The Cereal Grains: Focus on Vitamin E

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Abstract

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Tocopherols (T) and tocotrienols (T3) were analysed using HPLC and vitamin E content was calculated in selected cereal grains and their different morphological fractions. Wheat (*Triticum aestivum* L.) cv. Almari, barley (*Hordeum vulgare* L.) cv. Gregor, rye (*Secale cereale* L.) cv. Dańkowskie Złote, oat (*Avena sativa* L.) cv. Sławko and buckwheat (*Fagopyrum esculentum* Moench) cv. Kora were used in this study. The highest level of tocopherols was found in dehulled buckwheat and in its fraction of the endosperm with the embryo, where γ-T was found to be the main tocopherol (94.1% and 93.7% of total, respectively). α-T and β-T were the main isomers found in wheat, barley, rye and oat. β-T3 was the main tocotrienol found in wheat and oat, whereas α-T3 predominated in barley and rye. Small quantities of γ-T3 were only found in barley. No tocotrienols were found in buckwheat grains and their morphological fractions. The content of tocopherols and tocotrienols in the fractions of endosperm with embryo was 10–30% lower than in whole grain. The fraction originating from rye was the richest one in total tocols, followed by wheat and barley. Similarly, among the analysed fractions of pericarp and testa the richest fraction was that from rye, followed by wheat and barley. Extrusion cooking caused a significant decrease in tocopherols and tocotrienols, expressed in terms of biological activity of vitamin E, from 63 to 94%, depending on the cultivar examined. α-Tocopherol and α-tocotrienol were least resistant to hydrothermal processing. The remaining tocols were more stable, though the degree of their degradation reached up to 50%.

Keywords: tocopherols; tocotrienols; vitamin E; cereal grains; morphological fractions; hydrothermal process

Cereal grains are one of the main sources of vitamin E for humans. Vitamin E, a major biological antioxidant, quenches free radicals and acts as a terminator of lipid peroxidation, particularly in membranes that contain highly unsaturated fatty acids (Burton & Traber 1990; Martinez *et al*. 1996; Zielinski 1997). Vitamin E comprises all naturally occurring tocopherols (T) and tocotrienols (T3). Studies suggest that vitamin E may have immunostimulatory effects and possible clinical applications for the prevention of cardiovascular diseases (Gey 1993; Gruber 1994). Grains are a unique source because they contain more tocotrienols than other food products (Mc Laughlin & Weihrauch 1979). Biological activities of individual isomers of tocopherols differ and decline in the order α-T > β-T > γ-T > δ-T (Bourgeois 1992). Recent evidence suggests that under certain conditions the biological activity of α-T3 might be higher than the biological activity of α-T (Serbinowa *et al*. 1991). The bioavailability of dietary vitamin E is affected by differences among ingested forms (Acuff *et al*. 1994), processing methods, physiological factors (e.g. nutritional status), drugs and other dietary components (Erdman *et al*. 1988).

The first objective of this study was to determine the content of tocopherols and tocotrienols in cereal grains and their different morphological fractions. The second objective was to examine the effect of a hydrothermal process – extrusion cooking – on these compounds, providing a baseline of the vitamin E activity that is left after hydrothermal processing of cereals grains.

MATERIALS AND METHODS

Cereal Samples: Cereal grain samples harvested in 1998 and 1999 were obtained from a local plant breeding station in northeast Poland. The samples included wheat cv. Almari, barley cv. Gregor, rye cv. Dańkowskie Złote, oat cv. Sławko and buckwheat cv. Kora. Samples from two

replications were chosen for analysis. Whole-grain samples originated from 1998 were dehulled using a laboratory dehuller and fractions of hull, pericarp and testa, and endosperm with embryo were separated manually by sieving through a set of sieves. All samples were ground in a laboratory mill. Ground samples were stored at –30°C until extracted.

Hydrothermal Treatment: The extrusion cooking was applied as a model of hydrothermal technological processes. The extrusion process was conducted using the disintegrated cereal kernels of the moisture content reaching 20%, harvested in 1999, in a twin-screw laboratory extruder 2S-9/5 (type 2S-9/5 Metalchem, Poland). The following profiles of barrel temperatures were applied: 80/100/120/120, 100/130/160/160, 120/160/200/200, 500 rpm screw speed, and 225g/min mass flow rate. The raw and extruded material was ground in a laboratory mill type WZ-1. Ground samples were stored at –30°C until chemical analysis.

