Degradation of Selected Nutrients in Sunflower Oils during Long-Term Storage

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Abstract

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We investigated the influence of long-term storage (10 months) at an average ambient temperature of 25°C on oxidative stability of sunflower oils (made in Slovakia, Czech Republic, Austria, and Hungary) and their nutrients. Chemical properties were determined and changes in oxidative stability monitored. Oil samples were collected and analysed for the content of tocopherols and β-carotene. Degradation of nutrients depends on chemical composition of oils and storage conditions. It was found that the concentration of both antioxidants decreased in all the samples with the increase in storage time. According to the results, losses of total tocopherols and β-carotene in refined sunflower oils stored in transparent 5-l PET bottles and exposed to daylight at ambient temperature were found to be 52–64% and 63–65%, respectively. The country of origin had no statistically significant impact on the oxidative stability of stored sunflower oils.

Keywords: β-carotene; long-term storage; oxidative stability; sunflower oil; tocopherol

Lipid oxidation is responsible for a gradual decrease in nutritional and sensory quality of lipid-containing products (Hrádková *et al.* 2013). In fact, lipid autoxidation and inadequate storage contribute significantly to the deterioration and reduction of the shelf-life of vegetable oils causing changes in colour, texture, odour and flavour, and loss of vitamins (Milanez & Pontes 2014). Sunflower oil, like most vegetable oils, is composed mainly of triacylglycerols (98–99%) and a small fraction of phospholipids, tocopherols, carotenoids, sterols, and waxes. Regular sunflower oil is characterised by a high concentration of linoleic acid, followed by oleic acid (Grompone 2005). Oxidative stability of sunflower oils depends on their fatty acid composition and presence of antioxidants (Kamal-Eldin 2006). Antioxidants belong to a specific class of chemical compounds that possess the ability to prevent or reduce the rate of oxidative reactions of substrates in food and can thus increase the shelf-life of food products by retarding lipid oxidation (Pokorný & Schmidt 2001). They inhibit oxidation by reacting with free radicals, thus blocking the formation of fatty acid radicals and terminating the chain reaction (Khan *et al.* 2001). In the reaction, the antioxidant is consumed. When it has been completely consumed, autoxidation starts running as antioxidants are no longer present in the oil (Velíšek 2002a).

The important role of vitamin E compounds in food production and commercialisation is preventing lipids and lipid-containing foodstuffs from oxidation during storage, thus extending their stability and shelf-life (Pokorný *et al.* 2003; Gliszczyńska-Świgło *et al.* 2007). Their high solubility in oil is caused by a long alkyl side chain (Grompone 2005). Carotenoids are efficient singlet oxygen quenchers, mainly in low concentrations (Kamal-Eldin 2006). β-carotene is an oil-soluble and natural pigment of many oils and

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it also has a strong antioxidant activity under low temperatures and oxygen pressure conditions (Grompone 2005). However, storage causes oxidation and complete degradation of β-carotene (Velíšek 2002b). Because the vitamin degradation is affected by many factors, each combination of ingredients, processing procedures, packaging, and storage conditions may result in a different level of vitamin losses. Degradation of vitamins depends on contents and activities of antioxidants and also pro-oxidants (light, air, heat, presence of free fatty acids, transition metals, oxidation products) (Burch 2011). Vitamins are oxidised relatively rapidly by singlet oxygen while the latter reacts the fastest with β-carotene, more slowly with tocopherols, riboflavin, vitamin D, and ascorbic acid (Schmidt 2010). β-carotene is effective especially in combination with tocopherols which protect it from oxidation (Velíšek 2002b). Degradation patterns of vitamins and ways to minimise losses on storage are important for both the food industry and the consumer. Vitamin degradation occurs in food during processing as well as during storage. Numerous studies concentrated on losses of vitamins during processing; fewer studies have been published on losses during storage (Burch 2011). Storage takes place at ambient or still lower temperatures so that the extent of oxidation and the subsequent antioxidant damage is low. Nevertheless, after protracted storage times of several months or even years, they may become quite considerable (Pokorný & Schmidt 2000).

The aim of this work was to focus on monitoring of antioxidant degradation in sunflower oils, purchased at a market and stored in plastic bottles at room conditions for 10 months.

