

## Contents of tocopherols in different types of dry shell fruits

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**Abstract.** The aim of this study was to identify all forms of vitamin E in different kinds of dry shell fruits (generally called ‘nuts’) obtained from different sources and to perform their mutual comparison. All tocopherols were determined by reversed phase HPLC using isocratic elution with fluorescence detection. Almonds were evaluated as the most important source of  $\alpha$ -tocopherol (average value 1,132 mg kg<sup>-1</sup>),  $\beta$ -tocopherol was present in all samples as a minor component, its maximum content was found in hazelnuts (9.6 mg kg<sup>-1</sup>).  $\gamma$ -Tocopherol was found in all kinds of nuts and the highest content was found in pistachios (584 mg kg<sup>-1</sup>), very high amount of  $\delta$ -tocopherol was contained in Brazil nuts (2,298 mg kg<sup>-1</sup>). Tocotrienols were found in a smaller number of nut kinds than tocopherols.  $\alpha$ -Tocotrienol was found only in three kinds of nuts - Brazil nuts, which contained the highest amount (399 mg kg<sup>-1</sup>), pine nuts and hazelnuts grown in the Czech Republic, which were analyzed soon after harvest. Similarly,  $\gamma$ -tocotrienol was determined only in four kinds of nuts (pistachios, macadamias, cashew and walnuts grown in the Czech Republic), which were analyzed soon after harvest; the most significant amount was found in pistachios (34.8 mg kg<sup>-1</sup>),  $\beta$ - and  $\gamma$ -tocotrienols were below the detection limit. In the Czech Republic, walnuts and hazelnuts are the most widely cultivated and consumed nuts. While in walnuts the most important form is  $\gamma$ -tocopherol (459 ± 40 mg kg<sup>-1</sup>), in hazelnuts it is  $\alpha$ -tocopherol (863 ± 10 mg kg<sup>-1</sup>).

**Key words:** nuts, tocopherols, tocotrienols, storage, HPLC.

### INTRODUCTION

Dry shell fruits (generally called ‘nuts’) together with the other kinds of fruits and vegetable play an important role in human nutrition, in particular as a source of vitamins, minerals and fiber (Kader et al., 2001; Brufau et al., 2006; Tošić et al., 2015). The ideal recommended daily intake of dry nuts to protect human health is 30 g per day (Kornsteiner et al., 2006).

Vegetable fat is included among the most important macronutrients, its content in nuts is 46–76%. The fats represent about 20 to 30 kJ g<sup>-1</sup>, depending on the species. Nuts belong to the most important sources of vegetable fats due to its composition, in which the unsaturated fatty acids are predominant. The unsaturated fatty acids have a positive influence on a variety of lifestyle diseases (Ros & Mataix, 2006). The low ratio of n-6/n-3 fatty acids in macadamia nuts, walnuts, chestnuts and almonds is also important (Freitas & Naves, 2010). High content of vegetable fat in dry nuts is associated with a significant content of vitamin E (Wagner et al., 2004); therefore dry nuts are one of the

most important sources of vitamin E (tocopherols and tocotrienols) from all foods that form a usual part of the human diet. The tocopherols, which have antioxidant properties, provide protection against oxidation of unsaturated fatty acids at the same time (Amaral et al., 2005). Antioxidant activity of vitamin E consists mainly in the prevention of free radicals formation, leading to disabling of lipid peroxidation and thereby reducing the content of LDL cholesterol (Brigelius-Flohe & Traber, 1999). Similarly as in the case of fats, it was found that different types of nuts are fundamentally different both in content and in the composition of the various forms of vitamin E (Kornsteiner et al., 2006). Mainly almonds are a rich source of  $\alpha$ -tocopherol, which is the most active form of vitamin E, while walnuts contain a significant amount of its isomeric form that is  $\gamma$ -tocopherol (Ros, 2015).

