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Relationship Between Tocopherols Depletion and Polymerised Triacylglycerols Formation During Heating of Vegetable Oils

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Abstract

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Alpha tocopherol and total tocopherols depletion in relation to polymerised triacylglycerols formation were studied during the heating of nine vegetable oils (olive, palm, peanut, sunflower, rapeseed, soybean, grapeseed, maize and rice) at oil temperature 180°C (as a typical temperature during frying practise). It was observed that the rate of tocopherols depletion (expressed as the function of polymerised triacylglycerols content) decreased with the increasing degree of unsaturation and total antioxidant capacity of the tested oils ($P < 0.05$). Consequently, no tocopherols were present in olive, peanut and palm oil at their half lifespan (i.e., at 6% of polymerised triacylglycerols), whereas tocopherols in maize, sunflower, grapeseed, rice and, particularly, soybean oil were present even at the end of their lifespan, (i.e., at 12% polymerised triacylglycerols). These findings have not been published yet, or only little published.

Keywords: frying; lipid oxidation; plant oils; vitamin E

Tocopherols (vitamin E) are important lipophilic antioxidants *in vivo*, protecting fatty acids against oxidation in cell membranes and lipoproteins of blood serum (Eitenmiller & Lee 2004a). An adequate intake of vitamin E is crucial for a mammalian reproductive cycle and in the prevention of cardiovascular diseases (Eitenmiller & Lee 2004a,b).

During culinary processing of foods, tocopherol losses can be significant (Piironen *et al.* 1987; Wyatt *et al.* 1998; Kalogeropoulos *et al.* 2007a,b; Chiou *et al.* 2009; Fišnar *et al.* 2014) and may contribute to the insufficient intake of vitamin E (Rodríguez-Palmero *et al.* 1998; Steinhart & Rathjen 2003; Maras *et al.* 2004). Notably, during repeated frying and panfrying (when food and frying fat or oil are exposed to high temperature and oxygen access), considerable losses of tocopherols can occur (Chiou *et al.* 2009; Réblová *et al.* 2009; Fišnar *et al.* 2014). Therefore, fried foods can be a rich source of vitamin E (similarly to unheated vegetable oils) but also a source of very poor to zero (Fišnar *et al.* 2014).

In some works (Yuki & Ishikawa 1976; Normand *et al.* 2001; Verleyen *et al.* 2001; Barrera-Arellano *et al.* 2002; Eitenmiller & Lee 2004c,d; Steel *et al.* 2005; Aladedunye & Przybylski 2013; Fišnar *et al.* 2014), tocopherol losses were studied

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during frying of food and heating of fats and oils at frying temperature. However, in most of the studies, tocopherol depletion was examined as a function of heating time or the number of repeated fryings (Yuki & Ishikawa 1976; Piironen *et al.* 1987; Wyatt *et al.* 1998; Barrera-Arellano *et al.* 2002; Eitenmiller & Lee 2004c,d; Kalogeropoulos *et al*. 2007a,b; Chiou *et al.* 2009; Fišnar *et al.* 2014). From a practical perspective, it is useful to study tocopherol (vitamin E) depletion as a function of the content of polymerised triacylglycerols (pTAG) or total polar compounds, i.e., parameters determining the lifespan of fats and oils under frying conditions. This approach allows determining the tocopherols (vitamin E) content during the lifespan of frying fats and oils, typically at the end of the lifespan of the frying medium, which is given by pTAG content, i.e., 12% and/or total polar compounds, i.e., 24% (DGF 2000).

There is a lack of scientific research (Barrera-Arellano *et al.* 2002; Réblová *et al.* 2009) assessing tocopherol depletion as a function of pTAG content (or total polar compounds). In our previous study (Réblová *et al.* 2009), it was observed that tocopherols could disappear in some frying oils much earlier than these oils should be discarded, based on the increase of pTAG content at 12%. On the contrary, in other frying oils, tocopherols can be present at the end of the lifespan of the frying oils (Réblová *et al.* 2009).

This successive study aimed to clarify what factors describing the composition of fats and oils influence the rate of α-tocopherol and total tocopherols depletion (expressed as a function of pTAG content) during heating of vegetable oils at a typical frying temperature. In order to understand this issue in context, the influence of the composition of the tested vegetable oils on the rate of pTAG formation and tocopherols depletion (both expressed as a function of time) were also investigated.

MATERIAL AND METHODS

Vegetable oils. All vegetable oils were purchased from local shops. The chemical characteristics of all tested oils are given in Tables 1 and 2.

Oil heating. Aliquots of the oils (25 g ± 1%) were heated in beakers (internal diameter 47 mm and height 80 mm) on a hot plate set at 220 ± 1°C (Prazitherm PZ 28-2; Harry Gestigkeit GmbH, Germany), using a steel adapter with outlets (internal diameter 60 mm and depth 28 mm). Under these conditions, the oil temperature in the beaker (approximately 20 min after the beginning of heating) ranged between 175–185°C. The heating times of the respective oils were estimated so that no tocopherols were present in the oil at the end of the heating and from 7 to 10 samplings were always performed until these times. Sampling was always carried out by taking the whole beaker so that the amount of oil in the beaker was constant throughout the heating period. Upon completion of heating and cooling of the samples to ambient temperature, the samples were placed in a freezer (–18°C) for further determination of tocopherols and pTAG. Two parallel experiments were performed with each vegetable oil.

Analytical methods

Peroxide value. The peroxide value was measured in accordance with Commission Regulation (EEC) No. 2568/91 on the characteristics of olive oil

Table 1. Chemical characteristics of the used oils

Vegetable oil		Olive	Sunflower	Soybean	Rapeseed	Palm	Grapeseed	Rice	Maize	Peanut
Peroxide value (mEq O_2/kg)		6.8 ± 0.1	1.9 ± 0.2	3.1 ± 0.1	2.9 ± 0.3	1.7 ± 0.1	2.2 ± 0.1	4.2 ± 0.3	1.3 ± 0.1	2.6 ± 0.1
Tocopherol (mg/kg)	α-T	167 ± 1	539 ± 1	123 ± 8	266 ± 3	169 ± 1	130 ± 1	127 ± 4	223 ± 4	185 ± 2
	ß + γ-T	12 ± 3	24 ± 2	795 ± 1	320 ± 1	14 ± 2	27 ± 1	94 ± 4	796 ± 4	148 ± 2
	δ-T	n.d.*	16 ± 2	266 ± 1	7 ± 1	32 ± 1	4 ± 1	9 ± 4	31 ± 4	11 ± 1
AOC (mg α-T/kg)		366 ± 28	778 ± 5	1231 ± 15	903 ± 5	998 ± 19	675 ± 31	1277 ± 18	1326 ± 13	471 ± 9