Analysis: All samples were analysed in duplicate for dry matter, nitrogen and ash using the methods of the AOAC (1990). Lipids (crude fat) were extracted from 1 g samples by shaking with 10 ml of petroleum ether for 30 min. The solvent was decanted after centrifuging (2000 g, 10 min), and the residue was then extracted once again. The combined supernatants were evaporated in a rotary evaporator at 50°C under vacuum, and the petroleum ether-extractable lipid was weighted gravimetrically. The hull content of oat, barley and buckwheat was determined by hulling 5 g samples of seeds by hand and then calculating hull weight from the total weight. The pericarp with testa fraction contents were determined according to the Carra's method in respect to the 50 g of free caryopses of rye and wheat grain and according to the Luffa's method in relation to the 50 g of free caryopses of barley grain (JAKUBCZYK & HABER 1981). The results are the means of three replications.

Determination of Tocopherols and Tocotrienols by HPLC: Tocopherols (α-T, β-T, γ-T, δ-T) and tocotrienols (α-T3, β-T3, γ-T3) were extracted by 80% methanol (0.5 g of sample per 7 ml of solvent) and then evaporated extracts were redissolved in *n*-hexane. The tocols were separated by high-performance liquid chromatography (HPLC) on Lichrospher Si 60 5-µm particle size, 4 × 250-mm column, according to the method described by PATERSON and QURESHI (1993). Twenty microliters of each sample were injected into the column. The HPLC systems consisted of a Shimadzu model LC pump series 10 AD, and a Shimadzu RF-535 fluorescence spectrometer. The mobile phase was 0.5% isopropanol in hexane. Flow rate was 1 ml/min, and the peaks were detected using an excitation wavelength of 295 nm and emission wavelength of 330 nm. The tocols contents were calculated from the peak areas using standard curves of tocopherols (α-T, β-T, γ-T, δ-T) and tocotrienols (α-T3, β-T3, γ-T3) obtained from Merck and Sigma. The vitamin E content, expressed in mg of α-tocopherol-equivalents (α-TE) was calculated according to MC LAUGHLIN and WEIHRAUCH 1979) using biological activities of 1.0 for α-T, 0.3 for α-T3, 0.4 for β-T, 0.05 for β-T3, 0.1 for γ-T, 0.01 for γ-T3 and 0.01 for δ-T.

RESULTS AND DISCUSSION

Contents of protein, ash, fat and average percent content of hull and of the pericarp with testa fraction in the whole grain harvested in 1998 are presented in Table 1. The highest content of hull was found in buckwheat and oat. PRZYBYLSKI *et al*. (1998) and XING and WHITE (1997) reported a similar content of buckwheat and oat hulls. The hull content in barley grain was in the range reported by other authors (GĄSIOROWSKI 1997). The content of pericarp with testa fraction in wheat, barley and rye was higher than recorded in the literature. Using drastic chemical methods for their separation and possible transfer of certain part of aleurone layer into the pericarp fraction probably caused this.

The content of tocopherols and tocotrienols, and the biological activity of vitamin E in whole grains and their morphological fractions, are shown in Table 2. The high-

Table 1. The content of protein, ash, fat (% dry matter) and means of hull and pericarp with testa fraction (%) in the whole grain of selected cereals

Source	Protein	Ash	Fat	Hull content (%)	Pericarp testa content* (%)
Wheat cv. Almari	17.8	1.94	1.2	no contain	18.9
Barley cv. Gregor	17.3	2.08	1.5	9.8	8.3
Rye cv. Dańkowskie Złote	11.1	1.79	1.0	no contain	26.5
Oat cv. Sławko	13.9	2.37	3.1	23.8	5.1
Buckwheat cv. Kora	16.9	2.36	1.8	23.6	n.d.

n.d. – not determined

*Pericarp with testa fraction content was determined by chemical treatment of 50 g of free caryopses according to the Carra's and Luffa's method (JAKUBCZYK & HABER 1981)

