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Tocotrienols and tocopherols in colored-grain wheat, tritordeum and barley

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ABSTRACT

Colored-grain spring and winter wheat, spring tritordeum and barley (blue aleurone, purple pericarp, and yellow endosperm) from the harvests 2014 and 2015 were evaluated for tocol contents by HPLC-FD. Higher content of total tocols was found in spring wheat varieties compared with winter varieties. Four tocols (β-tocotrienol, α-tocotrienol, β-tocopherol, and α-tocopherol) were identified in wheat and tritordeum varieties. Dominant tocols in purple- and blue-grained wheat and yellow-grained tritordeum were α-tocopherol and β-tocotrienol, whereas spring barley varieties differed from wheat and tritordeum by high α-tocotrienol content. Tocol content was significantly affected by genotype and in a lesser extent in some varieties and lines also by rainfall and temperatures during crop year. Higher rainfall and lower temperatures caused in most varieties higher tocol contents. Purple- and blue-grained wheat lines with higher tocol, anthocyanin and phenolic acids with health benefits may be useful for breeding new varieties.

1. Introduction

Tocols (vitamin E) comprise a chromanol ring with a C16 phytol side chain and are classified into two types in which the side chain is either saturated (tocopherols) or contains three double bonds at carbons 3, 7 and 11 (tocotrienols). They exist in eight forms (α-, β-, γ- and δ- both tocopherols and tocotrienols), which differ in positions of methyl groups on the chromanol ring (Shewry & Ward, 2012). Tocols (especially unsaturated tocotrienols) have antioxidant properties which may be responsible for their health benefits.

In cereals, the major lipophilic secondary metabolites with antioxidant properties include tocols and carotenoids (Atanasova-Penichon, Barreau, & Richard-Forget, 2016). Tocol composition of cereals includes tocopherols (α-, β-, δ- and γ-tocopherol) and tocotrienols (α-, β-, δ- and γ-tocotrienol) (Panfili, Fratianni, & Irano, 2003). The α-forms are predominant (Gutierrez-Gonzalez, Wise, & Garvin, 2013). Tocopherols are mainly present in the germ fraction while tocotrienols are present in the pericarp and endosperm fractions (Falk, Krahnstöver, van der Kooij, Schlensog, & Krupinska, 2004). In small-grained cereals such as oat, barley and wheat, tocotrienols are the main tocols and their concentrations range between 40 and 60 mg/kg DW, depending on the cereal type and the variety (Falk et al., 2004).

Wheat and barley are a good source of tocopherols and tocotrienols, which are known to reduce serum LDL cholesterol through their antioxidant action (Baik & Ullrich, 2008). Soft wheat was found to contain high total tocol level, similarly to barley (≅75 mg/kg DW) (Moore et al., 2005). β-Tocotrienol was the main vitamer found in hulled and dehulled wheats (33–43 mg/kg DW). High levels of tocols are characteristic for einkorn and emmer wheat (Lachman, Hejtmánková, & Kotíková, 2013; Hejtmánková, Lachman, Hejtmánková, Pivec, & Janovská, 2010). The most abundant tocol in einkorn wheat was β-tocotrienol (48.22 mg/kg DW), followed by α-tocotrienol (12.77 mg/kg DW), α-tocopherol (12.18 mg/kg DW), and β-tocopherol (4.79 mg/kg DW). Mean tocotrienol/tocopherol ratio in einkorn was estimated at 3.68 (Tsao, 2008). In small-grained cereals, e.g. bread wheat ZP Zemunska rosa or ZP Zlatna higher concentrations of total tocopherols (13.85 mg/kg DW and 32.65 mg/kg DW, respectively) were found when compared with other small-grained durum wheat varieties and breeding lines with different α-

Abbreviations: α-T3, -tocotrienol; β-T3, -tocotrienol; γ-T3, γ-tocotrienol; δ-T3, δ-tocotrienol; α-T, α-tocopherol; β-T, β-tocopherol; γ-T, γ-tocopherol; δ-T, δ-tocopherol; CR, Czech Republic; DW, dry weight; HPLC-FD, high performance liquid chromatography with fluorescence detection; LOD, limit of detection

