridge, where two electron pairs of the oxygen atom of the water molecule are involved in the formation of bonds between two hydroxyl groups of one arabinoxylan chain. In another case, the one electron pair from water oxygen atom is coordinated to the hydrogen atom of an arabinoxylan hydroxyl group. In contrast, the second electron pair binds subsequent molecules of water. The maximum hydration of polymer occurs, and such polysaccharide structures, exhibit both the greatest freedom of movement of polysaccharide molecules and water molecules, as well as the highest hydrophilicity. The presence of polysaccharide carboxylic groups tends to increase the density of water because the distance between two oxygen atoms from carboxylic groups of arabinoxylans, coordinatively linked by a hydrogen atom (2.2 Å) is much smaller than the distance between two analogous oxygen atoms in water (2.8 Å) (Chaplin, 2003). Cross-linked arabinoxylan has been shown to bind much larger amount of water than its less cross-linked counterpart (Izydorczyk *et al.* 1990; Vinkx and Delcour, 1996), and cross-linked arabinoxylans can contain up to 100 g of water per gram of polymer; the addition of electrolytes does not change the degree of hydration of arabinoxylans (Döring *et al.* 2016; Izydorczyk and Biliaderis, 1995; Muralikrishna and Rao, 2007). Water-soluble arabinoxylans exhibit water retention in amounts up to 6.3 -fold of their weight, while the water-insoluble up to 10-fold (Courtin and Delcour, 2002; Jelaca and Hlynka, 1972; Kim and D'Appolonia, 1977).

Antioxidant potential

The antioxidative properties of arabinoxylans depend on the presence of ferulic acid residues and its isomeric dehydrodimers in their structure. It is believed that the strong antioxidant effect of arabinoxylan oligomers is caused

by the hydrolysis of ester bonds between arabinofuranose and ferulic acid residue by the bacterial flora of the large intestine (Broekaert *et al.* 2011). Recent reports, however, indicate the antiradical activity of fragments of arabinoxylans in which the presence of ferulic acid residues was found (Malunga and Beta, 2015); short chains of polysaccharides were found to have a stronger antioxidant effect than released phenolic compounds. One of the arguments favoring this theory is the fact that only part of arabinoxylans is fermented in the gastrointestinal tract; thus, there is an only partial release of ferulic acid residues and its dehydrodimers, while antioxidative activity in colonocytes would indicate on the action performed by bound phenolic compounds. This theory is confirmed by the fact that water-soluble arabinoxylans deprived ferulic acid residues do not show antioxidant activity. Also, by comparing antioxidant activity of free phenolic acids and ferulic acid attached in water-soluble arabinoxylans with the equimolar content of phenolic compounds, the higher antiradical potential was found for bound phenolic compounds. The explanation may be the fact that the phenolic compounds esterified with the soluble part of arabinoxylans are better soluble in body fluids. The reduction of the molecular weight of water-soluble arabinoxylans is also one of the factors that their antioxidant activity increases (Malunga *et al.* 2017). In other studies, there was an inverse relationship between the antioxidant activity of water-soluble arabinoxylans and their degree of arabinosylation, suggesting the attribution of the highest antioxidant activity to unsubstituted xylose residues. In this case, the antioxidant activity would be assigned to the interaction of hydroxyl groups of unsubstituted or monosubstituted xylose residues (Malunga and Beta, 2015). These results showed that the factor limiting the antioxidant activity of arabinoxylans is the mechanism of antioxidant action in addition to their molecular structure. The chelation of metal ions, particularly Cu^{2+}, responsible for catalytic oxidation of lipid, using hydroxyl groups, may explain the antioxidant activity of arabinoxylans. In the case of water-insoluble arabinoxylans, Serpen *et al.* (2007) pointed to a decrease in the antioxidant potential of them after alkaline hydrolysis and releasing the phenolic compounds from the polysaccharide complex.

METABOLISM IN THE HUMAN BODY

Arabinoxylans are the main component of dietary fiber, not digestible in the human gastrointestinal tract. The specific structure of rye arabinoxylans caused them resistant to modification and degradation by digestive enzymes in the small intestine (Cummings, 1987). In contrast, these degradative processes are performed by the bacterial enzymes of the human intestinal flora colonizing the lower part of the human digestive tract.

