

Variation of the total content of polyphenols and phenolic acids in einkorn, emmer, spelt and common wheat grain as a function of genotype, wheat species and crop year

MONIKA ZRCKOVÁ¹, IVANA CAPOUCHOVÁ¹, LUBOŠ PAZNOCHT², MARIE ELIÁŠOVÁ², PETR DVOŘÁK¹*, PETR KONVALINA³, DAGMAR JANOVSKÁ⁴, MATYÁŠ ORSÁK², LUCIE BEČKOVÁ¹

¹Department of Agroecology and Crop Production, Faculty of Agrobiological Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic

²Department of Chemistry, Faculty of Agrobiological Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic

³Department of Agroecosystems, Faculty of Agriculture, University of South Bohemia in České Budějovice, České Budějovice, Czech Republic

⁴Crop Research Institute, Prague, Czech Republic

*Corresponding author: dvorakp@af.czu.cz

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Abstract: The study aimed at evaluating the total content of polyphenols (TPC) and phenolic acids (TPA) in grain of 4 spring einkorn, 4 emmer, 4 spelt and 4 common wheat genotypes cultivated under organic cropping system in two-year trials. The TPC and TPA were significantly affected both by genotype, wheat species and crop year (weather conditions). At the same time, TPC and TPA were mainly affected by the crop year while the effect of genetic factors was lesser. The TPC ranged from 618 mg/kg DM (dry matter) (common wheat cv. Annie) to 792.37 mg/kg DM (*Triticum monococcum* GEO) and TPA from 700.66 mg/kg DM (cv. Annie) to 874.74 mg/kg DM (Schwedishes einkorn) in an average of two-year results. Related to the wheat species, total content of polyphenols was in order einkorn > emmer > common wheat > spelt, total content of phenolic acids in order einkorn > spelt > emmer > common wheat. Higher TPC and TPA were observed in the very dry year 2018.

Keywords: hulled wheat; organic farming; antioxidant compounds; cereal; phytochemicals

Wheat is the most widely grown food crop. The consumption of wheat grain and especially whole grain products is associated with several health benefits which may be related in part to the contents of different phytochemicals. They can act as antioxidants and belong to chemically different groups of antioxidant compounds such as polyphenols, carotenoids, phytosterols (Lachman et al. 2012) or phenolic acids (Liu et al. 2008). Phenolic compounds are considered

as a major group of compounds that contribute to the antioxidant activity of cereals (Fogarasi et al. 2015). They are excellent oxygen radical scavengers, with an electron reduction potential lower than the oxygen radicals. Strong antioxidant activity of phenolic compounds leads to beneficial anti-inflammatory, anti-microbial, anti-thrombotic, anti-atherogenic, vasodilatory and cardio-protective effects on human health (Brandolini et al. 2008). In recent years,

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many studies have dealt with antioxidant compounds in common wheat or durum wheat (Stracke et al. 2009, Ficco et al. 2014, Martini et al. 2015).

Current trends towards organic and low-impact agriculture, as well as an increase in the utilization of organic food products, provide wider possibilities also for use of hulled wheat species (Brandolini et al. 2008). Hulled wheat species *Triticum dicoccum* (Schränk) Schuebl, *T. monococcum* L. and *T. spelta* L., also known as emmer, einkorn and spelt, respectively, were among the earliest *Triticeae* domesticated by a human (Chrpvá et al. 2013). Despite their limited commercial availability, consumer's demand for einkorn, emmer and spelt is currently increasing because bakery and other cereal products derived thereof are widely perceived as 'healthy' alternatives to those made of bread wheat (Ziegler et al. 2016). Moreover, these wheat species could be an alternative to common wheat especially in organic farming with a wider diversity of crops. They are cultivated in organic farms in Europe not only because they are believed to have a higher nutritive value in comparison with common wheat, but also due to their higher resistance to unfavourable environmental conditions as well as lower fertilization and soil requirements (Konvalina et al. 2012). However, information regarding to antioxidant compounds in hulled wheat species cultivated under organic cropping system is still scarce, although this knowledge is important both for breeding and for organic farming.