Kornsteiner et al. (2006) dealt with the content of tocopherols ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ ) in oils pressed from 10 different kinds of nuts. The highest content of  $\alpha$ -tocopherol showed almond (31.4 mg in 100 g of extracted oil) and hazelnuts (24.2 mg in 100 g of extracted oil). The relatively high amount of  $\beta$ - and  $\gamma$ -tocopherols, determined together, contained pistachios, Brazil nuts, cashew nuts, peanuts, pecans, pine nuts and walnuts. Average values in 100 g of extracted oil moved in the range from 5.1 mg (cashew nuts) to 29.3 mg (pistachio). Traces of  $\delta$ -tocopherol (< 4 mg in 100 g of extracted oil) were determined in the cashew nuts, hazel nuts, peanuts, pecan nuts, pine nuts, walnuts, pistachios, and also in the walnuts.

A significant advantage of nuts compared with the other fruits is their good preservation and stability which was appreciated especially in the past (Fraser et al., 1992). The nuts are quite predisposed to mildew, above all in areas with high rainfall and humidity. The main risk factors are aflatoxins that are produced by the fungus *Aspergillus flavus* (Macrae et al., 1993). The risk associated with contamination by mycotoxins may be reduced by correct pre-harvest procedures, proper drying in an adequate time after the harvest and safe storage at the right temperature and humidity (Macrae et al., 1993; Ozilgen & Ozdemir, 2001).

Changes in the contents of tocopherols in peanuts during storage were studied by Silva et al. (2010). According to them the contents of all forms of tocopherols decreased during storage. In contrast, the indicators of lipid oxidation, such as peroxide number and intensity of the oxidized and cardboard taste increased during storage. At the same time a strong negative correlations were evaluated between the content of tocopherols and peroxide number. Chun et al. (2005) came to the conclusion that tocopherols decrease exponentially during storage with the current increase of peroxide number describes. According to the cited authors the contents of tocopherols could be used as an indicator of oxidative state in peanut products. According to Tsantili et al. (2011) the stability and total antioxidant capacity of nuts, which is made up by vitamin E as well, can have been influenced in addition to the external conditions also by selected cultivar.

The goal of this study was to determine the contents of various forms of vitamin E (tocols and tocotrienols) in different kinds of dry nuts purchased at different locations in the Czech Republic or grown and harvested in the Czech Republic and their mutual comparison.

## MATERIALS AND METHODS

### Analyzed nuts

Various types of nuts, namely walnuts (*Juglans regia* L.), hazelnuts (*Corylus avellana* L.), almonds (*Prunus dulcis* (Mill.) D. A. Webb), cashew nuts (*Anacardium occidentale* L.), pistachios (*Pistacia vera* L.), Brazil nuts (*Bertholletia excelsa* Humb. & Bonpl.), macadamia nuts (*Macadamia integrifolia* Maiden & Betche), pecans (*Carya illinoensis* (Wangenh.) K. Koch), pine nuts (*Pinus pinea* L.) and peanuts (*Arachis hypogaea* L.) of unknown origin were purchased at two different locations in the Czech Republic market network. In addition, three samples of walnuts and one sample of hazelnuts grown and harvested in different locations of the Czech Republic were collected. All samples of nuts were purchased or harvested in October 2015.

### Chemicals

For the preparation of analytical samples the following standards and chemicals were used: DL- $\alpha$ -tocopherol, 98.2% (CALBIOCHEM, Canada), tocopherol set (CALBIOCHEM, Canada), propan-2-ol (Lachner, Czech Republic), methanol super gradient, content min. 99.9% (Lachner, Czech Republic), and treated distilled water (Milipore, France).