*not detected; AOC – antioxidant capacity; α-T – α-tocopherol; ß + γ-T – ß- and γ-tocopherol; δ-T – δ-tocopherol; results are expressed as average ± standard deviation (n = 3)

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Table 2. Fatty acids composition of the used oils

Plant oil	Fatty acids composition (%)								Iodine value (g I_2/100 g)
	palmitic	stearic	other saturated	oleic	other mono-unsaturated*	linoleic	α-linolenic	other poly-unsaturated*	
Maize	11.30	1.85	0.89	28.91	1.27	54.67	0.58	0.53	123
Grapeseed	6.90	4.06	0.39	17.61	0.96	69.00	0.23	0.85	137
Olive	12.93	3.08	0.72	68.29	3.77	10.48	0.50	0.23	82
Palm	34.00	3.97	1.83	45.57	1.17	12.44	0.47	0.55	64
Peanut	5.99	1.70	5.95	67.47	3.93	13.86	0.92	0.18	88
Rapeseed	4.50	1.69	1.19	59.96	4.40	18.81	8.36	1.09	112
Sunflower	6.72	3.28	1.42	23.98	1.13	62.72	0.08	0.67	132
Soybean	10.73	4.13	1.27	22.98	1.84	51.60	6.37	1.08	130
Rice	18.33	2.29	2.10	40.04	1.60	34.10	1.10	0.44	99

*including *trans*-isomers

and olive-residue oil and on the relevant methods of analysis.

Antioxidant capacity. The antioxidant capacity was determined spectrophotometrically (Espín *et al.* 2000), based on the ability of the analysed samples to scavenge free 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals (Sigma Aldrich, USA). The oil samples (approximately 0.3 g) were weighed into volumetric flasks (25 ml) and dissolved in 10 ml ethyl acetate. Then, 5 ml of an ethyl acetate DPPH radical solution (0.2 mg/ml) was added, and the flasks were filled to the mark with ethyl acetate. After mixing, the closed volumetric flasks were incubated in the dark at room temperature for 1 hour. The absorbance was measured at 518 nm against a pure ethyl acetate blank, using a Cary 60 spectrophotometer (Varian, USA). The antioxidant capacity was expressed as mg α-tocopherol/kg sample, using a calibration curve generated by replacement of the oil samples with ethyl acetate solutions containing 0 to 500 μg of α-tocopherol.

Fatty acids composition. Fatty acids were determined by gas chromatography with flame ionisation detection (GC-FID) after derivatisation to the corresponding methyl esters (Zamora & Hidalgo 2015). The GC analysis was performed using an Agilent Technologies 6890N gas chromatograph (Agilent Technologies, USA), equipped with a Supelco SP 2560 capillary column with a bis(cyanopropyl) siloxane stationary phase (100 m × 0.25 mm i.d., 0.2 μm thickness; Supelco, USA). The injector (split ratio 75 : 1) and detector were held at 220°C. The column temperature was programmed from 175 to 210°C at a rate of 1°C/min, with a delay of 30 min at 175°C and 40 min at 210°C. Nitrogen, at a flow rate of 1 ml/min, was used as the carrier gas, and 1 μl of sample was injected. Confirmation of fatty acid identity was made by GC-mass spectrometry (MS), and the results obtained by GC-FID were expressed as the relative percentage of each fatty acid, calculated by the internal normalisation method using the chromatographic peak areas.

Iodine value. The iodine value was calculated from the fatty acid composition, according to the official AOCS method Cd 1c-85 (AOCS 1995).

Tocopherols. Tocopherols were determined by reverse-phase high-performance liquid chromatography (HPLC) with amperometric detection (Trojáková 2001), under the following conditions: mobile phase of methanol/acetonitrile (1 : 1, v/v) with $LiClO_4$ (0.02 mol/l) and NaCl (0.005 mol/l); 1 ml/min flow rate (LCP 4020.31 non-steel pump; Ecom, Czech Republic); 20 μl injection volume; Hypersil ODS column (200 × 4.6 mm, 5 μm particle size; Hewlett-Packard, USA); column temperature of 28°C (LCO 101 column heater; Ecom, Czech Republic), and +1.05 V detection potential (HP 1049A amperometric detector, equipped with a glassy-carbon working electrode and Ag/AgCl reference electrode; Hewlett-Packard, USA). Quantification was achieved by external calibration using the respective tocopherol standards. Under the chromatographic conditions used, β- and γ-tocopherol did not separate. Their common chromatographic peaks were quantified as γ-tocopherol due to prevalence of this isomer in common vegetable oils (Eitenmiller & Lee 2004e).

