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ESTIMATION OF TECHNOLOGICAL VALUE AND CHEMICAL COMPOSITION OF SELECTED COMMON WHEAT CULTIVARS (*TRITICUM AESTIVUM* L.)

ABSTRACT

The aim of the study was to estimate the technological value and chemical composition of new common wheat cultivars. The experimental material consisted of grain and flour obtained from 5 cultivars of common wheat, registered in 2017-2019, donated by Plant Breeding Strzelce - IHAR Group Ltd., Co., and harvested in years 2017-2018. The physical characteristics of the grain were determined: milling yield, falling number, gluten content, Zeleny sedimentation index as well as farinograph analysis and laboratory baking were performed. Furthermore, the content of grain nutrients and dietary fiber was determined. Significant differences between cultivars in terms of technological parameters and chemical composition have been demonstrated. The protein content in the grain was in the range of 13.3-15.2%, and the amount of gluten in the range of 28.6-36.8%. The cultivars were characterized by low alpha-amylase activity with an average falling number value 331s. The average water absorption of flour was 62.1%, and the volume of obtained bread was in the range 318-381cm^3. The bread of best quality was obtained from the Wilejka cultivar, that was also the richest source of protein. The average dietary fiber content from two years of research was 10.8%, including 7.7% of non-starch polysaccharides (NSP), of which 5.8% of the insoluble fraction (I-NSP) and 1.9% of the soluble fraction (S-NSP). The spring wheat Alibi has been recognized as the richest source of dietary fiber. The tested cultivars were characterized by high technological value and very good chemical composition and can be recommended for use in the food industry.

Key words: bread, falling number, flour, gluten, protein, wheat

INTRODUCTION

Wheat is the world's first consumer grain (OECD/FAO, 2020). The two most popular species of this cereal are common or bread wheat (*Triticum aestivum* ssp. *vulgare*), which covers about 93% of the total crop area, and durum wheat (*Triticum turgidum* ssp. *durum*) used for pasta, covering about 7% of the area (Guzman *et al.*, 2019; Stevenson *et al.*, 2012). Wheat is used for consumption mainly as a white flour, although most of the valuable grain components are found in the bran fraction removed during

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milling (Lærke and Bach Knudsen, 2011). However, the share of bread and cereal products in the diet is large enough, that wheat, like other cereals, is an important source of energy and nutrients. Wheat covers about 50% of the daily requirement for carbohydrates and 30% for protein. It is also a valuable source of B vitamins, minerals and bioactive substances such as dietary fiber, exerting a number of health-promoting effects. Consuming fiber-rich products reduces the risk of diseases such as obesity, atherosclerosis, coronary artery disease, type 2 diabetes, and prevents the onset of some cancers (Shewry and Hey, 2015; Kendall *et al.*, 2010).

Depending on the direction of use, the wheat grain must meet certain quality criteria. When used for milling and baking purposes, appropriate grain and flour parameters are required. The quality of wheat flour depends on many factors, such as growing and environmental conditions and chemical composition of grain, in particular with regard to protein and starch content (Tomić *et al.*, 2015). In breeding works and in laboratories of the cereal-milling industry the estimation of wheat grain quality is most often based on the following factors: protein content, gluten content, sedimentation index, falling number and rheological properties (Szafrańska, 2012). Recently the growing number of diet-related diseases in the society indicates the need to search for raw materials, rich in nutrients and pro-healthy components that are useful for the production of high-quality food. Breeding works on wheat besides the amount of the harvest yield also strives to improve the quality of grain (Skrzypek A., 2019). For that reason, it is necessary to monitor newly introduced wheat cultivars in terms of a wide range of quality features. The most desirable raw materials for use in food are cultivars, which except to high technological value are also distinguished by good chemical composition. Therefore, the purpose of the research was to assess the technological suitability and chemical composition of new cultivars of common wheat.

MATERIALS AND METHODS

The research material consisted of grain and flour obtained from 5 cultivars of common wheat including four winter and one spring, kindly donated by the Plant Breeding Strzelce Ltd, Co., and registered in 2017-2019. The Euforia, Poezja and Wilejka cultivars belong to the quality group (A), while the Alibi and Plejada cultivars are bread wheats (B). The material was cultivated in the same environmental conditions in 2017-2018.

To produce flour the wheat grain was conditioned to 14% moisture content and ground on the Quadrumat Senior Laboratory Mill (Brabender), according to AACC 26 -50 (2003) procedure. The obtained flour was subjected to farinographic analysis, in accordance with ICC 115/1 (2005) procedure. The laboratory baking was carried out in duplicate according to the standard ICC 131 (2005) method with modifications using 100g of flour, 1.5g of salt and 3% of yeast. The bread volume was measured using 3D laser scanner Next Engine (Santa Monica, USA) with Scan Studio HD software. The falling number (FN) was analysed using the Falling Number 1800 Perten apparatus (Hagerstern, Sweden) according to the ICC 107/1 (2005) procedure. The wet gluten content was measured according PN-EN ISO 2145-2:2015-2 method, whereas the Zeleny sedimentation index according to PN-EN ISO 5529:2010 procedure.

The material for chemical analysis has been ground in the Perten Laboratory Mill 3100 (Hagerstern, Sweden) with a sieve diameter of 0.5 mm. The protein content was analysed using the Dumas method in the Rapid N Cube apparatus (Elementar, Germany), according to the AACC 46-30 method (2003). The ash content was performed gravimetrically, according to AOAC 923.03 method (1995), and the starch content was measured using the colorimetric method, according to the AACC 76-13 procedure (2003). The lipids content was assessed gravimetrically by extraction with solvent consisting of 60:40:1 (v/v/v) chloroform, methanol and concentrated hydrochloric acid, as described by Marchello *et al.* (1971). Dietary fibre content (DF) was determined using the enzymatic-chemical method as a sum of nonstarch polysaccharides (NSP), lignin and associated polyphenols in accordance with AACC 32-25 procedure (2003). NSP content with its fractionation to insoluble (I-NSP) and soluble (S-NSP) fraction was determined using gas chromatography as previously described by Englyst and Cummings (1984), as a sum of individual monomers: arabinose, xylose, mannose, galactose and glucose. Lignin and other insoluble residues were determined gravimetrically as described by Theander and Westerlund (1986). All analyses were performed in duplicate and the results reported on dry weight basis [% of d.w.].

The weather conditions in the subsequent years of plant cultivation were characterized based on the Sielianinowa (K) hydrothermal coefficients (Table 1) and calculated on the basis of the sum of average daily temperatures and the sum of precipitation for each month. The months were classified as follows: $K \leq 0.4$-month extremely dry; $0.4 < K \leq 0.7$ - very dry; $0.7 < K \leq 1$ - dry; $1.0 < K \leq 1.3$ - quite dry; $1.3 < K \leq 1.6$ - optimal; $1.6 < K \leq 2.0$ - moderately moist; $2.0 < K \leq 2.5$ - moist; $2.5 < K \leq 3.0$ - very humid; $K > 3.0$ - extremely humid (Skowera, 2014). Meteorological data were obtained from the meteorological station of Plant Breeding Strzelce, located near the place where wheat was grown.

Table 1

Sielianinowa coefficients (K) for the months of spring vegetation in the two years of research.