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tocopherol and (β + γ)-tocopherol contents (Žilić, Hadži-Tašković Šukalović, Dodig, Maksimović, Maksimović, & Basić, 2011). Within evaluation of fifteen diploid, tetraploid and hexaploid accessions belonging to different *Triticum* species, focused on chemical composition of wild and feral diploid wheats, the highest total tocol content was found in *T. thaoudar, T. aegilopoides, T. monococcum* and *T. urartu* (75.1 ± 3.95, 70.8 ± 3.35, 66.8 ± 3.82 and 63.9 ± 2.91 mg/kg DW, respectively) (Brandolini, Hidalgo, Gabriele, & Heun, 2015; Hidalgo, Brandolini, Pompei, & Piscozzi, 2006). The highest contents were characteristic for β-tocotrienol (in diploid wheats in range 37.90 ± 3.22–45.90 ± 2.31 mg/kg DW) followed by α-tocotrienol (9.1 ± 0.24–17.5 ± 0.85 mg/kg DW) and α-tocopherol (9.9 ± 0.01–12.6 ± 0.12 mg/kg DW). The lowest contents were reported for β-tocopherol (2.9 ± 0.04–5.5 ± 0.70 mg/kg DW). For durum, bread wheat and triticale, with a total tocol content of 33.75, 36.85 and 33.00 mg/kg DW, respectively, (β + γ)-tocotrienols was the major isomer, followed by α-tocopherol, α-tocotrienol and (β + γ)-tocopherols (Irakli, Samanidou, & Papadoyannis, 2011). In wheat the range of tocol concentrations in the HEALTHGRAIN was evaluated in common bread wheat, durum wheat, spelt, einkorn, and emmer 27.6–79.7 mg/kg, 40.1–62.7 mg/kg, 40.2–50.6 mg/kg, 29.0–57.5 mg/kg, and 29.0–57.5-mg/kg, respectively; in barley 46.2–68.8 mg/kg (Shewry et al., 2013).

On average, the raw kernels of einkorn had higher contents of total tocols and α-tocotrienol but lesser content of β-tocopherol than those of bread wheat. Unlike carotenoids, tocols are heat-resistant antioxidant compounds. Moreover, as already observed by Hidalgo, Brandolini, and Pompei (2010) in bread crust and water biscuits after baking, puffing led to a slight increase of α-tocotrienol and of β-tocopherol contents. As a consequence, total tocol content augmented only slightly, but significantly, both in bread wheat and einkorn. It has to be remembered that in wheat and other cereals tocols are esterified to lipids and other molecules. Drastic heat conditions, such as puffing, may break the bonds and increase the tocols extractable by the heat saponification method (Hidalgo, Scuppa, & Brandolini, 2016).

Tritordeum (*x Tritordeum martinii* A. Pujadas nothosp. nov.) is a new hexaploid hybrid derived from the cross between a South American wild barley (*Hordeum chilense* Roem. & Schult.) and durum wheat (*Triticum durum* Desf.), where tocol concentrations have not been reported yet.

Lipophilic and hydrophilic antioxidants are important wheat and other cereals constituents beneficial for human health. In wheat genotypes with purple pericarp and blue aleurone there are contained antioxidant pigments anthocyanins (Lachman, Martinek, Kotíková, Orsák, & Šulc, 2017). But so far no lipophilic tocotrienols and tocopherols were analyzed and reported in purple- and blue-grained wheat varieties and yellow-grained spring tritordeums. Therefore, the main aim of this study was the determination of tocols in purple- and blue-grained wheats and breeding lines and their comparison with standard varieties. Tocols were also determined in three tritordeum and three barley varieties.

2. Materials and methods

2.1. Materials

2.1.1. Analyzed cereals

Analyzed spring and winter wheat, spring tritordeum and barley varieties were obtained from the collection of Agricultural Research Institute Kroměříž, Ltd., Czech Republic, from the harvests in 2014 and 2015. Their major characteristics are described in Table 1. Field trials were sown in locality Kroměříž (N 49 °17′52″, E 17 °23′35″, 235 m a.s.l., beet production region, luvisol, average year sum of precipitation 599 mm and average year temperature 9.8 °C). The weather conditions during crop years 2013/2014 and 2014/2015 such as sum of rainfalls and mean monthly air temperatures are depicted in Table 2. The data are compared with long-term average for the location. Harvest was carried out using a plot harvester. Samples were stored after harvest in paper bags in a box in the dark at room temperature of 25 °C for 2 months before being analyzed.