Table 2. The content of tocopherols and tocotrienols in different cereal grains and their morphological fractions (µg/g DM)

Material	Tocopherols (T)					Tocotrienols (T3)				T3/T	Vitamin E (IU/kg)
	α	β	γ	δ	total	α	β	γ	total		
Wheat											
Whole grain	6.06	4.23	–	–	10.29	1.05	23.68	–	24.73	2.4	13.8
Endosperm with embryo	3.65	1.13	–	–	4.78	0.92	21.60	–	22.52	4.7	8.1
Pericarp and testa	6.07	2.93	–	2.81	11.81	ślad	22.37	–	22.37	1.9	12.5
Barley											
Whole grain	3.84	–	–	–	3.84	17.09	1.32	4.73	23.14	6.0	13.5
Endosperm with embryo	1.94	–	–	–	1.94	14.93	1.54	6.11	22.58	11.6	9.8
Pericarp and testa	–	–	–	–	–	–	–	–	–	–	–
Rye											
Whole grain	11.46	2.28	–	–	13.74	5.94	8.19	–	14.13	1.0	21.2
Endosperm with embryo	1.89	ślad	–	–	1.89	3.97	12.80	–	16.77	8.9	5.5
Pericarp and testa	52.22	14.67	–	–	66.89	2.87	5.04	–	7.91	0.1	88.2
Oat											
Whole grain	0.85	0.78	–	–	1.63	2.68	10.8	–	13.48	5.02	3.73
Buckwheat											
Whole grain	0.85	–	51.42	2.35	54.62	–	–	–	–	–	8.9
Endosperm with embryo	1.88	–	60.35	2.21	64.44	–	–	–	–	–	17.4
Pericarp	–	–	–	–	–	–	–	–	–	–	–

est level of tocopherols was found in dehulled buckwheat and in its fraction of endosperm with embryo where γ-T was the main tocopherol (94.1% and 93.7% of total, respectively). α-T and δ-T were found only at a low level below 6.3% of total tocopherols. No β-T and none of tocotrienols were found in buckwheat grains and their morphological fractions. The data provided here are different from those reported by PRZYBYLSKI *et al*. (1998). They found the α-isomer to be the major tocopherol in buckwheat, constituting up to 70.5% of total tocopherols. The remaining β, γ, and δ tocopherols accounted for 3.1, 19.5 and 6.8% of the total, respectively. These differences may be explained by the fact that the above-mentioned authors used buckwheat seed, Mancan variety, originating from Canada, whereas we used Kora variety, harvested in Poland. Despite of the differences of these two buckwheat varieties in the content of respective tocopherols, the total tocopherols were at the same level. In contrast, α-T was the main isomer found among analysed tocopherols in wheat, barley, rye and oat. β-tocopherol was found in wheat, rye and oat. Small quantities of δ-T were found only in wheat whereas γ-T was not present in these cereal grains analysed. The total amount of tocopherols in rye and wheat was three to four times higher than in barley and oat and five times lower than in buckwheat whole grains. The rye grain was the richest one in α-T, which was concentrated in the fraction of pericarp and testa.

The level of total tocopherols in wheat and rye was three times lower than reported by GRELA *et al*. (1993), but they did not specify the varieties used in their study.

