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Antioxidant activity and content of selected antioxidant compounds in grain of different oat cultivars

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Abstract: The total antioxidant activity (TAA), total contents of polyphenols (TPC), phenolic acids (TPA) and tocols (TTC) were determined in the grain of 5 oat cultivars of Czech origin grown under organic and conventional cropping systems in two-year experiments (2018 and 2019). The TPC ranged from 772.9 mg/kg DM (dry matter) (hulled oat cv. Seldon) to 890.6 mg/kg (naked oat cv. Patrik); the TPA from 261.6 mg/kg (cv. Seldon) to 479.0 mg/kg (cv. Patrik); the TTC from 110.9 mg/kg (hulled oat cv. Korok) to 126.5 mg/kg (cv. Seldon). The TAA ranged from 427.1 mg/kg (cv. Korok) to 474.9 mg/kg (cv. Seldon). Besides the effect of the cultivar, the TAA and antioxidant contents were significantly affected also by year (weather conditions); higher values were observed in the drier and warmer the year 2019. The effect of the cropping system was statistically insignificant.

Keywords: *Avena sativa* L.; vitamin E; phytochemical; fertilisation; nutritional quality

Oat is a minor but very valuable crop cultivated, especially in European and North American colder regions (Brindzová et al. 2008). While the common oat (*Avena sativa* L.) with the hulled grain is the most used cultivated oat species, hulless (naked) oat (*Avena nuda* L.) with a hull that is loosely attached to the grain and can be easily removed during threshing is less widespread (Kouřimská et al. 2018).

Despite the fact that the positive effects of oat consumption on human health have been generally known, the use of oat in the human diet has decreased during the last 70 years (van der Broeck et al. 2016). Nevertheless, recently interest in oat production and consumption is growing again, especially due to increasing awareness of its health-related properties and the content of different health beneficial bioactive compounds, including β-glucans, well-balanced proteins, vitamins and unsaturated fatty acids (Martínez-Villaluenga and Peñas 2017).

Moreover, oat is a source of many phytochemicals with antioxidant effect that are associated with the prevention of many diseases, including cancer or cardiovascular diseases (Ben Halima et al. 2015). The most abundant of these phytochemicals in oat are tocols (vitamin E). They are not only essentials for humans, but they also have been associated with numerous other positive health benefits. Tocols are powerful antioxidants composed of four homologues of tocotrienol and tocopherol syn-

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thesised in photosynthetic organisms (Galli and Azzi 2010). Phenolic and polyphenolic compounds are another important phytochemicals occurring in oat grain. These secondary metabolites protect the plants against biotic and abiotic stress, like a pathogen attack, ultraviolet radiation, heat, water, or nutrient stress (Nicholson and Hammerschmidt 1992, Chen et al. 2018).

The growing interest in the production of oat is also associated with lower inputs needed, including lower fertilisation and pesticide requirements, as well as higher disease resistance compared to other cereals. The positive impact of oat on soil properties within crop rotation is known as well (Strychar 2011). Oat's advantages regarding the mentioned agronomic impacts can be another reason for its wider use. In view of the fact that environmental conditions in which cereals are cultivated can affect the chemical composition of grain very strongly (Redaelli et al. 2013), higher attention should also be given to the investigation of the effects of various environmental factors, including temperatures and precipitation (drought/flooding), that can act as abiotic stressors on the oat's quality and production (Stewart and McDougall 2014, Ben Halima et al. 2015).

The objective was to determine total antioxidant activity (TAA) and total content of polyphenols, phenolic acids, and tocols in selected hulled and naked oat cultivars of Czech origin in order to find the most promising materials for the potential improvement of the nutritional quality of various oat products. Besides the effect of cultivars, the effects of cropping system (organic × conventional) and weather conditions on the concentration of the mentioned antioxidant compounds were determined.

MATERIAL AND METHODS

Plant material. The selected set of four hulled oat cultivars (Korok, Kertag, Raven and Seldon) and one hulless (naked) oat cultivar (Patrik), all of these of Czech origin, was evaluated during 2018 and 2019 in field trials, established at the experimental station in Prague-Uhříněves (central Bohemia, 295 m a.s.l., average annual temperature 8.4 °C, the average sum of precipitation 575 mm).