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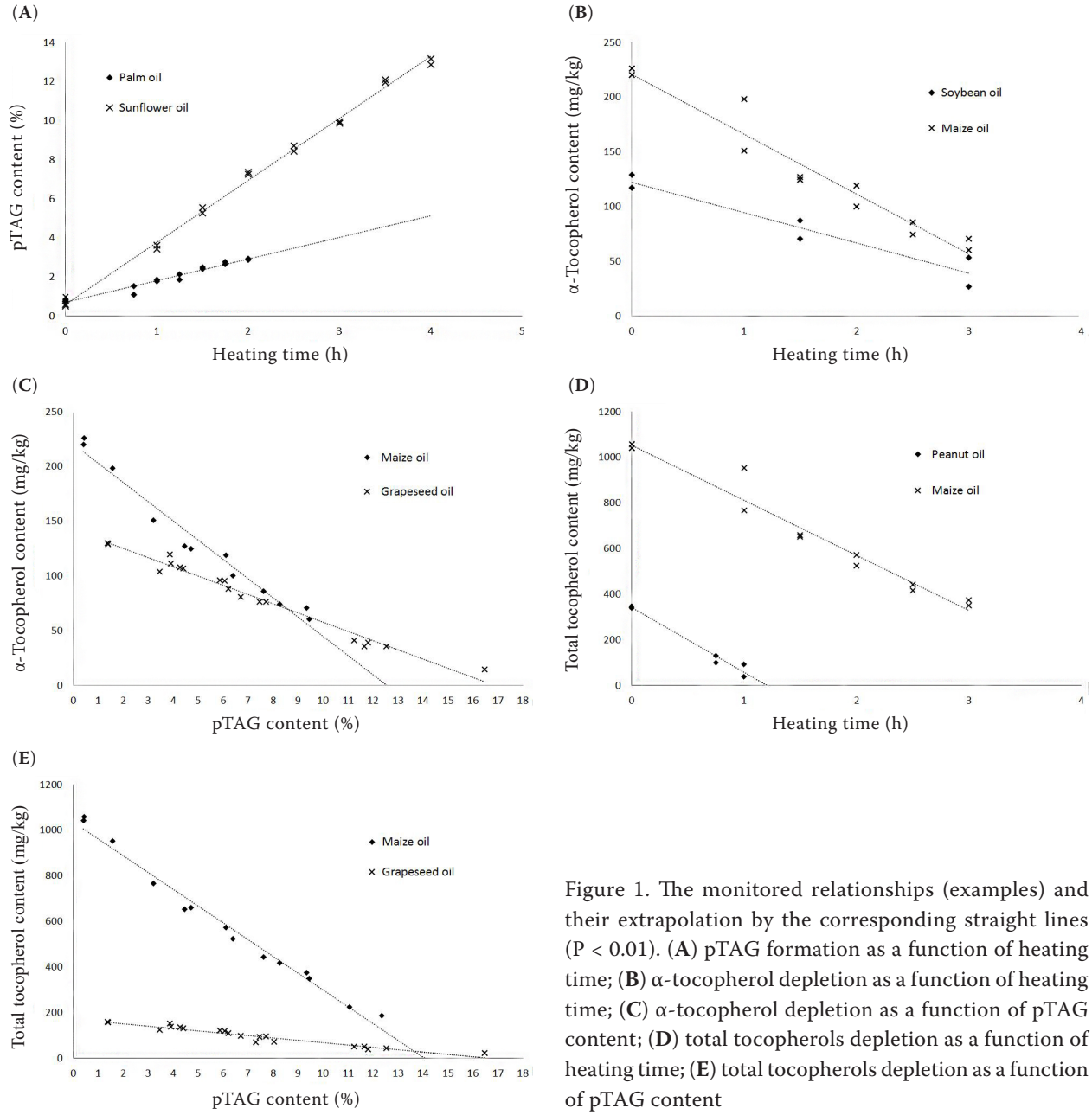

Figure 1. The monitored relationships (examples) and their extrapolation by the corresponding straight lines ($P < 0.01$). (**A**) pTAG formation as a function of heating time; (**B**) α-tocopherol depletion as a function of heating time; (**C**) α-tocopherol depletion as a function of pTAG content; (**D**) total tocopherols depletion as a function of heating time; (**E**) total tocopherols depletion as a function of pTAG content

For the determination of tocopherols, oil samples (about 2.5 g) were placed in a 25-ml volumetric flask and the volume was filled to the mark with acetone.

Polymerised triacylglycerols. The pTAG were determined by high-performance size-exclusion chromatography (HP-SEC), with refractometric detection (ISO 2009) under the following conditions: tetrahydrofuran mobile phase; 0.6 ml/min flow rate (LCP 4000.11 pump; Ecom, Czech Republic); 5 µl injection volume; PL-gel Mixed-E column (300 × 7.5 mm, 3 µm particle size; Agilent Technologies, USA); autosampler (HP 1050; Hewlett-Packard, USA), and refractometric detector (HP 1047A; Hewlett Packard, USA), temperature 30°C. The quantification was made by internal normalisation.

For the determination of pTAG, each oil sample was separately dissolved in tetrahydrofuran so that 1 ml of the resulting solution contained approximately 50 to 70 mg of oil. The resulting solution was dried by using anhydrous sodium sulphate and then analysed by HP-SEC.

Data processing. All calculations and statistical analysis of the obtained data were performed using Microsoft Excel 2010. All the monitored relationships

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(i.e., pTAG formation as a function of heating time, α-tocopherol depletion as a function of heating time, α-tocopherol depletion as a function of pTAG content, total tocopherols depletion as a function of heating time and total tocopherols depletion as a function of pTAG content) were replaced by the corresponding straight lines ($P < 0.01$; Figure 1). The average rates of pTAG formation and tocopherol depletion were calculated as directives of these regression lines, whereas the rates of tocopherol depletion were expressed for both α-tocopherol and total tocopherols in four different ways: depletion rate in mg/kg as a function of heating time, depletion rate in mg/ kg as a function of pTAG content, depletion rate in % of the original content as a function of heating time and depletion rate in % of the original content as a function of pTAG content. Differences between the rates of pTAG formation (tocopherol depletion) in various vegetable oils were tested using the Student's *t*-test ($P < 0.05$), and the relationship between the composition of the studied oils and the rate of pTAG formation (tocopherol depletion) was evaluated by Pearson's correlation coefficients (r; $P < 0.05$) (Eckschlager *et al.* 1980). The total tocopherols and α-tocopherol contents of each vegetable oil at 6 and 12% pTAG content were calculated using the regression dependencies (lines) described above.

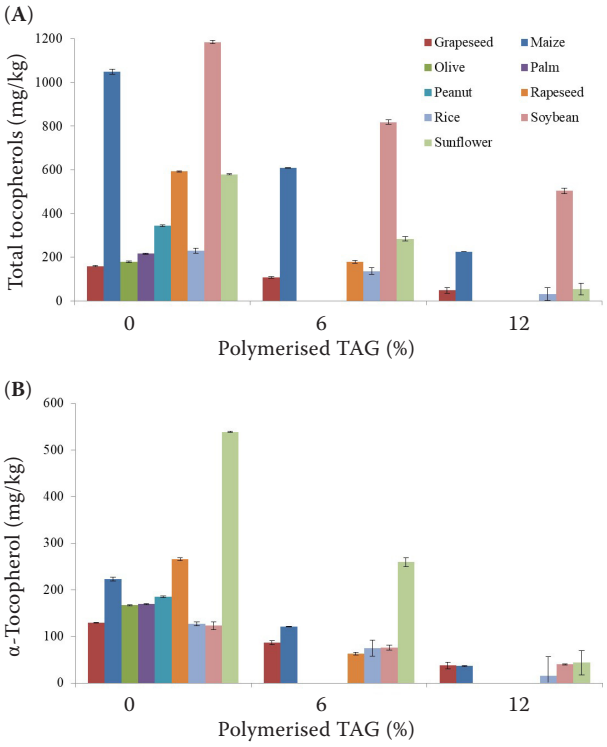

Figure 2. The content of total tocopherols (**A**) and α-tocopherol (**B**) at different level of frying oil lifespan (error bars are expressed as a standard deviation)

