Agronomy Research **15**(S2), 1287–1294, 2017

Total phenolic content and antioxidant activity of tritordeum wheat and barley

M. Eliášová* and L. Paznocht

Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Chemistry, Kamýcka 129, CZ16521, Prague 6, Czech Republic
*Correspondence: eliasovam@af.czu.cz

Abstract. Whole grains are a source of numerous antioxidant compounds such as phenolic compounds, anthocyanins, phenolic acids, proanthocyanidins, lignans and others which are able to scavenge free radicals. Thus cereals seem to be very useful in preventing chronic diseases like metabolic syndrome (obesity, high blood pressure, high blood triglyceride and glucose levels), diabetes, neurodegenerative diseases, cancer and chronic inflammatory diseases. Recently, there has been an increased consumer demand for cereal based foods, especially whole cereals. Such demand provides scope for innovations of which an important one is introduction of a completely new cereal cross called tritordeum. This alternative cereal, which is presented as a good source of health beneficial compounds, was assessed in this study and compared with wheat and barley. The total phenolic content (TPC) and related total antioxidant activity (TAA) were investigated via two spectrophotometric methods using a stable 2,2-diphenyl-1-picryhydrazyl radical (DPPH) and Folin-Ciocalteau reagent respectively. Both TPC and related TAA values of tritordeum and wheat were similar but were significantly lower compared to barley. Results have also revealed a close relationship between TAA and TPC ($R^2 = 0.93$, $p < 0.05$), which might suggest that increased antioxidant activity in those grains is caused by phenolic compounds contained in them. Tritordeum seems to represent a new cereal with good prospects; nevertheless more detailed analysis of its health related compounds is required.

Key words: cereals, DPPH, spectrophotometry, total antioxidant activity, total phenolic content, tritordeum

INTRODUCTION

Cereals represent a staple food group in the human diet. They are a major source of energy, essential and health promoting components (Tayyem et al., 2016). Especially whole grain products are a potential source of numerous antioxidant compounds, which can be found in bran or germ of kernels (Abozed et al., 2014; Laddomada et al., 2015). Yet they are very heterogenic groups of compounds belonging to several classes such as phenolic compounds, anthocyanins, phenolic acids, proanthocyanidins, lignans and others (Okarter et al., 2010). Thanks to their antioxidant properties they can significantly contribute to the total antioxidant activity of cereal based foods (Liu, 2007; Shao et al., 2014). Nowadays, such foods are gaining importance while they seem to be very useful for prevention of human chronic diseases. Due to the increasing prevalence of metabolic syndrome (obesity, high blood pressure, high blood triglyceride and glucose levels), diabetes, neurodegenerative diseases, cancer, chronic inflammatory diseases, insufficient fibre intake and only minimal physical activity, the overall demand for nutritionally beneficial products is growing. Consumers are interested in buying new sources (or in re-discovering the old ones) of such foods, which can help them to maintain good health. Since whole cereal products represent such commodities, grain breeders all over the world are seeking and breeding various cereal varieties/lineages with increased health benefits (Espín et al., 2007; Dykes and Roony, 2007; Syed Jaafar et al., 2013).

Genetic breeding led to creation of, besides many others, tritordeum, a cross between durum wheat (*Triticum durum* L.) and wild barley (*Hordeum chilense* L.). While wheat and barley have been common crops worldwide for millennia, tritordeum is a relatively new crop presented as an alternative cereal with unique properties and major health benefits (www.tritordeum.com). These are associated with content of nutritionally significant constituents, such as carotenoids and phenolic acids. Above all, tritordeum shows high content of carotenoids, especially lutein, which is estimated to be 5-6 times higher than in common wheat (Mellado-Ortega & Hornero-Méndez, 2015). Furthermore, another recent study identified the composition of phenolic acids in tritordeum, suggesting their contribution to health benefits of tritordeum (Navas-Lopez et al., 2014). Nonetheless, little is known about other nutritionally important constituents in tritordeum or its health associated characteristics.