The field trials were performed in both organic and conventional cropping systems using red clover as a preceding crop for oat. The design of randomised blocks in 3 replicates with an average area of an experimental plot of 10 m^2 was used. Treatment of the oat by weeding harrows was used during the vegetation; no additional fertilisers and pesticides were applied to oat grown organically on experimental field certified for organic farming. Nitrogen fertilisation in the total dose of 80 kg N/ha, herbicidal and fungicidal treatment were applied to oat in a conventional cropping system. The grains of hulled cultivars were dehulled after the harvest; a laboratory dehulling machine was used.

Regarding the weather conditions (Table 1), the time from the oat heading to maturation exceeded the average temperature of the long-term standard in this period by 22% in 2018 and by 27% in 2019. With regard to precipitation, the evaluated period was very dry both in 2018 and 2019 and reached only 35% of the precipitation in 2018 and 21% of the precipitation in 2019 compared to the long-term standard.

Grain samples. Obtained grain samples were prepared for analyses by grinding; an analytical mill to

Table 1. The survey of average temperatures and sums of precipitation in decades from the oats heading to maturity

Decade	Month	Average temperature (°C)		Σ of precipitation (mm)	
		2018	2019	2018	2019
1st	June	17.7	22.1	11.4	6.4
2nd	June	18.6	22.9	6.6	0.6
3rd	July	19.4	19.2	15.0	0.0
4th	July	22.5	20.8	6.0	9.4
5th	July	25.7	24.4	9.6	2.8
6th	August	21.6	21.3	0.0	10.6
Average temperature		20.9	21.8		
Σ of precipitation				48.6	29.8
Average temperature (long-term standard)		17.11			
Σ of precipitation (long-term standard)				140.9	

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pass through a 0.5 mm screen was used. Then the meal was dried at 105 °C for 24 h, and dry matter (DM) was determined. All analyses were performed in triplicates.

Total antioxidant activity using DPPH. The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical cation scavenging activity of methanolic extracts was measured using the method published by Eliášová and Paznocht (2017). The results were quantified using external calibration and expressed as mg of trolox equivalent antioxidant activity per kg of DM.

Total polyphenols content (TPC). The method for TPC evaluation was described by Eliášová and Paznocht (2017). The results were quantified using external calibration and expressed as mg of gallic acid equivalent per kg of DM.

Total phenolic acids content (TPA). The TPA analysis was carried out according to the method of isolation of bound phenolic acids previously published by Paznocht et al. (2020). This extraction method is suitable for the determination of the TPA when applied to the raw cereal material without previous steps specific to other phenolic acid fractions (free and conjugated). The results were expressed as the sum of individual phenolic acids in mg per kg of DM.

Total tocols content (TTC). The method described by Gutierrez-González et al. (2013) with some modifications was used for the TTC determination. These modifications consist in using the different temperatures of sample incubation (80 °C) and another kind of reconstituting solution (methanol). The identification and quantification were performed using an Ultimate 3000 HPLC system (Thermo Fisher Scientific, USA) with fluorescence detection under the conditions published by Lachman et al. (2018). The results were expressed as the sum of individual tocols in mg per kg of DM.

Statistical analysis. The obtained data were statistically analysed by the ANOVA (complete three-factor model "cultivar" (C), "cropping system" (S) and "year" (Y) with all interactions, Table 2) in the SAS program (SAS Institute, Carry, USA), version 9.4 at the level of significance $P = 0.05$. The differences between means were evaluated by the Tukey's *HSD* (honestly significant difference) test.

RESULTS AND DISCUSSION

The effect of cultivar on the content of evaluated antioxidant compounds. A very significant influence ($^{**}P < 0.01$) of the cultivar was found for all evaluated traits (TAA, TPC, TPA, TTC). However, only the TPA content was mostly affected by the cultivar, probably due to a great difference in the TPA content among the naked oat cv. Patrik and other (hulled oat) cultivars. The effect of interactions C × Y and C × Y × S was mostly very significant ($^{**}P < 0.01$) or significant ($^{*}P < 0.05$). On the other hand, the effect of C × S was non-significant, apart from the TPA content (Table 2).

The results of Tukey's test describing the statistical significance between the mean values of cultivars, cropping systems, and years are given in Table 3. Detailed values of each cultivar are shown in Table 4.