MATERIAL AND METHODS

Samples. Commercially refined sunflower oils purchased from four various producers were used: PALMA Group a.s., Bratislava, Slovak Republic (SK); LUKANA OIL a.s., Praha, Czech Republic (CZ); DELIKATESSA GmbH, Neudorf, Austria (A); BUNGE Zrt, Budapest, Hungary (H) while they were produced two months before the beginning of storage experiment. They were stored in transparent polyethylene terephthalate (PET) bottles of 5 l volume without protective atmosphere covered with the cup. They were stored for 307 days at room temperature ranging from 21°C to 34°C, exposed to daylight and measured with DVM 1300 digital light meter (Velleman, Fort Worth, USA) with average illumination of 1061 lux. At the beginning of the experiment, the volume of air in the upper part of the bottle above the oil level was 150 cm^3 and at the end it increased approximately 20 times. Before each sampling, the oil was agitated and poured into the flask (within less than 1 min), thereafter used for analyses.

Chemicals. Mikrochem (Pezinok, Slovak Republic): chloroform p.a.; diethyl ether p.a.; ethanol p.a.; glacial acetic acid p.a.; potassium iodide p.a.; starch; sodium thiosulphate p.a.; potassium hydroxide p.a. (Lachema, Brno, Czech Republic): *n*-heptane for UV (VWR International, Wien, Austria): *n*-hexane ≥ 99%; isopropanol ≥ 99% (Sigma-Aldrich Chemie GmbH, Steinheim, Germany): α-, β-, γ-, and δ-tocopherols 90–96%, sodium methoxide ≥ 95%, methanolic HCl for GC derivatisation.

Determination of chemical parameters. The iodine value was determined iodometrically and expressed in g I_2/100 g of oil (AOCS Official Method Cd 1-25 1993). The peroxide value was estimated iodometrically using sodium thiosulphate and expressed in 0.5 mmol O_2/kg of oil (AOCS Official Method Cd 8-53 1993). The acid value was investigated by alkalimetric titration and expressed in mg KOH/g of oil (AOCS Official Method Cd 3d-63 1993).

Determination of fatty acid composition. The determination of fatty acid composition was carried out according to the IUPAC 2.302 (1992) method. The fatty acids were determined from their methyl esters by gas chromatography using a HP 5890 series II (Hewlett Packard, Palo Alto, USA) with a Supelcowax 10 capillary column with the dimensions 30 m × 0.53 mm; 1.0 μm film. The sample was analysed under the following conditions: injector temperature at 250°C; flame-ionisation detector temperature at 260°C; helium as carrier gas with 7 cm^3/min flow rate (IUPAC 1992).

Determination of oxidative stability. The oxidative stability of the investigated oil samples was determined by the Rancimat test (AOCS Official Method Cd 12b-92 1993) performed on a 743 Rancimat apparatus (Methrom, Herisau, Switzerland). The oil samples (3 g) were subjected to a constant temperature of 110°C at an air flow rate of 20 l/hour. The determination was based on the conductometric detection of volatile acids. The results were expressed as the induction period (IP) in hours (Vidrih *et al*. 2010).

Determination of tocopherol content. Tocopherol content was measured according to the IUPAC 2432 (1992) method. Oils were dissolved in *n*-hexane, filtered through a microfilter (0.45 nm) and injected directly onto the analytical silica gel column Nucleosil 100 Si (250 × 4 mm) packed with microparticulate

silica (5 µm particle size) (Macherey-Nagel, Düren, Germany). *n*-hexane and isopropanol (98.8 : 1.2 v/v) were used as a mobile phase and pumped at the flow rate of 1.0 cm^3/min by the HPLC pump (DeltaChrom™ SDS 030 Watrex model, Prague, Czech Republic). The absorbance was measured at 292 nm (DeltaChrom™ UVD-250 detector). The results were expressed as mg of tocopherol per kg of oil.

Determination of β-carotene. The changes in β-carotene content were measured by the Lovibond spectrophotometer, PFXi 995 model (Amesbury, UK) according to the BS 684-2.20 method. The results were expressed as mg of β-carotene per kg of oil.