Months	Growing season	
	2017	2018
March	1.9	4.6
April	2.4	0.5
May	0.7	1.3
June	2.4	0.7
July	1.3	2.7
Entire growing season	1.6	1.4

The obtained results were subjected to statistical analysis. To determine the variability of individual technological parameters and chemical components between cultivars and years of cultivation, a two-way fixed model of analysis of variance (ANOVA) and Tukey's contrast analysis were performed. The significance level was set to $p < 0.01$ and $p < 0.05$. Statistical analyses were performed using Statistica 13.3 software (TIBCO Software, USA).

RESULTS AND DISCUSSION

The grain milling process significantly affects flour yield and its chemical composition. The milling yield did not differ significantly between wheat cultivars, and the values ranged from 68.7% to 73.8%, for the Poezja and Plejada cultivars respectively. However, significant differences were observed between subsequent harvest years, which confirms the impact of environmental conditions on this parameter. Higher values were found for cultivars harvested in 2018 (Table 2). Other researchers obtained wheat flour yields at a similar level, and the values ranged from 68.0% to 72.2% (Kweon *et al.*, 2009). The falling number, which is a measure of the activity of amylolytic enzymes, increases due to grain sprouting and is largely dependent on weather conditions, which is confirmed by significant differences in the value of this feature between harvest years (374s vs. 288s). Considering the hydrothermal conditions in July, being the pre-harvest period, 2018 was distinguished by two-fold higher value of the K factor (Table 1), which may result in greater amylolytic activity of grain from this year. The analysed wheat cultivars were characterized by low alpha-amylase activity in the range from 246s for the Alibi cultivar to 443s for the Euforia cultivar. Similar values in the range from 253s to 476s were previously described by Szafrańska (2014) and Ktenioudaki *et al.* (2011). The protein content and the amount of gluten significantly determine the usefulness of grain in breadmaking. Klockiewicz-Kamińska and Brzeziński (1997) reported that the protein content in grain intended for technological purposes should be in the range of 11.0-15.0% for winter cultivars and 12.0-16.0% for spring cultivars, while the minimum gluten content should be at a level of 20%. Considering the protein substances, significant differences were found between the harvest years in protein content (Table 4) and the amount of gluten (Table 1) with better values for the year 2017. The cultivars differed significantly in terms of these characteristics. The highest average protein and gluten content was observed in the Wilejka cultivar (15.2% and 36.8%, respectively), while the lowest content was found in the Plejada cultivar (13.3% and 28.6%, respectively). The grain of common wheat tested by other authors showed similar content of these parameters (Kiczorowska *et al.*, 2015; Warechowska *et al.*, 2013; Szafrańska 2012). One of the preliminary methods for assessing the baking value of wheat flour is determination of the Zeleny sedimentation index, which allows quantitative and qualitative assessment of gluten in grain and flour. The higher value of this parameter, the greater the share of gluten proteins in flour, especially glutenin, which is characterized by high swelling capacity and affect good baking value (Szafrańska, 2012; Gąsiorowski, 2004). The analysed wheat cultivars were significantly diversified and were characterized by a high value of this parameter in the range from 42cm^3 for the Alibi cultivar to 59 cm^3 for the Wilejka cultivar, which proves their high breadmaking usefulness. There were also significant correlations between the protein content and the amount of gluten (0.965) and the sedimentation index (0.934), as well as between the amount of gluten and the sedimentation index (0.875). Szafrańska (2012) obtained comparable values of this parameter for common wheat cultivars, in the range from 48 cm^3 to 55 cm^3, while values described by Jaskulska *et al.* (2018) and Harasim and Wesołowski (2013) were not higher than 40 cm^3.

Table 2

Physical properties of grain of common wheat cultivars

Parameter	Milling yield [%]	Falling numer [s]	Gluten content [%]	Zeleny sedimentation index [cm^3]
Cultivars				
Alibi	71.9^a	246^d	29.0^c	42^c
Euforia	73.5^a	443^a	30.8^b	47^b
Plejada	73.8^a	292^c	28.6^c	45^b
Poezja	68.7^a	300^c	28.8^c	48^b
Wilejka	71.1^a	374^b	36.8^a	59^a
F-Statistic	2.57	215.35	214.82	107.55
p-value	0.1032	0.0000	0.0000	0.0000
Growing seasons				
2017	69.9^b	374^a	33.5^a	51^a
2018	73.7^a	288^b	28.2^b	46^b
F-Statistic	11.57	326.72	621.85	76.43
p-value	0.0067	0.0000	0.0000	0.0000

Water absorption of flour ranged from 60.2% for the Plejada cultivar to 64.5% for the Euforia cultivar (Table 3). There were no significant differences between both cultivars and harvest years. Szafrańska (2012) showed in her research an increase in water absorption along with the amount of gluten, which was also reflected in the results described in our study for all samples except the Wilejka cultivar. Other literature reports have shown that this parameter may be dependent on the protein content and sedimentation index, but no such relationships were found for the tested cultivars (Caffe-Treml *et al.*, 2010; Hruskova *et al.*, 2006). Szafrańska (2012) also described that wheat cultivars belonging to the quality group (A) according to the COBORU classification were characterized by greater water absorption than cultivars (B). It was confirmed only for the Euforia cultivar, for which the result of this parameter was the highest. Other farinographic parameters were characterized by significant differences both between the examined cultivars and the harvest years. The dough development time ranged from 3.5 min to 5.0 min, for the Alibi and Poezja as well as Euforia and Plejada cultivars, respectively and was significantly correlated with the water absorption of flour (0.464). The highest value of dough stability, which expresses the dough tolerance to kneading, was observed for the Poezja cultivar (12.5 min) and the smallest for the Alibi cultivar (4.0 min). The dough softening ranged from 40 BU (Brabender units) for the Poezja cultivar to 105 BU for the Alibi cultivar. For this parameter, the lower its value, the higher the dough's resistance to kneading and the better quality of gluten (Klockiewicz-Kamińska and Brzeziński, 1997). In relation to the research years, significantly higher values for dough development time and stability were reported for materials cultivated in 2017, while the higher average value of the degree of dough softening was characteristic for cultivars from 2018. The values of rheological features obtained from the farinographic analysis were similar to results described by other authors for wheat flour (Szafrańska 2012; Ktenioudaki *et al.*, 2011; Ktenioudaki *et al.*, 2010). The baking value of flour is determined directly by laboratory baking. The technological parameters of grain and flour discussed above had a significant impact on the volume of obtained bread. The analysed cultivars differed significantly in this parameter. The highest average volume was found for bread obtained from flour of the Wilejka cultivar, and the lowest for the Poezja cultivar, 381 cm^3 and 318 cm^3, respectively. Similar values of bread volume obtained from common wheat flour were reported by Radomski *et al.* (2007), while in other studies lower volumes in the range 150-190 cm^3 as well as (Ktenioudaki *et al.*, 2010) higher in the range 415– 517 cm^3 (Jaskulska *et al.*, 2018; Gambuś *et al.*, 2011) were showed. The differences in the obtained results could have been influenced by factors such as the grain milling method and baking technology. For analysed material the significant correlations were obtained between the bread volume and the amount of gluten (0.680), as well as such rheological parameters as water absorption of flour (0.613), dough development time (0.458) and a negative relationship with dough stability (-0.624). The obtained wheat breads had good organoleptic characteristics. They were all well-baked, with a smooth crust, a light crumb, and a typical wheat bread taste. The grain of the Wilejka cultivar had the best technological value among all tested samples. Except the largest volume it was distinguished by a golden, smooth crust and a delicate, non-sticky and elastic crumb with uniform porosity. Despite the low amount of gluten and the sedimentation index, the second best-rated cultivar in terms of technology was spring wheat Alibi. The Poezja cultivar was characterized by the weakest technological parameters and the lowest quality of bread.

