



ORIGINAL PAPER

EVALUATION OF THE CONTENT OF PHENOLIC ACIDS AND THEIR ANTIOXIDANT ACTIVITY IN WINTER CEREAL SEEDS

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ABSTRACT

Grains of three cultivars of each cereal: winter wheat (*Triticum aestivum* L. – Tonacja, Bogatka, Satyna), winter rye (*Secale cereale* L. – Stanko, Dańkowskie Złote, Amilo) and winter barley (*Hordeum sativum* L. – Laverola, Mertada, Merk) were examined. In addition, phenolic acids were identified in these grains, and the content of the identified acids, i.e. ferulic, vanillic, *p*-coumaric, protocatechuic, *p*-hydroxybenzoic, syringic, sinapic and caffeic, was determined. The antioxidant properties of phenolic acids were examined using the free radical scavenging method against a stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). The antiradical efficiency of phenolic acids depended on the duration of a reaction and genotype-specific properties. The data showed that different winter cereal seed extracts were able to quench 15-47% of DPPH radical solution and to exhibit potent radical scavenging activity. Results of the determinations of the content of phenolic acids in grains of the cultivars correlated with the activity of their extracts. The tested cereal grains were characterized by different levels of the identified phenolic acids, depending on the generic and specific characteristics. Differences in the content of phenolic acids may be subject to genetic traits, environmental factors and different analytical procedures. Cereal kernels with a higher phenolic acid content also exhibited a higher antioxidant activity of extracts with these compounds. Kernels of winter barley cv. Metaxa with a higher content of phenolic acids were also characterized by higher antioxidant activity than those of cvs. Merle and Laverola. The lowest content of phenolic acids was observed in winter rye cv. Stanko. 5-day seedlings showed a statistically significant increase in phenolic acids (ferulic, *p*-coumaric, protocatechuic, *p*-syringic, sinapic and caffeic) and the high antioxidant activity. Winter cereal seed extracts were able to quench 15-47% of DPPH radical solution and exhibited potent radical scavenging activity.

Keywords: antioxidant activity, DPPH; early stage germination, phenolic acids, winter barley, winter rye, winter wheat.

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INTRODUCTION

Phenolic acids are very widely distributed in the plant world. They exist mostly as esters of organic acids or glycoside or are bound to a number of cell wall polymers, with only a small share of present in the free form (ZHAO et al. 2008, MOHSEN, AMMAR 2009, TABART et al. 2009). Particularly noteworthy are the biological functions of these compounds, expressed through antioxidant and anti-radical activity, which substantiates their classification as antioxidants in the form of natural food ingredients (MELO et al. 2006, CÁCERES et al. 2014). Antioxidant properties of phenolic acids consist in elimination of reactive oxygen species, blocking (sweeping) of free radicals, inhibition of enzymes from the oxidase group and chelation of metal ions (iron, copper). This way antioxidants protect the human body from oxidative stress and prevent the development of diseases (ZHAO et al. 2006).

Cereal grain is rich in phenolic compounds, mainly phenolic acids, such as ferulic, vanillin, *p*-coumaric and caffeic acids (KILCI, GOCMEN 2014). Ferrulic acid is the most common among phenolic acids, ester-bound to the rest of the α -L-arabinose of arabinoxylane chain of cell walls (PEREZ-GARRIDO et al. 2012). Most of polyphenols including phenolic acids occur in the outer layer of the grain (SERPEN et al. 2008). During technological processes it is removed together with the husk resulting in a reduction of potential antioxidant properties of the final product (LIU, YAO 2007, INGLETT et al. 2010, ZHU et al. 2011). Several studies have been conducted concerning antioxidant action of phenolic acids in *in vitro* conditions (MELO et al. 2006). Caffeic acid has been shown to be a potent antioxidant *in vitro* in different oxidation systems (ZHAO et al. 2014). Ferulic acid is present in considerable amounts in outer layers of cereal grains (RIVAS et al. 2013). Ferulic acid and *p*-coumaric acid present in barley and malt in free and insoluble, bound forms are very potent and interesting antioxidants (HUANG et al. 2014). Other authors also pointed out cinnamic acid derivatives as very potent antioxidants in wort and beer (ZHAO et al. 2008), especially scavenging hydroxyl radicals in beer. The most active phenolic acids turned out to be caffeic acid, which also showed strong α -tocopherol-sparing activity.