The main tocotrienol found was β-T3 in wheat and oat, and α-T3 in barley and rye. Small quantities of γ-T3 were found only in barley. The tocotrienol content was higher than the tocopherol content in all analysed whole grains. The level of tocotrienols in wheat and barley was twofold than in rye and oat. The content of tocopherols and tocotrienols in the fractions of endosperm with embryo was 10–30% lower than in whole grain. The fraction originating from rye was the richest one in total tocols, followed by wheat and barley. Similarly among the analysed fractions of pericarp and testa, the highest content was found in rye, followed by wheat and barley. The content of tocopherols and tocotrienols in wheat and rye was similar to that reported by HOLASOVÁ *et al*. (1995), whereas in barley and oat it amounted only about 50%. However, these data are compared to cereal grain harvested in the Czech Republic. The differences between our tocol content data and those reported by other researchers may be caused by differing genotypes of the cultivars used and by environmental effects (PATERSON & QURESHI 1993; HOLASOVÁ *et al*. 1995; PRZYBYLSKI *et al*. 1998). The results provided in this study confirm differences in tocopherol and tocotrienol concentrations among grain kernels and their different morphological parts, and are in good
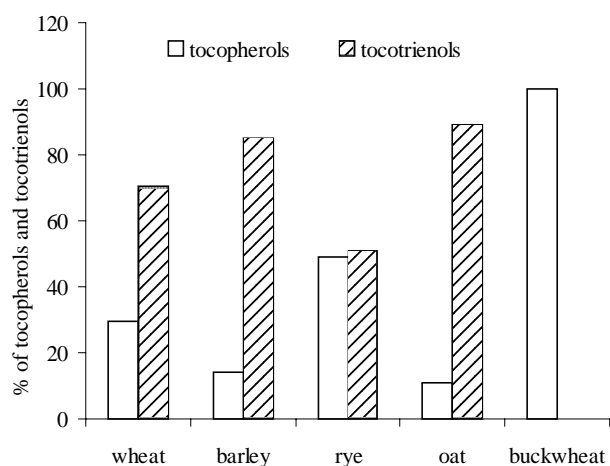
Fig. 1. Contribution of tocopherols and tocotrienols to the tocol content in the whole grain of different cereals

agreement with published data (MORRISON *et al*. 1982; PIIRONEN *et al*. 1986; PATERSON & QURESHI 1993; GĄSIO-ROWSKI 1995; HOLASOVÁ 1997). The contribution of tocopherols and tocotrienols to the tocol content in whole grain of cereals studied is shown on Fig. 1.

When all analysed tocopherols and tocotrienols were expressed in term of biological activity of vitamin E and activities of individual isomers were taken into account, then the highest activity was found in whole-grain of rye, then in wheat, barley, buckwheat and oat. The highest vitamin E activity was found in pericarp and testa fraction of rye (Table 2).

Extrusion cooking was performed on the samples harvested in 1999. Extrusion caused a significant decrease in tocopherols and tocotrienols, expressed in terms of biological activity of vitamin E, from 63 to 94%, depending on the cultivar used (Fig. 2). α-Tocopherol and α-tocotrienol were least resistant to hydrothermal processing. The remaining tocols were more stable, though the degree of their degradation reached up to 50%. A positive fact was found, namely that the ratio of tocotrienols to tocopherols increased after extrusion cooking. This indicates that tocotrienols remain the main isomers of vitamin E left (Table 3). However, this was not observed in oat grain. This fact indicates that tocotrienols are more resistant to hydrothermal conditions than tocopherols. It was reported that α-tocotrienol had opposite effects on the cholesterol metabolism in chickens, indicating that a higher ratio of tocotrienols to tocopherols in the diet may be important in metabolic regulation (QURESHI *et al*. 1989). MILLAUER *et al*. (1984) studied the effect of extrusion cooking on the degradation of vitamin E. Similar losses of vitamin E, up to 66%, were found in germinated wheat grains indicating that tocopherols and tocotrienols are thermo labile compounds. The data provided by other authors (GRELA *et al*. 1993) indicate that losses of vitamin E after soaking or short thermal treatment of cereal grains are smaller, ranging between 15–20%. Moreover, other technological processes such as milling and pearling cause a loss of vitamin E when compared to whole grains. But these processes are also effective in grain processing to concentrate total tocols, suggesting a possible use of milling or pearling fractions as by-products that are nutrient-rich and health-promoting food ingredients (WANG *et al*. 1993).
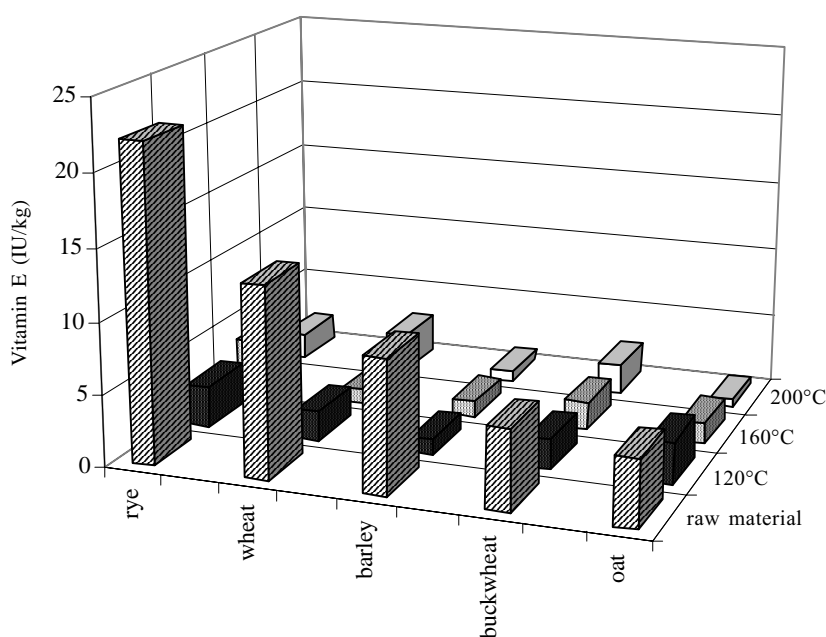