Table 3

Rheological properties of dough obtained from flour of common wheat cultivars.

Parameter	Water absorbing [%]	Dough development time [min]	Stability [min]	Degree of softening [BU]*	Bread volume [cm^3]
			Cultivars		
Alibi	63.2^a	3.5^c	4.0e	105^a	374^{ab}
Euforia	64.5^a	5.0^a	10.0^b	50^c	371d
Plejada	60.8^a	5.0^a	8.0^c	60^b	354^b
Poezja	60.2^a	3.5^c	12.5^a	40d	318^c
Wilejka	61.6^a	4.5^b	6.0d	65^b	381^a
F-Statistic	2.60	92.00	446.41	418.64	19.52
p-value	0.1008	0.0000	0.0000	0.0000	0.0001
			Growing seasons		
2017	63.0^a	5.0^a	8.5^a	56^b	374^a
2018	61.1^a	3.5^b	8.0^b	72^a	345^b
F-Statistic	3.67	392.00	8.44	216.95	30.76
p-value	0.0845	0.0000	0.0157	0.0000	0.0002

*BU – Brabender units

The content of nutrients and dietary fiber in all wheat cultivars were also evaluated (Table 4). The average protein content was 13.9% and was described above. Determination of ash content in grain provides information on the amount of mineral compounds that are important from a nutritional point of view, as well as allows to determine the suitability of grain as a raw material for flour production. The content of ash below 1.85% in wheat grain indicates that the studied cultivars constitute a valuable raw material for the production of white and wholemeal flour (Szafrańska, 2012). This parameter content differed significantly between cultivars and the years of research. The highest amount of ash, 1.8%, was found in the spring wheat cultivar Alibi, while the lowest, 1.5% in the Wilejka cultivar. In relation to the harvest years, significantly higher values were obtained for the 2017. Kiczorowska *et al.* (2015) obtained the ash in common wheat grain in the range of 1.8-1.9%, while Rachoń *et al.* (2012) showed its amount at the level of 2.2%. Spring wheat cultivars were characterized by a higher ash content compared to winter wheat cultivars, as confirmed in research described by Szafrańska (2012), and Woźniak and Staniszewski (2007) who obtained results at the level of 1.8% for spring wheat grain and 1.6% for winter form. The lipid content ranged from 1.9% for the Plejada cultivar to 2.6% for the Alibi and Euforia cultivars. The average starch content was 67.9%, its extremes were obtained for the Alibi and Plejada cultivars, 66.4% and 70.4%, respectively. Both parameters were also significantly different in the subsequent harvest years. Their content was higher for cultivars from the 2018 harvest year, the average lipids content was 2.5% whereas the starch content and was 69.3%. The obtained values were characteristic for common wheat grain and were consistent with the results described by other authors. Andersson *et al.* (2013) determined lipid content in the range of 2.04-3.6%, while Boros *et al.* (2015) described their average amount in winter wheat grain at a level of 2.4% and in spring wheat grain 2.7%. The same authors also provided an average starch content of 63.9% and 63.5% for winter and spring forms, respectively. Research described by Coles *et al.* (1997) indicates starch content in the range of 57.4-64.6%, while Shewry and Hey (2015) report its content at the level of 60-70%. For some determined chemical components, links between their content and technological parameters were observed. The protein content was significantly correlated with the bread volume (0.567), while in the case of starch a correlation with grain yield (0.501) and a negative relationship with the bread volume (-0.470) were found. The basic bioactive component of cereal grains is dietary fiber. From a nutritional point of view, it is important that the wheat cultivars available on the market contain as much of fibre as possible. The content of dietary fiber varied significantly and ranged from 9.8% for the Wilejka cultivar to 11.8% for the Alibi cultivar (Table 4). There were no significant differences in the content of this component between harvest years, and its average amount was at the level of 10.8%. The largest part of wheat fiber constitutes the non-starch polysaccharide fraction (NSP), of which about 70-80% is the insoluble part (I-NSP). In wheat grain, arabinoxylans are a key part of the NSP fraction. The NSP content ranged from 6.7% to 8.5%, including the content of the I-NSP fraction in the range from 4.7% to 6.6%. The extreme values for both parameters, as in the case of dietary fiber content, were observed for the Wilejka and Alibi cultivars. The average content of lignin, which is a part of insoluble fiber fraction, was 3.1%, and extreme values were found for the Plejada and Alibi cultivars. The content of insoluble dietary fiber components varied significantly between harvest years. The grain of cultivars grown in 2018 was characterized by a higher content of NSP and I-NSP, 7.8% and 5.9%, respectively, and a lower amount of lignin (3.0%) compared to 2017. The soluble fraction of dietary fiber, expressed as the content of soluble non-starch polysaccharides (S-NSP), was significantly different between cultivars and ranged from 1.6% for the Plejada cultivar to 2.0% for the Euforia and Wilejka cultivars. However, no significant differences were found for the S-NSP content between harvest years and the average value of this parameter was 1.9%. In the earlier studies Boros *et al.* (2015) showed an average fiber content in winter wheat grain at a level of 11.6% and in spring wheat grain at a level of 12.3%. According to the authors, the average content of the I-NSP and S-NSP fraction was 7.2% and 1.4% in winter wheat and 7.6% and 1.7% in spring wheat, respectively while the average level of lignin was 2.8%. Similar studies in common wheat grain were carried out by Gebruers *et al.* (2010) who showed a fiber content in the range of 9.6-14.4%, including 7.8-11.4% NSP, 1.25-2.25% S-NSP and an average lignin content of 2.1%. Stevenson *et al.* (2012) determined the amount of dietary fiber in wheat grain at a level of 13.2% and 1.9% of lignin while Barron *et al.* (2007) analysing the content of individual monosaccharides included in NSP received their total amount at the level of 7.1%. According to literature data, particular fractions of dietary fiber, especially arabinoxylans, which are the main part of NSP, have a significant impact on the baking process of wheat bread (Courtin and Delcour, 2002). In the case of dietary fiber fractions, a significant relationship was found between the content of S-NSP fraction and flour water absorption (0.491) and between TDF, NSP and I-NSP and the degree of softening (0.490, 0.492, 0.470, respectively). In addition, negative relationships were observed between the content of NSP and the I -NSP fraction and the amount of gluten ((-0.549) and (-0.619), respectively), as well as between TDF, NSP and the I-NSP fraction and the Zeleny sedimentation index ((-0.597), (-0.729) and (-0.793), respectively).

Based on the assessment of the chemical composition, the Wilejka cultivar was the richest source of nutrients, in particular protein among the tested cultivars, while the Alibi spring wheat cultivar were characterised by the highest content of dietary fibre.

