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ORIGINAL PAPER

Effects of α-Tocopherol on Oxidative Stability and Phytosterol Oxidation During Heating in Some Regular and High-Oleic Vegetable Oils

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Abstract The first part of this study evaluated oxidative stability in high-oleic rapeseed oil, palm olein, refined olive oil, low erucic acid rapeseed oil and sunflower oil. The results showed oxidative stability in the order: palm olein > high-oleic rapeseed oil > refined olive oil > low erucic acid rapeseed oil > sunflower oil, as determined by the Rancimat method. Addition of α-tocopherol at high levels of up to 0.2% increased the oxidative stability of refined olive oil, whereas the opposite effect was generally observed in the other oil samples. In the second part of the study, high-oleic rapeseed oil, palm olein, refined olive oil and refined olive oil containing 0.2% α-tocopherol were heated for 3, 6, 9 and 12 h at 180 °C. The peroxide and *p*-anisidine values generally increased over time in the samples, including olive oil containing 0.2% α-tocopherol. High-oleic rapeseed oil contained the highest amount of total sterols and total phytosterol oxidation products (POPs), but during heating the total POPs content increased moderately (~10%), in contrast to the threefold increase after 12 h of heating in palm olein and refined olive oil. Very high levels of 6-hydroxy derivatives of brassicastanol, campestanol and sitostanol and of 7-ketobrassicasterol were observed in high-oleic rapeseed oil samples. Addition of 0.2% α-tocopherol during heating significantly decreased POPs formation in refined olive oil ($P < 0.05$).

Keywords Frying oil · Lipid oxidation · Oxidative stability · Phytosterols · Phytosterol oxidation products · POPs · PV · AV · α-Tocopherol · Vegetable oil

Introduction

Oxidative stability is an important factor in oil quality, and is particularly significant for oils used for frying because of the high temperature and long duration of the frying process. The chemistry of oxidation at high temperatures is very complex since both thermal and oxidative reactions are involved [1]. Frying oil must have high oxidative stability during long-term use. Each vegetable oil has a characteristic stability against oxidation depending on the fatty acid composition and the content and composition of antioxidants and other minor components [2, 3].

Polyunsaturated fatty acids are responsible for oxidation and off-flavours in fried food, and therefore to improve oxidative stability during deep-fat frying, the use of unsaturated oils should be avoided. However, from a nutritional point of view, it should be taken into account that oils with high amounts of saturated fatty acids and fats containing *trans* fatty acids are less desirable for good health than mono- or polyunsaturated fatty acids because of their correlation to chronic degenerative diseases. Therefore, good frying oil should be low in saturated fatty acids, low in linoleic acid, very low in linolenic acid and very high in oleic acid. New vegetable oils containing high levels of oleic acid would provide alternative and rather ideal frying oils considering the health and stability issues [4].

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Phytosterols, like other unsaturated fatty acids, are prone to oxidation, especially when subjected to heat treatment or long-term storage [5]. During the past decade, interest in safety evaluations of phytosterol oxidation products (POPs) and their metabolic absorption in studies performed in vivo and in vitro has increased, but data on their biological effects are still inadequate [6]. Studies on the formation and levels of POPs in vegetable oils are not numerous, but are steadily on the rise [5, 7–12]. Soupas et al. [13] showed that the interaction between lipid matrices and temperatures may have an effect on the stability of phytosterols in food. A study on the POPs contents of olive oil, peanut oil and maize oil heated at 180 °C for 0–2 h reported an increase in total POPs content during heating for olive oil, but a slight decrease in the levels of total POPs in peanut oil and maize oil [10].

