Short communication

Tocol Content in Barley

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Received: 26-09-2006

Paper based on a presentation at the 12th International Symposium on Separation Sciences, Lipica, Slovenia, September 27–29, 2006.

Abstract

Green barley and malting barley are sources of numerous compounds with positive effects on the human organism – especially substances with antioxidant effects, e.g. vitamin E. We determined tocol content in barley caryopses in dependence of varietal and genetic properties of materials. Using chromatography, we studied vitamin E content in different growth phases of green barley and in varietal lines of malting barley. The content of vitamin E was the highest in the first growing phase (14.4–18.0 mg kg^{-1}) in the variety Sebastian. Green plants contain significantly less tocotrienols than barley caryopses.

Keywords: vitamin E, tocols, barley, green barley, HPLC analysis

1. Introduction

Vitamin E is one of the most important antioxidants in the organism. Unlike vitamin C, it is soluble in fats, which is of special importance as free radicals in the organism may damage cell membranes and lipoproteins with a lower density. The positive role of vitamin E was recently proven in coronary artery disease.[1] It belongs among the most significant lipophilic antioxidants that protect unsaturated lipids in eukaryotic cells against free radicals that damage DNA and cause e.g. age-related pigmentation and skin ageing, cataract, neuritic plates of the Alzheimer's disease, arteriosclerosis, stroke, heart attack, tumors.[2]

Vegetable oils are the main source of vitamin E, but of it is also contained in cereals in significant amounts. Vitamin E occurs in alpha, beta, gamma, delta isomers – tocopherols and tocotrienols.[3] and barley is apparently the only commonly grown cereal, the caryopsis of which contains vitamin E in all eight forms of tocopherols and tocotrienols.[4,5] The determination of these substances was performed using alkaline saponification and extraction of nonsaponified portion with diethyl ether with subsequent liquid-chromatographic determination.[6,7] Green barley plants are also commercially used as a source of natural antioxidants.[8] The content of vitamin E was determined in selected varieties of malting barley caryopses and also in different growth phases of green barley.

2. Materials and Methods

2.1. Green Barley

We used spring two-row barley varieties Sebastian, Malz and the line KM1910, grown in 2005 in plots of MUAF in Žabčice and Agrotest Kroměříž. Three growing phases were selected for screening the content of active components (according to a decadic scale – DC): I in DC 29, II in DC 31 and III in DC 32-33 in which plant green matter was taken.

2.1. Barley Lines with Increased Vitamin E Content

Seed samples used for analyses were from hand sown plants grown in field trials in spacing 15 × 10 cm on the field of the experimental station of MUAF in Žabčice near Brno. This station lies in the maize production area, at the altitude of 184 m above the sea level.

The varieties of American provenience Wabet (Wb), Wanubet (Wnb), Washonubet (Wsnb) were used as donors of vitamin E. Lines with higher vitamin E content and other antioxidants in comparison with malting-type varieties were attained by their suitable combinations with the malting varieties Kompakt (Ko), Krona (Kr).

Totally 12 varieties and lines of spring barley were used for the analyses: standard hulled malting varieties re-

gistered in the CR Kompakt and Krona, experimental lines were waxy types, Wabet, Wanubet and Washonubet, obtained from the Montana Agricultural Experiment Station, Bozeman MT USA and seven hulled lines, obtained from crossing the waxy types and Kompakt and Krona. Wabet is a hulled cultivar and Wanubet and Washonubet are hull-less cultivars.

The sampled grain from the manually harvested ears of individual plants were cleaned, homogenized and chemically analyzed. The content of vitamin E and of its isomer, α-, $\beta\gamma$-, δ-tocopherols and α-, $\beta\gamma$-, δ-tocotrienols were determined.

2.2. Tocol Analysis

Total contents of tocols – tocopherol (T) and tocotrienol (T3) isomers, α, $\beta + \gamma$ and δ were determined as follows: grain samples (2 g) were homogenized and then 100 mg of ascorbic acid, 50 mL of ethanol and 10 mL of 50% KOH were added. Saponification was performed at room temperature (18–25 °C) in a dark room in a nitrogenous atmosphere overnight. Subsequently, the samples were extracted with diethyl ether, washed with water, dried with anhydrous sodium sulphate and evaporated. The evaporated residue was dissolved in a defined volume with reagent grade methanol. The extract was analyzed using HPLC with fluorescence detection according EN 12822:2000. We used an HPLC SpectraSystem (Thermo Separation Products, Inc., USA), Pump P2000, fluorescence detector FL 3000, column stationary phase Nucleosil 120-5 C 18, 250 × 4 mm, mobile phase: methanol, 1.0 mL min^{-1}, injection volume 20 μL, detection: λ_{EX} = 290 nm, λ_{EM} = 330 nm.