Table 4

Chemical composition of grain of common wheat cultivars

Parameter	Protein [%]	Ash [%]	Lipids [%]	Starch [%]	I-NSP [%]	S-NSP [%]	NSP [%]	Lignin [%]	Dietary fibre [%]
Cultivars									
Alibi	13.4^{c}	1.8^{a}	2.6^{a}	66.4^{c}	6.6^{a}	1.9^{a}	8.5^{a}	3.3^{ab}	11.8^{a}
Euforia	13.9^{b}	1.6^{c}	2.6^{a}	67.4^{bc}	5.7^{c}	2.0^{a}	7.7^{b}	3.3^{a}	10.9^{b}
Plejada	13.3^{c}	1.7^{b}	1.9^{d}	70.4^{a}	6.2^{b}	1.6^{b}	7.7^{b}	2.9^{c}	10.7^{b}
Poezja	13.7^{b}	$1.6b^{c}$	2.3^{c}	67.8^{b}	5.7^{c}	1.9^{a}	7.6^{b}	3.0^{c}	10.6^{b}
Wilejka	15.2^{a}	1.5^{d}	2.5^{b}	67.7^{b}	4.7^{d}	2.0^{a}	6.7^{c}	3.1^{b}	9.8^{c}
F-Statistic	161.8	82.06	390.8	28.2	85.74	22.88	46.08	35.14	45.54
p-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000
Growing seasons									
2017	14.4^{a}	1.8^{a}	2.3^{b}	66.6^{b}	5.7^{b}	1.9^{a}	7.6^{b}	3.3^{b}	10.8^{a}
2018	13.4^{b}	1.5^{b}	2.5^{a}	69.3^{a}	5.9^{a}	1.9^{a}	7.8^{a}	3.0^{b}	10.7^{a}
F-Statistic	400.7	409.96	99.7	120.4	10.73	0.95	5.03	188.37	1.50
p-value	0.0000	0.0000	0.0000	0.0000	0.0084	0.3537	0.0488	0.0000	0.2485

CONCLUSIONS

1. All common wheat cultivars were characterized by high technological suitability. The grain of the Wilejka cultivar was distinguished by the best technological parameters and the quality of obtained bread.
2. Evaluation of the chemical composition of grain showed that the highest content of protein and starch was characteristic for the Wilejka cultivar, while the grain of spring wheat Alibi was the richest source of dietary fibre.
3. In terms of determined technological parameters as well as nutrients and bioactive components, the significant differences were observed between analysed wheat cultivars. Many significant relationships were also found between selected chemical components of wheat grain and the analysed technological parameters.
4. The harvest year had a significant impact on most technological parameters and chemical components except for water absorption of flour and the content of S-NSP fraction and dietary fiber.

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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 glycosidic 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 μg × g^{-1} of soluble dietary fiber; degree of cross-linking DiFA/Xyl = 0.2) than the water-insoluble arabinoxylans (DiFATot = 3647 μg × 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 × mol^{-1}, the water extract viscosity was 11.9 mPa and intrinsic viscosity was 376 ml × g^{-1}. In contrast, a slight, almost 5% increase in average molecular weight ($4.4 \cdot 10^5$ g × 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).

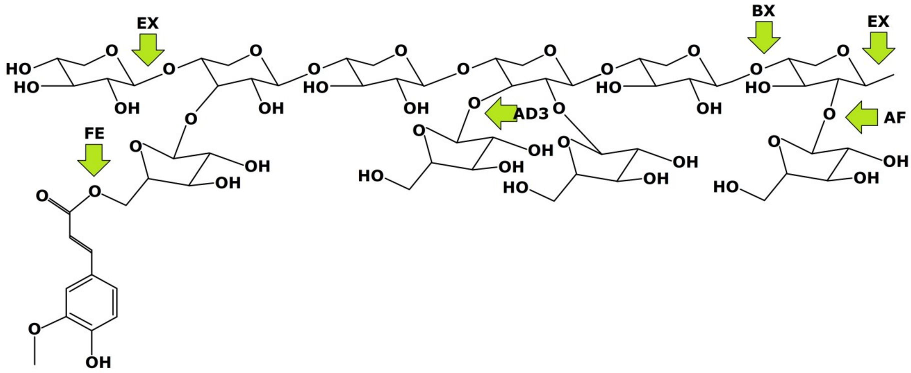

Fig. 1. The structure of arabinoxylan and its susceptibility to hydrolytic enzymes: EX – endo-β-D-xylanase; BX – β-xylosidase; AF – α-L-arabinofuranosidase; FE – feruloyl esterase; AD3 – arabinoxylan arabinofuranohydrolase-D3

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

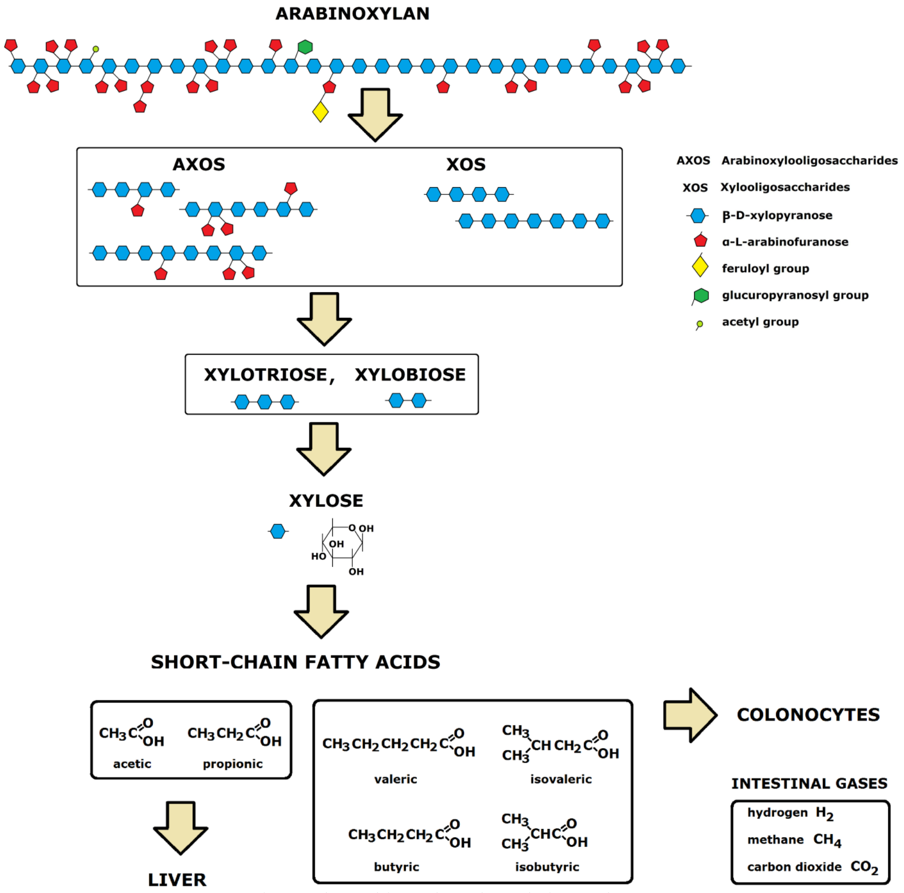

Fig. 2. The arabinoxylans degradation pathway

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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THE EFFECT OF MATURATION STAGE AND AFTER-RIPENING ON SEED QUALITY IN ORGANICALLY- AND CONVENTIONALLY PRODUCED PEPPER (*CAPSICUM ANNUUM* L.) SEEDS