RESULTS AND DISCUSSION

Internal factors affecting the rate of pTAG formation and the rate of tocopherols depletion. Table 3 provides the rates of the increase of pTAG content and tocopherols depletion (expressed in various ways) found during heating of the studied vegetable oils at 180°C. Table 4 summarises the correlation coefficients, identifying the relationship between these rates and selected parameters characterising the composition of the studied vegetable oils. As shown in Table 4, the rate of pTAG formation increases with the linoleic acid content, i.e., the system unsaturation. The rate of tocopherols depletion then generally decreases with the system unsaturation, as well as the antioxidant capacity and probably (at least in some instances) increases with the peroxide value. These results fundamentally concur with previous findings (see below), although (as noted in the introduction) tocopherols depletion expressed as a function of pTAG content has not been studied in detail.

In pTAG, the potential relationship betweenthe rate of formation of these fatty acid reaction products and the degree of unsaturation of the tested fats and oils has been observed, particularly in experiments performed with TAG (triacylglycerols) isolated from different fats and oils (Steel *et al.* 2005; Marmesat *et al.* 2012). For real fats and oils, the rate of pTAG formation is likely to be affected by other factors (especially those present in the unsaponifiable fraction of fats and oils (Gertz *et al.* 2000; Barrera-Arellano *et al.* 2002), although the effect of these factors was not evidenced by the correlation analysis in this study. However, the influence of other factors on the rate of pTAG formation was confirmed in some studies conducted on real fats and oils, where no statistically significant relationship between the degree of unsaturation of these fats and oils and the rate of pTAG formation was described (Gertz *et al.* 2000; Barrera-Arellano *et al.* 2002).

Faster tocopherols depletion (expressed as a function of time) in more saturated oils (than in unsaturated oils) has been reported in a relatively large number

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Table 3. Rates of tocopherols depletion and polymerised triacylglycerols (pTAG) formation during heating of plant oils at an oil temperature of 180°C

Plant oil	pTAG (%/h)	α-T	Total tocopherols	α-T	Total tocopherols	α-T	Total tocopherols	α-T	Total tocopherols
		(mg/kg per h)		(% IC/h)		(mg/kg per % pTAG)		(% IC per % pTAG)	
Olive	2.2 ± 0.2^{de}	175.2 ± 33.7^{a}	187.0 ± 34.3^{ae}	105.0 ± 20.2^{a}	1.104.4 ± 19.1^{a}	118.0 ± 42.1^{ab}	126.1 ± 44.1^{ab}	70.7 ± 25.2^{a}	70.4 ± 24.4^{a}
Palm	1.1 ± 0.1^{a}	79.2 ± 7.9^{b}	102.7 ± 9.1^{b}	46.8 ± 4.7^{b}	47.5 ± 4.2^{b}	74.0 ± 4.0^{a}	95.6 ± 5.5^{a}	43.7 ± 2.4^{a}	44.2 ± 2.5^{a}
Peanut	2.0 ± 0.2^{cd}	184.5 ± 27.9^{a}	230.2 ± 27.5^{a}	99.7 ± 15.1^{a}	65.1 ± 8.5^{c}	136.1 ± 37.6^{b}	195.3 ± 31.2^{b}	73.6 ± 21.2^{a}	56.4 ± 8.7^{a}
Rapeseed	1.5 ± 0.1^{b}	48.9 ± 10.0^{c}	102.8 ± 15.7^{b}	8.4 ± 3.2^{ce}	17.3 ± 2.6^{d}	31.1 ± 5.1^{c}	65.4 ± 8.8^{c}	11.7 ± 1.9^{b}	11.0 ± 1.4^{b}
Sunflower	3.2 ± 0.1^{g}	114.0 ± 19.3^{d}	120.7 ± 20.3^{b}	21.2 ± 3.6^{ce}	20.4 ± 3.5^{d}	36.0 ± 5.6^{c}	38.1 ± 5.9^{d}	6.7 ± 1.0^{c}	6.6 ± 1.0^{cd}
Grapeseed	2.1 ± 0.1^{cd}	16.9 ± 1.0^{e}	20.4 ± 1.1^{c}	13.1 ± 0.8^{d}	13.0 ± 0.8^{e}	8.0 ± 0.6^{d}	9.7 ± 0.7^{e}	6.2 ± 0.5^{c}	6.2 ± 0.4^{d}
Rice	2.4 ± 0.3^{e}	26.0 ± 2.4^{f}	41.6 ± 3.0^{d}	20.5 ± 1.9^{c}	18.1 ± 1.4^{d}	10.5 ± 1.1^{e}	17.5 ± 1.8^{f}	8.3 ± 0.9^{d}	7.6 ± 0.8^{c}
Maize	2.8 ± 0.1^{f}	39.5 ± 4.5^{g}	179.3 ± 18.7^{e}	17.7 ± 2.0^{e}	17.2 ± 1.8^{d}	14.0 ± 1.3^{f}	63.8 ± 5.1^{c}	6.3 ± 0.6^{c}	6.1 ± 0.5^{d}
Soybean	1.9 ± 0.2^{c}	12.1 ± 1.3^{h}	105.5 ± 7.8^{b}	9.8 ± 1.0^{f}	8.9 ± 0.7^{f}	6.0 ± 0.7^{g}	52.8 ± 3.5^{g}	4.9 ± 0.5^{e}	4.5 ± 0.4^{e}

IC – initial content; α-T – α-tocopherol; results are expressed as average ± standard deviation; values with the same mark in the same column are not significantly different ($\alpha = 0.05$)

of researches (Yuki & Ishikawa 1976; Verleyen *et al.* 2001, 2002; Barrera-Arellano *et al*. 2002; Steel *et al.* 2005). For example, Verleyen *et al*. (2002) observed a faster relative α-tocopherol depletion in the purified TAG of high oleic sunflower oil and palm oil compared to α-tocopherol depletion in the TAG of classical sunflower oil and linseed oil during heating to 240°C. In work by the Spanish-Brazilian collective (Barrera-Arellano *et al.* 2002), six vegetable oils (palm, olive, high oleic sunflower, rapeseed, soybean and sunflower) were heated at 180°C. After 8 h of heating, almost no tocopherols were detected in olive, palm and high oleic sunflower oils, but in rapeseed, sunflower and soybean oils, the corresponding total tocopherols were 192, 258 and 601 mg/kg (Barrera-Arellano *et al.* 2002).

