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THE ANTIOXIDANT ACTIVITY OF ANCIENT WHEAT VARIETIES AND MODERN WHEAT VARIETIES

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Abstract: Wheat is a crucial dietary stable and economic commodity around the globe. It plays an important role in health benefits to combat oxidative stress in the human body by maintaining a balance between antioxidants and oxidants. The objective of this study was to determine the contents of antioxidant activity (tocopherols) in varieties of einkorn, emmer, spelt and Triticum aestivum L. and identify the richest sources for improving the nutritional value of bread, pasta and other wheat products. The field experiment were arranged in Ceske Budejovice from 2010 to 2012 with 26 wheat varieties. 2,2-diphenyl-1-pycrylhydrazyl assay was used to evaluate the level of antioxidant activity. The results revealed that antioxidant activity (AOA) ranged from 225.45 mg/kg Trolox DM to 400.83 mg/kg Trolox DM and its values were significantly different among varieties, ploidy level and wheat accessions. Also, modern wheat varieties showed higher AOA than ancient wheat varieties apart from emmer varieties.

Key Words: Antioxidant activity, wheat, food grain sources, phytochemical, reactive oxygen species.

INTRODUCTION

Wheat is plant grown on more land area than any other commercial crop. It is also one of the most important food grain sources for people all over the world because of the universal use of wheat for a wide variety of products such as bread, noodles, cakes, biscuits, etc. Wheat kernel is composed of endosperm (81–84%), bran (14–16%), and germ (2–3%) (Pomeranz 1988). Endosperm is the inner part playing a role as storage of energy and functioning protein. Bran is outer layer protecting the grain and germ is the kernel’s reproduction system. Whereas wheat endosperm contains mostly starch and protein, bran and germ are rich in dietary fiber, vitamins, minerals and phytochemicals playing an important role in nutrition and health benefits for humans (Pomeranz 1988). The customers are, therefore, strongly recommended to consume whole-grain foods with at least three servings per day. The recent studies have showed that regular consumption whole wheat grain has been found to be associated with reduced total mortality, as well as reduced risk of coronary heart disease, ischemic stroke, type 2 diabetes (Archie et al. 2006), hypertension in women and colorectal cancer (Schatzkin 2007).

The aim of this study was to determine the level of antioxidant activity (tocopherols) in varieties of einkorn (T. monococcum L.), emmer (T. dicoccum Schuebl [Schrank]), spelt (T. spelta L.) and T. aestivum L. and identify the richest sources for improving the nutritional value of bread, pasta and other wheat products.

MATERIAL AND METHODS

Used varieties

The varieties came from the Gene bank of the Crop Research Institute in Prague-Ruzyné. In the precise three-year field experiments in 2010, 2011 and 2012 four varieties of wheat einkorn, eight varieties of emmer, seven varieties of spelt, four varieties of landraces of bread wheat and three varieties of spring wheat as control (SW Kadrilj, Vanek, Jara) were used.
Field Trials
The field experiments were arranged in a randomized complete design with two replications. Plot size of this treatment was 10 m². Varieties were sown on the organic certified research area of the University of South Bohemia in Ceske Budejovice, the Czech Republic. The seeding rate was adjusted for a density of 350 germinable grains per m². The crop stands were treated in compliance with the European legislation (the European Council Regulation (EC) No. 834/2007, the European Commission Regulation (EC) No. 889/2008. Characteristics of the conditions of the University of South Bohemia in Ceske Budejovice research area: Mild warm climate, soil type – pseudo gley cambisols, kind of soil – loamy sandsoil, altitude of 388 m.