ABSTRACT

High-quality seed production is essential in organic production as well as in conventional production. Fruit maturity can be observed at different times due to the continuous flowering of pepper plant. Consequently, seeds with different maturity stages were obtained as the fruits were collected during once over-harvesting period. Immature seeds collected in once over-harvest may cause quality losses in the seed lot. Hence, this study was conducted to determine the effect of after-ripening on mature and immature pepper seeds produced in organic and conventional production systems. To see the effect of after-ripening treatment, seeds were harvested in two different periods (immature 45-50 days after anthesis(DAA) and mature 60-65 DAA). After-ripening (AR) was performed by keeping the seeds in fruits for 7 days after harvesting the fruits. Effect of production systems and after-ripening on immature and mature pepper seed lots were assessed for four cultivars harvested in 2015 and 2016. After-ripening increased germination (AR:76.3%, C:28% for organic and AR:88%, C:53.8% for conventional), seedling emergence (AR:70.8%, C:44.3% for organic and AR:82.5%, C:53.8% for conventional) percentages and mean weight of 1000 seeds (AR:6.5, C:6.0g for organic and AR:6.5, C:6.2g for conventional) in both production systems of immature seed lots compared to control (C)($P<0.05$) but did not have a similar effect on mature ones. Moreover, organically-produced seed lots have the same quality as conventionally-produced seeds. Consequently, obtained results indicate that after-ripening can be used to enhance the quality of immature seeds of pepper cultivars and seeds can be produced organically without any loss of quality.

Key words: after-ripening, germination, harvesting periods, organic seed, pepper

INTRODUCTION

Seed is one of the most important elements in terms of increasing yield and obtaining high quality crops. Organic crop growing systems may claim to produce even higher quality reproduction material compared to conventional growing methods

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(Sripathy *et al.*, 2012). Seeds and other production materials used in organic farming should be produced organically (Anonymous, 2005). Thus, organic seed is the initial material for organic plant production. The demand for organic seed has grown rapidly in recent years. Accordingly, sales of organic products were worth nearly $43 billion in 2015, an 11% increase compared to 2014. The food share of this amount was $39.7 billion (Anonymous, 2016). Accelerated understanding of health and environmental matters accompanied by the intense use of chemicals attracted attention to different methods of agriculture in the world. Organic farming is defined as a production method that protects the natural balance and includes human and environmentally friendly production systems, ensuring soil has sustainable efficiency, increasing the resistance of the plant, and recommending biological methods in plant protection (Tasbasli *et al.*, 2003).

Organic seed production usually involves higher costs than conventional seed production because of losses throughout the seed production period or inadequate quality of seed production (van der Zeijden, 2003). When research about organic vegetable seed production is examined, it was observed that the studies were limited to some vegetable species and this was related to the vegetation period (Bonina and Cantliffe, 2004). The longer vegetation period in vegetable seed production causes the plants to be exposed to ecological conditions for a longer period of time. Hence, research is required to handle these problems and support seed companies in advancing their organic seed production and seed treatment techniques (Groot *et al.*, 2004).

Determination of appropriate harvest time during seed development for high quality is of great importance for seeds that contain high seed moisture during maturation (Vidigal *et al.*, 2006). Pepper (*Capsicum annuum* L.) seeds contain seed moisture at maturity (Demir and Ellis, 1992a) and a high germination rate is not easily achieved due to the indeterminate flowering seed maturation on the plant. For this reason, the ideal harvest time in terms of fruit maturation directly affects the seed quality of the pepper seed lots (Sinniah *et al.*, 1998; Demir and Samit, 2001; Dias *et al.*, 2006; Vidigal *et al.*, 2011; Caixeta *et al.*, 2014; Nogueira *et al.*, 2017; Demir *et al.*, 2018).

Fruit maturity can be observed at different times due to the continuous flowering of the pepper plant. As a result, seeds with different maturity are obtained as the fruits are collected during one over-harvesting period in commercial seed production. When the seeds with different maturity are mixed, the immature seeds may cause a decrease in the quality of the seed lots by adversely affecting seedling emergence and uniformity (Demir and Ellis, 1992a). For this reason, pepper seed lots can be composed of immature, mature, and over mature seeds due to differences in flowering time and one over-harvesting.

Earlier studies indicate that seed quality was enhanced by keeping seeds in harvested fruits for a while (Edwards and Sundstrom, 1987; Sanchez *et al.*, 1993; Desai *et al.*, 1997; Vidigal *et al.*, 2006; Dias *et al.*, 2006; Vidigal *et al.*, 2009; Passam *et al.*, 2010; Kalyanrao and Singh, 2014; Santos *et al.*, 2015). Accordingly, if the vigor potential of the immature seeds is increased, it may have a positive effect on the overall quality of the seed lot (Vidigal *et al.* 2009). This may be more important when seed harvesting is done during once-over (harvesting all fruits at different maturation stages due to sequential flowering).

In previous reports, the effect of after-ripening treatment on seed quality was determined under conventional production conditions. However, to the best of our knowledge, there were no studies found about the effects of organic seed production system with after-ripening treatment on seed quality development and seed vigor performance. Hence, this study was carried out to assess the effect of after-ripening treatment linked to the maturation levels on organically- and conventionally-produced pepper seed lots. In addition, organic and conventional production systems were compared in terms of seed quality criteria.

MATERIALS AND METHODS

Plant cultivation and seed harvest in organic and conventional production systems

The study was conducted in 2015 and 2016 in the organic and conventional production parcels of Atatürk Horticultural Central Research Institute – Yalova / TURKEY. “Surmeli”, “Kandil Dolma”, “Yaglik” and “Corbaci” open-pollinated pepper cultivars were used as plant materials.

During the vegetation period for seed production, different fertilizers were used depending on plant growth periods and the amount of plant nutrients in the soil. 'Gentasol' (30% total organic matter, 5% N, 3% K_2O, 2% P_2O_5), 'Ormin K' (30% K_2O, %5 organic matter), and 'Biofarm' organic fertilizers were used in organic seed production parcels and 20:20:20 (NPK), potassium sulfate (50% K_2O) and 15:15:15 (NPK) compound fertilizers were used in conventional seed production parcels.

Table 1

Soil properties of organic and conventional seed production parcels in 2015-2016

Parameter	Year	Production system	
		Organic	Conventional
Depth [cm]		0-30	0-30
Water holding capacity [%]	2015	53	59
	2016	52	56
EC25 [1:2.5] [mmhos × cm^{-1}]	2015	0.10	0.11
	2016	0.07	0.10
pH	2015	7.73	7.79
	2016	7.28	7.09
Lime [%]	2015	0.40	0.40
	2016	0.40	0.40
Organic matter [%]	2015	2.03	3.51
	2016	1.41	2.22
Available phosphorus [ppm]	2015	20.0	17.0
	2016	8.0	7.2
Exchangeable potassium [ppm]	2015	233.0	310.0
	2016	202.5	232.5

According to the results of soil analysis, the soil structures of the production parcels belonging to both vegetation periods (2015-2016) have a clayey structure. The soil pH was determined to be between 7.09-7.79. Therefore, the structures of the production parcels have slightly alkaline character. Soil salinity is between 0.07% and 11.11%, while the available phosphorus values in the soil range from 7.2 to 20.0 ppm. The amount of organic matter in parcels was found to be between 1.41-3.51% and the amount of exchangeable potassium in the parcels was between 202.5-310.0 ppm (Table 1).