The phenomenon of faster tocopherols depletion in more saturated fats and oils (compared to unsaturated fats and oils) during heating at about 180°C and higher temperatures has not yet been sufficiently explained. At low temperatures, typical for food storage conditions and moderate temperatures during oxidation-accelerated tests, typically using the Rancimat or Oxipres apparatus (Méndez *et al.* 1996; Sabolová *et al.* 2017), tocopherols depletion is faster in more unsaturated oils (Marmesat *et al.* 2010). This behaviour is probably due mainly to the selective oxidation of tocopherols by peroxyl radicals, which are formed to a greater extent by more reactive unsaturated fatty acids.

At temperatures above about 180°C, hydroperoxides are more likely to decompose into hydroxyl and alkoxy radicals, which (unlike peroxyl radicals) do not selectively react with tocopherols (antioxidants) and consequently, the reactions of these radicals with unsaturated fatty acids can predominate (Verleyen *et al.* 2001, 2002). However, this mechanism does not sufficiently explain the slower depletion of tocopherols in unsaturated oils at higher temperatures, considering the formation of hydroperoxides must always comprise the formation of peroxyl radicals, which react highly selectively with tocopherols and other antioxidants, due to the slow reaction rate of these radicals with unoxidised fatty acids (Kamal-Eldin & Appelqvist 1996).

The faster depletion of tocopherols as a function of pTAG content in less unsaturated oils results from the dependencies discussed above and has also been observed by Réblová *et al.* (2009). If pTAG are produced faster in more unsaturated oils, and (at the same time) tocopherols decrease slower

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Table 4. Pearson's correlation coefficients characterising relationship between rate of polymerised TAG (pTAG) formation (tocopherols depletion) and parameters describing composition of studied vegetable oils

	pTAG (%/h)	α-T (mg/kg per h)	Total tocopherols (mg/kg per h)	α-T (% IC per h)	Total tocopherols (% IC per h)	α-T (mg/kg per % pTAG)	Total tocopherols (mg/kg per % pTAG)	α-T (% IC per % pTAG)	Total tocopherols (% IC per % pTAG)
Total tocopherols	0.258	–0.390	0.202	–0.470	–0.463	–0.458	–0.158	–0.488	–0.514
α-T	0.538	0.257	0.152	–0.150	–0.127	–0.032	–0.113	–0.210	–0.215
ß + γ-T	0.121	–0.461	0.195	–0.410	–0.409	–0.440	–0.107	–0.416	–0.443
δ-T	–0.124	–0.392	–0.065	–0.325	–0.331	–0.336	–0.133	–0.290	–0.296
AOC	0.073	–0.788[b]	–0.376	–0.750[b]	–0.749[b]	–0.755[b]	–0.563	–0.715[a]	–0.703[a]
Peroxide value	–0.040	0.381	0.136	0.542	0.699 [a]	0.384	0.212	0.458	0.522
SFA	–0.468	–0.012	–0.129	0.134	0.087	0.173	0.100	0.255	0.323
Oleic acid	–0.396	0.710[a]	0.583	0.806[b]	0.765[b]	0.813[b]	0.786[b]	0.812[b]	0.797[b]
Linoleic acid	0.603[a]	–0.569	–0.446	–0.715[a]	–0.663[a]	–0.744[b]	–0.721[a]	–0.774[b]	–0.786[b]
Iodine value	0.539	–0.537	–0.347	–0.695[a]	–0.635[a]	–0.721[a]	–0.636[a]	–0.773[b]	–0.805[b]

AOC – antioxidant capacity; IC – initial content; SFA – saturated fatty acids; α-T – α-tocopherol; ß + γ-T – ß- and γ-tocopherol; δ-T – δ-tocopherol; [a]$P < 0.05$; [b]$P < 0.01$. No significant correlation was observed between studied rates and monounsaturated fatty acid content, polyunsaturated fatty acid content, α-linolenic acid content, stearic acid content and palmitic acid content

in such oils (see earlier), tocopherols depletion (expressed as a function of pTAG content) have to be slower in more unsaturated oils than in saturated fats. Concurrently, the tocopherols depletion shown as a function of the pTAG content is more dependent on the degree of unsaturation of frying fats and oils than tocopherols depletion shown as a function of time. This behaviour is apparent from Table 3, wherein the rates of tocopherols depletion expressed in various ways as a function of time are 3.6–5 times higher in palm oil (with the lowest iodine value of 64) than in grapeseed oil, which has the highest iodine value (i.e., 137). However, the rates of tocopherols depletion expressed as a function of pTAG content are 7–10-fold higher in palm oil than in grapeseed oil. Réblová *et al.* (2009) evaluated the total tocopherols, α-tocopherol and vitamin E depletion as a function of pTAG content during the heating of olive, sunflower, rapeseed and soybean oils at 180°C. It was found that total tocopherols, α-tocopherol and vitamin E in olive oil disappeared at a much lower content of pTAG than in rapeseed, sunflower and soybean oils , although (due to the small number of vegetable oils studied) the results obtained (unlike the article presented here) could not be statistically processed (Réblová *et al.* 2009).

The observed negative relationship between the rate of tocopherols depletion and the antioxidant capacity of unheated oils indicates the protective effect of some antioxidants naturally present in the studied oils against tocopherols. The ability to protect tocopherols (primarily α-tocopherol) has already been described for numerous antioxidants (Kajimoto *et al.* 1988a; Zhang *et al.* 2001; Raneva *et al.* 2002) under various conditions, including frying or heating of vegetable oils at frying temperatures (Kajimoto *et al.* 1988b, 1991, 1992; Tomaino *et al.* 2005; Réblová *et al.* 2012). However, some substances can protect tocopherols but do not protect fatty acids under the given conditions (Psomiadou & Tsimidou 2002; Réblová & Okrouhlá 2010). For example, Réblová and Okrouhlá (2010) noted that gallic, caffeic and gentisic acid had the ability to protect α-tocopherol during heating of sunflower oil at 180°C but, simultaneously, did not slow (or only slightly) the formation of pTAG. For example, polyphenols of olive oil (i.e., hydroxytyrosol and others) (Pellegrini *et al.* 2001; Mateos *et al.* 2003) or procyanidins of grapeseed oil (Lourenço *et al.* 2008) can be considered as the substances naturally present in the studied oils with the ability to protect tocopherols.

