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CONTENT

TOCOPHEROL CONTENT IN COLD-PRESSED OIL FROM DIFFERENT SUNFLOWER HYBRIDS GROWN IN SERBIA

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Vegetable oils rich in antioxidant are of great interest in the food industry as they decelerate oxidative degeneration of lipids and therefore promote the quality as well as the nutritional value of food. Sunflower (*Helianthus annuus* L.) is one of the most abundant annual crops in the world that is grown for edible oil which is a good source of bioactive compounds. Tocopherols belong to the most important bioactive compounds in oil which have many health benefits for human. These compounds preserve oil from peroxidation by scavenging lipid peroxyl radicals. In humans, tocopherols play an important role as vitamin E, contributing to maintaining the immune system and delaying the pathogenesis of a variety of degenerative diseases. Over the last few years, increased interest for natural antioxidant in cold-pressed plant oils has been observed since they have better nutritive properties in comparison with refined ones. In this work, three cold-pressed sunflower oils obtained from sunflower hybrids cultivated in Serbia (2017 harvest) were studied for their contents. Quantification of tocopherols was carried out using high performance liquid chromatography on a column Nucleosil 100-5 NH_2 with fluorescence detection (λex=280 nm, λem=340 nm). The mobile phase was n-hexane/ethyl acetate (70/30%v/v) with a flow rate of 1 ml/min. The relative retention value and maximum values of absorption at the given relative retention time were used to identify and confirm the presence of tocopherolsin injection volume of 20 µl solution samples obtained by diluting of oil (300 µL) in n-hexane (2 ml). This research verified the dominant presence of α-tocopherol in cold-pressed sunflower oils. The content of α-tocopherol ranged between 497.40 mg/kg oil in NS Oliva hybrid and 691.46 mg/kg oil in NS Romeohybrid. The results have shown that there were differences in content of tocopherols among tested hybrids, indicating the great genetic potential for improvement.
***Keywords**: tocopherols, sunflower, cold-pressed oil, HPLC, fluorescence detector*

INTRODUCTION

Natural antioxidants present in food have obtained substantial attention because of their health and nutritional effects. Antioxidants can scavenge free radicals before they cause damage, or prevent oxidative damage from spreading (Czerwinski et al., 2004). Plants produce of antioxidant compounds such as tocopherols and tocotrienols to prevent oxidation of the susceptible substrate. These plant-based antioxidants are believed to have a better biological effect than the synthetic one, are to have better compatibility with human body. Crops and vegetable are significant sources of antioxidants in human nutrition either via consumption oils or in the form of vegetable juices.

Sunflower (*Helianthus annuus* L.) is one of important annual crops in the world that is grown for edible oil. Sunflower oil is considered to be healthy oil, because it contains high levels of essential fatty acids and vitamin E. Standard sunflower oil contains linoleic (C18:2), oleic (C18:1), palmitic (C16:0) and stearic (C18:0) ranged between 62-72%w/w, 15-25%w/w, 5-7%w/w and 4-6%w/w, respectively,as well as some other higher fatty acid in traces (Purdy, 1986; Grompone, 2005).

Vitamin E is the important non-enzymatic antioxidant, which reacts with free radicals to form radicals themselves which are less reactive than the radicals. It is a key lipid-soluble antioxidant and a most effective chain-breaking antioxidant within the cell membrane where it defends membrane fatty acids from lipid peroxidation. Besides that, this antioxidant can transfer its phenolic hydrogen to a peroxyl free radical of a peroxidized PUFA (n-3 and n-6 polyunsaturated fatty acids), so it interruption the radical chain reaction and inhibit the

peroxidation of PUFA in cellular membrane phospholipids. The vitamin E is represented by a family of structurally related compounds, have isolated from vegetable oils and other parts plant materials. Tocopherols and tocotrienols (tocochromanols), grouped as tocols, composed of chemical analogues: α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol and their unsaturated tocotrienols: α-tocotrienol, β-tocotrienol, γ-tocotrienol, and δ-tocotrienol (Panfili et al., 2008). *In vivo* studies have shown that this antioxidant improves various parameters of oxidative stress in both animals (Golestani et al., 2006) and humans. In addition, their antioxidant properties the tocols content of oils can give human health benefits like modulating cancer, cardiovascular diseases and a protective effect by lowering LDL cholesterol by inhibiting cholesterol biosynthesis (Tiwari and Cummins, 2009). The total tocopherol contents in sunflower oil of different hybrids, ranged between 271-2188 mg/g oil (Gunstone et al., 1994).