Seeds of four different pepper cultivars were sown in the second week of March in both vegetation periods. The medium used for seedling production is organic origin and contains 1:1:1 burned sheep manure, loam soil, and biofarm chicken manure. These materials were steam sterilized before use. The study was conducted according to the randomized complete block design with 4 replicates and each replicate contained 150 plants. Seedlings were planted in organic and conventional production fields in the second week of May in both seasons. Plant spacing was 0.8 m between rows and 0.4 × 0.5 m between plants with double rows. Before planting, biofarm chicken manure at a rate of 50 kg per 1000 m^2 was added to the organic parcels and 15:15:15 NPK at a rate of 50 kg per 1000 m^2 was applied to conventional parcels. Weekly fertilization was done depending on the plant growth and the amount of plant nutrients in the soil. “Gentasol” 20 L per 1000 m^2 was applied to the organic seed production parcels and 20:20:20 (N-P-K) 2 kg per 1000 m^2 of compound fertilizer was given by drip irrigation to the conventional seed production parcels. Potassium fertilization was done during the fruit phase. Accordingly, “Ormin K” 2 kg per 1000 m^2 was used in organic production parcels and potassium sulfate 1.5 kg per 1000 m^2 was given to conventional production parcels.

Plants began flowering in June in both production systems and both vegetation periods. Flowers were labeled on the day of flowering and fruits were harvested with two different maturities; 45-50 (immature) and 60-65 (mature) days after anthesis for each cultivar in both vegetation periods. The seeds from each harvest were kept in the fruits for 7 days under indoor conditions (about 23 °C and 70% RH). Afterward seeds were extracted and dried at 30 °C until the seed moisture content declined to 8%. Moisture content of dried seeds was determined according to the ISTA (2016) method. The mean weight of 1000 seeds was determined after the drying process. The dried seeds were kept in the dark at 4 °C in hermetically-sealed aluminum foil packets until use.

Seed viability and vigor tests

Germination test

The experiment was established with 4 replicates and each replicate had 50 seeds for each production system, after-ripening and harvest periods (i.e. 4 cultivars = 2 production systems × 2 harvests × 1 after-ripening and 1 control). Two pieces of Whatman No: 1 blotting paper were placed in 9 cm Petri dishes. Then 4 ml of 0.2% KNO_3 solution was added after the seeds were placed in the Petri dishes (ISTA, 2016). The germination test was carried out at 25 ºC in the dark and the percentage of germination was calculated using daily counts for 14 days. The germination papers were moistened as needed. Normal and abnormal seedlings were determined at the end of the germination experiment. Mean germination time was calculated based on the daily counts made during the experimental period (Ellis and Roberts, 1980).

Seedling emergence test

The seedling emergence test was conducted with the same protocol as the germination test (described above). Seeds from organic and conventional systems were sown in vials at a depth of 2 cm including a mixture of peat and perlite (1:0.5). The vials were put in a climate-controlled chamber at 23 ± 2 °C and 60-70% relative humidity with 16 hours of light and 8 hours dark conditions. Emergence counts were made daily for 25 days. The seedling emergence percentages (the appearance of hypocotyls at the surface) were determined after 25 days. Mean emergence time was recorded by virtue of the daily counts made during the experimental period (Ellis and Roberts, 1980).

Weight of 1000 seed

Using a seed counting machine, 1000 seeds from each seed lot were counted with four replicates and subjected to statistical analysis.

Statistical analysis

The laboratory tests in the study were established in accordance with the experimental randomized parcel design. Field trials were conducted according to the randomized complete block design. The percentage of values obtained from these tests were subjected to $\sqrt{n}$ transformation. JMP 8.0 Statistical package program was used for analysis. Data were assessed with analysis of variance for the main effects, whereby the means of values were compared using the Duncan Multiple Range Test and Least Significant Difference test (p=0.05).

RESULTS

There were statistically triple interactions found between production systems, harvest periods and after-ripening treatment for all viability and vigor tests. The difference between organic and conventional production systems was found to be statistically insignificant using germination and seedling emergence tests in consecutive years.

The mean germination rate (all cultivars) of after-ripening (AR) treated seeds belonging to immature harvests were 76.3% and 86.5% for the organic production system and 88.8% and 91.5% for the conventional production system in 2015 and 2016, respectively (Table 3). Nevertheless, control seed lots from immature harvests were 28 and 64.3% for the organic system and 53.8 and 70.3% for the conventional system in both years, respectively. In accordance with these results, AR treatment increased germination percentages compared to the control group of immature seed lots up to 48.3% and 22.2% for the organic system and 35% and 21.2% for the conventional system in subsequent years (P<0.05). AR treatment was found to be statistically insignificant for mature seed lots regardless of the production system in both vegetation periods. The mean germination percentages of the organically-produced control group of mature seed lots were 90.8% and 90.3 and seeds of a conventional production system were 92% and 90.3% in 2015-2016, respectively, while AR-treated seed lots from the organic system were 89.3% - 90% and 91.5% - %88 for the conventional production system.

Table 3

Germination percentages for four cultivars of after-ripening(AR) and control(C), immature and mature pepper seeds belonging to organic and conventional production systems. Means with different letters in the same line (i.e. cultivars and production systems) are significantly different at 5% level with Duncan's multiple range test(P<0.05). Values are means ± S.D. (n = 4)

Year	Cultivars	Organic				Conventional				CV	LSD
		Immature		Mature		Immature		Mature			
		AR	C	AR	C	AR	C	AR	C		
2015	Surmeli	55±5.7b	34±3.3c	89±1.7a	89±1.5a	89±3.0a	49±4.4b	86±1.4a	91±2.1a	5.23%	9.4
	Kandil D.	85±3.2a	10±2.3c	84±2.8a	88±1.7a	89±3.0a	28±2.2b	93±0.6a	90±1.0a	4.69%	6.9
	Yaglik	84±1.4b	33±3.7d	93±0.5a	91±2.2ab	83±2.4b	63±5.8c	91±1.7ab	94±1.2a	3.45%	6.9
	Corbaci	81±1.9b	35±3.7d	91±1.7a	95±0.6a	94±1.4a	75±2.2c	96±0.8a	93±1.3a	2.51%	5.1
2016	Surmeli	76±2.2bc	37±4.4d	85±2.9ab	87±1.7a	88±1.9a	70±2.6c	85±1.7a	87±3.6a	4.12%	8.1
	Kandil D.	91±1.0ab	57±1.8d	96±1.3a	91±2.1ab	91±2.7ab	72±1.8c	95±1.0a	89±1.7b	2.02%	5.0
	Yaglik	92±2.1ab	82±1.2c	87±1.7b	89±1.0ab	91±1.0ab	62±1.0e	76±1.2d	94±2.5a	1.82%	4.6
	Corbaci	87±3.3bc	81±1.7cd	92±1.0ab	94±0.8a	96±1.0a	77±2.4d	96±0.8a	91±3.1ab	2.31%	5.9

Table 4

Emergence percentages for four cultivars of after-ripening(AR) and control(C), immature and mature pepper seeds belonging to organic and conventional production systems. Means with different letters in the same line (i.e. cultivars and production systems) are significantly different at 5% level with Duncan's multiple range test(P<0.05). Values are means ± S.D. (n = 4)