Fig. 2. Vitamin E activity in different cereal grains before and after extrusion cooking

The knowledge of the content of vitamin E in raw and processed cereal grains is important from the dietary point of view. Currently, the Recommended Dietary Allowance for vitamin E, expressed as α-tocopherol equivalents, is 10 mg for a man and 8 mg for a woman. A healthy man is rarely deficient in vitamin E because of its wide distribution in food. However, vitamin E deficiency may still arise in some rare cases, such as in patients with lipid malabsorption syndromes (MULLER 1994) or in patients with familial isolated vitamin E deficiency (FIVE deficiency) (TRABER *et al*. 1992). A prolonged and severe deficiency of vitamin E gives rise to a neurological syndrome characterised by ataxia, tendon areflexia and muscle weakness (HARDING 1987). In the case of significantly increased

risk of chronic diseases such as cardiovascular disease and cancer the level of 23–100 mg is required (DIPLOCK 1987; ANDLAUER & FURST 1999). The findings, outlined above, highlight two important areas of current vitamin E research. First, the public health benefit that arises from a high intake of vitamin E from diet (20–50 mg) over a long period of time (lifestyle habit) and second the potential of vitamin E, albeit in very high doses (250–500 mg) to help treat patients following the onset of clinical symptoms (antioxidant therapy).

Taking into account the evidence provided here and the level of consumption of cereal based products, which amounts 210 g/day per person, it can be calculated that unprocessed cereal grains contribute about 14–17% of

Table 3. The content of tocopherols and tocotrienols in different cereal grains after extrusion cooking (µg/g DM)