Finally, the ability of hydroperoxides to initiate tocopherols losses results from the general mechanism of lipid oxidation (Yanishlieva-Maslarova 2001) and has been experimentally demonstrated (Yamauchi *et al.* 1998, 2002). The main mechanism involved is the decomposition of hydroperoxides to the corresponding radicals

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and the reaction of these radicals with α-tocopherol (Yamauchi *et al.* 1998, 2002; Yanishlieva-Maslarova 2001).

Practical impacts of observed relationships. As mentioned above, investigating the association between the formation of pTAG (or total polar compounds), which restrict the lifespan of fats and oils during repeated frying (DGF 2000), and the tocopherols depletion has significant practical implications. The expression of tocopherols (vitamin E) depletion as a function of pTAG content allows determining (or at least estimate) the content of tocopherols (vitamin E) at different stages during the lifespan of the frying bath, typically at the end of the lifespan, given by pTAG of 12% or total polar content of 24% (DGF 2000). The respective total tocopherols and α-tocopherol contents in the various vegetable oils heated at 180°C and the pTAG level of 6 and 12% (i.e., half or end of the frying bath lifespan, respectively) are illustrated in Figure 2, together with the initial contents of the monitored analytes in the tested vegetable oils.

In Figure 2, no tocopherols existed in olive, peanut and palm oils, at half of the lifespan of the individual oils, i.e., at 6% pTAG. In contrast, in the maize, sunflower, grapeseed, rice and, primarily, soybean oil, tocopherols were identified at the end of the lifespan of these oils, i.e., at 12% pTAG (Figure 2). Olive, palm and peanut oils had the lowest iodine values (Table 2), peanut and olive (commercial blend of refined and virgin olive oil) oils had the lowest antioxidant capacities, and the olive oil had the highest peroxide value (Table 1). The combination of these factors resulted in the slowest pTAG formation in palm oil (among the tested oils) and, in the olive and peanut oils, the fastest tocopherols depletion (expressed as a function of heating time). Consequently, there was a considerably more rapid rate of tocopherols depletion (expressed as a function of the pTAG content) in all three mentioned oils than in the other studied oils (Table 3).

From the dependencies found in this study between the formation of pTAG and tocopherols depletion it can also be concluded that the content of tocopherols (vitamin E) can be significantly influenced by the fats or oils used in frying and, furthermore, will be generally lower in foods fried in fats or oils with a higher degree of saturation and/or a lower initial content of antioxidants. This assumption has been confirmed in some studies (Eitenmiller & Lee 2004d; Sabolová *et al.* 2018). For example, Sabolová *et al.* (2018) documented that vitamin E content in α-tocopherol equivalents (as well as α-tocopherol) in commercially prepared French fries and potato chips fried in palm oil was typically lower than in the above products fried in rapeseed or sunflower oils (Sabolová *et al.* 2018).

Consumption of fried foods should be as limited as possible, as these foods can contain high amount of fat (often with unsuitable fatty acids composition) and salt and also can contain some processing contaminants, for example acrylamide or 3-MPCD (Boskou & Andrikopoulos 2011). At the same time, however, in view of the generally high and rising consumption of fried foods, it is necessary to search for ways to ensure high nutritional quality and safety of these products, including efforts to increase the vitamin E content of these foods. If we consider ways to increase vitamin E content in fried foods, several solutions are offered (regarding the results of this study). These include: (i) to prefer frying in more unsaturated oils (e.g., in sunflower, corn or soybean oils) (White 2008); (ii) to prefer frying in oils with a higher initial natural content of antioxidants (i.e., especially in unrefined oils (Matthäus & Spener 2008)); (iii) to enhance frying oils with antioxidants capable of protecting tocopherols, especially those that do not also protect the fatty acids (i.e., do not slow down the formation of pTAG), and (iv) to change the frying bath medium at the lower content of pTAG (or total polar compounds) than presently used in the literature (i.e., 12 and 24% (DGF 2000)), especially for oils with a higher degree of saturation and/or a lower initial antioxidant content, such as olive, peanut and palm oils, where all tocopherols already disappeared at the content of pTAG 1.7, 2.7 and 3.1%, respectively, in this study. However, it is a question of which of these possible ways would be the most appropriate in practice, namely, at least problematic.

A relatively easy solution seems to be the addition of antioxidants that can protect tocopherols while not also protecting fatty acids. However, it is necessary to consider an overall negative attitude of ordinary consumers towards food additives (Bearth *et al.* 2014). A substantial use of unrefined oils as a frying medium also appears as a simple solution. However, the use of virgin oils for pan-frying and repeated frying is not typically recommended (Matthäus & Spener 2008). Food frying in more unsaturated oils is appropriate given the typical flavour of fried foods is derived from linoleic acid (Warner 2009). However, more than 3% linolenic acid is un-

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desirable (Warner 2009). Therefore, frying in more unsaturated oils (containing predominantly linoleic acid) may worsen the unfavourable intake ratio of *n*-6 and *n*-3 fatty acids (Simopoulos 2002). The decrease in the limit of the pTAG content (or total polar compounds) in the frying bath (probably selectively for different oils, depending on a degree of saturation) seems to be a highly suitable solution. However, this solution would be organisationally exceptionally demanding and would increase the cost of the frying medium. It is also necessary to realise that the existing limits (i.e., 12% for pTAGand 24% for total polar compounds) have not yet been fully enforced in practice (Dobarganes & Márquez-Ruíz 1995; Andrikopoulos *et al.* 2003; Mekhanoshina & Réblová 2016).

CONCLUSIONS

The results of this work showed that rate of tocopherols depletion (as a function of pTAG content) decreases with increasing unsaturation and with total antioxidant capacity of system. Although this study has clarified the reasons for the low content of tocopherols (vitamin E) in some fried foods and has suggested possible ways to solve this underestimated nutritional problem, definitive conclusions in this regard must provide wider discussions beyond this study. However, the obtained results confirm the need to reduce the intake of fried foods, as well as the need for more regulation of their production.

References

Aladedunye F., Przybylski R. (2013): Frying stability of high oleic sunflower oils as affected by composition of tocopherol isomers and linoleic acid content. Food Chemistry, 141: 2373–2378.

Andrikopoulos N.K, Boskou G., Dedoussis G.V.Z., Chiou A., Tzamtzis V.A., Papathanasiou A. (2003): Quality assessment of frying oils and fats from 63 restaurants in Athens, Greece. Food Service Technology, 3: 49–59.