Despite relatively high genetic weighing, some environmental factors such as the specific climate parameters (rainfall and temperature) influence the antioxidant compounds development, too (Ficco et al. 2014). Some authors have observed an increased antioxidants synthesis in different cereals grown under water deficit and higher average temperatures during the grain filling (Paznocht et al. 2018). The results of Lu et al. (2015) showed that environment factors (E), including precipitation and temperature stress, had a stronger influence on the selected health-beneficial components and antioxidant properties of soft winter wheat than genotype (G) or $G \times E$ interaction. Related to the phenolic compounds, they are secondary metabolites synthesized during plant development in response to stress conditions. Therefore, environmental stresses that induce oxidative damage often promote the synthesis of phenolic metabolites, which act as phytoalexins and safeguard cell wall integrity (Brandolini et al. 2013).

The objective of this study was to determine total contents of polyphenols and phenolic acids in the

collection of spring einkorn, emmer, spelt and, for comparison, common wheat genotypes grown under organic cropping system to identify the richest sources for potential improving the nutritional value of different wheat products. The understanding of the genotype, year (weather conditions) and $G \times Y$ effects on evaluated antioxidant compounds could also be used for improving the breeding efforts to produce hulled wheats grain rich in selected health components. Therefore, in addition to the effect of genotypes and wheat species on polyphenols and phenolic acids content, the effect of weather conditions (i.e. temperature and precipitation) was investigated, too.

MATERIAL AND METHODS

Plant material. The collection of 12 spring hulled wheat genotypes was cultivated in the exact field plot trials, carried out during the 2017 and 2018 growing seasons at the experimental station of the Czech University of Life Sciences in Prague-Uhřetěves (central part of Bohemia, 295 m a.s.l., average annual temperature 8.4°C, average sum of precipitation 575 mm). The collection involved 4 einkorn wheat, 4 emmer wheat and 4 spelt wheat genotypes (both present cultivars, old landraces and accessions obtained from the Gene Bank of the Crop Research Institute Prague). The collection was supplemented by 4 common wheat genotypes (as a control).

The field trials with evaluated wheat genotypes were established using random blocks, in 3 replicates, with an experimental plot average area of 10 m². The trials were carried out under an organic cropping system. Red clover was used as a preceding crop of wheat. Treatment of the wheat stands by weeding harrows was used during the vegetation; no fertilizers nor pesticides were applied. After the harvest, the yield of a grain of evaluated genotypes was observed, and hulled spikelets of einkorn, emmer and spelt were dehulled using a dehulling laboratory machine.

As for the weather conditions (Table 1), the period of wheat heading, flowering, grain filling and maturing both in 2017 and 2018 was generally similar in temperatures. As to precipitation, the year 2018 was very dry and reached only 33% of precipitation in the evaluated period compared to 2017.

Grain samples. Grain samples obtained after the field plot trials harvest were ground using IKA analytical mill (Janke & Kunkel Co., Staufen, Germany) to pass through 0.5 mm screen (35 mesh) and ho-

Table 1. Weather conditions in decades from heading to grain maturity

| Decade | Month | 2017 | | 2018 | |
|--|--------|--------------------------|-------------------------|--------------------------|-------------------------|
| | | average temperature (°C) | Σ of precipitation (mm) | average temperature (°C) | Σ of precipitation (mm) |
| 1 st | June | 20.0 | 13.2 | 17.7 | 11.4 |
| 2 nd | June | 19.8 | 62.8 | 18.6 | 6.6 |
| 3 rd | July | 20.5 | 13.2 | 19.4 | 15.0 |
| 4 th | July | 19.2 | 12.6 | 22.5 | 6.0 |
| 5 th | July | 19.8 | 41.8 | 25.7 | 9.6 |
| 6 th | August | 24.8 | 3.0 | 21.6 | 0.0 |
| Average temperature and Σ of precipitation in the evaluated period | | 20.7 | 146.6 | 20.9 | 48.6 |
| Long-term standard | | | | 17.3 | 74.0 |

mogenised well. Dry matter (DM) was determined by drying of a meal at 105°C for 24 h. Three replicates were made in all of the following analyses.

Total polyphenols content (TPC). The TPC was evaluated according to Eliášová and Paznocht (2017). Briefly, 2.5 g of the meal was extracted with 25 mL of 0.1% HCl in methanol. 2 mL of extract was reacted with 2.5 mL of Folin-Ciocalteu reagent with the addition of 7.5 mL of 20% sodium carbonate and filled up with pure water to 50 mL. After 2 h the solution was measured spectrophotometrically at 765 nm. The results were quantified using external calibration and expressed as mg of gallic acid equivalent per kg of DM.