Year	Cultivars	Organic				Conventional				CV	LSD
		Immature		Mature		Immature		Mature			
		AR	C	AR	C	AR	C	AR	C		
2015	Surmeli	53±2.5b	42±5.3b	69±4.7a	73±1.9a	79±3.4a	44±4.9b	84±6.7a	82±5.3a	7.34%	13.4
	Kandil D.	79±6.4a	24±5.9c	77±4.4a	77±5.3a	84±5.9a	38±1.2b	72±4.9a	80±2.3 a	7.86%	13.8
	Yaglik	77±1.9bc	53±3.0d	86±6.0ab	87±3.0ab	72±2.8c	52±2.8d	86±2.6ab	93±4.4a	4.66%	10.3
	Corbaci	74±2.6cd	58±2.6f	90±3.5ab	76±4.3cd	95±3.5a	81±1.9bc	64±4.0ef	69±3.8de	4.28%	9.5
2016	Surmeli	79±5.3bc	39±2.7d	90±3.5a	86±1.2ab	86±3.0ab	75±3.0c	81±3.9bc	84±1.6ab	3.75%	8.5
	Kandil D.	88±5.4a	65±1.9d	86±3.8ab	72±6.3cd	85±5.0a-c	72±2.8b-d	77±6.0a-d	79±5.0a-c	6.13%	13.9
	Yaglik	92±2.3ab	73±5.0c	94±3.5a	90±3.5ab	89±3.0ab	80±2.3bc	62±6.0d	89±4.1ab	4.97%	11.5
	Corbaci	86±2.0c	62±2.0e	93±1.9ab	90±1.2a-c	91±3.4a-c	75±2.7d	95±1.9a	87±1.0bc	2.35%	6.0

Table 5

Mean germination time (day) for four cultivars of after-ripening(AR) and control(C), immature and mature pepper seeds be longing to organic and conventional production systems. Means with different letters in the same line (i.e. cultivars and production systems) are significantly different at 5% level with Duncan's multiple range test(P<0.05). Values are means ± S.D. (n = 4)

Year	Cultivars	Organic				Conventional				CV	LSD
		Immature		Mature		Immature		Mature			
		AR	C	AR	C	AR	C	AR	C		
2015	Surmeli	53±2.5b	42±5.3b	69±4.7a	73±1.9a	79±3.4a	44±4.9b	84±6.7a	82±5.3a	7.34%	13.4
	Kandil D.	79±6.4a	24±5.9c	77±4.4a	77±5.3a	84±5.9a	38±1.2b	72±4.9a	80±2.3 a	7.86%	13.8
	Yaglik	77±1.9bc	53±3.0d	86±6.0ab	87±3.0ab	72±2.8c	52±2.8d	86±2.6ab	93±4.4a	4.66%	10.3
	Corbaci	74±2.6cd	58±2.6f	90±3.5ab	76±4.3cd	95±3.5a	81±1.9bc	64±4.0ef	69±3.8de	4.28%	9.5
2016	Surmeli	79±5.3bc	39±2.7d	90±3.5a	86±1.2ab	86±3.0ab	75±3.0c	81±3.9bc	84±1.6ab	3.75%	8.5
	Kandil D.	88±5.4a	65±1.9d	86±3.8ab	72±6.3cd	85±5.0a-c	72±2.8b-d	77±6.0a-d	79±5.0a-c	6.13%	13.9
	Yaglik	92±2.3ab	73±5.0c	94±3.5a	90±3.5ab	89±3.0ab	80±2.3bc	62±6.0d	89±4.1ab	4.97%	11.5
	Corbaci	86±2.0c	62±2.0e	93±1.9ab	90±1.2a-c	91±3.4a-c	75±2.7d	95±1.9a	87±1.0bc	2.35%	6.0

Table 6

Mean emergence time (day) for four cultivars of after-ripening(AR) and control(C), immature and mature pepper seeds belonging to organic and conventional production systems. Means with different letters in the same line (i.e. cultivars and production systems) are significantly different at 5% level with Duncan's multiple range test(P<0.05). Values are means ± S.D. (n = 4)

Year	Cultivars	Organic				Conventional				CV	LSD
		Immature		Mature		Immature		Mature			
		AR	C	AR	C	AR	C	AR	C		
2015	Surmeli	7.4±0.29b	7.5±0.13ab	7.4±0.09b	7.5±0.03ab	6.9±0.11c	7.8±0.12a	6.2±0.08d	7.0±0.05c	2.87%	0.30
	Kandil D.	7.8±0.10c	8.4±0.14b	8.7±0.08a	8.0±0.08c	8.0±0.13c	8.5±0.06ab	8.7±0.09ab	8.7±0.14ab	2.54%	0.31
	Yaglik	6.0±0.05e	7.1±0.10c	5.3±0.13f	6.3±0.09d	7.5±0.07b	8.0±0.10a	5.2±0.16f	6.1±0.04de	2.97%	0.28
	Corbaci	7.6±0.17bc	7.7±0.21bc	7.8±0.16b	8.3±0.12a	7.7±0.12bc	7.8±0.03b	7.3±0.08c	7.7±0.17bc	3.69%	0.42
2016	Surmeli	7.2±0.15e	8.7±0.07b	6.9±0.13f	7.5±0.03d	7.9±0.14c	9.2±0.09a	5.9±0.08h	6.5±0.03g	2.60%	0.28
	Kandil D.	8.8±0.09e	10.4±0.13b	9.5±0.10cd	9.9±0.09c	9.6±0.10cd	10.8±0.14a	9.2±0.14d	9.8±0.19c	2.60%	0.37
	Yaglik	6.0±0.12e	7.2±0.04b	5.8±0.05e	6.5±0.08d	6.5±0.07d	7.7±0.05a	5.8±0.11e	6.8±0.11c	2.58%	0.25
	Corbaci	7.3±0.05b	7.9±0.23a	7.3±0.10b	7.3±0.06b	7.3±0.08b	8.1±0.17a	7.1±0.13b	7.4±0.06b	3.03%	0.33

Table 7

Mean weight of 1000 seed (g) for four cultivars of after-ripening(AR) and control(C), immature and mature pepper seeds belonging to organic and conventional production systems. Means with different letters in the same line (i.e. same year, cultivar and production systems) are significantly different at 5% level with Duncan's multiple range test(P<0.05). Values are means ± S.D. (n = 4).

Year	Cultivars	Organic				Conventional				CV	LSD
		Immature		Mature		Immature		Mature			
		AR	C	AR	C	AR	C	AR	C		
2015	Surmeli	6.4±0.02bc	6.1±0.02e	6.4±0.04bc	6.7±0.04a	6.5±0.01b	6.3±0.01d	6.4±0.04c	6.4±0.02bc	%0.74	0.08
	Kandil D.	6.3±0.02c	5.8±0.01d	6.7±0.06a	6.8±0.08a	6.5±0.02b	5.9±0.03d	6.5±0.01b	6.6±0.03b	%1.10	0.12
	Yaglik	6.5±0.04bc	6.1±0.04e	6.5±0.04b	6.7±0.04a	6.4±0.05cd	6.2±0.02e	6.3±0.03d	6.7±0.01a	%0.95	0.11
	Corbaci	6.6±0.02c	6.1±0.04e	6.7±0.01b	6.9±0.02a	6.4±0.00d	6.2±0.02e	6.9±0.04a	6.8±0.05b	%0.81	0.09
2016	Surmeli	6.8±0.02e	6.5±0.04f	7.0±0.01bc	7.1±0.05a	7.0±0.04ab	7.0±0.01ab	6.9±0.01cd	6.9±0.02de	%0.70	0.08
	Kandil D.	7.4±0.02bc	7.3±0.04c-e	7.1±0.02f	7.2±0.09ef	7.6±0.06a	7.5±0.08ab	7.2±0.02d-f	7.4±0.06 b-d	%1.30	0.17
	Yaglik	7.4±0.04a	7.3±0.03a	7.1±0.03c	7.4±0.04a	7.4±0.05a	7.2±0.03b	7.4±0.02a	7.4±0.03a	%0.80	0.10
	Corbaci	6.8±0.02b	6.8±0.04b	7.0±0.01a	7.0±0.03a	6.8±0.06b	6.8±0.05b	7.1±0.03a	7.0±0.03a	%0.94	0.11

Similar results were obtained from the seedling emergence test. AR treatment improved seedling emergence performance in terms of immature seed lots of both production systems ($P<0.05$). Emergence percentages of organically-produced AR-treated immature seed lots were 70.8% and 86.3%, whereas the control groups were 44.3% and 59.8% in 2015 and 2016. Corresponding findings were seen in conventionally-produced immature seed lots. Accordingly, AR treatment increased the mean emergence percentages (all cultivars) of immature seeds compared to the control group from 53.8% to 82.5% in 2015 and 75.5% to 87.8% in 2016. However, mature seed lots from both production systems were not affected by AR treatment (Table 4).