Barrera-Arellano D., Ruiz-Méndez V., Velasco J., Márquez-Ruiz G., Dobarganes C. (2002): Loss of tocopherols and formation of degradation compounds at frying temperatures in oils differing in degree of unsaturation and natural antioxidant content. Journal of the Science of Food and Agriculture, 82: 1696–1702.

Bearth A., Cousin M.E., Siegrist M. (2014): The consumer's perception of artificial food additives: Influences on acceptance, risk and benefit perceptions. Food Quality and Preference, 38: 14–23.

Boskou G., Andrikopoulos K.N. (2011): Food hazards associated with frying In: Boskou D., Elmadfa I. (eds): Frying of Food: Oxidation, Nutrient and Non-Nutrient Antioxidants, Biologically Active Compounds, and High Temperatures. Boca Raton, CRC Press: 267–288.

Chiou A., Kalogeropoulos N., Salta F.N., Efstathiou P., Andrikopoulos N.K. (2009): Pan-frying of French fries in three different edible oils enriched with olive leaf extract: Oxidative stability and fate of microconstituents. LWT-Food Science and Technology, 42: 1090–1097.

Dobarganes M.C., Márquez-Ruíz G. (1995): Calidad de las grasas de fritura en el sector de restauración de Andalucía. Grasas y Aceites, 46: 115–120.

Eckschlager K., Horsák I., Kodejš Z. (1980): Vyhodnocování analytických výsledků a metod. Prague, SNTL.

Eitenmiller R., Lee J. (2004a): Nutrition and health implication of vitamin E. In: Eitenmiller R., Lee J. (eds): Vitamin E: Food Chemistry, Composition, and Analysis. New York, CRC Press: 39–88.

Eitenmiller R., Lee J. (2004b): Vitamin E: chemistry and biochemistry. In: Eitenmiller R., Lee J. (eds): Vitamin E: Food Chemistry, Composition, and Analysis. New York, CRC Press: 1–38.

Eitenmiller R., Lee J. (2004c): Effect of food preparation and storage on the vitamin E content of food. In: Eitenmiller R., Lee J. (eds): Vitamin E, Food Chemistry, Composition, and Analysis. New York, CRC Press: 231–284.

Eitenmiller R., Lee J. (2004d): Analysis of tocopherols and tocotrienols in foods. In: Eitenmiller R., Lee J. (eds): Vitamin E: Food Chemistry, Composition, and Analysis. New York, CRC Press: 285–322.

Eitenmiller R., Lee J. (2004e): Food composition–vitamin E. In: Eitenmiller R., Lee J. (eds): Vitamin E: Food Chemistry, Composition, and Analysis. New York, CRC Press: 425–505.

Espín J.C., Soler-Rivas C., Wichers H.J. (2000): Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-picrylhydrazyl radical. Journal of Agricultural and Food Chemistry, 48: 648–656.

Fišnar J., Doležal M., Réblová Z. (2014): Tocopherol losses during pan-frying. European Journal of Lipid Science and Technology, 116: 1694–1700.

Gertz C., Klostermann S., Kochhar S.P. (2000): Testing and comparing oxidative stability of vegetable oils and fats at frying temperature. European Journal of Lipid Science and Technology, 102: 543–551.

https://doi.org/10.17221/73/2018-CJFS

Kajimoto G., Takaoka M., Yoshida H., Shibahara A. (1988a): Decomposition of tocopherol in oils by oxidized products during autooxidation, and the preventive effects of gallic acid. Journal of Japan Society of Nutrition and Food Sciences, 41: 405–410.

Kajimoto G., Okajima N., Takaoka M., Yoshida H., Shibahara A. (1988b): Effects of catechins on thermal decomposition of tocopherol in heated oils. Journal of Japan Society of Nutrition and Food Sciences, 41: 213–218.

Kajimoto G., Shibata M., Kanomi Y., Yoshida H., Shibahara A. (1991): Effects of mixtures of gallic acid, soya lecithin or catechin and ascorbic acid on the thermal decomposition of tocopherol in vegetable oils. Journal of Japan Society of Nutrition and Food Sciences, 44: 45–50.

Kajimoto G., Kanomi Y., Kawakami H., Hamatani M., Maeda H. (1992): Effects of antioxidants on the thermal oxidation of oils and decomposition of tocopherol in vegetable oils. Journal of Japan Society of Nutrition and Food Sciences, 45: 291–295.

Kalogeropoulos N., Chiou A., Mylona A., Ioannou M.S., Andrikopoulos N.K. (2007a): Recovery and distribution of natural antioxidants (α-tocopherol, polyphenols and terpenic acids) after pan-frying of Mediterranean finfish in virgin olive oil. Food Chemistry, 100: 509–517.

Kalogeropoulos N., Mylona A., Chiou A., Ioannou M.S., Andrikopoulos N.K. (2007b): Retention and distribution of natural antioxidants (α-tocopherol, polyphenols and terpenic acids) after shallow frying of vegetables in virgin olive oil. LWT-Food Science and Technology, 40: 1008–1017.

Kamal-Eldin A., Appelqvist L.Å. (1996): The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids, 31: 671–701.

Lourenço C.F., Gago B., Barbosa R.M., de Freitas V., Laranjinha J. (2008): LDL isolated from plasma-loaded red wine procyanidins resist lipid oxidation and tocopherol depletion. Journal of Agricultural and Food Chemistry, 56: 3798–3804.

Maras J.E., Bermudez O.I., Qiao N., Bakun P.J., Boody-Alter E.L., Tucker K.L. (2004): Intake of α-tocopherol is limited among US adults. Journal of the American Dietetic Association, 104: 567–575.

Marmesat S., Morales A., Velasco J., Dobarganes M.C. (2010): Action and fate of natural and synthetic antioxidants during frying. Grasas y Aceites, 61: 333–340.

Marmesat S., Morales A., Velasco J., Dobarganes M.C. (2012): Influence of fatty acid composition on chemical changes in blends of sunflower oils during thermoxidation and frying. Food Chemistry, 135: 2333–2339.

Mateos R., Domínguez M.M., Espartero J.L., Cert A. (2003): Antioxidant effect of phenolic compounds, α-tocopherol, and other minor components in virgin olive oil. Journal of Agricultural and Food Chemistry, 51: 7170–7175.

Matthäus B., Spener F. (2008): What we know and what we should know about virgin oils – a general introduction. European Journal of Lipid Science and Technology, 110: 597–601.