A possible way of assessing cereals' health benefits is analysis of compounds contained in grains or potential health effects evaluation. Although these effects are difficult to determine they can be reflected in some detail by certain methods. For example measuring of total phenolic content (TPC) or total antioxidant activity (TAA) represents a simple way to evaluate potential nutritional significance. Total phenolic content quantifies phenolic compounds using Folin-Ciocalteu reagent assay, which is based on electron transfer and measures the ability of an extract to reduce the reagent (Margraf et al., 2015). Total antioxidant activity can be measured by various methods, for example common antioxidant assay using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). It is based on the significant ability of phenolic compounds to scavenge the DPPH free radical (Antolovich et al., 2002).

So far, no study quantifying phenolic compounds (TPC) in tritordeum and their antioxidant activity (TAA) has been conducted. Therefore the first aim of this study was to assess the overall amount of phenolic compounds in tritordeum by determination of TPC and to obtain the TAA value of phenolic compounds. The subsequent aim of this study was to verify the correlation between TPC and TAA as presented in some studies (Ivanišová et al., 2014; Zhao et al., 2006) and to compare these values to those measured in wheat and barley.

MATERIALS AND METHODS

Grain samples

This study evaluated several various cultivars and genotypes of three types of cereal grains – wheat, barley and tritordeum (Table 1). The cereals included red wheat, blue wheat, purple wheat, light-coloured barley, dark-coloured barley (*Hordeum vulgare* var. *nudimelanocrithon* L.) and tritordeum. The description of all cereal genotypes used in this study is shown in Table 1. The cereal varieties were grown in the Agricultural Research Institute Kromeriz (Czech Republic) over the same period and were harvested in 2014. The analyses were performed using ground samples prepared according to Hosseinian et al. (2008). Briefly, the grain samples were ground in an IKA analytical mill (Janke & Kunkel Co., Stanfen, Germany) to pass through 0.5 mm screen (35 mesh) and homogenised well.

Table 1. Description of investigated cereal genotypes

Cultivar	Species	Growth type	Country of origin*	Variety status	Grain colour
JB 1	× *Tritordeum* Ascherson et Graebner	spring	ESP	released variety	yellow endosperm
JB 3	× *Tritordeum* Ascherson et Graebner	spring	ESP	released variety	yellow endosperm
HT 439	× *Tritordeum* Ascherson et Graebner	spring	ESP	breeding line	yellow endosperm
UC 66049	*Triticum aestivum* L.	spring	USA	genetic resource	blue aleurone
Tschermaks B.S.	*Triticum aestivum* L.	spring	AUT	research germplasm	blue aleurone
KM 131-15**	*Triticum aestivum* L.	winter	CZE	breeding line	blue aleurone
Skorpion	*Triticum aestivum* L.	winter	CZE	released variety	blue aleurone
RU 687-12	*Triticum aestivum* L.	spring	CZE	breeding line	purple pericarp
Konini	*Triticum aestivum* L.	spring	NZL	research germplasm	purple pericarp
Vanessa	*Triticum aestivum* L.	winter	CZE	released variety	red (standard)
PS Karkulka	*Triticum aestivum* L.	winter	SVK	released variety	purple pericarp
Bohemia	*Triticum aestivum* L.	winter	CZE	released variety	red (standard)
Hordeum nudimelanocrithon	*Hordeum vulgare* L.	spring	ETH	genetic resource	black grain
AF Cesar***	*Hordeum vulgare* L.	spring	CZE	released variety	standard
AF Lucius***	*Hordeum vulgare* L.	spring	CZE	released variety	standard

* CZE Czech Republic, ESP Spain, AUS Austria, NZL New Zealand, SVK Slovakia, USA United States, AUT Austria, ETH Ethiopia
** origin of KM 131-15 is: (Skorpion × PS Karkulka) × (Citrus × Bona Dea)
*** barley varieties have naked (hulless) caryopsis

Total phenolic content

The TPC was evaluated according to Lachman et al. (2011) with minor modifications. Briefly, 2.5 g of ground grain were extracted with 25 ml of 0.1% HCl in methanol overnight at -20 °C. Each extract was centrifuged at 8,000 rpm for 10 min and 2 ml volume was reacted with 2.5 ml of Folin-Ciocalteu reagent with addition of 7.5 ml of 20% sodium carbonate and was filled up with pure water to 50 ml. After 2 hours the solution was centrifuged at 8000 rpm for 2 min and absorbance at 765 nm was measured. The results were expressed as mg of gallic acid per 100 g of dry matter (mg GA 100 g^{-1} DM).