Material	Tocopherols (T)					Tocotrienols (T3)				T3/T	Vitamin E
	α	β	γ	δ	total	α	β	γ	total		(IU/kg)
Wheat											
before extrusion	6.39	2.05	–	–	8.44	2.83	16.54	–	19.37	2.3	13.3
after extrusion at (°C):											
80–100–120–120–120	0.46	1.32	–	–	1.78	0.23	6.61	–	6.84	3.8	2.1
100–130–160–160–120	0.02	0.97	–	–	0.99	0.02	5.22	–	5.24	5.3	1.0
120–160–200–200–120	0.84	1.28	–		2.12	0.52	6.69		7.21	3.4	2.7
Dehulled barley											
before extrusion	2.85	0.11	–	0.19	3.15	10.61	2.27	2.70	15.58	4.9	9.3
after extrusion at (°C):											
80–100–120–120–120	0.12	0.03	–	0.08	0.23	1.75	0.77	0.79	3.31	14.4	1.1
100–130–160–160–120	0.20	0.04	–	0.11	0.35	1.64	0.92	0.91	3.47	9.9	1.1
120–160–200–200–120	0.13	0.04	–	0.10	0.27	1.11	0.86	0.86	2.83	10.5	0.8
Rye											
before extrusion	11.04	2.07	–	0.03	13.14	8.69	5.95	–	14.64	1.1	22.0
after extrusion at (°C):											
80–100–120–120–120	0.95	0.57	–	0.02	1.54	2.05	2.49	–	4.54	2.9	2.9
100–130–160–160–120	1.31	0.65	–	0.02	3.52	2.70	2.63	–	5.33	1.5	3.7
120–160–200–200–120	0.95	0.36	–	0.02	1.33	2.33	1.66	–	3.99	3.0	1.9
Oat											
before extrusion	2.14	0.46	–	–	2.60	1.38	7.61	–	8.99	3.4	4.7
after extrusion at (°C):											
80–100–120–120–120	1.13	0.45	–	–	1.58	1.55	4.19	–	5.74	3.6	2.9
100–130–160–160–120	0.82	0.37	–	–	1.19	1.23	2.25	–	3.48	2.9	1.5
120–160–200–200–120	0.22	0.17	–	–	0.39	0.01	0.60	–	0.60	1.5	0.5
Dehulled buckwheat											
before extrusion	0.78	–	29.88	–	30.66	0.09	–	–	0.02	< 0.01	5.6
after extrusion at (°C):											
80–100–120–120–120	0.30	–	10.62	–	10.92	0.08	–	–	0.08	< 0.01	2.1
100–130–160–160–120	0.29	–	10.05	–	10.34	0.06	–	–	0.06	< 0.01	2.0
120–160–200–200–120	0.35	–	10.99	–	11.34	0.07	–	–	0.07	< 0.01	2.2

the total requirement for vitamin E in humans (EU-Air Concerted Action 1998). Moreover, the contribution of vitamin E from extruded grains to human RDA is significantly lower. Hydro thermally treated grains are no good source of vitamin E. Fortunately, there is a possibility to supplement vitamin E to the cereal-based products at the one of the final stages of technological processing. Then, the data provided in this study may be used as a baseline of the vitamin E activity that is left after hydrothermal processing of cereal grains and as a starting point for the vitamin E fortification of cereal-based products.

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Souhrn

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Pomocí HPLC jsme provedli analýzu tokoferolů (T) a tokotrienolů (T3) v zrnech vybraných obilnin a v jejich jednotlivých morfologických frakcích a stanovili obsah vitaminu E. Analyzovali jsem zrna následujících odrůd: pšenice (*Triticum aestivum*) Almari, ječmen (*Hordeum vulgare* L.) Gregor, žito (*Secale cereale* L.) Dańkowske Złote, oves (*Avena sativa* L.) Sławko a pohanka (*Fagopyrum esculentum* Moench) Kora. Nejvyšší hladinu tokoferolů jsme zjistili ve vyluštěném zrnu pohanky a v jeho frakci endosperm s embryem, kde je převládajícím tokoferolem γ-T (94,1 %, resp. 93,7 %). α-T a β-T byly hlavními izomery v pšenici, ječmeni, žitu a ovsu. β-T3 byl převládající tokotrienol v pšenici a ovsu, zatímco v ječmeni a žitu byl dominantní α-T3. V ječmeni jsme našli malá množství γ-T3. V zrnech pohanky a v jejich morfologických frakcích se žádné tokotrienoly nevyskytovaly. Obsah tokoferolů a tokotrienolů ve frakcích endosperm s embryem byl o 10–30 % nižší než v celém zrnu. Frakce pocházející z žita byla nejbohatší na celkové tokoly, dále následovala pšenice a ječmen. Obdobně z analyzovaných frakcí oplodí a osemení pocházela nejbohatší frakce z žita, následovaná pšenicí a ječmenem. Extruzní opracování vedlo k významnému poklesu obsahu tokoferolů a tokotrienolů, vyjádřenému jako biologická aktivita vitaminu E (v závislosti na odrůdě z 63 na 94 %). Tokoferol a α-tokotrienol vykazovaly nejnižší odolnost k hydrotermálnímu opracování. Ostatní tokoly byly stabilnější, ačkoliv stupeň jejich degradace dosahoval až 50 %.

Klíčová slova: tokoferoly; tokotrienoly; vitamin E; zrna obilnin; morfologické frakce; hydrotermální proces

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