Mekhanoshina L., Réblová Z. (2016): Content of polymerised triacylglycerols in fat of fried foods. Czech Journal of Food Sciences, 34: 211–216.

Méndez E., Sanhueza J., Speisky H., Valenzuela A. (1996): Validation of the Rancimat test for the assessment of the relative stability of fish oils. Journal of the American Oil Chemists' Society, 73: 1033–1037.

Normand L., Eskin N.A.M., Przybylski R. (2001): Effect of tocopherols on the frying stability of regular and modified canola oils. Journal of the American Oil Chemists' Society, 78: 369–373.

Pellegrini N., Visioli F., Buratti S., Brighenti F. (2001): Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating. Journal of Agricultural and Food Chemistry, 49: 2532–2538.

Piironen V., Varo P., Koivistoinen P. (1987): Stability of tocopherols and tocotrienols in food preparation procedures. Journal of Food Composition and Analysis, 1: 53–58.

Psomiadou E., Tsimidou M. (2002): Stability of virgin olive oil. 2. Photo-oxidation studies. Journal of Agricultural and Food Chemistry, 50: 722–727.

Raneva V., Shimasaki H., Furukawa Y., Ueta N., Yanishlieva N., Aaseng J.E., Partali V., Sliwka H.R., Yoshida Y., Niki E. (2002): Action of 1-(11-selenadodecyl)-glycerol and 1-(11-selenadodecyl)-3-trolox-glycerol against lipid peroxidation. Lipids, 37: 633–640.

Réblová Z., Tichovská D., Doležal M. (2009): Heating of plant oils – fatty acid reactions versus tocopherols degradation. Czech Journal of Food Sciences, 27: S185–S187.

Réblová Z., Okrouhlá P. (2010): Ability of phenolic acids to protect α-tocopherol. Czech Journal of Food Sciences, 28: 290–297.

Réblová Z., Fišnar J., Tichovská D., Doležal M., Joudalová K. (2012): Effect of temperature and oil composition on the ability of phenolic acids to protect naturally present α-tocopherol during the heating of plant oils. Czech Journal of Food Sciences, 30: 351–357.

Rodríguez-Palmero M., Castellote-Bargalló A.I., López-Sabater C., de la Torre-Boronat C., Rivero-Urgell M. (1998): Assessment of dietary nutrient intakes: Analysed vs calculated values. Food Chemistry, 61: 215–221.

Sabolová M., Johanidesová A., Hasalíková E., Fišnar J., Doležal M., Réblová Z. (2017): Relationship between the composition of fats and oils and their oxidative stability at different temperatures, determined using the Oxipres

https://doi.org/10.17221/73/2018-CJFS

apparatus. European Journal of Lipid Science and Technology, 119: 1600454. doi.org/10.1002/ejlt.201600454

Sabolová M., Czornyj Š., Fišnar J., Doležal M., Sosnová M., Matějková K., Réblová Z. (2018): Tocochromanol content in commercially prepared fried foods. Czech Journal of Food Sciences, 36: 392–402.

Simopoulos A.P. (2002): The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedicine & Pharmacotheraphy, 56: 365–379.

Steel C.J., Dobarganes M.C., Barrera-Arellano D. (2005): The influence of natural tocopherols during thermal oxidation of refined and partially hydrogenated soybean oils. Grasas y Aceites, 56: 46–52.

Steinhart H., Rathjen T. (2003): Dependence of tocopherol stability on different cooking procedures of food. International Journal for Vitamin and Nutrition Research, 73: 144–151.

Tomaino A., Cimino F., Zimbalatti V., Venuti V., Sulfaro V., De Pasquale A., Saija A. (2005): Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. Food Chemistry, 89: 549–554.

Trojáková L. (2001): Study of selected natural antioxidants [Doctoral thesis]. Institute of Chemical Technology Prague, Prague.

Verleyen T., Kamal-Eldin A., Dobarganes C., Verhe R., Dewettinck K., Huyghebaert A. (2001): Modeling of α-tocopherol loss and oxidation products formed during thermoxidation in triolein and tripalmitin mixtures. Lipids, 36: 719–726.

Verleyen T., Kamal-Eldin A., Mozuraityte R., Verhé R., Dewettinck K., Huyghebaert A., De Greyt W. (2002): Oxidation at elevated temperatures: Competition between α-tocopherol and unsaturated triacylglycerols. European Journal of Lipid Science and Technology, 104: 228–233.

Warner K. (2009): Flavor changes during frying. In: Sahin S., Sumnu S.G. (eds): Advances in Deep-Fat Frying of Foods. Boca Raton, CRC Press: 201–214.

White P.J. (2008): Fatty acids in oilseeds (vegetable oils). In: Chow C.K. (eds): Fatty Acids in Foods and their Health Implications. Boca Raton, CRC Press: 227–262.

Wyatt C.J., Carballido S.P., Méndez R.O. (1998): α- and γ-Tocopherol content of selected foods in the Mexican diet: Effect of cooking losses. Journal of Agricultural and Food Chemistry, 46: 4657–4661.

Yamauchi R., Goto K., Kato K. (1998): Reaction of α-tocopherol in heated bulk phase in the presence of methyl linoleate (13*S*)-hydroperoxide or methyl linoleate. Lipids, 33: 77–85.

Yamauchi R., Ozaki K., Shimoyamada M., Kato K. (2002): Iron-catalyzed reaction products of α-tocopherol with 1-palmitoyl-2-linoleoyl-3-*sn*-phosphatidylcholine (13*S*)-hydroperoxide. Chemistry and Physics of Lipids, 114: 193–201.

Yanishlieva-Maslarova N.V. (2001): Inhibiting oxidation. In: Pokorný J., Yanishlieva N., Gordon M.H. (eds): Antioxidants in Food: Practical Applications. Cambridge, Woodhead Publishing: 22–70.

Yuki E., Ishikawa Y. (1976): Tocopherol contents of nine vegetable frying oils, and their changes under simulated deep-fat frying conditions. Journal of the American Oil Chemists' Society, 53: 673–676.

Zamora R., Hidalgo F.J. (2015): fatty acids. In: Nollet L.M.L., Toldrá F. (eds): Handbook of Food Analysis. Boca Raton, CRC Press: 413–430.

Zhang Z., Chang Q., Zhu M., Huang Y., Ho W.K.K., Chen Z.Y. (2001): Characterization of antioxidants present in hawthorn fruits. The Journal of Nutritional Biochemistry, 12: 144–152.

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