The upper part of the human digestive tract

It has been assumed that in the mouth, stomach, and small intestine, the hydration processes prevail, causing the dispersion and dissolution of small molecular weight polymers. In consequence, the arabinoxylans are swelling and increasing the volume of dietary fiber. The low pH of gastric juice causes partial

degradation of arabinoxylans; however, this process has a negligible extent (Salyers *et al.* 1977).

The lower part of the human digestive tract

A colon is a place of intense bacterial degradation of water-soluble arabinoxylans as well as partial, dependent from a degree of binding to lignin, a water-insoluble fraction (Knudsen *et al.* 1991; Cyran *et al.* 1995), where the intestinal bacterial flora intensively participates in this process (Stephen and Cummings, 1980). The breakdown of arabinoxylans by fermentation is largely dependent on their molecular structure, especially the degree of arabinosylation, the manner of arabinose substitution as well as cross-linking by ferulic acid dehydrodimers and combination with other diet components (Glitsø and Knudsen, 1999).

Enzymes of colon microflora depolymerize arabinoxylans to xylooligosaccharides (XOS), arabinoxylooligosaccharides (AXOS), xylotrioses and xylobioses, which are then hydrolyzed to free xylose (Broekaert *et al.* 2011; Grootaert *et al.* 2007). Released arabinose was likely to be taken up rapidly by the microbes (Feng *et al.* 2018). Previous studies have shown the higher extend of degradation of arabinoxylans with a low degree of cross-linking than those being more cross-linked (Glitsø and Knudsen, 1999). Next to xylanases, Andreasen *et al.* (2001) also indicated the presence of intestinal esterases hydrolyzing the esters of hydroxycinnamic acids. Free phenolic acids and ferulic acid dehydrodimers releasing from arabinoxylans are then degraded by *Lactobacillus brevis* to vinylphenols.

The final products of arabinoxylans degradation are short-chain fatty acids, mainly butyric acid, propionic acid, and acetic acid (Fig. 2); their presence decreases the pH of intestinal juice. The low intestinal pH inhibits the growth of putrefactive bacteria, contributing to the improvement of the intestinal flora composition (Döring *et al.* 2016), which makes it possible to classify arabinoxylans as prebiotics (Delcour *et al.* 2016; Slavin, 2000). The content and profile of short-chain fatty acids depend on the kind of substrate as well as intestinal bacterial flora. Resulted organic acids are quickly absorbed from the intestine. It was found that they have a significant impact on the absorption of both water and sodium ions. Butyric acid is the primary source of energy to colonocytes and is metabolized by these cells, which accounts for 70% of the total energy demand of the colon mucosa. Also, this compound is essential in the prevention and treatment of colon mucosal diseases, such as distal ulcerative colitis and cancer. It has been found that reduced oxidation of butyric acid in colonocytes contributes to ulcerative colitis (Henningsson *et al.* 2001). Unlike butyric acid, propionic acid is metabolized in the liver, where it has the inhibitory effect in the processes of gluconeogenesis and cholesterogenesis, i.e., enzymatic transformation non-sugars precursors into glucose and cholesterol, respectively, and also increases the efficiency of the glycolysis – the enzymatic pathway in hepatocytes where glucose is transformed to pyruvate. Acetic acid is also utilized in the liver, and their metabolite is an essential precursor in lipogenesis (esterification of fatty acids to triacylglycerols), but also stimulates gluconeogenesis (Henningsson *et al.* 2001). The end-products of the arabinoxylans me-

tabolism are also intestinal gases such as hydrogen, carbon dioxide, and methane (Knudsen *et al.* 1991; Cummings, 1984).