Laboratory analysis
Finely ground wheat samples (ca 5.0 g) were weighed into 100 mL volumetric flasks and dissolved in methanol. The flasks were filled up with methanol to volume of 100 mL. For AOA determination, 100 μL aliquots of sample solutions were pipetted. Determination of AOA with DPPH assay. Indirect method described by Roginsky and Lissi (2005) was used. Sample containing antioxidants reacts with a solution of stable synthetic radical being converted to a colourless product (DPPH assay). Methanolic DPPH solution [absorbance (t₀) 0.600 ± 0.01] was prepared and 100 μL of the sample were added. Reaction time was 20 min. Absorbency was measured at wavelength λ = 515 nm. AOA was calculated as the decrease of absorbency according to the equation (1): AOA (%) = 100 − [A_{t20}/A_{t0}] × 100

$$\text{(1)} \quad \text{AOA (mg Trolox/kg DM)}$$

Where: At₂₀—absorbency in time 20 min; A₀—absorbency in time 0 min. Calculated AOA was expressed in mg Trolox/kg DM. At₀ and A₁₂₀ were determined from the standard calibration curve (r² ≥ 0.9945). Calibration curves were prepared using working solutions of Troloxin methanol between 5-25 μg Trolox/mL (LOD = 0.601 μg Trolox/mL, LOQ = 2.000 μg Trolox/mL, RSD = 1.83%). All samples were analysed in duplicates.

Statistical analysis
The data were subjected to analysis by using software Minitab 17.0. Specifically, ANOVA multiple factorial analysis, Turkey’s HSD test and t-test were used for analyzing the parametric data and non-parametric data.

RESULTS AND DISCUSSION
Whole grain phytochemicals have antioxidant activity, the ability to scavenge free radicals that may oxidise biologically relevant molecules (Liu 2007). Thank to this, whole wheat foods could to contribute to the health benefits of people such as reducing the risk of heart disease, diabetes type 2, cancer and etc. In the present study, there were highly significant differences (p < 0.05) among 26 varieties for antioxidant activity (Table 1).

<table>
<thead>
<tr>
<th>Variety</th>
<th>D11*</th>
<th>D12*</th>
<th>D13*</th>
<th>D14*</th>
<th>D17*</th>
<th>D18*</th>
</tr>
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<tbody>
<tr>
<td>AOA (mg Trolox/kg DM)</td>
<td>400.83a</td>
<td>364.15ab</td>
<td>341.60bc</td>
<td>288.36c-g</td>
<td>304.56c-f</td>
<td>351.62b</td>
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<tr>
<th>Variety</th>
<th>D19*</th>
<th>RUDICO*</th>
<th>J1**</th>
<th>J2**</th>
<th>J4**</th>
<th>J6**</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOA (mg Trolox/kg DM)</td>
<td>339.92bc</td>
<td>332.90b-d</td>
<td>247.42gh</td>
<td>306.16c-f</td>
<td>293.23df</td>
<td>327.73b-c</td>
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<tr>
<th>Variety</th>
<th>P1***</th>
<th>P2***</th>
<th>P3***</th>
<th>P4***</th>
<th>SP1****</th>
<th>SP2****</th>
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<tbody>
<tr>
<td>AOA (mg Trolox/kg DM)</td>
<td>345.88bc</td>
<td>362.25ab</td>
<td>365.26ab</td>
<td>360.95ab</td>
<td>225.45h</td>
<td>226.55h</td>
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<tr>
<th>Variety</th>
<th>SP3****</th>
<th>SP6****</th>
<th>SP7****</th>
<th>SP8****</th>
<th>SP9****</th>
<th>JARA</th>
</tr>
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<tbody>
<tr>
<td>AOA (mg Trolox/kg DM)</td>
<td>232.63h</td>
<td>265.56f-h</td>
<td>280.63fg</td>
<td>281.10fg</td>
<td>248.82gh</td>
<td>357.36ab</td>
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<tr>
<th>Variety</th>
<th>SW</th>
<th>VANEK</th>
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<tr>
<td>AOA (mg Trolox/kg DM)</td>
<td>336.98b-d</td>
<td>353.70b</td>
</tr>
</tbody>
</table>