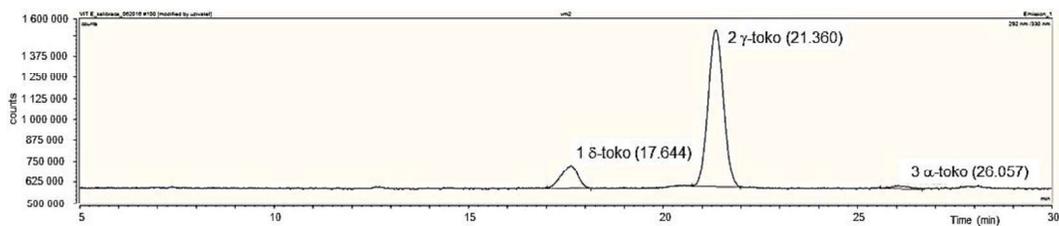
### Sample preparation

The nuts were shelled and afterwards homogenized in a laboratory grinder. Approximately 0.3 g of homogenized sample was weighed into a plastic test tube with a plastic stopper (Falcon type). 10 mL of propan-2-ol was added. The tube was slightly shaken and then placed for 10 minutes into an ultrasound bath. Subsequently, the sample was centrifuged for 5 minutes (Eppendorf 5810R, 20 °C at 11.5 rpm) and the supernatant was decanted. The extraction was 3 times repeated. After that the supernatants were collected and refilled up to 50 mL. Afterwards the tubes were placed for 2 hours into a freezer box (-20 °C) to separate fat layer. Final supernatant was transferred through a syringe filter (nylon, 0.22  $\mu$ m) 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 into the lab). The efficiency of extraction of all vitamers was checked by analyses of all the following extracts of the identical extracted samples. The content of all individual vitamers was close to the limit of detection in the fourth extract, and therefore three consecutive extractions have been evaluated as sufficient approaching to 100% efficiency. Extractions of all analyzed samples were performed in 3 replicates.

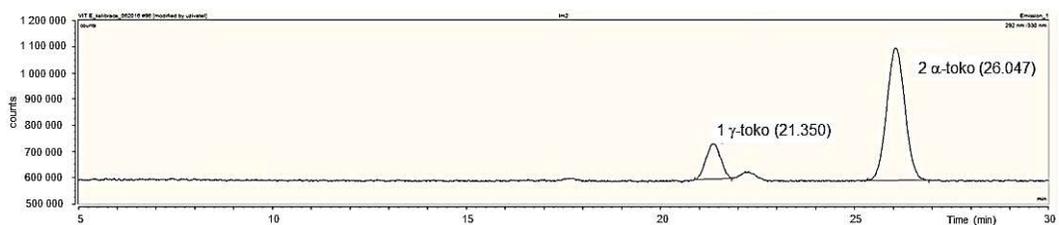
### Tocopherol and tocotrienol chromatographic determination

HPLC-FD analyses were performed under the following conditions: analytical column Develosil® 5  $\mu$  RP-Aqueous (250  $\times$  4.5 mm), (Phenomenex, Torrance, CA, USA), mobile phase H<sub>2</sub>O : methanol (3 : 97, v/v), flow 1.0 mL min<sup>-1</sup>, column temperature 30 °C, injection volume 10.0  $\mu$ L, time of analysis 30 min. Conditions of detection: fluorescence detector, excitation wavelength  $\lambda$  = 292 nm, emission wavelength  $\lambda$  = 330 nm. The contents of analytes in the samples were evaluated by an external standard method. Calibration curve of all tocopherols was linear in the range from 0.05 to 10  $\mu$ g mL<sup>-1</sup>. Limits of detection (LODs), expressed as a ratio of three times the value of the signal-to-noise ratio, for individual tocopherols  $\delta$ -T3,  $\gamma$ -T3,  $\beta$ -T3,  $\alpha$ -T3,  $\delta$ -T,  $\gamma$ -T,  $\beta$ -T and  $\alpha$ -T were 0.056,

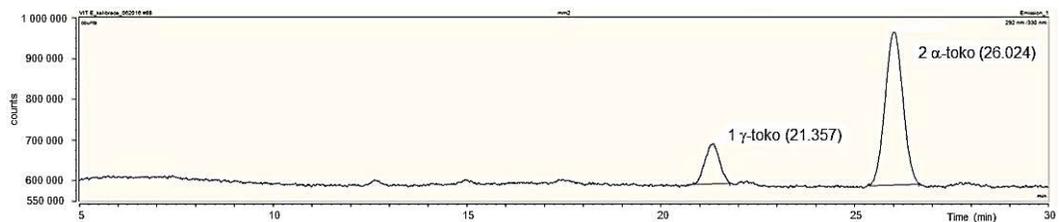
0.111, 0.111, 0.167, 0.056, 0.111, 0.111, and 0.167  $\mu\text{g g}^{-1}$ , respectively. The results were processed with chromatography software Chromeleon and MS Excel. Examples of chromatograms of are given in the Figs 1–3.