Whole grain cereals have been found to be a good source of nutritionally valuable substances, such as antioxidants, minerals, vitamins and dietary fiber. A wide range of these compounds is affected by germination (LIU, YAO 2007, ZHAO et al. 2008).

Germination starts when a dry seed begins to take up water and is completed when the embryonic axis elongates. At this point, reserves within the storage tissues of the seed are mobilised to support the seedling's growth. It is not clear how the level of phenolics, especially phenolic antioxidants, vary throughout seed germination. It is hypothesised that phenolic synthesis and their antioxidant activity will change throughout germination stages. Changes in phenolic synthesis and antioxidant activity would indicate the seed's

preparation towards adverse conditions. Identifying the germination stage when the level of phenolic antioxidants is optimised would be useful for the growth of edible sprouts with enhanced nutraceutical properties (LIU, YAO 2007).

The aim of the present research was to identify and analyse phenolic acids isolated from the grain and 5-days-old seedlings of winter cereals at early germination stages and to determine antioxidant activity of extracts of these compounds using the 2,2-diphenyl-1-picrylhydrazyl reagent (DPPH radical).

MATERIALS AND METHODS

The research material consisted of cereal grains: winter wheat (*Triticum aestivum* L. – cultivars: Tonacja, Bogatka, Satyna), winter barley (*Hordeum sativum* L.– cultivars Laverola, Mertada and Merk) and also winter rye (*Secale cereale* L. – cultivars Stanko, Dańkowskie Żłote, Amilo). Seeds and early stage germinated grain of winter cereals were subject to tests. The content of eight phenolic acids was analysed in winter cereal seeds and seedlings. The chamber tests were carried out in controlled conditions of the plant growth period. The experiment was carried out in a laboratory of the Department of Agriculture Microbiology (Institute of Soil Science and Plant Cultivation – State Research of Institute in Pulawy, Poland) in 2012. Cereal seedlings were growing for 5 days on sterile agarose medium (Hoagland nutrient solution) (PAJAK et al. 2014). After that time, grain together with roots was analysed for the content of phenolic acids.

Preparation of extracts from cereal grain

The extraction and hydrolysis of the ester-linked phenolic acids in the analysed grain were conducted according to the method described by the HUNG et al. (2011). Freeze-dried cereal grains were milled in a laboratory mill IKA A10 (3 × 5 s). Milled cereal grains (100 mg) were subject to 4-hour-long hydrolysis in 5 cm³ of 2 M NaOH containing 1% of ascorbic acid as an antioxidant. After the onset of hydrolysis, 4 µg of m-hydroxybenzoic acid was added to the samples as an internal standard. After the completion of hydrolysis, the samples were acidified with 6 M HCl solution to a pH of approximately 2. The acidified hydrolysate was then centrifuged (6500 × g, 20 min) and the resulting supernatant was subjected to extraction with ethyl acetate (3 × 7 cm³) in Falcon type tubes (15 cm³). The collected extract was evaporated to dry in a vacuum evaporator (35°C) and the precipitate was dissolved in 4 cm³ of 25% methanol. Diluted extracts were stored in a freezer until analysis. In the case of cereal grain, the seeds and roots were used for extraction.

Determination of phenolic acids content

The content of phenolic acids in the samples was determined by Ultra Performance Liquid Chromatography, carried out in the reverse phase on an apparatus ACQUITY UPLC® Systems by Waters. Chromatographic separations were performed on a Waters ACQUITY UPLC® HSS C18 column (1.0 × 100 mm, 1.8 μ) at 30°C. The mobile phase consisted of 0.1% solutions of formic acid in water (solution A) and in acetonitrile (solution B). The elution procedure included isocratic and gradient steps: 0-0.07 min 5% B, 0.07-8.33 min 5-15% B; 8.33-8.67 min 15-60% B; 8.67-9.33 min 60% B, 9.33-9.40 min 40 - 5% B, 9.40 - 12.00 min 5% B. A photodiode array detector (PDA) and triple quadrupole (TQ) were used for the detection of analytes. The concentrations of phenolic acids in the samples were determined from calibration curves. Sigma phenolic acid standards were used for the identification.