Statistical analysis. The chemical parameters and content of individual nutrients were determined for each sample three times. The results were expressed as mean values ± SD (the corresponding error bars were displayed in the graphical plots). A statistical analysis was carried out by the Statgraphics Plus, software version 3.0 for Windows (Manugistic Inc., Rockville, USA). The statistical significance was performed by one-variable analysis of data (ANOVA) at a confidence level of 95.0%. The figures were carried out in Origin, software version 8.1 (Origin Lab Corp., Northampton USA).

RESULTS AND DISCUSSION

Four sunflower oils obtained from different producers were stored under ambient conditions for a period of 10 months. At the beginning of storage, oils were characterised by chemical parameters and fatty acid composition. The main aim of this study was to monitor changes in oxidative stability, tocopherol and β-carotene losses during long-term storage.

The quality markers of sunflower oils are given by chemical parameters. Iodine, acid and peroxide values of the initial oils are listed in Table 1. Regular sunflower oil is characterised by a high concentration of linoleic acid and oleic acid. Saturated fatty acids (mainly palmitic and stearic acid) do not amount to more than 12% of the fatty acid content. The determined fatty acid profile of fresh sunflower oils is shown in Table 2. Some authors show a similar range of major fatty acids in regular sunflower oil (Vidrih *et al*. 2010). Aladedunye and Przybylski (2013) mentioned that some researchers variously reported the influence of fatty acid composition of oils on stability. In general, oils that are more unsaturated oxidise more readily than less unsaturated ones. Worded differently, as the number of double bonds in a fatty acid increases, so does both the rate of formation and the amount of degradation compounds accumulated at the end of the induction period increase (Aladedunye & Przybylski 2013).

Oxidative stability, known as the resistance to oxidation under defined conditions, is one of the most important indicators of maintaining the quality of edible oils (Vidrih *et al*. 2010). Determination of the oxidative stability index by Rancimat is the

Table 1. Basic quality parameters of purchased sunflower oils used as the initial oils in storage experiments

Chemical parameter	Sunflower oils			
	SK	CZ	A	H
Iodine value (g I_2/100 g)	132.2 ± 1.0	128.5 ± 0.2	126.2 ± 0.6	124.0 ± 1.2
Peroxide value (0.5 mmol O_2/kg)	3.72 ± 0.10	3.89 ± 0.08	2.44 ± 0.05	2.41 ± 0.06
Acid value (mg KOH/g)	0.54 ± 0.01	0.55 ± 0.01	0.54 ± 0.01	0.54 ± 0.01

SK – Slovak Republic; CZ – Czech Republic; A – Austria; H – Hungary; mean values ± standard deviation (SD)

Table 2. Fatty acid composition (% of total fatty acids) determined in purchased sunflower oils used as the initial oils in storage experiments

Fatty acid	Sunflower oils			
	SK	CZ	A	H
Palmitic acid (C16:0)	5.86	6.30	6.22	5.99
Stearic acid (C18:0)	3.14	3.51	3.55	3.23
Oleic acid (C18:1-9*c*)	26.32	23.98	23.23	27.56
Linoleic acid (C18:2-9*c*,12*c*)	61.96	63.80	64.55	60.70
Linolenic acid (C18:3-9,12,15*c*)	0.34	0.11	0.12	0.13
Others	2.38	2.30	2.33	2.39

SK – Slovak Republic; CZ – Czech Republic; A – Austria; H – Hungary

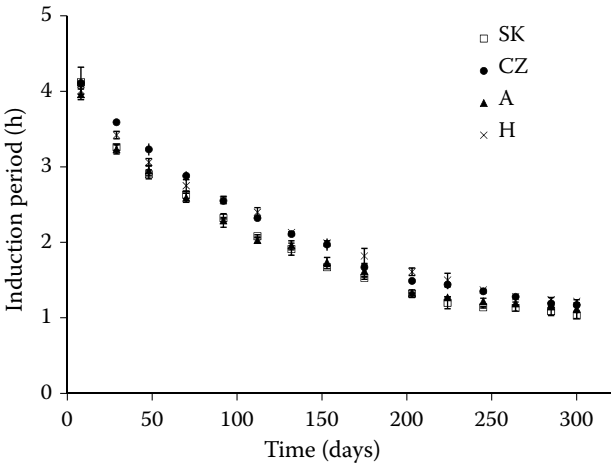

Figure 1. Oxidative stability of investigated sunflower oils expressed as induction periods determined by the Rancimat test under accelerated conditions (110°C, 20 l/h air flow)