Normal phase chromatography (*n*-hexane eluent) enabled the separation of all isomers and at the same time the ratio between β and γ-isomers was determined. However, the column was extremely sensitive to humidity, thus creating resolution problems. Therefore, reverse phase chromatography was used for the analyses. Using this procedure, we could not distinguish between the β and γ-isomers, therefore the sum of these isomers is shown as a combined value for both T and T3.[9] Total contents of tocols and the vitamin E equivalent (VEeq) were calculated for each cultivar as described by McLaughlin and Weibrauch.[10]

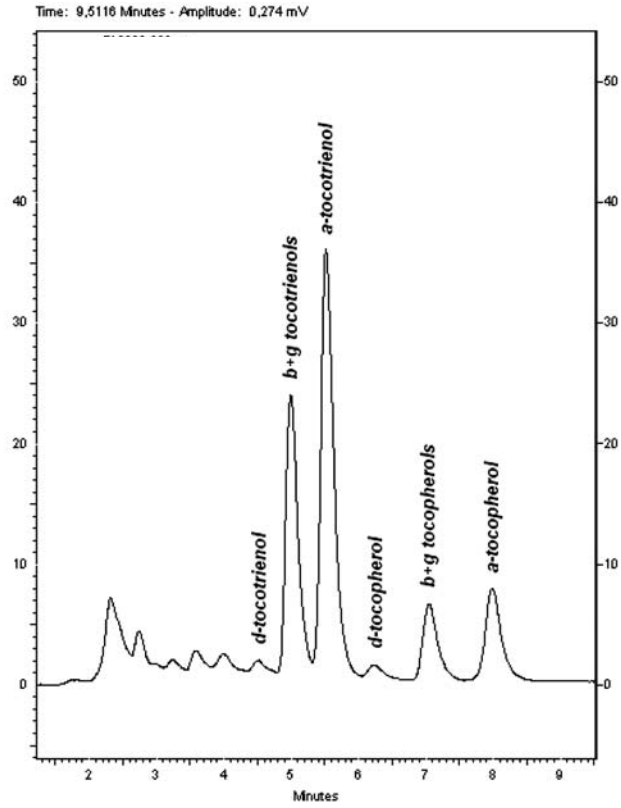

Figure 1. Chromatogram of tocopherols and tocotrienols in barley.

Table 1: Content of vitamin E in green barley.

Variety (Linie)	α-T mg kg^{-1}	$(\beta+\gamma)$-T mg kg^{-1}	δ-T mg kg^{-1}	α-T3 mg kg^{-1}	$(\beta+\gamma)$-T3 mg kg^{-1}	δ-T3 mg kg^{-1}
1st sampling						
KM 1910	16.3	3.1	0.45	–	–	–
Sebastian	17.6	2.7	0.32	–	–	–
Malz	14.0	3.0	0.25	–	–	–
2nd sampling						
KM 1910	6.8	2.0	0.36	–	–	–
Sebastian	8.5	2.1	0.29	–	–	–
Malz	8.7	1.2	0.34	–	–	–
3rd sampling						
KM 1910	6.5	0.8	0.11	4.07	–	–
Sebastian	9.1	1.7	0.24	4.21	–	–
Malz	9.3	1.3	0.21	4.11	–	–

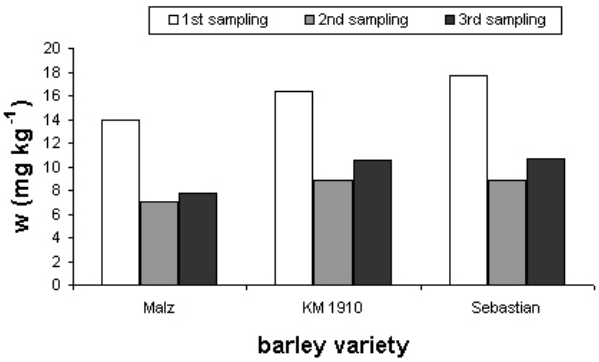

Figure 2: Content of vitamin E in barley varieties.