Total phenolic acids content (TPA). For extraction and chromatographic separation, a method published by Martini et al. (2015) with some modifications was used. Briefly, 0.25 g of the meal was hydrolysed with 14 mL of 2 mol/L aqueous sodium hydroxide for 1 h at the room temperature. 7 mL of 4 mol/L HCl was added to adjust acidic pH (1–2). Two mL of hydrolysate were transferred into 8 mL glass vial and twice extracted with 2 mL of ethylacetate. Combined supernatants of upper organic phase were removed to another glass vial, evaporated to dryness under the nitrogen stream and reconstituted with 1 mL of 70% aqueous methanol, filtered through a syringe filter into an amber glass vial and analysed by HPLC-DAD. The analysis was carried out using an Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, USA) with a diode array detector. The analytes were separated by an Omnispher C18 HPLC column (250 × 4.6 mm; particle size 5 µm; Agilent, Inc., Santa Clara, USA) and detected at two different wavelengths 280 nm and 325 nm. The results were expressed in mg of the analyte per kg of DM.

Statistical analysis. The results were statistically analysed by the analysis of variance (ANOVA) method. The differences between mean values were evaluated by the Tukey's *HSD* (honestly significant difference) test in the SAS program (SAS Institute, Carry, USA), version 9.4 at the level of significance $P = 0.05$.

RESULTS AND DISCUSSION

Total polyphenols content. The results of ANOVA related to the 16 wheat genotypes belonging to the four wheat species and grown over two trial years are given in Table 2. The analysis shows that the TPC was significantly affected both by genotype, wheat species (S), crop year (Y) and interaction (G × Y). However, differences in the impact of individual factors were observed. The TPC was mostly affected by Y (58.44%) and wheat species (20.92%), while the effects of genotype (12.97%) and interaction G × Y (7.68%) were lesser.

Table 2. The effect of genotype, wheat species, crop year and interaction G × Y (% of the total mean square) on the content of evaluated antioxidants in the wheat grain (ANOVA)

| | TPC | TPA |
|-------------------|----------|----------|
| | (%) | |
| Genotype (G) | 12.97*** | 1.23*** |
| Wheat species (S) | 20.92*** | 28.57*** |
| Year (Y) | 58.44*** | 69.25*** |
| G × Y | 7.68*** | 0.95*** |

*** $P < 0.001$; TPC – total content of polyphenols; TPA – total content of phenolic acids

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The results of the Tukey's *HSD* test describing significance between the mean values of genotypes, wheat species and years is shown in Table 3, a more detailed view on individual genotypes is given in Table 4. The TPC ranged from 556.57 mg/kg DM (common wheat cv. Annie, 2017) to 849.73 mg/kg DM (*T. monococcum* GEO, 2018) (Table 4).

The differences for the TPC of the wheat genotypes in previous studies might be due to differences of genotypes as well as differences in environmental conditions and environment × genotype interaction. Our results are similar to those of Lachman et al. (2012) who recorded the TPC from 502 to 748 mg/kg DM in the collection of einkorn, emmer and bread wheat cultivars and accessions. On the other hand,

total polyphenol contents in the collection of *T. monococcum*, *T. dicoccum*, *T. durum*, *T. spelta* and *T. aestivum* genotypes evaluated by Brandolini et al. (2013) were higher in comparison with our results and varied from 1075 to 1374 mg/kg DM. On the contrary, the TPC values in grain samples of different cereal species, including einkorn, were relatively low and ranged from 349 to 593 mg/kg DM (Fogarasi et al. 2015).

Our results confirmed the existence of differences among wheat species – einkorn reached the highest content of total polyphenols (744.97 mg/kg DM), followed by emmer (705.28 mg/kg DM) common wheat (702.15 mg/kg DM) and spelt (694.99 mg/kg DM). However, the only einkorn differed from other wheat species significantly (Table 3).