Evaluation of seed vigor with the mean germination and emergence times gave analogous outcomes to the germination and seedling emergence percentages. AR treatment for immature seed lots shortened the germination and emergence time in both production systems and years (Table 5 and 6). Consequently, seed vigor of immature seed lots was enhanced by AR treatment in organic and conventional production systems. On the other hand, mature seed lots were not affected by AR treatment in both production systems. The mean germination times for AR-treated immature seed lots were 4.9 and 4.2 (days), while it was 5.8 and 5.4 (days) in the control group for the organic system in 2015 and 2016. AR-treated immature seed lots were 4.6 and 4.3 (days), whilst the control group was 6.0 and 5.5 (days) for the conventional system.

There was no statistical difference found between organic and conventional production systems in terms of mean germination and emergence times for all the cultivars in both years (Tables 5 and 6).

The mean weight of 1000 seed (all cultivars) values remained constant for AR-treated mature seed lots from organic and conventional systems (6.6 -7.1 and 6.5-7.2 g respectively) compared to control groups (6.8-7.2 and 6.6 -7.2 g respectively) in 2015-2016 (Table 7). Yet, AR treatment significantly improved the accumulation of dry matter content in immature seed lots for both production systems (C: 6.0, AR: 6.5 for organic and C: 6.2, AR: 6.5g for conventional) in the first year ($P<0.05$). But, this influence was not seen statistically in seed lots from the second year (C: 7.0, AR: 7.1 for organic and C: 7.1, AR: 7.2 g for conventional).

There was no statistical difference found between organic and conventional production systems for the means of the weight of 1000 seeds for all the cultivars in subsequent years as well.

DISCUSSION

Our research study examined the effect of AR treatment for different seed maturity levels from organically- and conventionally-produced pepper seed lots. In addition, the comparison was made with a conventional production system in order to determine whether the organic production system has an effect on quality of pepper seeds.

Each species and cultivars has specific needs for seed germination including certain features of seeds and environmental conditions (Baskin and Baskin, 1998). Our study indicates that similarities were recorded in the responses of all the cultivars to AR treatment, harvest periods, and production systems. This trend is seen in the results obtained by Yanmaz *et al.* (1994), Mavi *et al.* (2010), Kenanoglu *et al.* (2013), Demir *et al.* (2016), and Demir *et al.* (2018) who worked with more than one cultivar to see the influence of different treatments on these cultivars.

Many studies have been done to reveal the effect of after-ripening treatment on many species under conventional production conditions. Seed maturation of these species can continue for some time especially when harvested in the green fruit phase (Barbedo *et al.*, 1994; Dias *et al.*, 2006; Vidigal *et al.*, 2009; Passam *et al.*, 2010; Kalyanrao and Singh, 2014; Kumar *et al.*, 2014; dos Santos *et al.*, 2016). Yet, seed production using organic growing methods may influence seed quality (Yildirim and Demir, 2018). Thus, evaluating the treatment under organic production conditions has to be considered. Our findings indicate that the after-ripening treatment enhanced the germination and seedling emergence percentages and decreased the mean germination and emergence time of immature seed lots of all the cultivars belonging to organic and conventional production systems in both harvest years compared to the control group ($P<0.05$). However, the same effect was not monitored in mature seed lots. It was observed that the application of after-ripening had no effect on the mature pepper seed lots in both production systems. This could be due to the continuous growth of immature embryos reaching the physiological maturity of seed during after-ripening (Weston *et al.*, 1992).

There was no statistical difference found between organic and conventional production systems in terms of germination and seedling emergence tests. Consequently, Duman (2009) reported similar results that differences between seeds produced in organic conditions and conventionally-produced seeds were insignificant according to viability tests.

Seeds achieve their maximum quality some days after the maximum seed dry weight is reached (Welbaum and Bradford, 1988; Demir and Ellis, 1992b). Therefore, maximum seed germination and vigor is observed some days after maximum dry weight in fleshy-fruited species such as pepper. In our study, the data for the mean weight of 1000 seeds states that AR treatment slightly increased the seed weight of all the cultivars from the immature harvest period in both production systems in the first year. But this effect was not seen in the second year. Carvalho and Nakagawa (2000) reported that owing to high moisture content when seeds are stored in fleshy fruits, they are prone to respire more by consuming the endosperm; hence decreasing dry matter content. Conversely, some other reports suggest that considerable enhancement was recorded for a dry matter of seeds through nutrient transfer from fruit to seed during storage in fleshy fruits (Passam *et al.*, 2010; dos Santos *et al.*, 2016). It can also be claimed, and our results indicate that this may be the case, that it is caused by the climatic differences between the vegetation periods (Table 2).

Table 2

Changes in air temperature values during vegetation periods of 2015 and 2016

Month	Average temperature [°C]		Average highest temperature [°C]		Average lowest temperature [°C]		Instant highest temperature °C]		Instant lowest temperature [°C]	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	**2016**
March	9.0	11.3	12.5	15.4	5.5	6.7	20.5	24.9	-1.1	1.8
April	11.9	16.0	16.9	21.2	7.0	10.3	27.2	27.9	1.6	4.5
May	18.5	18.3	23.4	22.4	13.4	13.9	28.4	30.4	9.1	10.0
June	21.3	23.5	25.5	28.4	17.1	18.1	31.1	34.3	12.1	11.2
July	24.9	25.1	30.1	30.0	19.3	19.9	36.9	33.1	15.8	16.3
August	25.9	25.5	30.9	29.9	21.4	21.4	33.5	33.2	17.9	15.8
September	23.4	21.2	27.8	26.4	19.5	16.5	35.9	31.0	17.1	9.5
October	18.2	19.1	21.7	24.8	15.0	12.2	27.3	30.7	12.1	11.0

CONCLUSION

In this study, triple interaction of production systems, harvesting periods and after-ripening treatment on the quality of pepper seed lots was demonstrated. It is suggested that the production of pepper seeds consider these interactions. It was concluded that pepper seed production can be done effectively with the use of organic inputs and there will be no significant losses in the viability and quality characteristics of the seed lots compared to the conventional production systems. AR treatment induced the development of immature seeds to a certain level, but the same effect was not seen in mature seed lots. Therefore, in order to improve the overall quality of pepper seed lots, the harvested fruits should be left for 7 days of after-ripening treatment before seed extraction in both production systems.

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Content