2.1.2. Chemicals and equipment used for analyses

Methanol, super gradient (min. 99.9%, Lach-Ner, Neratovice, Czech Republic), adjusted deionized ultra-pure (Type 1) water obtained with Milli-Q® Type 1 Ultrapure Water System (EMD Millipore SAS Corp., Molsheim, France), pyrocatechol, > 99.5% (Sigma–Aldrich, Inc., Saint Louis, MO, USA), and hexane, pure min. 95.0% (Penta, Prague, Czech Republic), potassium hydroxide, min. 85% (Lachema, Neratovice, Czech Republic) were purchased and used. DL-α-tocopherol, 98.2% (GC) and tocopherol set (Calbiochem, La Jolla, Canada) were used as standards.

A magnetic stirrer IKA RET control-visc C and the Vortex IKA MS 3 Basic (both from ILABO, Ltd., Kyjov, Czech Republic) were used for the homogenization of samples. Chromatographic determination was performed using a chromatographic system for HPLC – Dionex UltiMate 3000 RS (Thermo Fisher Scientific, Inc., Sunnyvale, CA, USA) with a fluorescence detector Dionex UltiMate 3000 RS (Thermo Fisher Scientific, Inc., Sunnyvale, CA, USA).

2.2. Methods

2.2.1. Sample preparation

Samples were prepared according to the method of Sánchez-Machado, López-Cervantes, and Ríos Vázquez (2006) with minor modifications. Briefly, approximately 0.4 g of homogenized wheat caryopses was weighed into long glass test tubes with plastic stoppers. Solutions of pyrocatechol (200 μL, conc. 2 mg/mL) and methanolic KOH (5 mL, 0.5 mol/L) were added to the wheat samples. The mixture was stirred on a Vortex apparatus to obtain complete homogenization (1 min). Then the test tube was placed into a water bath for 15 min at a temperature of 80 °C and it was shaken on the Vortex every 5 min (i.e. in 5, 10 and 15 min). Afterwards the test tube was rapidly cooled in a beaker with water and ice and after cooling 1 mL deionized water and 5 mL hexane were added. The mixture was stirred for 1 min on the Vortex and then 3 mL hexane aliquot was transferred into an evaporation flask and hexane was evaporated in a rotation vacuum evaporator at 30 °C. Dry residue was redissolved in 0.5 mL methanol and filtered through a syringe filter (nylon, 0.22 μm) into a dark vial for HPLC analysis. All steps were carried out at low light intensity (the windows were darkened with blinds with no direct lighting in the lab).

2.2.2. Tocopherol and tocotrienol chromatographic determination

HPLC-FD analyses were performed under the following conditions: analytical column Develosil® 5 μ RPAQUEOUS (250 × 4.5 mm), (Phenomenex, Torrance, CA, USA), mobile phase H_2O: methanol (3:97), (v/v), flow 1.0 mL min^{-1}, column temperature 30 °C, injection volume 10.0 μL (for samples with little analyte content it is possible to increase injection volume to 20.0 μL), time of analysis 30 min. The measurements were performed in three replicates. Conditions of detection: fluorescence detector, excitation wave length λ = 292 nm, emission wave length λ = 330 nm. Limits of detection (LOD) for individual tocols δ-T3, γ-T3, β-T3, α-T3, δ-T, γ-T, β-T and α-T were 0.056, 0.111, 0.111, 0.167, 0.056, 0.111, 0.111, and 0.167 μg/g, respectively. Chromatograms of blue-grained wheat EF02-54-9, V1-135-15, tritordeum HT 439 and barley cv. Lucius are given in Supplementary material (Figs. S1–S4). Repeatability of individual tocols α-T, β-T, γ-T, δ-T, α-T3, β-T3, γ-T3 and δ-T3 was 8.6%, 8.4%, 9.7%, 12.0%, 8.8%, 8.2%, 6.3% and 6.5%, respectively; percentage is dependent on their levels in measured samples.