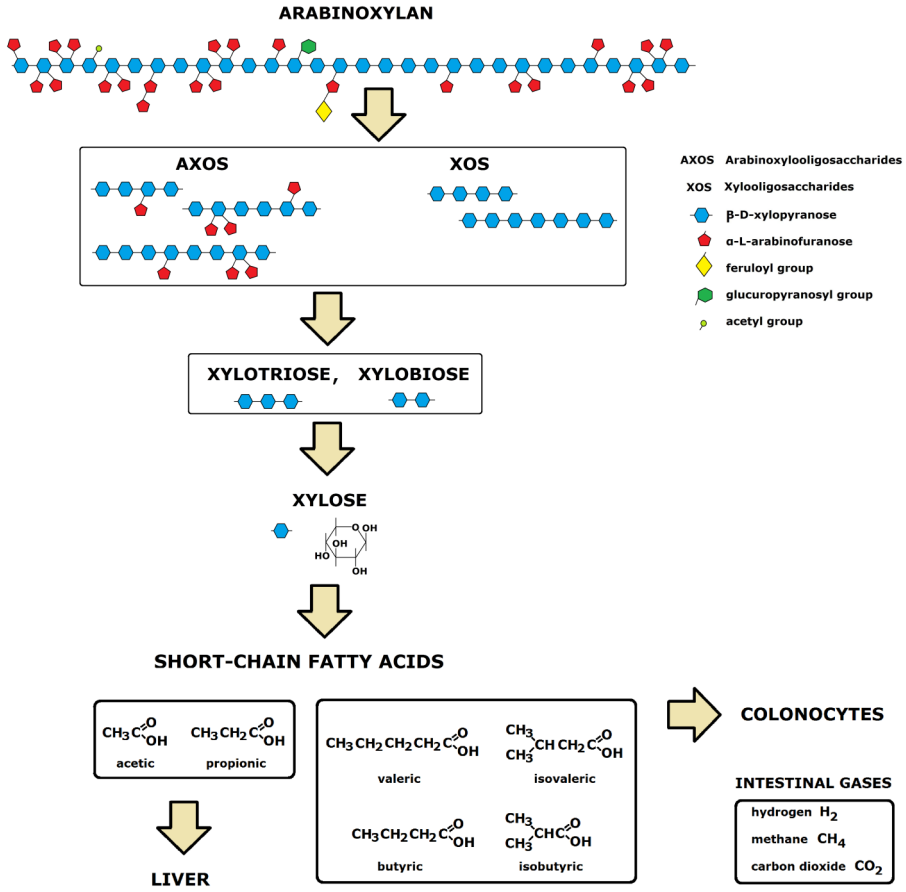

Fig. 2. The arabinoxylans degradation pathway

The role of water-insoluble arabinoxylans whose are not degraded to smaller fragments of oligosaccharide thus not undergo intestinal fermentation, mainly consist of the increasing fecal bulk, which results in improved bowel peristalsis, accelerated intestinal transit time of meal as well as reduced the susceptibility of constipation (Muralikrishna and Rao, 2007; Slavin, 2013). According to Cummings (1984), the specificity of the action of non-degraded arabinoxylans consists in retaining intestinal water and some of the intestinal gases in the structure of water-insoluble arabinoxylans and other components of insoluble dietary fiber, whereby the mechanical pressure of increased fecal masses on the intestinal wall leads to irritation of nerve receptors causing increased bowel peristalsis.

The knowledge about the fermentation of arabinoxylans in the human large intestine is mainly based on indirect studies such as the analysis of the content of intestinal gases and short-chain fatty acids in the feces. Differences between species and individuals in the composition of intestinal flora and gastrointestinal

tract functionality cause that the studies on animals provide only general knowledge about dietary fiber metabolism even though they are supported by in *vitro* analysis (Henningsson *et al.* 2001). Also, thermal treatment of the consumed product and combining it with other components of daily diet makes it difficult to understand and clearly define both the direction of chemical alteration and health-promoting effects of the action of arabinoxylans and their metabolites.

HEALTH-PROMOTING ACTIVITY

Despite the small content of these compounds in the total weight of the whole cereal grain, their biological functions related to their physicochemical contribute to a large extent to the proper functioning of the body and keep it in good health.

The main places of the health-promoting effect of arabinoxylans are the small intestine and colon. The structural specificity of water-soluble arabinoxylans, especially the ability to form viscous solutions and oxidative cross-linking, makes it possible to describe this food component as a barrier to starch hydrolyzing enzymes. Confirming clinical studies indicated a reduction in the level of postprandial glucose in the blood after consumption of cereal products, in particular those obtained from rye flour. The reason of observed action was the molecular weight of arabinoxylan and its spatial structure, including the degree of cross-linking but not its concentration (Brennan, 2005; Juntunen *et al.* 2003; Malunga and Beta, 2015; Slavin, 2004; Vinkx and Delcour, 1996)

The high viscosity of digestive content caused by the presence of water-soluble arabinoxylans has a negative effect on the activity of the digestive enzymes. It initiates the formation of a viscous, sticky layer adhering to the mucosa, which blocks on the physical way the absorption of nutrients (Boros and Bedford, 1999). Increased intestinal viscosity is considered to be the fundamental element of the mechanism that leads to lowering the cholesterol and blood glucose as well as bile acid sorption after consumption of cereal products (Brennan, 2005; Slavin, 2013; Slavin, 2004; Topping, 1991). Consequently, it reduces the risk and supports the treatment of heart diseases, type 2 diabetes, and obesity (Jenkins *et al.* 2004; Lu *et al.* 2004).