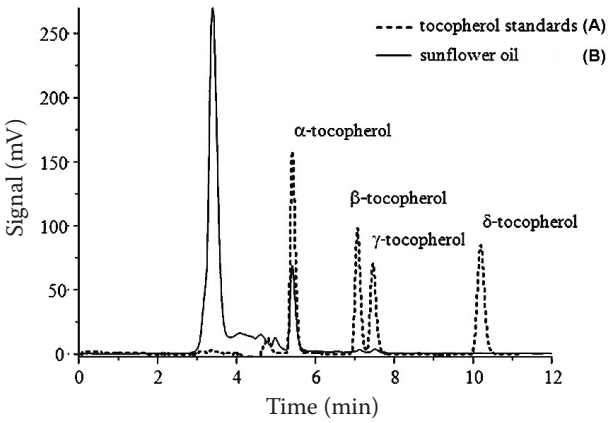

Figure 2. Determination of individual tocopherols by HPLC: (A) chromatogram of individual tocopherols determined in a model mixture composed of tocopherol standards and (B) chromatogram of individual tocopherols naturally present in the sample of initial sunflower oil (SK) used in the storage experiment

most widely applied standard method to determine susceptibility to oxidation of edible fats and oils under accelerated conditions (Velasco *et al.* 2009). It has been shown that the oxidation of edible oils proceeds through a series of chemical reactions that essentially starts with an induction stage (Vidrih *et al*. 2010). In Figure 1, it can be seen that oils stored for 10 months had shorter induction periods than initial oils. The values of IP measured at 110°C at the beginning of storage were 3.3–4.0 times higher in initial oils compared to oils that were stored for 10 months. As proved statistically using the ANOVA test, the oxidative stability measured in the examined samples of sunflower oils significantly depended on the storage time ($P = 0.8776$, $P > 0.05$), while no effect of the country of origin was observed ($P < 0.05$).

It is well known that the presence of antioxidants can improve the oxidative stability. At the beginning

Table 3. Contents of individual tocopherols (mg/kg) determined by HPLC in the samples of sunflower oils during storage experiments

Individual tocopherols	Sunflower oils	Storage time (days)						Losses of individual tocopherols (%)
		10	41	112	169	224	304	
α-T	SK	235.7	159.8	124.0	111.1	94.3	87.8	62.8
	CZ	257.9	196.0	156.5	146.5	119.8	127.6	50.5
	A	260.3	182.2	138.2	128.4	126.1	125.5	51.8
	H	256.6	198.8	160.9	140.0	129.0	123.2	52.0
β-T	SK	17.4	12.2	6.5	6.2	6.1	5.8	66.7
	CZ	13.8	10.7	9.7	9.6	9.3	8.3	40.0
	A	15.6	11.5	10.8	10.1	9.1	8.7	44.1
	H	18.8	12.2	10.8	10.5	7.3	6.1	67.4
γ-T	SK	28.3	18.1	12.0	10.8	8.0	7.3	74.2
	CZ	11.0	7.1	2.4	2.2	2.2	2.0	81.8
	A	6.2	4.1	2.9	2.4	2.0	1.8	71.2
	H	8.8	8.0	6.0	2.9	2.5	2.2	75.4
δ-T	SK	2.0	1.0	nd	nd	nd	nd	–
	CZ	1.9	1.0	nd	nd	nd	nd	–
	A	2.1	1.0	nd	nd	nd	nd	–
	H	nd	nd	nd	nd	nd	nd	–

SK – Slovak Republic, CZ – Czech Republic, A – Austria, H – Hungary; nd – not detected