Determination of antioxidant activity (DPPH· scavenging activity)

Antioxidant properties of phenolic acids were determined in an investigation that involved a decrease in the absorbance values occurring during a reduction reaction of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH·) by the acid extracts isolated from grain (PAJAK et al. 2014). To 0.1 cm³ of the extract, 3.9 cm³ of a radical was added at a concentration of 6·10⁻⁵ M dm⁻³ (stock solution) and the absorbance was measured at α = 515 nm wavelength in 5-min intervals. Calibration curves were prepared based on the calibration solution ranging from 1·10⁻⁵ mol dm⁻³ to 6·10⁻⁵ M dm⁻³. Antioxidant activity expressed as a percentage of inhibition was calculated from the formula:

inhibition % = [(ACo – AAt) / ACo] 100, where ACo – absorbance of the control sample in time 0, AAt – absorbance of a tested sample measured every 5 min for 40 minutes.

The above measurement was repeated three times. The content of polyphenols and antioxidant activity in grains were performed in triplicate. The results were subjected to one-way analysis of variance (ANOVA). The assessment of the significance of differences between means was performed using the Duncan multiple test at the level of significance $P < 0.05$. The relationship between the content of polyphenols and antioxidant activity is expressed as a correlation coefficient. Standard deviation is also given for all the results.

RESULTS AND DISCUSSION

Determination of phenolic acids content

Given the scale of consumption, cereal products can be a significant source of antioxidants, including phenolic acids (MOHSEN, AMMAR 2009).

Varietal characteristics, year of harvest, as well as the location of a field may affect the concentration of these compounds (INGLETT et al. 2010, KILCI, GOCMEN 2014).

After alkaline hydrolysis of the phenolic acid from ester forms, qualitative and quantitative analysis of the extracts of these acid were carried out by HPLC. Table 1 shows the content of identified phenolic acids in cereal

Table 1

The content of phenolic acids in cereal seeds and 5-day-old seedlings of cereal (grain + roots) in Hoagland medium ($\mu\text{g g}^{-1}$), $n = 9$

Plant (variety)	The content of phenolic acids ($\mu\text{g g}^{-1}$)								
	PRO	POH	VAN	CAF	SIR	PCO	FER	SIN	$\Sigma 8$
The content of phenolic acids in 5-day-old seedlings of cereal									
Winter wheat									
Satyna	3.3	6.9	12.0	13.5	11.7	55.5	1006.2	68.0	1177.1
Bogatka	2.9	7.0	13.4	19.3	7.9	39.5	898.2	75.8	1064
Tonacja	2.5	8.9	11.8	10.2	6.9	43.3	897.4	71.2	1052.2
Winter barley									
Merle	2.0	5.3	13.4	33.9	3.4	129.6	1390.6	39.1	1617.3
Laverola	1.6	6.1	17.5	27.1	4.2	217.4	1475.7	33.0	1782.6
Metaxa	3.1	4.0	22.0	18.5	4.1	122.0	1817.1	52.3	2043.1
Winter rye									
Stanko	1.1	5.6	22.1	2.3	2.8	3.6	68.5	35.6	141.6
Dańkowskie złote	1.5	4.8	26.4	2.8	1.8	3.8	65.7	58.7	165.5
Amilo	1.8	5.2	12.8	3.2	2.6	3.4	56.8	65.4	151.2
The content of phenolic acids in winter cereal seeds									
Winter wheat									
Satyna	2.9*	6.5	11.5	12.4	10.6	45.5	898.7*	56.4	1044.5
Bogatka	2.3*	6.7	12.4	18.4	6.8	36.2	785.2*	68.2	936.2
Tonacja	2.1	8.2	10.8	8.7	5.7	41.2	845.6	62.4	984.7
Winter barley									
Merle	1.3*	5.1	13.1	28.7	2.8	111.5	1158.2*	32.5	1353.2
Laverola	1.2	5.4*	16.4	25.6	3.6	201.41	1168.4*	32.7	1454.71
Metaxa	2.4*	3.1*	18.5	15.7	3.7	98.6	1434.2*	48.7	1624.9
Winter rye									
Stanko	0.9	5.1	21.8	2.1	2.4	3.1	52.4*	31.2	119
Dańkowskie złote	1.2	4.2	25.4	2.2	1.4	2.8	52.8*	48.5*	138.5
Amilo	1.2*	4.2*	12.2	2.8	1.8	2.7	41.2*	53.2*	119.3