Total antioxidant activity using DPPH

The DPPH radical cation scavenging activity of methanolic extracts was evaluated according to Lachman et al. (2012) with minor modifications. Briefly, 2.5 g of ground grain were extracted with methanol and shaken for 2 hours at room temperature. The extracts were filled up with methanol to 25 ml and stored in darkness at room temperature for one week. The extracts were then centrifuged at 8000 rpm for 2 min. The solution of DPPH was prepared in methanol in a concentration responding to absorbance of 0.600 ± 0.01 AU at 515 nm. 100 µl of extract was mixed with 1 ml of DPPH solution, incubated for 20 min and measured at 515 nm. The results were expressed as mg of Trolox equivalent antioxidant activity per 100 g of dry matter (mg TEAC 100 g^{-1} DM).

Statistical analysis

All experimental data were analysed using Statistica software, version 12 (Dell Software). Analysis of variance (ANOVA) was performed with 5 replicates for each sample. Statistical significance was declared at $p < 0.05$.

RESULTS AND DISCUSSION

Total phenolic content (TPC) and total antioxidant activity (TAA) were evaluated in varieties of tritordeum as well as wheat and barley. The measured values of TPC and TAA proved distinctions among cereal types (Fig. 1).

Tritordeum, the new cereal with possible major health benefits, showed the lowest values of both TPC and TAA. Their average values were 65.86 ± 2.68 mg GA 100 g^{-1} DM and 16.30 ± 0.21 mg TEAC 100 g^{-1} DM. Contrary to the JB 1 and JB 3 released varieties, the HT 439 breeding line reached higher values of TPC and TAA. Its higher content of health beneficial compounds therefore offers potential for subsequent breeding. Overall, evaluation of tritordeum showed just a little difference between tritordeum and wheat varieties, which gave slightly higher results of 73.68 ± 3.87 and 21.98 ± 1.23 for TPC and TAA respectively. These results indicate that the amount of phenolic compounds possessed by tritordeum is similar to that in wheat. Such finding therefore supplements the study of Navas-Lopez et al. (2014) who reported that the composition of phenolic compounds in tritordeum and wheat is alike. Nevertheless, as mentioned before, Mellado-Ortega & Hornero-Méndez (2015) stated that tritordeum has above all a significant content of carotenoids, particularly lutein, the amount of which can reach 5-6 times that of the amount in wheat. Since the extraction method used in this study reflects only the antioxidant activity of phenolic compounds, we can still assume that carotenoid content in tritordeum can contribute to overall antioxidant activity more than phenolic compounds.

Compared to these results, barley reached substantially higher values of both TPC ($p = 0.039$, $\alpha = 0.05$) and TAA ($p = 0.028$, $\alpha = 0.05$) than tritordeum and wheat. The average TPC of barley was 129.49 ± 5.81 mg GA 100 g^{-1} DM and 98.86 ± 2.82 mg

TEAC 100 g^{-1} DM. The TPC and TAA values were significantly different not only between barley on one side and wheat and tritordeum on the other side, but also among single barley varieties.

Moreover, the results showed a noticeable trend among TPC and TAA values, which were assessed by Fisher's linear correlation analysis to evaluate their relationship. The measurement proved a very close correlation between TPC and TAA as the correlation analysis gave $R^2 = 0.93$ ($p < 0.05$).
These results suggest that phenolic compounds in cereals directly affect antioxidant capacity. This fact supports the previous findings of Ivanišová et al. (2014) who reported significant correlation between TCP and AOA ($R^2 = 0.87$, $p < 0.05$) as well as those of Zhao et al. (2006) who determined an even closer relationship ($R^2 = 0.94$, $p < 0.01$) very similar to the one published in this study.