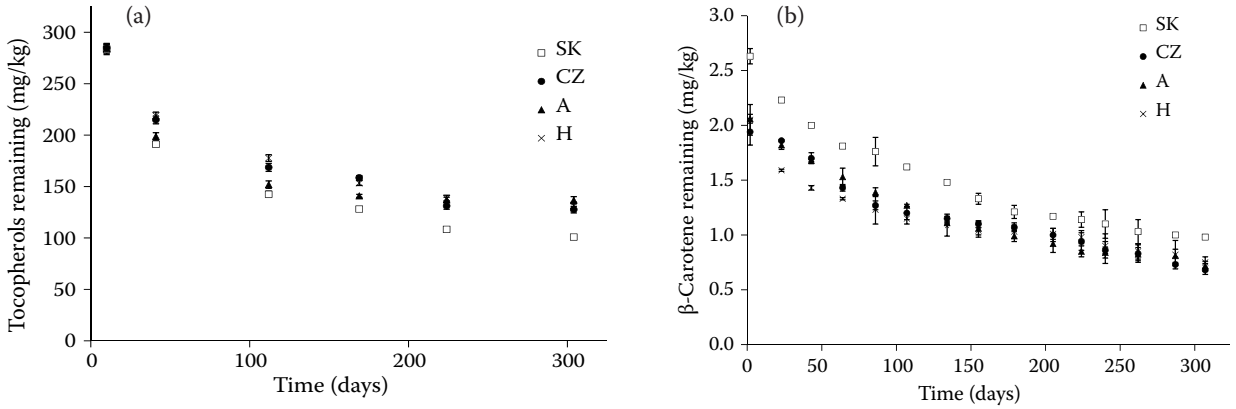

Figure 3. Degradation of (a) total tocopherols and (b) β-carotene in sunflower oils during storage

of simulated oxidation, the rate of oxidation reaction is low because of free radicals which activate autoxidative reactions that are quenched by natural antioxidants present (Davídek *et al.* 1983). Prior to the development of autoxidation of fats takes place, there is an induction period during which the antioxidants are gradually consumed by a rising of free radicals (Schmidt 2010). Under storage conditions, tocopherols are more effective antioxidants in oils with oleic acid as the main component compared to oils that contain a higher amount of linoleic acid (Velíšek 2002b). Schmidt (2010) showed that usually an optimal concentration of tocopherols (from 250 mg/kg to 500 mg/kg) in vegetable oils was in relation with the fatty acid composition but at a higher content they have a pro-oxidative effect. The range of total tocopherol content was from 283.4 mg/kg to 284.7 mg/kg of initial oils. Literature data show that sunflower oil is rich in α-tocopherol. However, the concentration of β-tocopherol, γ-tocopherol reaches only a value of 45 mg/kg and δ-tocopherol is found only in traces (Grompone 2005). Figure 2 presents a chromatogram of individual tocopherols determined in a model mixture composed of tocopherol standards (A) and of individual tocopherols naturally present in the sample of initial sunflower oil (SK) used in the storage experiment (B). The relative protective effect of antioxidants against oxidative rancidity depends on storage time (Khan *et al.* 2001). Figure 3a describes the degradation of total tocopherols in sunflower oils during storage. We determined that the stored sunflower oil had by 50% lower content of total tocopherols compared to the original amount of total tocopherols in initial oils. The losses of tocopherols in the oils ranged from 51% to 63 % of oil for α-tocopherol. Unfortunately, the highest antioxidant stability of γ-tocopherol resulted in the greatest losses of approximately 71–82%. δ-Tocopherol was present in small quantities (Table 3). Koski *et al.* (2002) confirmed a decrease of the total tocopherol content in vegetable oils during storage. Our investigation found a good correlation (r = 0.972–0.993) between the total tocopherol content and IP determined by the Rancimat method at 110°C.

β-carotene is a pigment partly removed by bleaching process together with other minor components. It acts as a strong natural antioxidant. A high level of β-carotene offers greater stability of oil against rancidity (Ahmed *et al.* 2011). The results (Figure 3b) indicate a decreasing trend in the β-carotene content in all the tested samples. At the end of the storage experiment, 35–37% amount of β-carotene remained in analysed sunflower oils. The correlation between IP and β-carotene content was statistically significant at 0.05 level for all sunflower oils (r = 0.974–0.997).

CONCLUSION

We confirmed that the time of storage affected the shelf-life of oils and caused losses of antioxidants. The extent of oxidation changes monitored in all examined samples of sunflower oils stored under the same packaging and storage conditions was almost identical and the country of origin had no statistically significant effect on the oxidation stability. In terms of oxidative stability of edible oils, the transparent PET is not a suitable material for long-term oil storage, because of a considerable degradation of tocopherols and β-carotene. To reduce the extent of adverse changes during storage, sunflower oil should be stored at a lower temperature and in the dark. This work is the preliminary study to create a mathematical model for prediction of the shelf-life of sunflower oils stored under real-life conditions.

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