Table 3. The content of total polyphenols (TPC) and phenolic acids (TPA) in the wheat genotypes, species and years (means ± standard deviation and the Tukey's *HSD* test results)

| | TPC | TPA |
|--|--------------------------------|---------------------------------|
| | (mg/kg DM) | |
| <i>Triticum monococcum</i> 44* | 757.93 ± 56.92 ^{ab} | 779.43 ± 109.64 ^{efg} |
| <i>T. monococcum</i> 38* | 792.37 ± 59.07 ^a | 817.54 ± 43.29 ^{bcd} |
| <i>T. monococcum</i> No. 8910* | 721.07 ± 19.36 ^{bcde} | 837.44 ± 53.00 ^b |
| Schwedisches einkorn* | 708.50 ± 12.35 ^{cde} | 874.74 ± 76.03 ^a |
| <i>T. dicoccum</i> Brno** | 695.95 ± 35.02 ^{cde} | 792.66 ± 35.87 ^{cdef} |
| <i>T. dicoccum</i> Dagestan** | 692.62 ± 43.42 ^{de} | 773.29 ± 49.06 ^{fg} |
| Weiser Sommer** | 724.80 ± 24.25 ^{bcde} | 816.29 ± 24.29 ^{bcde} |
| Rudico** | 707.75 ± 17.29 ^{cde} | 777.49 ± 37.46 ^{fg} |
| <i>T. spelta</i> No. 8930*** | 715.47 ± 25.88 ^{bcde} | 823.26 ± 62.83 ^{bc} |
| Špalda bílá jarní*** | 695.60 ± 26.64 ^{cde} | 802.46 ± 57.17 ^{bcdef} |
| <i>T. spelta</i> Kew*** | 682.77 ± 16.03 ^e | 783.89 ± 20.97 ^{def} |
| <i>T. spelta</i> VIR St. Petersburg*** | 686.13 ± 18.38 ^e | 805.74 ± 40.08 ^{bcdef} |
| Izzy**** | 736.70 ± 45.45 ^{bc} | 742.62 ± 52.44 ^{gh} |
| Jara**** | 718.17 ± 12.45 ^{bcde} | 705.07 ± 44.87 ⁱ |
| Astrid**** | 734.90 ± 46.92 ^{bcd} | 715.48 ± 62.72 ^{hi} |
| Annie**** | 618.83 ± 62.92 ^f | 700.66 ± 62.57 ⁱ |
| <i>HSD</i> _{0.05} | 43.82 | 37.19 |
| Einkorn | 744.97 ± 53.79 ^a | 827.29 ± 82.50 ^a |
| Emmer | 705.28 ± 34.03 ^b | 789.93 ± 39.95 ^c |
| Spelt | 694.99 ± 25.60 ^b | 803.84 ± 50.10 ^b |
| Common wheat | 702.15 ± 66.83 ^b | 715.96 ± 58.62 ^d |
| <i>HSD</i> _{0.05} | 16.21 | 13.76 |
| 2017 | 693.05 ± 24.85 ^b | 734.60 ± 45.46 ^b |
| 2018 | 730.65 ± 63.41 ^a | 833.91 ± 60.36 ^a |
| <i>HSD</i> _{0.05} | 8.68 | 7.37 |

*einkorn genotypes; **emmer genotypes; ***spelt genotypes; ****common wheat genotypes; *HSD* – honestly significant difference; DM – dry matter

<https://doi.org/10.17221/134/2019-PSE>Table 4. The effect of crop year on the content of total polyphenols (TPC) and phenolic acids (TPA) in a grain of individual wheat genotypes (means \pm standard deviation and Tukey's *HSD* test results)