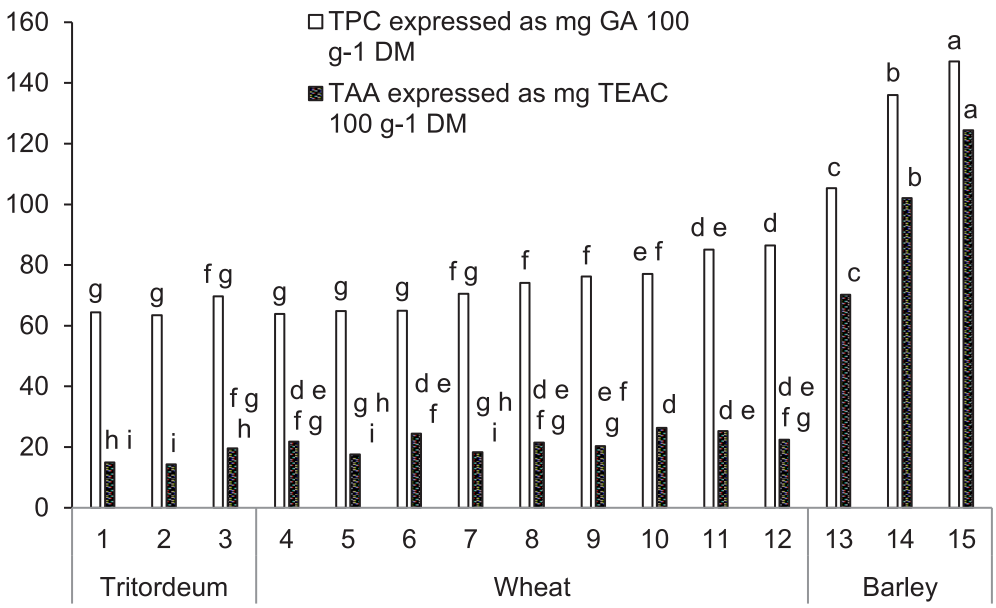

Figure 1. TPC and TAA values of specific cereals and their varieties. Values with the same letters are significantly different ($P < 0.05$). Different small letters indicate significant difference among samples. Tritordeum: 1 – JB 1, 2 – JB 3, 3 – HT 439. Wheat: 4 – UC 66049, 5 – Tschermak B.S., 6 – KM 131-15, 7 – Skorpion, 8 – RU 687-12, 9 – Konini, 10 – Vanessa, 11 – PS Karkulka, 12 – Bohemia. Barley: 13 – Hordeum nudimelanocrithon, 14 – AF Cesar, 15 – AF Lucius.

TPC values measured in this study are similar to results published by other researchers. Wheat varieties showed value of 73.68 ± 3.87 mg GA 100 g^{-1} DM, which correspond well with Moore et al. (2005) and Lachman et al. (2011) who reported mean value of TPC in wheat 60.0 and 55.2 respectively, but are slightly lower than the values of Adom et al. (2002) and Abozed et al. (2014) who measured 136.0 and 112.0 respectively. This distinction is not substantial and might be caused by the differences between analysed varieties and the extraction methods used in these studies. The distinction of TPC between barley and wheat, which was demonstrated in this study, was also published by Fogarasi et al. (2015).

The literature reports significant discrepancies in cereals' TAA measured using DPPH. Some researchers such as Li et al. (2007), Fogarasi et al. (2015) and Mazzoncini et al. (2015) obtained TAA mean values of wheat (as mg TEAC 100 g^{-1} DM) to be 147.0, 250.0, and 2753.2 respectively. On the contrary, other authors such as Brandolini et al., Lachman et al. (2012) Yilmaz et al. (2015) reported TAA mean values of wheat as 13.8, 16.4 and 30.0 respectively. The last stated results correspond with those measured in this study. Such distinctions could be caused by diverse sample preparation or by a different method procedure. Particularly the time of incubation of the extract with DPPH can significantly affect the results – for example Fogarasi et al. (2015) let DPPH react for 60 min while Brandolini et al. (2013) for only 30 min. The shorter time of incubation quantifies rather fast reacting antioxidants while a longer time reflects the slow reacting antioxidants to a greater extent. Another significant parameter could be the presence of acid in the extracting solution. DPPH is reduced by accepting of the hydrogen atom (Mishra et al., 2012) and its decrease can be therefore negatively affected by the presence of hydrogen releasing acid. This fact might explain the distinction in values measured by Mazzoncini et al. (2015) who used acidified extract and Yilmaz et al. (2015) who used non-acidified extract.