* – values in columns are statistically important, $P < 0.05$,

PRO – protocatechic acid,
 POH – *p*-hydroxybenzoic acid,
 VAN – vanillic acid,
 CAF – caffeic acid,

SIR – syringic acid,
 PCO – *p*-coumaric acid,
 FER – ferulic acid,
 SIN – sinapic acid.

seeds and in 5-day-old seedlings. The highest content of phenolic acids in cereal seeds was in winter barley cv. Metaxa ($1624.9 \mu\text{g g}^{-1}$) and the lowest one appeared in winter rye variety Stanko ($119 \mu\text{g g}^{-1}$). Analogously, the highest content of phenolic acids in 5-day-old seedlings of cereals was in winter barley Metaxa cv. ($2043.1 \mu\text{g g}^{-1}$) and the lowest one occurred in winter rye cv. Stanko ($141.6 \mu\text{g g}^{-1}$). Similar results were presented by other authors (PEREIRA et al. 2006, KLEPACKA et al. 2011, LEITAO et al. 2012). The difference in the content of total phenols depended on several factors such as a cultivar, climatic and ecological factors, agricultural practice and harvesting method (ZHAO et al. 2008, ZHU et al. 2011). However, the wide range of differences among the cultivars in this study may be due mainly to the different genetic background of these cultivars. Other researchers confirm that the content of phenolic compounds that prevails in products made from cereals may depend on some pertinent factors, the cultivar, the method applied for extracting the compounds and the storage conditions.

The amount of phenolic compounds vary dependng on such factors as the climate, soil conditions, the variety of cereal, pH, soils, and others. KLEPACKA et al. (2011) determined an average content of this acid in a number of barley cultivars at the level of 477.5 mg g^{-1} and the amount of ferulic acid at 401.2 mg g^{-1} per kilogram of wheat grain. ZHU et al. (2011) show that the content of that acid in the ester-bound form is at a level of 870 mg g^{-1} of grain. The differences in the content of ferulic acid given in the cited papers can be induced by genetic and environmental factors as well as the different analytical procedures. Among the analyzed cereal grains, the winter barley cultivars (Merle, Laverola, Metaxa) had the highest content of phenolic acids with predominantly ferulic, *p*-coumaric and caffeic acids, while the lowest values of total phenolic acids were present in winter rye cultivars (Stanko, Dańkowskie Żłote, Amilo) – Table 1.

The concentration of phenolic acids in cereal grains may be affected by varietal characteristics, year of harvest as well as the location of a cultivation field (ZHAO et al. 2006, PEREZ-GARRIDO et al. 2012, SIAH et al. 2014).

From the above discussion it may be concluded that at initial germination stages phenolics may serve as radical scavengers or antioxidants, while later they could become part of the structural framework of the growing plant and lose some of their antioxidant efficiency. The phenols synthesised during seed germination could help obtain enhanced levels of phenols and antioxidant activity resulting in their improved nutraceutical properties. Seed germination could also serve as models for applying similar nutraceutical enhancement strategies to other crops. This study shows that germinated cereal seeds are an excellent source of dietary phenolic antioxidants. There have also been where the authors reported no significant differences in the initial phase of plant growth and only during the flowering phase (RIVAS et al. 2013, PAJAK et al. 2014). This relationship can translate kind of plant and grain structure different for each group of plants. Among the phenolic acids, ferulic acid occurred in the greatest amount, the concentration of which in

the studied seedlings of cereals ranged from 56.8 mg g⁻¹ (winter rye cultivar Amilo) to 1817.1 mg g⁻¹ (winter barley cv. Metaxa) – Table 1.

Results of the content of phenolic acids in grain and also in seedlings of the cultivars correlated with the antioxidant activity of their extracts (Table 2). Grains and 5-day-old seedlings of the barley cultivars Merle,

Table 2

The values of correlation coefficients (*r*) between the content of phenolic acids (Σ8) and their antioxidant activity