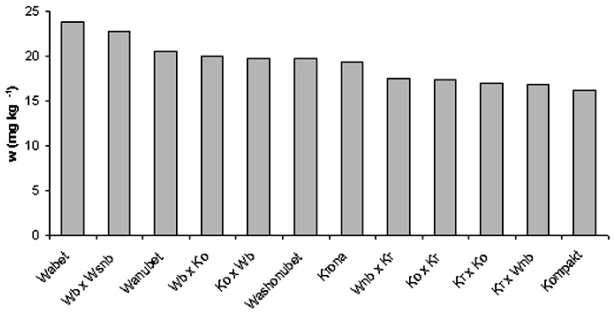

Table 2. Content of vitamin E in barley grain.

3. Results and Discussion

Contents of individual isomers of tocopherols and tocotrienols in three growing phases of green barley were determined and on the basis of these, the content of vitamin E was determined (Table 1, Figure 1). It was the highest at the first sampling (14.4–18.0 mg kg^{-1}) and in the variety Sebastian.

Dependence of vitamin E content on the variety was confirmed. The varieties with the highest tocol content and the highest content of vitamin E (Wabet) can serve as donors when breeding new lines (Table 2, Figure 2). Vitamin E content in barley was determined in the range of 16.2–23.8 mg kg^{-1}, which is in agreement with literature data. Peterson and Quereshi[11] and Holasová et al.[12] found out that in some cases vitamin E content exceeds even the amount detected in other cereals even four times (wheat 15.6 , triticale 11.8 , rye 17.1 , oats 14.7 and barley 23.7 mg kg^{-1} VEeq). Holasová et al.[13] found that the content of vitamin E in barley caryopses was significantly higher (21.9–25.5 mg kg^{-1}) compared to triticale (4.8–18.8 mg kg^{-1}), wheat (13.5–17.6 mg kg^{-1}), oats (13.6–17.6 mg kg^{-1}) and rye (15.5–18.7 mg.kg^{-1}). Green plants contain significantly less tocotrienols than barley caryopses.

4. Conclusion

Liquid chromatography with fluorimetric detection was used for determination of vitamin E content in green barley and malt barley. The highest content of vitamin E was found in green barley after the first sampling. Considerable variability in tocol content in barley varieties was found, ranging from 16–24 mg kg^{-1}.

5. Acknowledgement

The authors acknowledge the financial support of the Ministry of Education, Youth and Sports of the Czech Republic, Project RC No. MSM6019369701 and 1M0570 and the Grant Agency of the Czech Republic, Project No. 525/05/0781.

Table 1: Content of Vitamin E in Barley Grain

Variety (Linie)	α-T mg kg^{-1}	(β + γ)-T mg kg^{-1}	δ-T mg kg^{-1}	α-T3 mg kg^{-1}	(β + γ)-T3 mg kg^{-1}	δ-T3 mg kg^{-1}
Wabet	9.8	5.3	1.3	43.0	8.2	0.5
Wb x Wsnb	9.5	5.0	1.1	40.9	7.8	0.6
Wanubet	8.6	5.4	1.2	36.6	7.5	0.8
Wb x Ko	8.8	4.8	1.4	33.9	9.4	0.3
Ko x Wb	8.2	6.9	1.5	33.9	8.4	0.5
Washonubet	6.9	4.4	1.1	39.6	8.4	0.5
Krona	8.5	7.4	1.5	31.9	8.0	0.5
Wnb x Kr	7.9	4.5	1.1	29.1	7.5	0.4
Ko x Kr	8.0	8.4	1.4	26.3	8.8	0.7
Kr x Ko	8.6	7.1	1.2	23.6	9.2	0.7
Kr x Wnb	7.9	5.8	0.9	26.2	7.7	0.6
Kompakt	8.2	7.0	1.0	22.5	6.7	0.4

6. References

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Povzetek

Ječmen je vir mnogih spojin s pozitivnim učinkom na zdravje, še posebej antioksidantov, kot je vitamin E. V zrnju smo določili vsebnost tokola v kultivarjih različnega genetskega izvora. S pomočjo kromatografske analize s fluorimetrično detekcijo smo preučevali vsebnost vitamina E v različnih rastnih fazah ječmena. Največja vsebnost je bila v prvi rastni fazi (14,4–18,0 mg kg^{-1}) pri kultivarju Sebastian. Zelene rastline vsebujejo bistveno manj tokotrienolov kot zrnje ječmena.