CONCLUSION

Tritordeum varieties showed similar total phenolic content and antioxidant activity to wheat. However, the results indicate that the breeding line HT 439 possesses slightly higher content of health beneficial compounds. More attention should be paid to this breeding line since it offers potential for further genetic breeding. Yet more analyses of tritordeum are required as this study gave just a brief overview of its characteristics.

The overall results provide evidence that cereals have significant content of antioxidants from the group of phenolic compounds, although their amounts in individual cereal types differ. The highest contents of total phenolics and related TAA were found especially in barley varieties, while values of wheat and tritordeum were significantly lower. The proven relationship between TPC and TAA suggest direct responsibility of phenolic compounds for antioxidant activity. It can be assumed that cereals are an important source of phenolics and other antioxidant compounds and that their consumption can considerably contribute to consumers' health through increase of antioxidant intake.

ACKNOWLEDGEMENTS. This work has been supported by the University Internal Grant Competition (CIGA) at the Czech University of Life Sciences in Prague, No. 20162002 and No. 20162005.

REFERENCES

Abozed, S.S., El-kalyoubi, M., Abdelrashid, A. & Salama, M.F. 2014. Total phenolic contents and antioxidant activities of various solvent extracts from whole wheat and bran. *Annals of Agricultural Science* **59**(1), 63–67.