**Figure 1.** Chromatogram of tocopherols in walnuts.



**Figure 2.** Chromatogram of tocopherols in hazelnuts.



**Figure 3.** Chromatogram of tocopherols in almonds.

### Statistical evaluation

Statistical analysis was done in *Statistica* Version 12.0 (Statsoft). The measured values were processed by the analysis of one-way variance method (*ANOVA*), using *post-hoc Scheffé's* test for more detailed evaluation, considering  $\alpha = 0.05$  and the level of significance  $P < 0.05$  as significant.

## RESULTS AND DISCUSSION

### Contents of tocopherols in different types of nuts

The determination of individual forms of vitamin E was done in 10 selected types of nuts purchased at 2 different markets (Table 1). Only 6 different forms of vitamin E were determined,  $\beta$ -tocotrienol and  $\delta$ -tocotrienol in all analyzed types of nuts were below the limit of detection ( $\text{LOD} = 3.33 \text{ mg kg}^{-1}$  and  $1.67 \text{ mg kg}^{-1}$ , resp.) but the composition and contents of the other particular forms of vitamin E in analyzed types of nuts were statistically very different.

**Table 1.** The contents of individual tocopherols (mg kg<sup>-1</sup>) in different types of nuts

Nuts	$\alpha$ -TcP	$\beta$ -TcP	$\gamma$ -TcP	$\delta$ -TcP	$\alpha$ -TcT	$\gamma$ -TcT
Walnuts 1	32.7 ± 4.6	< 3.33	432 ± 6.5	44.8 ± 2.3	< 5.00	< 3.33
Walnuts 2	51.8 ± 4.1	< 3.33	453 ± 6.8	59.9 ± 1.3	< 5.00	< 3.33
<b>Mean</b>	<b>42.2 ± 13.5</b>	<b>&lt; 3.33</b>	<b>442 ± 14.7</b>	<b>52.4 ± 10.7</b>	<b>&lt; 5.00</b>	<b>&lt; 3.33</b>
Hazelnuts 1	838 ± 6	7.22 ± 0.1	31.7 ± 0.8	3.12 ± 1.3	< 5.00	< 3.33
Hazelnuts 2	769 ± 26	9.56 ± 0.8	31.3 ± 2.4	< 1.7	< 5.00	< 3.33
<b>Mean</b>	<b>803 ± 34</b>	<b>8.39 ± 1.16</b>	<b>31.5 ± 1.4</b>	<b>2.39 ± 1.1</b>	<b>&lt; 5.00</b>	<b>&lt; 3.33</b>
Almonds 1	1,125 ± 52	4.74 ± 0.2	12.2 ± 0.7	< 1.67	< 5.00	< 3.33
Almonds 2	1,139 ± 7.4	< 3.33	30.6 ± 2	< 1.67	< 5.00	< 3.33
<b>Mean</b>	<b>1,132 ± 10</b>	<b>4.04 ± 1</b>	<b>21.4 ± 13</b>	<b>&lt; 1.67</b>	<b>&lt; 5.00</b>	<b>&lt; 3.33</b>
Cashew 1	< 5.00	< 3.33	116 ± 1.9	6.32 ± 0.5	< 5.00	3.72 ± 0.6
Cashew 2	< 5.00	< 3.33	75.3 ± 5.6	6.60 ± 0.3	< 5.00	< 3.33
<b>Mean</b>	<b>&lt; 5.00</b>	<b>&lt; 3.33</b>	<b>95.5 ± 28.7</b>	<b>6.46 ± 0.2</b>	<b>&lt; 5.00</b>	<b>3.52 ± 0.3</b>