The minimum value of TPC was 772.9 mg/kg (hulled oat cv. Seldon); the maximum was 890.6 mg/kg (naked oat cv. Patrik) on average of two-year results from two cropping systems. While cv. Patrik differed significantly from other cultivars, the difference in TPC among hulled oat cvs. Korok, Kertag, and Seldon were statistically insignificant (Table 2). In total, the lowest TPC (716.3 mg/kg) was detected in cv. Seldon (2018), the highest value (966.6 mg/kg)

Table 2. The effect of genotype, cropping system and year on the content of evaluated antioxidant compounds in the oat grain (ANOVA, Fisher's *F*-values)

	Total antioxidant activity	Total polyphenols content	Total phenolic acids content	Total tocols content
Cultivar (C)	26.53**	51.64**	654.90**	21.88**
Cropping system (S)	1.72	1.97	2.77	75.93**
Year (Y)	1982.98**	331.21**	1.37	1837.56**
C × Y	13.82**	5.22**	13.74**	6.81**
C × S	1.39	0.39	14.66**	2.57
Y × S	33.87**	106.19**	5.76*	105.74**
C × Y × S	3.94**	4.29**	8.92**	1.29

$^{*}P < 0.05$; $^{**}P < 0.01$; non-significant factors are not labelled

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Table 3. The content of evaluated antioxidant compounds and antioxidant activity in the oat cultivars, cropping systems and years – Tukey's *HSD* (honestly significant difference) test at the level of $P < 0.05$

		TAA	TPC	TPA	TTC
		(mg/kg dry matter)			
Cultivar	Korok	427.1 ± 85.0^{c}	774.3 ± 66.7^{c}	294.0 ± 15.7^{c}	110.9 ± 60.5^{c}
	Kertag	452.7 ± 82.5^{b}	799.3 ± 68.1^{c}	304.9 ± 15.9^{c}	118.6 ± 27.8^{b}
	Raven	449.9 ± 81.6^{b}	828.5 ± 60.4^{b}	358.9 ± 20.3^{b}	122.0 ± 29.1^{ab}
	Seldon	474.9 ± 62.2^{a}	772.9 ± 65.9^{c}	261.6 ± 16.8^{d}	126.5 ± 30.7^{a}
	Patrik	463.7 ± 57.8^{ab}	890.6 ± 95.5^{a}	479.0 ± 35.0^{a}	124.6 ± 22.0^{a}
	$HSD_{0.05}$	13.98	27.48	13.48	5.32
Cropping system	ECO	455.7 ± 66.6^{a}	817.4 ± 104.3^{a}	342.2 ± 68.1^{a}	115.4 ± 32.8^{b}
	CONV	451.6 ± 81.7^{a}	808.8 ± 54.6^{a}	337.2 ± 91.3^{a}	125.6 ± 21.0^{a}
	$HSD_{0.05}$	6.26	12.30	6.03	2.38
Year	2018	384.7 ± 30.9^{b}	757.7 ± 67.7^{b}	341.4 ± 74.8^{a}	95.3 ± 13.8^{b}
	2019	522.6 ± 18.0^{a}	868.5 ± 54.5^{a}	337.9 ± 86.0^{a}	145.8 ± 7.9^{a}
	$HSD_{0.05}$	6.26	12.30	6.03	2.38

TAA – total antioxidant activity; TPC – total polyphenols content; TPA – total phenolic acids content; TTC – total tocols content; ECO – organic cropping system; CONV – conventional cropping system; all means with the same letters in columns by factors are not significantly different

in cv. Patrik (2019) (Table 3). These results are in accordance with observations of Fernández-Acosta et al. (2019), who recorded in the oat cultivars collection the highest TPC value of 970 mg/kg and are higher than 520 mg/kg, verified by Chen et al. (2016). The results reported by Chen et al. (2018) showed that the mean value of TPC in the oat cultivars grown in China was 613 mg/kg DM.

Just as in the case of TPC, the highest TPA content (479.0 mg/kg) was reached with naked oat cv. Patrik, on average of two-year results from two cropping systems. The lowest TPA content (261.6 mg/kg) was observed in hulled cv. Seldon (Table 2). It is also evident from the results (Table 3), that naked oat cv. Patrik exceeded the TPA content of evaluated hulled oat cultivars very strongly and reached the highest value of 498.6 mg/kg in total. Kováčová and Malinová (2007) observed a very strong genetically determining effect on the content of TPA in oat, thus the substantial variation of the TPA content of our hulled oats and naked cv. Patrik is in accordance with its results. Our results for the total content of phenolic acids were lower than those reported by Multari et al. (2018) – in their study, the TPA content in the evaluated Finnish oats varied from 1 202 to 1 687 mg/kg.