Values marked with different small letters are significantly different at P ≤ 0.05
* Emmer varieties; ** Einkorn varieties; *** Landrace of T. aestivum; **** Spelt varieties
Mean antioxidant activity among varieties ranged from 225.45 mg Trolox/kg DM to 400.83 mg Trolox/kg DM. This demonstrates a broad range of antioxidant content in wheat species. There were eight groups in which the means were not significantly different from one another. Having 400.83 mg Trolox/kg DM, D11 variety belonged to lead group and was significantly different from all other varieties except P3, D12, P2, P4 and JARA. In contrast, the varieties containing the lowest content of antioxidant were SP6, SP9, J1, SP3, SP2 and SP1 with 266.57 mg Trolox/kg DM, 248.82 mg Trolox/kg DM, 247.42 mg Trolox/kg DM, 232.63 mg Trolox/kg DM, 226.55 mg Trolox/kg DM and 225.45 mg Trolox/kg DM, respectively.

According to the findings of Lachman et al. (2012) the antioxidant activity content of 7 varieties ranged between 134.0 and 197.5 mg Trolox/kg DM. Obviously, our results are approximately two-time higher than these ones. This means that the varieties in our experiment are potential to breeding new wheat varieties, as well as its essential as a source of functional food ingredients.

It is known that antioxidant activity content can be influenced by stress factors of the weather conditions during the vegetation period and genotype effects. Comparing the data collected from 2010 to 2012 of four species (Figure 1) show that there is a decrease gradually the mean of antioxidant during the three-year period with 23.26 mg Trolox/kg DM. These differences are, however, not statistically significant.

*Figure 1 Antioxidant activity in 26 varieties harvested in 2010, 2011 and 2012*

The cultivated diploid (einkorn), tetraploid (durum wheat), hexaploid (bread wheat) and varieties possess antioxidant activity due to their content of hydrophilic (phenolics, selenium) and lipophilic (carotenoids, tocopherols) antioxidants (Hidalgo et al. 2008).
Analysing ANOVA Tukey’s HSD revealed statistically significant differences between Tetraploid and Diploid as well as between Tetraploid and Hexaploid (Figure 2). The mean antioxidant activity of tetraploid from 2010 to 2012 (340.49 ± 39.11 mg Trolox/kg DM) was higher than the value of diploid and hexaploid (293.64 ± 34.82 mg Trolox/kg DM) and (303.08 mg Trolox/kg DM), respectively. Our results are different to those of Lachman (2012). While antioxidant values in our findings increase from diploid (einkorn) to tetraploid, the reverse is true for Lachman’s results. This is because our experiment used 26 varieties in three years compared to 7 varieties in two years of Lachman’s experiment.

Figure 3 Antioxidant activity values of four species
The figure 3 illustrates the differences of four varieties. *T. aestivum* and emmer wheat shared the highest value with 354.44 ± 24.97 mg Trolox/kg DM and 340.49 ± 39.11 mg Trolox/kg DM, respectively. The second high value belonged to *T. monococcum* (293.64±34.82 mg Trolox/kg DM). With 251.54 ± 29.60 mg Trolox/kg DM), *T. spelta* had the lowest value in total four species (P < 0.05).

**CONCLUSION**

Wheat contains a huge essential antioxidants such as dietary fiber, tocopherols, tocotrienols, and etc. The consumption of wheat is associated with reducing risk of chronic diseases including type 2 diabetes, obesity, and cardiovascular disease. In this study, the content antioxidant activity of 26 varieties of whole wheat are reported. Antioxidant activity ranged from 225.45 mg Trolox/kg DM to 400.83 mg Trolox/kg DM. The antioxidant activity values were significantly different among varieties, ploidy level and wheat accessions. Also, this study showed a genotypical variation in the antioxidant activity of einkorn, emmer, spelta and *T. aestivum*.

**ACKNOWLEDGEMENTS**

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