Pistachios 1	42.2 ± 6.4	< 3.33	590 ± 4.8	11.1 ± 1.4	< 5.00	36.3 ± 0.9
Pistachios 2	< 5.00	< 3.33	579 ± 8.4	10.0 ± 1.1	< 5.00	33.3 ± 1.7
<b>Mean</b>	<b>23.6 ± 26</b>	<b>&lt; 3.33</b>	<b>585 ± 8.1</b>	<b>10.6 ± 0.8</b>	<b>&lt; 5.00</b>	<b>34.7 ± 2.2</b>
Brazil nuts 1	175 ± 26	< 3.33	472 ± 11.3	2,932 ± 167	391 ± 39.3	< 3.33
Brazil nuts 2	149 ± 3.7	< 3.33	381 ± 12.8	1,664 ± 154	< 5.00	< 3.33
<b>Mean</b>	<b>162 ± 18</b>	<b>&lt; 3.33</b>	<b>427 ± 64.1</b>	<b>2,298 ± 897</b>	<b>202 ± 279</b>	<b>&lt; 3.33</b>
Macadamia 1	464 ± 22	< 3.33	187 ± 8.46	6.96 ± 0.61	< 5.00	14.0 ± 1.13
Macadamia 2	406 ± 13	< 3.33	< 3.33	< 1.67	< 5.00	< 3.33
<b>Mean</b>	<b>435 ± 41</b>	<b>&lt; 3.33</b>	<b>95.1 ± 129</b>	<b>4.31 ± 3.74</b>	<b>&lt; 5.00</b>	<b>8.67 ± 7.54</b>
Pecans 1	32.5 ± 0.76	< 3.33	586 ± 9.96	39.9 ± 0.71	< 5.00	< 3.33
Pecans 2	97.3 ± 24.3	< 3.33	188 ± 1.28	16.1 ± 0.36	< 5.00	< 3.33
<b>Mean</b>	<b>64.9 ± 46</b>	<b>&lt; 3.33</b>	<b>387 ± 282</b>	<b>28.0 ± 16.8</b>	<b>&lt; 5.00</b>	<b>&lt; 3.33</b>
Pine nuts 1	< 5.00	< 3.33	< 3.33	< 1.67	78.9 ± 7.21	< 3.33
Pine nuts 2	26.7 ± 1.0	< 3.33	67.5 ± 1.27	< 1.67	< 5.00	< 3.33
<b>Mean</b>	<b>15.8 ± 15</b>	<b>&lt; 3.33</b>	<b>35.4 ± 45.4</b>	<b>&lt; 1.67</b>	<b>41.9 ± 52.2</b>	<b>&lt; 3.33</b>
Peanuts 1	267 ± 2.9	< 3.33	124 ± 1.32	5.83 ± 0.52	< 5.00	< 3.33
Peanuts 2	281 ± 6.8	< 3.33	99.8 ± 1.61	6.72 ± 0.53	< 5.00	< 3.33
<b>Mean</b>	<b>274 ± 10</b>	<b>&lt; 3.33</b>	<b>112 ± 17.4</b>	<b>6.27 ± 0.63</b>	<b>&lt; 5.00</b>	<b>&lt; 3.33</b>

TcP – tocopherol, TcT – tocotrienol.

The variability in the content of individual tocopherols between 2 groups of samples of the same type (purchased in different locations of the Czech Republic) was strongly divergent and ranged from 0.08% (almonds,  $\alpha$ -tocopherol) to 138% (Brazil nuts,  $\alpha$ -tocotrienol). In general, the highest differences were determined in the content of  $\alpha$ -tocotrienol, which was found only in Brazil nuts and pine nuts. In comparison, the average variability of parallel determination of the same sample was 5.10% and only exceptionally (in two cases) was close to 20% (Table 2). According to Tsantili et al. (2011) external conditions during growing play an important role in the content of individual substances. The origin of purchased samples is not known and may be different.