The objective of this study was to assess tocopherol content in oils of three selected sunflower hybrids, in order to improve the nutritional value of investigated oils and other sunflower products. This information can be important for improving tocopherols content in sunflower breeding programs. Genetic variation of tocopherol contents in sunflower oils is substantial for genetic improvement of the oils quality and developing new hybrids.

MATERIAL AND METHODS

Samples of sunflower cold-pressed oil

Analysed three sunflower hybrids were cultivated on the experimental field of the Institute of Field and Vegetable Crops, Rimski Šančevi (Vojvodina Province, Serbia). Sunflower oils were obtained by pressing of sunflower seeds in a hydraulic press (Sirio, Mikodental 10 tons force, cc 400 bars). The oil samples were immediately stored in the dark at 0°C until the moment of analysis. The analysis of tocopherols was carried out a few days after pressing oil.Tocopherols were analysed by high performance liquid chromatography (HPLC) according to the method of Lazzez et al., 2008 with a slight modification.Samples oil of 300µl were placed into 2 ml volumetric flasks. A quantity of n-hexane was added, swirling to dissolve the sample and making up to volume with the same solvent. An aliquot of 1 ml of this solution was filtered through regenerated cellulose filter (0.22 µm, Machery-Nagel, Germany) and transferred into the vial for further HPLC analysis.

High performance liquid chromatography analysis

The amount of tocopherols in the samples was determined by normal-phase HPLC analysis. The HPLC system consisted of Sykam (Germany), S 5200 sample injector, S 4011 column thermo controller and a LC 305 fluorescence detector. An aliquot of 20µl from each vial was injected onto a Nucleosil 100-5 NH_2 column (25 cm ×4.6 mm, particle size 5 µm, pore size 100 Å, Machery-Nagel, Germany). The mobile phase was a mixture of n-hexane with ethyl acetate (70:30%v/v). The flow rate was 1 ml/min.The mobile phase was previously degassed bysonication for 20 minutes. The eluant was monitored using the fluorescence detector set at excitation wavelength 280 nm and emission wavelength 340 nm. The detector temperature was30°C. The measurements were performed in three replicates.Individual tocopherols were identified by comparison of their retention times with those of authentic standards (Supelco, USA) and were quantified by the external standard method. Reference standards from Supelco, USA (Cat. No. 4-7783; 46401-U; 4-7784; 4-7785) which containing tocopherols (α-, β-, γ-, δ-tocopherol) were used. Chromatograms were recorded and processed using ChemStation chromatography software.

RESULTS AND DISCUSSION

The chromatographic separation of tocopherols in solution of commercial standards is reported in Figure 1A, whereas the separation of tocopherols from sunflower sample (NS Oliva hybrid) is shown in Figure 1B. A typical run lasted 10 minutes. The content of tocopherols was determined by measuring the peak area from 4.70 to 4.92 min for α-

tocopherol. A linearity test with a standard solution was carried out over concentration range close to the tocopherol contents found in oils of investigated sunflower hybrids. The concentration of tocopherols are expressed as mg tocopherol/kg of oil. The results of tocopherol contents are shown in Table 1. Values are displayed as the mean ± standard deviation (SD) of three replicates. The limits of detection (LODs) and quantification (LOQs) were determined for investigated tocopherols as the lowest concentration yielding a signal-to-noise ratio (S/N) of 3 and 10, respectively. The LODs and the LOQs of all the investigated tocopherols were 0.30 µg/kg and 1.00 µg/kg, respectively.

Table 1. α-, β-, γ-, δ-tocopherol contents (mg/kg) of seed oil from investigated sunflower hybrids

Sunflower hybrids	**α-tocopherol**	**β-tocopherol**	**γ-tocopherol**	**δ-tocopherol**
NS Oliva	497.40 ± 1.79	<LOQ	<LOQ	<LOQ
NS Horizont	509.07 ± 2.29	<LOQ	<LOQ	<LOQ
NS Romeo	691.46 ± 2.35	<LOQ	<LOQ	<LOQ

Results are given as mean ± standard deviation (n=3)