| Genotype | Origin | Year | TPC | | TPA | | <i>HSD</i> _{0.05} | |
|--|--------|------|---------------------------------|--|---------------------------------|-----|----------------------------|-------|
| | | | (mg/kg DM) | | TPC | TPA | TPC | TPA |
| <i>Triticum monococcum</i> 44 | ALB | 2017 | 731.87 \pm 12.70 ^b | | 670.29 \pm 8.96 ^b | | 42.50 | 29.13 |
| | | 2018 | 784.00 \pm 70.72 ^a | | 888.56 \pm 11.82 ^a | | | |
| <i>T. monococcum</i> 38 | GEO | 2017 | 735.00 \pm 19.71 ^b | | 777.05 \pm 7.83 ^b | | 39.14 | 42.51 |
| | | 2018 | 849.73 \pm 3.03 ^a | | 858.03 \pm 20.19 ^a | | | |
| <i>T. monococcum</i> No. 8910 | DNK | 2017 | 707.00 \pm 7.87 ^a | | 786.14 \pm 15.97 ^b | | 36.92 | 37.01 |
| | | 2018 | 735.13 \pm 17.08 ^a | | 888.74 \pm 10.02 ^a | | | |
| Schwedisches einkorn | SWE | 2017 | 711.10 \pm 9.49 ^a | | 802.03 \pm 23.82 ^b | | 33.53 | 48.65 |
| | | 2018 | 705.90 \pm 14.20 ^a | | 947.46 \pm 20.47 ^a | | | |
| <i>T. dicoccum</i> Brno | CSK | 2017 | 662.37 \pm 3.39 ^b | | 735.66 \pm 2.19 ^b | | 27.50 | 21.66 |
| | | 2018 | 729.53 \pm 13.62 ^a | | 849.67 \pm 5.52 ^a | | | |
| <i>T. dicoccum</i> Dagestan | RUS | 2017 | 649.73 \pm 5.43 ^b | | 711.86 \pm 3.98 ^b | | 18.98 | 26.65 |
| | | 2018 | 735.50 \pm 7.94 ^a | | 834.73 \pm 7.49 ^a | | | |
| Weiser Sommer | DEU | 2017 | 703.53 \pm 14.33 ^b | | 767.56 \pm 8.51 ^b | | 32.37 | 26.63 |
| | | 2018 | 746.07 \pm 8.15 ^a | | 865.02 \pm 10.56 ^a | | | |
| Rudico | CZE | 2017 | 692.90 \pm 2.01 ^b | | 734.40 \pm 3.08 ^b | | 24.57 | 24.08 |
| | | 2018 | 722.60 \pm 12.36 ^a | | 820.57 \pm 11.87 ^a | | | |
| <i>T. spelta</i> No. 8930 | DNK | 2017 | 693.40 \pm 9.55 ^b | | 762.15 \pm 18.59 ^b | | 37.56 | 40.56 |
| | | 2018 | 737.53 \pm 16.58 ^a | | 884.36 \pm 9.01 ^a | | | |
| Špalda bílá jarní | CSK | 2017 | 677.57 \pm 21.35 ^b | | 746.73 \pm 4.58 ^b | | 31.15 | 35.42 |
| | | 2018 | 713.63 \pm 17.70 ^a | | 858.19 \pm 17.45 ^a | | | |
| <i>T. spelta</i> Kew | GBR | 2017 | 675.03 \pm 6.11 ^a | | 769.80 \pm 10.22 ^b | | 38.98 | 31.12 |
| | | 2018 | 690.50 \pm 18.89 ^a | | 797.97 \pm 19.45 ^a | | | |
| <i>T. spelta</i> VIR St. Petersburg | CSK | 2017 | 676.27 \pm 8.20 ^a | | 769.18 \pm 15.07 ^b | | 37.65 | 45.63 |
| | | 2018 | 696.00 \pm 20.33 ^a | | 842.29 \pm 17.69 ^a | | | |
| Izzy | CZE | 2017 | 692.10 \pm 4.81 ^b | | 694.12 \pm 19.66 ^b | | 27.53 | 43.79 |
| | | 2018 | 781.30 \pm 13.17 ^a | | 791.11 \pm 19.08 ^a | | | |
| Jara | CSK | 2017 | 710.60 \pm 13.36 ^a | | 668.62 \pm 15.51 ^b | | 27.44 | 34.81 |
| | | 2018 | 725.73 \pm 4.08 ^a | | 741.51 \pm 8.60 ^a | | | |
| Astrid | CZE | 2017 | 689.17 \pm 3.54 ^b | | 679.86 \pm 18.33 ^b | | 29.12 | 42.59 |
| | | 2018 | 780.63 \pm 14.40 ^a | | 751.10 \pm 32.11 ^a | | | |
| Annie | CZE | 2017 | 556.57 \pm 6.16 ^b | | 620.66 \pm 13.57 ^b | | 25.05 | 33.03 |
| | | 2018 | 681.10 \pm 11.17 ^a | | 780.66 \pm 9.94 ^a | | | |

HSD – honestly significant difference; DM – dry matter

Our results are in agreement with those of Fogarasi et al. (2015) who observed higher TPC in einkorn compared to other wheat species. Also, the results of Şahin et al. (2017) showed that TPC of einkorn was significantly higher than TPC of bread and durum wheat. Lachman et al. (2012) recorded that the highest TPC was found for emmer genotypes, but also some einkorn and bread wheat genotypes were rich in the TPC. Similarly, Serpen et al. (2008) stated

that emmer had higher TPC than einkorn and the bread wheat controls. Abdel-Aal and Rabalski (2008) reported that einkorn, emmer and spelt had similar values, significantly higher than those of bread wheat.