The most contained forms of vitamin E, which are represented in most of the analysed species of dry nuts were  $\alpha$ -tocopherol and  $\gamma$ -tocopherol (Table 1). The content of  $\alpha$ -tocopherol ranged from 26.7 to 1,139 mg kg<sup>-1</sup> depending on the type of dry nuts. The highest average amount of  $\alpha$ -tocopherol was found in almonds (1,132 ± 10) mg kg<sup>-1</sup> and hazelnuts (863 ± 109) mg kg<sup>-1</sup>; these two kinds of dry nuts were the most important

source of  $\alpha$ -tocopherol also in studies by Maguire et al (2004) and Kornsteiner et al. (2006).

In contrast, the lowest content of  $\alpha$ -tocopherol was found in cashew nuts and in one sample of pine nuts, where the presence of  $\alpha$ -tocopherol was below the limit of detection ( $5 \mu\text{g g}^{-1}$ ). These findings are in agreement with the analysis of Kornsteiner et al. (2006) again.

The presence of  $\gamma$ -tocopherol was found in all of the analyzed species of dry nuts (Table 1). Contents of  $\gamma$ -tocopherol ranged between  $2.23$ – $590.4 \text{ mg kg}^{-1}$  depending on the kind of nuts. The highest amount of  $\gamma$ -tocopherol was found in pistachio ( $584.7 \pm 8.1 \text{ mg kg}^{-1}$ ) and next to in the samples of walnuts, Brazil nuts and pecan nuts, which is in good accordance with the study by Kornsteiner et al. (2006), where the amount of  $\gamma$ -tocopherol was found in the same descending order in different samples of nuts.

Miraliakbari & Shahidi (2008) reported that the highest amount of  $\gamma$ -tocopherol contained in pecan nuts was  $440.2$ – $472.9 \text{ mg kg}^{-1}$ . In this study various values of  $\gamma$ -tocopherol in pecan nuts were established in two analyzed samples ( $586 \pm 9.96 \text{ mg kg}^{-1}$  and  $(188 \pm 1.28) \text{ mg kg}^{-1}$ , resp. It means that other nuts, especially pistachios, have been found as more significant resources of  $\gamma$ -tocopherol.

$\beta$ -Tocopherol above the limit of detection has been found in only one sample of almonds ( $4,744 \text{ mg kg}^{-1}$ ), and in one sample of hazelnuts ( $9,556 \text{ mg kg}^{-1}$ ). It can therefore be stated that  $\beta$ -tocopherol is a minor form of vitamin E. This statement is in agreement with the work by Miraliakbari & Shahidi (2008), where  $\beta$ -tocopherol has been found only in samples of almonds and pine nuts. The founded content was in the range  $10.9$ – $22.6 \text{ mg kg}^{-1}$ .

The highest levels of  $\delta$ -tocopherol were contained in Brazil nuts ( $2297 \pm 89 \text{ mg kg}^{-1}$ ). The differences in the content of this vitamer among the various type of nuts were the biggest, because the content of the  $\delta$ -tocopherol in some next analyzed samples was very low and sometimes even below the limit of detection ( $< 1.67 \mu\text{g g}^{-1}$ ). In contrary only small amount of  $\delta$ -tocopherol in 10 different kinds of nuts were detected by Kornsteiner et al. (2006) that found the biggest content  $3.8 \text{ mg}$  in  $100 \text{ g}$  extracted oil from walnuts.

A significant higher amount of this form of vitamin E contained also walnuts ( $52.4 \pm 10.7 \text{ mg kg}^{-1}$ , which is more than twice higher value than determined Miraliakbari & Shahidi (2008) in their study. According to this authors the content of  $\delta$ -tocopherol in walnuts is ranging from  $19.8$ – $23.4 \text{ mg kg}^{-1}$ . This difference could probably be in consequence of different origin and varieties of nuts, or different period of storage before analysis.

In general, the contents of tocotrienols in comparison with tocopherols were found in significantly lower quantities in all dry nut types. Brazil nuts were evaluated as the most important source of  $\alpha$ -tocotrienol; in one sample of these nuts the content ( $399.1 \pm 37 \text{ mg kg}^{-1}$ ) was found.  $\alpha$ -Tocotrienol was also determined in only one sample of pinenuts ( $78.9 \pm 6.3 \text{ mg kg}^{-1}$ ). The content of  $\alpha$ -tocotrienol in all next analyzed samples was below the limit of detection ( $< 5.00 \mu\text{g g}^{-1}$ ).