Plant (variety)	Correlation coefficients (<i>r</i>)		% RSA	
	grain	5 day seedlings	grain	(-) 5 day seedlings
Winter wheat				
Satyna	0.421	0.561	12.85 ± 0.12 ^c	17.85 ± 1.42 ^c
Bogatka	0.431	0.458	13.85 ± 0.32 ^c	16.84 ± 0.84 ^c
Tonacja	0.387	0.417	12.85 ± 0.17 ^c	15.26 ± 0.52 ^c
Winter rye				
Stanko	0.785*	0.967*	22.85 ± 0.12 ^c	25.58 ± 1.12 ^b
Dańkowskie złote	0.845*	0.924*	21.85 ± 0.14 ^c	31.54 ± 0.62 ^b
Amilo	0.762*	0.964*	22.85 ± 0.42 ^c	26.13 ± 0.52 ^b
Winter barley				
Merle	0.842*	0.951*	34.85 ± 0.82 ^a	32.52 ± 0.42 ^a
Laverola	0.863*	0.874*	32.85 ± 0.52 ^a	41.34 ± 0.36 ^a
Metaxa	0.789*	0.958*	35.85 ± 0.44 ^a	47.56 ± 1.12 ^a

± SEM – the standard error, * – values in columns are statistically important, $P < 0.05$, % RSA – free radical DPPH· scavenging activity, % RSA = $(Ab_{516\text{ nm}}[\text{start}] - Ab_{516\text{ nm}}[6\text{ min}]) / Ab_{516\text{ nm}}[\text{start}] \cdot 100$. Values within a column followed by the same letter (s) are not significantly different at $p = 0.05$.

Metaxa, Laverola had a higher content of ferulic, *p*-coumaric and caffeic acids compared with winter wheat (Satyna, Bogatka, Tonacja) and showed a higher antioxidant effect of these compounds. The results obtained in this study confirmed the findings of other authors, showing a positive correlation between the antioxidant activity of phenolic compounds of cereals and their content (SERPEN et al. 2008, SOUSA et al. 2008, TABART et al. 2009, ZHAO et al. 2014).

Determination of antioxidant activity (DPPH· scavenging activity)

The scavenging activity on DPPH radicals has been widely used to determine the free radical-scavenging activity of different matrices (LIU, YAO 2007, ZHAO et al. 2008, PRADEEP, GUHA 2011, PEREZ-GARRIDO et al. 2012). DPPH is a stable free radical that is dissolved in methanol and its purple colour shows characteristic absorption at 515 nm. Antioxidant molecules scavenge the free

radical by hydrogen donation and the colour of the DPPH assay solution becomes light yellow, causing a decrease in absorbance. Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation (SERPEN et al. 2008, CHANDRASEKARA et al. 2012). In this assay, results are expressed as the per cent ratio of the absorbance decrease of DPPH radical solution in the presence of an extract at 515 nm to the absorbance of DPPH radical solution at the same wavelength.

The data showed that the different winter cereals were able to quench 15-47% of DPPH radical solution and exhibited potent radical scavenging activity. The strongest radical scavenging activity was shown by kernels of winter barley cv. Metaxa, whereas the winter rye cultivar Stanko showed the lowest value of the inhibition of DPPH. Significant differences were noticed among all the cultivars regarding antioxidant activity (Table 2).

Because of the complicated mechanism involved in the neutralization of free radicals in different plant raw materials, a correlation between the content of phenolic acids and their antioxidant activity is not always confirmed (ALRAHMANY et al. 2012, CARDOSO et al. 2014). In a study on the content and antioxidant activity of phenolic compounds in oat, MARTINEZ-VILLALUENGA (2010) found a significant influence of a variety and the location of a cultivation field on the level of phenolic acids. MOHSEN and AMMAR (2009) reached a similar conclusion in the case of ferulic acid in wheat. The above authors did not notice any significant impact of these factors on the antioxidant activity of these compounds.

Germination of cereal seeds increases their nutritive value (MARTINEZ-VILLALUENGA et al. 2010, HUNG et al. 2011). Several studies have reported higher levels of nutrients and a lower content of antinutrients in sprouts compared to the ungerminated seeds (ZIELIŃSKI et al. 2005, MARTINEZ-VILLALUENGA et al. 2010). However, information about free and bound phenolics and sprouted seeds is scarce. The content and composition of bioactive compounds in cereal kernels and seeds depends on many factors, e.g. climatic and agronomic conditions of growth, storage conditions, level of their maturity, and also on their cultivar.