Our results showed that average TTC ranged from 110.9 mg/kg (hulled oat cv. Korok) to 126.5 mg/kg (hulled oat cv. Seldon); the differences in TTC among cvs. Seldon, Patrik, and Raven were statistically insignificant (Table 2). In total, the lowest TTC (83.1 mg/kg) was found in cv. Korok (2018), the highest (154.8 mg/kg) in cv. Seldon (2019) (Table 3). These values were higher than those reported in some other studies. Gutierrez-González et al. (2013) detected an average tocol concentration of 60.0 mg/kg in oats. In a study with 12 oat genotypes from three sites in the USA, the concentration of tocols varied from 19.0 to 30.3 mg/kg (Peterson and Quereschi 1993). Redaelli et al. (2016) observed the total tocols concentration between 16 and 94 mg/kg, depending on the oat genotype. Contrary to the results of the authors mentioned above, our sample preparation method consists of hot saponification step (80 °C; 10 mol/L KOH), which results in a much higher recovery of tocols from grain matter.

The TAA ranged from 427.1 mg/kg (hulled oat cv. Korok) to 474.9 mg/kg (hulled oat cv. Seldon) on average; the differences in TAA between the cv. Seldon and Patrik were statistically insignificant (Table 2). Our results for TAA showed, just as for the TPC, TPA, and TTC, that naked oat cv. Patrik exceeded most of the evaluated hulled oat cultivars. Our previous experiments (Zrcková et al. 2018) showed that TAA of wheat was more than two times lower when compared to oats and ranged from 162.7 to 226.7 mg/kg DM. These results showed a higher antioxidant potential of oats compared to wheat and their value for human nutrition.

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Table 4. The antioxidant activity and the content of evaluated antioxidant compounds in individual oat cultivars – Tukey's *HSD* (honestly significant difference) test at the level of $P < 0.05$

		Korok	Kertag	Raven	Seldon	Patrik
Total antioxidant activity (mg/kg DM)	2018	347.4 ± 12.7^b	374.3 ± 6.4^b	373.5 ± 19.7^b	416.9 ± 19.3^b	411.6 ± 23.0^b
	2019	506.8 ± 22.0^a	531.1 ± 13.1^a	526.2 ± 16.5^a	532.8 ± 7.9^a	515.9 ± 17.6^a
	$HSD_{0.05}$	18.02	14.93	18.21	16.06	11.69
	ECO	424.5 ± 75.2^a	451.3 ± 88.7^a	456.9 ± 73.6^a	479.8 ± 56.9^a	465.8 ± 38.8^a
	CONV	429.7 ± 101.0^a	454.0 ± 84.3^a	442.9 ± 95.5^a	469.9 ± 72.2^a	461.6 ± 76.4^a
	$HSD_{0.05}$	18.02	14.93	18.21	16.06	11.69
Total content of polyphenols (mg/kg DM)	2018	719.0 ± 31.9^b	742.5 ± 39.7^b	796.3 ± 58.2^b	716.3 ± 29.5^b	814.6 ± 65.1^b
	2019	829.6 ± 37.6^a	856.0 ± 29.7^a	860.7 ± 46.5^a	829.4 ± 31.8^a	966.6 ± 44.3^a
	$HSD_{0.05}$	37.31	34.27	27.49	35.28	18.82
	ECO	774.1 ± 87.1^a	803.9 ± 93.8^a	834.6 ± 86.0^a	773.4 ± 83.6^a	900.8 ± 137.2^a
	CONV	774.5 ± 46.8^a	794.6 ± 36.5^a	822.4 ± 23.5^a	772.3 ± 50.6^a	880.4 ± 31.6^b
	$HSD_{0.05}$	37.31	34.27	27.49	35.28	18.82
Total content of phenolic acids (mg/kg DM)	2018	294.4 ± 14.5^a	314.2 ± 7.7^a	376.7 ± 9.5^a	257.2 ± 11.1^a	464.7 ± 14.4^b
	2019	293.7 ± 18.2^a	295.6 ± 16.9^b	341.2 ± 7.7^b	266.0 ± 21.3^a	493.4 ± 44.7^a
	$HSD_{0.05}$	17.92	16.85	11.03	13.38	16.71
	ECO	303.7 ± 15.3^a	310.5 ± 16.6^a	362.9 ± 20.6^a	274.2 ± 14.2^a	459.5 ± 15.4^b
	CONV	284.4 ± 9.2^b	299.2 ± 14.2^a	354.9 ± 21.1^a	249.0 ± 6.0^b	498.6 ± 39.3^a
	$HSD_{0.05}$	17.92	16.85	11.03	13.38	16.71
Total content of tocols (mg/kg DM)	2018	83.1 ± 13.0^b	93.8 ± 13.4^b	96.2 ± 13.8^b	98.2 ± 11.2^b	105.0 ± 11.5^b
	2019	138.6 ± 5.6^a	143.4 ± 6.8^a	147.7 ± 8.9^a	154.8 ± 5.3^a	144.3 ± 2.3^a
	$HSD_{0.05}$	6.73	6.71	8.08	4.48	2.95
	ECO	105.5 ± 37.3^b	114.9 ± 36.1^b	113.6 ± 33.0^b	123.6 ± 38.7^b	119.3 ± 27.1^b
	CONV	116.2 ± 24.4^a	122.3 ± 19.2^a	130.4 ± 24.5^a	129.4 ± 23.6^a	129.9 ± 16.1^a
	$HSD_{0.05}$	6.73	6.71	8.08	4.48	2.95