The other vitamer of vitamin E from the tocotrienols group,  $\gamma$ -tocotrienol, was found only in three types of purchased nuts namely cashew, pistachios and macadamia

nuts. The highest amount of  $\gamma$ -tocotrienol has been found in samples of pistachios ( $34.7 \pm 2$ ) mg kg<sup>-1</sup>.

The contents of tocotrienols could not be compared to the literature data, because the cited studies probably due to minor representation of tocotrienols deal only with tocopherols. Recently, however, more and more information has been available about tocotrienols that are very powerful antioxidants and their antioxidant activity could be even more significant than the antioxidant activity of tocopherols (particularly  $\alpha$ -tocopherol), even though their contents in food are usually smaller than the contents of tocopherols (Theriault et al., 1999; Choi & Lee, 2009; Comitato et al., 2017).

### Contents of tocols in walnuts and hazelnuts of different origin

Walnuts and hazelnuts are the most cultivated and consumed nuts in the Czech Republic. The contents of tocopherols and tocotrienols were evaluated in five samples of walnuts and three samples of hazelnuts that were either purchased in the market, or grown in different places of the Czech Republic (Table 2).

The average content of all tocols was ( $563.7 \pm 53.1$ ) mg kg<sup>-1</sup> in walnuts and ( $914.2 \pm 124.9$ ) mg kg<sup>-1</sup> in hazelnuts. The major tocopherol in walnuts was  $\gamma$ -tocopherol, whose average content was ( $459.6 \pm 40.0$ ) mg kg<sup>-1</sup>, which is in agreement with data given by Savage et al. (1999), Li et al. (2007) and Oliviera et al. (2002), who established the content of  $\gamma$ -tocopherol in different samples of walnuts in the range of 205–525 mg kg<sup>-1</sup>.

**Table 2.** The contents of individual tocols (mg kg<sup>-1</sup>) in walnuts and hazelnuts of different origin

Nuts	$\alpha$ -TcP	$\beta$ -TcP	$\gamma$ -TcP	$\delta$ -TcP	$\alpha$ -TcT	$\gamma$ -TcT
Waln - A	$51.4 \pm 1.7$	< 3.33	$419 \pm 10$	$50.3 \pm 2.7$	< 5.00	$14.6 \pm 0.61$
Waln - B	$42.1 \pm 8.4$	< 3.33	$473 \pm 9.8$	$50.5 \pm 0.95$	< 5.00	$16.1 \pm 0.28$
Waln - C	$53.0 \pm 3.52$	< 3.33	$521 \pm 8.5$	$67.6 \pm 2.4$	< 5.00	$15.2 \pm 1.3$
Waln - D	$32.7 \pm 4.6$	< 3.33	$432 \pm 6.5$	$44.8 \pm 2.3$	< 5.00	< 3.33
Waln - E	$51.8 \pm 4.1$	< 3.33	$453 \pm 6.8$	$59.9 \pm 1.3$	< 5.00	< 3.33
Hazel - A	$983 \pm 20$	< 3.33	$36.6 \pm 1.2$	$4.1 \pm 0.87$	$29.0 \pm 5.3$	< 3.33
Hazel - D	$769 \pm 26$	$9.56 \pm 0.77$	$31.3 \pm 2.4$	< 1.67	< 5.00	< 3.33
Hazel - E	$838 \pm 6.0$	$7.22 \pm 0.1$	$31.7 \pm 0.8$	$3.12 \pm 1.3$	< 5.00	< 3.33

TcP – tocopherol; TcT – tocotrienol; Waln – walnuts; Hazel – hazelnuts; A – harvested in CR (Poteč, location A); B – harvested in CR (Poteč, location B); C – harvested in CR (Valašské Klobouky); D – purchased on the market (Prague–Suchdol); E – purchased on the market (Kolín).