Adom, K.K. & Liu, R.H. 2002. Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry* **50**(21), 6182–6187.
Antolovich, M., Prenzler, P.D., Patsalides, E., McDonald, S. & Robards, K. 2002. Methods for testing antioxidant activity. *Analyst* **127**(1), 183–198.
Brandolini, A., Castoldi, P., Plizzari, L. & Hidalgo, A. 2013. Phenolic acids composition, total polyphenols content and antioxidant activity of *Triticum monococcum*, *Triticum turgidum* and *Triticum aestivum*: A two-years evaluation. *Journal of Cereal Science* **58**(1), 123-131
Dykes, L. & Rooney, L.W. 2007. Phenolic compounds in cereal grains and their health benefits. *Cereal Foods World* **52**(3), 105–111.
Espín, J.C., García-Conesa, M.T. & Tomás-Barberán, F.A. 2007. Nutraceuticals: Facts and fiction. *Phytochemistry* **68**(22-24), 2986–3008.
Fogarasi, A.L., Kun, S., Tankó, G., Stefanovits-Bányai, É. & Hegyesné-Vecseri, B. 2015. A comparative assessment of antioxidant properties, total phenolic content of einkorn, wheat, barley and their malts. *Food Chemistry* **167**, 1–6.
Hosseinian, F.S., Li, W. & Beta, T. 2008. Measurement of anthocyanins and other phytochemicals in purple wheat. *Food Chemistry* **109**(4), 916–924
Ivanišová, E., Ondrejovič, M., Chmelová, D., Maliar, T., Havrlentová, M. & Rückschloss, L’. 2014. Antioxidant activity and polyphenol content in milling fractions of purple wheat. *Cereal Research Communications* **42**(4), 578–588.
Lachman, J., Miholova, D., Pivec, V., Jírů, K. & Janovská, D. 2011. Content of phenolic antioxidants and selenium in grain of einkorn (*Triticum monococcum*), emmer (*Triticum dicoccum*) and spring wheat (*Triticum aestivum*) varieties. *Plant, Soil and Environment* **57**(5), 235–243.
Lachman, J., Orsák, M., Pivec, V. & Jírů, K. 2012. Antioxidant activity of grain of einkorn (*Triticum monococcum* L.), emmer (*Triticum dicoccum* Schuebl [Schrank]) and spring wheat (*Triticum aestivum* L.) varieties. *Plant, Soil and Environment* **58**(1), 15–21.
Laddomada, B., Caretto, S. & Mita, G. 2015. Wheat Bran Phenolic Acids: Bioavailability and Stability in Whole Wheat-Based Foods. *Molecules* **20**(9), 15666–15685.
Li, W.D., Pickard, M.D. & Beta, T. 2007. Effect of thermal processing on antioxidant properties of purple wheat bran. *Food Chemistry* **104**(3), 1080–1086.
Liu, R.H. 2007. Whole grain phytochemicals and health. *Journal of Cereal science* **46**(3), 207–219.
Margraf, T., Karnopp, A.R., Rosso, N.D. & Granato, D. 2015. Comparison between Folin-Ciocalteu and prussian blue assay to estimate the total phenolic content of juices and teas using 96-well microplates. *Journal of Food Science* **80**(11), C2397–C2403.
Mazzoncini, M., Antichi, D., Silvestri, N., Ciantelli, G. & Sgherri, C. 2015. Organically vs conventionally grown winter wheat: Effects on grain yield, technological quality, and on phenolic composition and antioxidant properties of bran and refined flour. *Food Chemistry* **175**, 445–451.
Mellado-Ortega, E. & Hornero-Mendez, D. 2015. Carotenoids in cereals: an ancient resource with present and future applications. *Phytochemistry reviews* **14**(6), 873–890.
Mishra, K., Ojha, H. & Chaudhury, N.K. 2012. Estimation of antiradical properties of antioxidants using DPPH center dot assay: A critical review and results. *Food Chemistry* **130**(4), 1036–1043.
Moore, J., Hao, Z.G., Zhou, K.Q., Luther, M., Costa, J. & Yu, L.L. 2005. Carotenoid, tocopherol, phenolic acid, and antioxidant properties of Maryland-grown soft wheat. *Journal of Agricultural and Food Chemistry* **53**(17), 6649–6657.
Navas-Lopez, J.F., Ostos-Garriro, F.J., Castillo, A., Martin, A., Gimenez, M.J. & Piston, F. 2014. Phenolic content variability and its chromosome location in tritordeum. *Frontiers in Plant Science* **5**(10).

Okarter, N., Liu, Ch. S., Sorrells, M.E. & Liu, R.H. 2010. Phytochemical content and antioxidant activity of six diverse varieties of whole wheat. *Food chemistry* **119**(1), 249–257.

Shao, Y., Xu, F., Sun, X., Bao, J. & Beta, T. 2014. Identification and quantification of phenolic acids and anthocyanins as antioxidants in bran, embryo and endosperm of white, red and black rice kernels (*Oryza sativa* L.). *Journal of Cereal Science* **59**(2), 211–218.

Syed Jaafar, S.N., Baron, J., Siebenhandl-Ehn, S., Rosenau, T., Böhmdorfer, S. & Grausgruber, H. 2013. Increased anthocyanin content in purple pericarp × blue aleurone wheat crosses. *Plant Breeding* **132**(6), 546–552.

Tayyem, R.F., Bawadi, H.A., Shehadah, I., Agraib, L.M., Al-Awwad, N.J., Heath, D.D. & Bani-Hani, K.E. 2016. Consumption of whole grains, refined cereals, and legumes and its association with colorectal cancer among jordanians. *Integrative Cancer Therapies* **15**(3), 318–325.

Yilmaz, V.A., Brandolini, A. & Hidalgo, A. 2015. Phenolic acids and antioxidant activity of wild, feral and domesticated diploid wheats. *Journal of Cereal Science* **64**, 168–175.

Zhao, H.F., Dong, J.J., Lu, J., Chen, J., Li, Y., Shan, L.J., Lin, Y., Fan, W. & Gu, G.X. 2006. Effects of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in barley (*Hordeum vulgare* L.). *Journal of Agricultural and Food Chemistry* **54**(19), 7277–7286.