All means with the same letters in columns are not significantly different by factors; ± values represent standard errors. DM – dry matter; ECO – organic cropping system; CONV – conventional cropping system

The effect of year. The results (Table 2) show a very significant ($^{**}P < 0.01$) prevailing influence of the year on the TAA, TPC, and TTC. The insignificant effect of year on the TPA content may be surprising; it is probably connected with a very strongly prevailed effect of cultivar on the TPA. The results also show mostly a very significant effect of interaction Y × S; this effect for TAA, TPC, and TTC was even higher compared to the effect of the cultivar.

In both evaluated years, there were substantially lower precipitation and higher temperatures during the evaluated season compared to the long-term standard (Table 1). Nevertheless, in 2019, when the content of most of the evaluated antioxidant compounds was higher, oat cultivars were exposed to higher temperatures and lower precipitation compared to 2018. It is already known that plants increase the synthesis of many antioxidants in stress conditions, including abiotic stresses like drought and/or heat stress (Ficco et al. 2014, Ben Halima et al. 2015, Zrcková et al. 2018). However, in view of the fact that the differences in weather conditions between both evaluated years were not too high, we would expect that differences in the content of evaluated antioxidants would be lower.

The effect of the cropping system. Investigation of the effects of cropping systems on the content of different phytochemicals was the aim of many studies. Most of them agree with the conclusion that crops from organic farming usually contain higher concentrations of antioxidant compounds compared to crops cultivated conventionally (Zuchowski et al. 2011, Barański et al. 2014, Zrcková et al. 2018). One of the reasons is probably the fact that in organic farming

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where it is not possible to use industrial pesticides, plants can be exposed to higher pest and disease attack. Thus, it is possible to expect a more intensive synthesis of antioxidant compounds by plants' defense systems (Nicholson and Hammerschmidt 1992). Another reason could be the possible worse availability of some nutrients, especially nitrogen, where no synthetic fertilisers are allowed. This situation can also result in a higher synthesis of some phytochemicals that are a part of the plants' defense systems (Rühmann et al. 2002). Nevertheless, as reported by Zuchowski et al. (2011), a higher concentration of antioxidants in organic cereal grain can also be connected with a lower size (thousand kernels weight) of organic kernels. So, it is possible to suppose that in smaller kernels is a lower share of endosperm and a higher share of outer seed layers rich in antioxidants.

The results are given in Table 2 as the same as the results in Table 3 and a more detailed view in Table 4 shows that the effect of cropping system (organic × conventional) on the TAA just as on the TPC and TPA content was statistically insignificant. Only the TTC was affected by the cropping system significantly. These results are in contrast to our previous experiments regarding wheat, according to which the cropping system affected the concentration of the evaluated antioxidants significantly. However, the effect of the cropping system was smaller in comparison with the effects of wheat genotype and year (Zrcková et al. 2018). Compared to wheat, oat typically has lower requirements for the intensity of cultivation, including lower fertilisation requirements and higher disease resistance (Strychar 2011). Thus, it is possible to presuppose that the effect of the cropping system will be lower, in our case, even statistically insignificant.

In conclusion, our findings confirmed the high antioxidant potential of oat. Antioxidant activity and the contents of the evaluated antioxidants depended mainly on cultivar and weather conditions, while the effect of cropping system was very low. Naked oat cv. Patrik exceeded hulled oat cultivars both in the TPC and TPA and was also among the cultivars with the highest TAA and TTC. Despite usually lower grain yields, the excellent nutritional quality of naked oats is a compelling reason for their cultivation and utilisation in the food industry.

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