In hazelnuts unlike walnuts the major form of tocols was  $\alpha$ -tocopherol, whose average content was ( $863.3 \pm 109.2$ ) mg kg<sup>-1</sup>. It is more than was reported by Miraliakbari & Shahidi (2008) who have determined the content of  $\alpha$ -tocopherol in hazelnuts in the range of 365.0–372.4 mg kg<sup>-1</sup>. With respect to the total content of vitamin E and to the proportion of  $\alpha$ -tocopherol as well, which is considered to be the most active biological form of vitamin E (Jiang et al., 2001; Ros et al., 2004; Wagner et al., 2004; Ros et al., 2015), hazelnuts are even better food source of this substance than the walnuts.  $\beta$ -Tocopherol was found only in purchased samples. According to Lavedrine et al. (1999) and Amaral et al. (2005), there is a link between the content of the major vitamers of vitamin E and the variety of walnuts. Probably there is also a link

between the content of other vitamers of vitamin E and the variety of other types of dry nuts. Unfortunately, nothing is known about the varieties of analyzed hazelnuts.

In samples of walnuts and hazelnuts grown and harvested in the different localities of the Czech Republic have been also determined tocotrienols but in significantly smaller quantities than tocopherols. In sample of hazelnut the most important and the only one detected form of tocotrienols was  $\alpha$ -tocotrienol ( $29.0 \pm 5.3$ ) mg kg<sup>-1</sup>. On the contrary, in all samples of walnuts the most important and the only one detected form of tocotrienols was  $\gamma$ -tocotrienol whose average content was  $5.32 \pm 0.75$  mg kg<sup>-1</sup>. In the purchased samples these vitamers were below the limit of detection.  $\beta$ - and  $\gamma$ -tocotrienols were below the limit of detection even in all samples of both walnuts and hazelnuts.

It seems likely that the duration and way of storage of nuts has a significant influence on the content of various forms of vitamin E, because the nuts grown in the Czech Republic were analyzed immediately after harvest. Nothing is known about the time of harvest and the way of storage of the purchased nuts. Silva et al. (2010) and Chun et al. (2005) came to the same conclusion that the contents of various forms of vitamin E depend on the duration of nuts storage.

On the basis of statistical analysis it was shown that in five tested samples of walnuts and three tested samples of hazelnuts differing in the place of origin, a statistically significant difference was found between the various forms of vitamin E, both between the purchased samples on the market and samples originating from the home production, as well as among the samples inside each group.

While statistically the highest content of majority form  $\alpha$ -tocopherol was set in hazelnut nuts grown in the Czech Republic, in samples of walnuts both statistically the highest and the lowest content of majority form  $\gamma$ -tocopherol were always established in samples of nuts grown in the Czech Republic. According to Lavedrine et al. (1999) and Amaral et al. (2005), there is a link between the content of the major vitamers of vitamin E and the variety of walnuts. In addition to the different geographic origin of nuts this fact could also be the cause of the differentiation in the content of tocopherols in different samples of walnut and hazelnut.

## CONCLUSIONS

Significant differences in the content and composition of the various forms of vitamin E among the 10 kinds of dry nuts were detected. The most important and the most represented vitamers of this lipophilic vitamin have been found  $\alpha$ -tocopherol (almonds) and  $\gamma$ -tocopherol (pistachios). High amount of  $\delta$ -tocopherol contained Brazil nuts. Tocotrienols ( $\alpha$  and  $\gamma$ ) were found only in a few selected species of dry nuts and  $\gamma$ - and  $\delta$ -tocotrienols have been in all the samples below the limit of detection.

In the Czech Republic are the most cultivated and consumed walnuts and hazelnuts. Statistically significant differences were found in the content of the various forms of vitamin E among samples of walnut and hazelnut, which came from different geographical areas or were purchased in different location in the Czech Republic.  $\alpha$ - and  $\gamma$ -tocotrienols have been also represented in walnuts and hazelnuts in minority quantity, but only in samples of nuts grown and harvested in the Czech Republic and analysed soon after harvest.

In conclusion it can be stated that dry nuts are an excellent source of vitamin E in human diet, but the content and mutual ratio of individual vitamers varies considerably according to the type of nut.

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