As mentioned, the intestinal fermentation of water-soluble fraction of dietary fiber results in the formation of short-chain fatty acids, in particular butyric, propionic, and acetic. Depending on the substrate fermented, the isobutyric and isovaleric acids may also be formed (Tungland and Meyer, 2002). A group of these compounds regulates energy homeostasis and glucose metabolism and also supports the immune system. Their production lowers the intestinal pH, which is associated with the stimulation of mineral absorption and reduced production of bile acid in the colon (Henningsson *et al.* 2001). Above all, short-chain fatty acids are absorbed by colonocytes and are metabolized in the liver modulating cholesterol synthesis (Delcour *et al.* 2016). It is also assumed that

short-chain fatty acids are a factor conducive to the multiplication of intestinal microflora and simultaneously blocking the growth of harmful bacteria; it has been shown that butyric acid intensifies the apoptosis of human colon cancer cells (Tungland and Meyer, 2002).

According to some authors, long-term consumption of food rich in components caused the elevation the viscosity of alimentary tract content and induced the adaptive changes in the digestive system through the excessive growth of some organs, as shown by studies in rats (Ikegami *et al.* 1990, Ikegami *et al.* 1983). This phenomenon was explained through the mechanism of compensation for the insufficiency of digestion and reduced absorption of nutrients to the organism. At the moment, there is no evidence for the hyperplasia action of arabinoxylans in the human body.

The water-insoluble dietary fiber has a slightly different health-promoting significance in the human body. Arabinoxylans, which undergo bacterial degradation to a small degree, due to their high water-binding capacity, affect the increasing fecal bulk, thus becoming an environment for substances not digested in the digestive system, especially toxins that are diluted in fecal masses. The volume of accumulated fecal masses results in intensified intestinal motility, and as a result, the intestinal transit time is reduced; thus, the water absorption is decreased (Cummings, 1984).

IMPACT OF THE QUALITY OF BREAD

The content in rye arabinoxylans concerning the amount of protein in rye flour determines the method of preparing bread dough as well as the appearance and organoleptic properties of rye bread (Heiniö *et al.* 2003). The volume of bread depends on the amount of water added to the dough, the time of dough kneading, and the degree of hydration of arabinoxylans (Vinkx and Delcour, 1996). Rye bread usually has a smaller volume and is more humid than wheat bread. Hydration capacity of arabinoxylans contributes to increasing the volume of rye bread and also delaying its aging (Izydorczyk and Biliaderis, 1995). The degree of cross-linking of arabinoxylans influences the water binding, thus implies the formation of viscous solutions and, in consequence, improves the parameters of rye dough. Water absorption of arabinoxylans in rye flour does not allow for the development of a gluten matrix, as is in the case of wheat flour, which results in these polymers being the main structure-forming factor of rye dough (Gąsiorowski, 1994).

The three-phase bread-making method is traditionally used to obtain rye bread. The interactivity of native enzymes of rye grain with the enzymes *Lactobacillus brevis* strain allows obtaining bread with pro-health activity due to the increase of water-soluble arabinoxylans in the liquefaction process from water-insoluble dietary fiber. The fermentation process used in the three-phase bread-making also contributes to increasing the amounts of phenolic bioactive substances, including phenolic acids. Increased content of the phenolic compounds may influence the insulin response of the human body (Zamaratskaia *et al.* 2017).

CONCLUSIONS

Stopping the development of civilization diseases such as obesity, type 2 diabetes, or cardiovascular diseases is one of the main challenges in modern science. Based on the message that "prevention is better than cure", one of the preventive measures is to supplement the daily diet with cereal-based products with increased dietary fiber. The unique properties of rye arabinoxylans predispose this cereal as a valuable component of the daily diet. The use of modern analytical techniques allowed for a more accurate description of these substances useful for health. However, due to the limited ability to reproduce the conditions prevailing in the human gastrointestinal tract, the knowledge about the action of these compounds and their metabolism, both individually and interaction with other food components should still be complemented by indirect tests, involving experimental animals as well as the direct ones, using volunteers involvement.

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