PAJAŁ et al. (2014) investigated the effect of germination on the profiles of phenolic acids and flavonoids and on the antioxidant activity in selected edible seeds of mung beans, radish, broccoli and sunflower. Germination increased the total phenolic and flavonoid levels, as well as the antioxidant activity of the seeds, and influenced the profile of free and bound phenolic compounds.

Phenolic acids isolated from grain and from 5-day-old seedlings of three cultivars of each wheat, winter barley and winter rye showed diverse abilities to neutralize free radicals. The highest activity was detected in the cultivars of winter barley (Figure 1).

Antioxidant activity of phenolic acids in these varieties expressed as % of inhibition was the highest and reached 27.5% in the final period of incubation (Figure 2). The highest antioxidant activities were obtained in the case

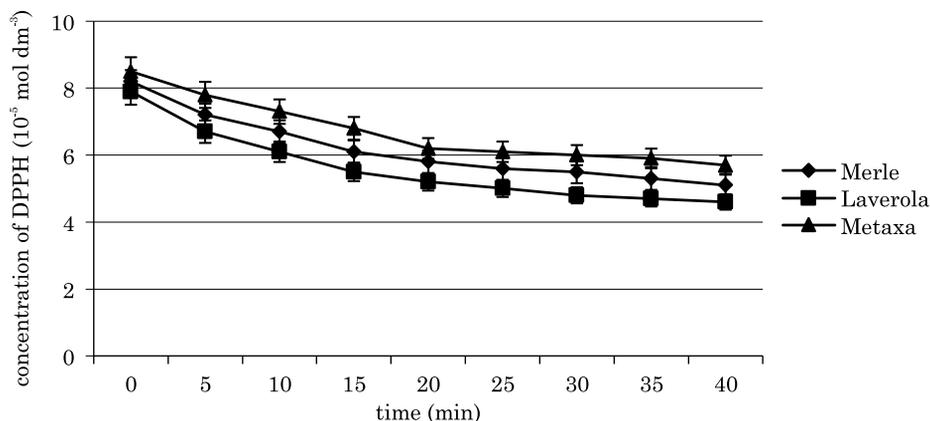


Fig. 1. Time course of scavenging of a DPPH free radical by extracts of phenolic acid from three winter wheat cultivars (\pm SD, $n = 6$)

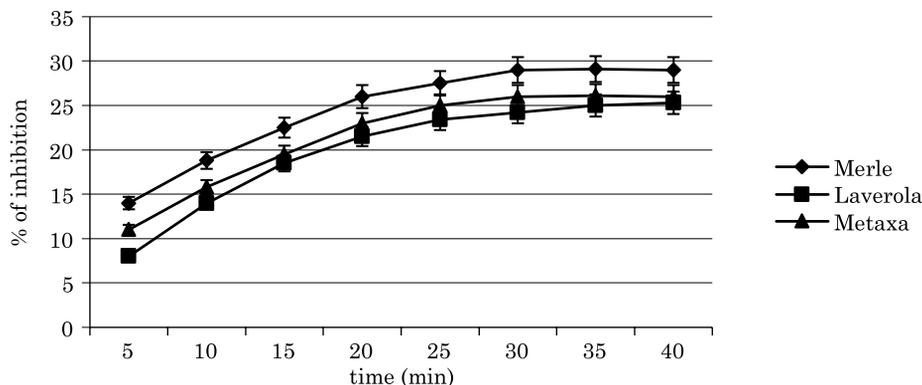


Fig. 2. Effect of the incubation time on antioxidant activity of phenolic acid extracts from seeds of three winter barley cultivars against DPPH (\pm SD $n = 6$)

of winter barley. During the whole incubation period of phenolic acids with DPPH, the ability to inhibit free radicals was the strongest during the first 15-25 min, when the DPPH concentration was visibly reduced. Simultaneously, the antioxidant activity increased from 4.5% to 22.6%. These results confirm the research of other authors who pointed to that the fact that the reduction of DPPH concentration by plant antioxidants is particularly characteristic of the initial reaction stage (PAJAK et al. 2014). ASHRAF et al. (2012) report that the ability of phenolic acids to eliminate the free radical DPPH is dependent on the location and number of hydroxyl (-OH) and methoxyl (-OCH₃) groups.