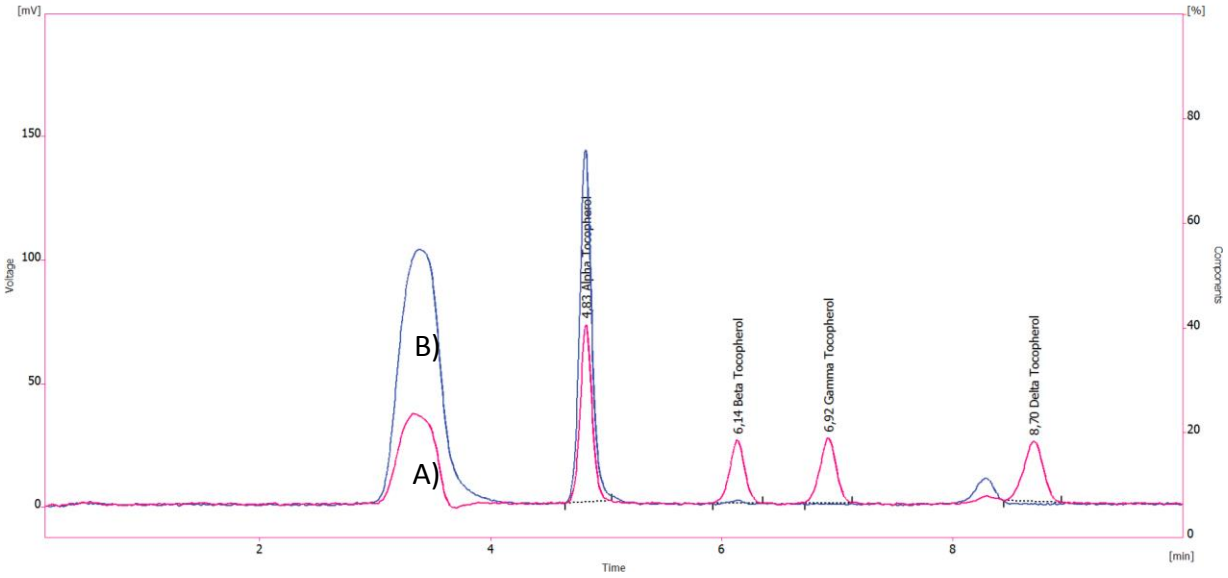

Figure 1. Typical chromatograms of a mixture of commercial standards (A) and tocopherol in oils of investigated sunflower hybrid NS Oliva (B)

The α-tocopherol isomer was observed in seed oil from all investigated sunflower hybrids and no β-, γ-, δ-tocopherols were present. The average contents of α-tocopherol were in range from 497.40 mg/kg (in NS Oliva hybrid) to 691.46 mg/kg (in NS Romeo hybrid) (Table 1). For instance, Gunstoneet al., 1994 reported a similar result in that the total tocopherol content of sunflower oil ranged between 271-2188 mg/kg oil. Differences between genotypes was similarly reported (Velasco et al., 2002) and within a genotype grown under different environmental conditions (Velasco and Fernández-Martínez, 2012).

Tocopherols are known to be important antioxidants that have a positive effect on the oxidative stability of oils. The oxidative stability of edible oils is largely determined by their tocopherol contents and fatty acid composition (more unsaturated fatty acids are very susceptible to oxidation). It is significant for their sensory and nutritional quality (Nawar, 1996). The primary and secondary oxidation products which have been formed as a result of the degradation of the hydroperoxides negatively affects their flavor.

Usually, the tocopherol content in oils is close to their optimal amount needed for their stabilization (Kamal-Eldin, 2006). Researchers were noted that the reaction kinetics and stability of the four tocopherols (α-, β-, γ-, δ-) were not identical. Among the tocopherols, α-

tocopherol is the most efficient in scavenging peroxyl radicals. The fast reacting α-tocopherol reacted more rapidly and trapped free radicals more thoroughly and was therefore only available as an antioxidant for a short period of time as compared with the slowly reacting δ-, β-and γ-tocopherols.Further, α-tocopherol obtained recognition as the most important lipophilic radical chain breaking antioxidant in tissues (*in vivo*). Deficiency of α-tocopherol in membranes made them highly permeable and therefore vulnerable to degradation. It seemed also to influence other important biophysical membrane characteristics, such as fluidity.

CONCLUSIONS

The tocopherol contents in sunflower oils of different hybrids were determined by high performance liquid chromatography with fluorescence detector. The information obtained in this study will be helpful to estimate the effect of detected tocopherol on the oxidative stability of analysed oils. Taking into consideration the biological activities of the isomers of vitamin E, quantitative data on individual tocopherol may be useful in the evaluation of the healthy and nutritional values of analysed sunflower oils. In particular, due to the variability in the content of tocopherol in investigated oils of sunflower hybrids indicates the possibility for an additional enhancement with α-tocopherol.

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