Significantly, the prevailing impact of weather conditions on TPC values was determined. The 2018 season was marked by similar temperatures and substantially lower precipitation during the time from heading to the grain maturity compared to 2017

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(Table 1). Therefore, it could be possible to suppose that in 2018, when the TPC was significantly higher, evaluated wheat genotypes were exposed to higher weather stress. The fact that many antioxidants are produced by plants in response to abiotic stress, like water stress and heat stress, is known (Lu et al. 2015). Our results are in agreement with the findings of Lachman et al. (2012) who recorded significant changes between two cropping years in their study of emmer, einkorn and bread wheat genotypes and concluded that the superior total polyphenol contents were a consequence of lower rainfall and higher temperatures during the ripening stages of cereals. Similarly, Stracke et al. (2009) registered significant differences in TPC of the wheat grain in dependence on the year (weather conditions).

Total phenolic acids content. Similarly to total polyphenols content, total phenolic acids content was significantly affected by genotype, wheat species, crop year and interaction ($G \times Y$) (Table 2). The results confirmed a strong effect of the year (69.25%) and wheat species (28.57%), while the effect of genotype (1.23%) was relatively low, probably due to lower genotypes variability within individual wheat species; the low effect was observed in interaction $G \times Y$ (0.95%), too.

Phenolic acids represent the most common phenolic compounds in cereal grains (Martini et al. 2015). Our results showed that TPA content was in total higher compared to the total polyphenols content and ranged from 700.66 mg/kg DM (common wheat cv. Annie) to 874.74 mg/kg DM (Schwedishes einkorn) in an average of two-year results (Table 3). In total, the lowest TPA value (620.66 mg/kg DM) was observed in cv. Annie (2017), the highest (947.46 mg/kg DM) in Schwedishes einkorn (2018) (Table 4). Brandolini et al. (2013), on the basis of their investigation of different wheat species, reported that phenolic acids (conjugated + bound fractions) content varied from 477 mg/kg DM to 687 mg/kg DM. The total content of conjugated and bound phenolic acids evaluated by Hidalgo and Brandolini (2017) in three einkorn accessions during three years ranged from 524.00 to 672.20 mg/kg DM. Li et al. (2008) recorded the highest phenolic acids content in emmer (779 ± 109 mg/kg DM), durum (699 ± 51 mg/kg DM) and bread wheat (664 ± 15 mg/kg DM); nevertheless, these values did not differ significantly from those of spelt (579 ± 57 mg/kg DM) and einkorn (615 ± 74 mg/kg DM). The content of total phenolic acids (sum of free, conjugated and bound phenolic acids) in 10 durum wheat cultivars grown during 3 crop

years ranged from 856.6 to 1464.0 mg/kg DM (Martini et al. 2015). The method for TPA content evaluation described by Martini et al. (2015) was used in our study as well. Slightly lower TPA values of our wheat samples compared to those of Martini et al. (2015) could be probably connected with different environmental conditions as well as different wheat species and genotypes.

Our results confirmed the existence of differences among wheat species – einkorn reached the highest content of total phenolic acids (827.29 mg/kg DM), followed by spelt (803.84 mg/kg DM), emmer (789.93 mg/kg DM) and common wheat (715.96 mg/kg DM). There were significant differences among all of the wheat species (Table 3). Contradictory results related to the TPA content in different wheat species are described in different studies. Brandolini et al. (2013) reported that *T. durum* and *T. aestivum*, followed by *T. spelta*, *T. monococcum* and *T. dicoccum* showed the highest total phenolic acids content, while *T. turanicum* reached the lowest TPA concentration. Serpen et al. (2008) stated that emmer has more total phenolic acids than einkorn and two bread wheat controls.

Significantly prevailing impact of weather conditions on TPA was determined; higher TPA content in the very dry year 2018 was observed (Tables 3 and 4). The influence of the crop year on phenolic acids concentration has been scantily studied. A strong influence of the year on the content of phenolic acids was reported by Lachman et al. (2011) for *T. monococcum*, *T. dicoccum* and *T. aestivum*. Also, Stracke et al. (2009) studying the effects of two production methods (traditional and organic) for three years on the TPA content stated that the year effect was the most important.

In conclusion, the results indicate a high antioxidant potential of the hulled wheat species. Thus, they could be an opportunity for wheat breeders as well as commercial organic wheat growers. As to the stress factors, the usually decrease yield; however, on the other hand they provide opportunities for production of nutritionally profitable secondary metabolites. Therefore, further studies on these subjects need to be carried out.

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