The antioxidant activity of the grain cultivars was estimated using the quenching intensity of the DPPH. Although it is well established that phenols are the main compounds responsible for antioxidant activity of cereals, the present result showed no correlation between the total phenols and the antioxidant activity. The relationship between phenolic compounds and anti-

oxidant capacity was also inconsistent in other studies. That values antioxidant activity were statistically correlated with the total content of phenols in the analysed samples.

Numerous studies on antioxidant properties of phenolic acids showed a significant dependence of these properties on the chemical structure (PEREIRA et al. 2006). In compounds with one hydroxyl group, antioxidant activity is increased additionally by the presence of one or two methoxyl groups in the ring. The substitution in the *ortho* position with an electron donor group, an alkyl or methoxyl, increases the stability and antioxidant properties of phenolic acids. Different levels of antioxidant activity of caffeic, ferulic and *p*-coumaric acids are related to their chemical structure, namely antioxidant capacity depends on the number of hydroxyl groups in a molecule and is higher when they are esterified (PEREIRA et al. 2006). Synapic acid with two methoxyl groups is more active than ferulic acid (one methoxyl group), and in turn ferulic acid is more active than coumaric acid (one hydroxyl group).

Chlorogenic acid, derivative of caffeic acid, shows high antioxidant activity, which occurs mainly in the plant kingdom. It has been demonstrated in the research on the inhibition of tyrosine nitration by peroxide nitrate (III) that caffeic and chlorogenic acids, i.e. dihydroxyl derivatives, are more active than single hydroxyl derivatives, i.e. ferulic and *p*-coumaric acids. Ferulic acid, which has an electron donor group in position 3, shows more ability to stabilize a phenoxy radical than *p*-coumaric acid does. Hydroxycinnamic acids can be arranged in the following order with respect to their ability to protect cells from damage by nitrogen peroxides (ONOO⁻): caffeic acid; chlorogenic acid; ferulic acid > *p*-coumaric acid > *o*-coumaric acid > *m*-coumaric acid (SOUSA et al. 2008).

While analyzing antioxidant properties of hydroxycinnamic acids effecting the reduced propagation of generated peroxy radicals, it was found that dihydroxyl derivatives, i.e. caffeic acid and chlorogenic acid, had a stronger ability to inhibit free radicals than *p*-coumaric single hydroxyl acid. Methoxylation of a hydroxyl group in the *ortho*-position causes a decrease in the antioxidant activity. Ferulic acid is more effective than *p*-coumaric acid owing to the possession of the methoxyl group. This group acting as an electron donor improves the ability to stabilize aryloxy radicals, which are formed after a hydrogen atom is released by a hydroxyl group. Hydroxylation instead of methoxylation makes molecules with such a structure becoming much more effective antioxidants (SOUSA et al. 2008). MELO et al. (2006) analyzed the ability to neutralize the peroxides of linoleic acid, generated under the influence of 2,2'-azobis (2,4-dimethylvaleronitrile) (AMVN), by phenolic acids isolated from fruits of *Boreave orientalia* plant from *Cruciferae* family, traditionally used in folk medicine, and discovered a higher activity of ferulic, caffeic and sinapic acids than that of vanillic and syringic acids. The authors suggest that the presence of an unsaturated radical in a molecule is responsible for the strengthened antioxidant effect.

CONCLUSIONS

1. Antiradical efficiency of phenolic acids depended on the time of the reaction and genotype-specific traits.

2. Differences in the content of phenolic acids may be subject to genetic factors, environmental factors and differences between analytical procedures.

3. Results of determinations of the composition and content of phenolic acids in grain of the analysed cultivars correlated with the antioxidant activity of the extracts.

4. Cereal kernels with a higher phenolic acid content also exhibited a higher antioxidant activity of extracts of these compounds.

5. Antioxidant properties of phenolic acids depend on the time of the reaction and the phenotypic traits of a plant variety.

6. The tested cereal grains were characterized by different levels of the identified phenolic acids, depending on the generic and specific characteristics.

7. Winter cereal seed extracts were able to quench 15-47% of DPPH radical solution and to exhibit potent radical scavenging activity.

8. Results of determinations of the content of phenolic acids in grains of the tested cultivars correlated with their extracts activity. Kernels of winter barley cv. Metaxa with a higher content of phenolic acids and were also characterized by a higher antioxidant activity than, for example, kernels of the cultivars Merle and Laverola.

9. 5-day-old seedlings showed a statistically significant increase in the content of phenolic acids and antioxidant activity.

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