Studies have been conducted on the antioxidant effects of tocopherols in vegetable oil stability, but the mechanism of the pro-oxidant activity of α-tocopherol at high concentrations is not clearly understood [14]. Reports concerning the effects of antioxidants on the formation of POPs under accelerated oxidation conditions, e.g. heating at a high temperature, are scarce [9, 15]. Oxidation of stigmasterol in purified triacylglycerols of sunflower oil has been investigated in the presence of different natural and synthetic antioxidants (α-tocopherol, ethanolic extracts of rosemary and green tea and butylated hydroxytoluene). Those authors demonstrated that of the antioxidants studied, α-tocopherol was the most effective at preventing stigmasterol oxidation, except for the formation of stigmastentriol [15]. In a recent study on the effects of adding 10% rosemary oil to extra virgin olive oil while heating at 180 °C for 6 h, the levels of individual and total POPs were found to be considerably lower than those for pure extra virgin olive oil [9].

Recently we showed that commercial French fries and potato crisps available in Sweden are prepared in frying oils dominated by saturated and monounsaturated fatty acids [16, 17]. The main aim of the present study was to identify frying oils, preferably monounsaturated fatty acid-dominated refined edible vegetable oils, that can minimise heat-induced lipid oxidation and particularly the formation of POPs during food preparation. Among the dominant monounsaturated vegetable oils, refined olive oil was of particular interest because of its potential as a more healthy frying oil. We investigated the effect of varying α-tocopherol concentration, in order to minimise lipid oxidation and increase oxidative stability, by Rancimat monitoring in some common and oleic acid-rich vegetable oils. In addition, some other criteria such as peroxide value (PV), *p*-anisidine value (*p*-AV) and formation of POPs were determined in some selected vegetable oils under heating at 180 °C for different time intervals.

Materials and Methods

Samples

Five different vegetable oils were used for the study: high-oleic rapeseed oil, palm olein, refined olive oil, low erucic acid rapeseed oil and sunflower oil, all of which were gifts from AarhusKarlshamns Sweden AB, (Karlshamn, Sweden).

Chemicals

α-Tocopherol was obtained from Sigma-Aldrich (Steinheim, Switzerland). The purity of tocopherol was at least 97%. Standard samples of sterols and 5α-cholestane were obtained from Steraloids (Newport, RI, USA); reference samples of tocopherols and tocotrienols (α, β, γ and δ) were obtained from Merck (Darmstadt, Germany); Tri-Sil reagent was from Pierce Chemical Co. (Rockford, IL, USA). The standard mixture of fatty acid methyl esters (FAME) F-07 was from Larodan Fine Chemicals AB (Malmö, Sweden). The solid-phase extraction (SPE) column (1 g silica, 6 mL) was from International Sorbent Technology Ltd. (Mid Glamorgan, UK). All solvents and reagents were either HPLC or pro-analysis grade and obtained from VWR International (Stockholm, Sweden), unless otherwise stated.

Oil Stability

The oxidative stabilities of all oil samples were determined by the Rancimat 679 (Metrohm KEBO Lab AB, Stockholm, Sweden). In brief, 2.5 g of vegetable oil were weighed into the reaction vessel in triplicate and heated to 110 °C with an air flow of 10 L/h. Volatile products released during the oxidation process were collected in a flask containing distilled water. The oxidation process was recorded automatically by measuring the change in conductivity of the distilled water due to the formation of volatile compounds. The oxidative stability index (OSI) is defined as the point of rapid change in the rate of oxidation, and the results are expressed in hours (h).

Analysis of Fatty Acid Methyl Esters

Fatty acid composition was determined following the method described previously with slight changes (16). In brief, ca. 10 mg of vegetable oils were methylated with 2 mL of 0.01 M NaOH in dry methanol and the sample tubes were heated in a water bath at 60 °C for 15 min under shaking. Thereafter, 2 mL of boron trifluoride in methanol were added to the tubes and heated again at 60 °C for 15 min. The tubes were cooled under running tap water, and 2 mL of 20% NaCl and 1 mL hexane were added. The tubes

were shaken vigorously and centrifuged briefly. The hexane layer containing FAME was transferred to glass tubes and stored at −20 °C until further GC analysis.

A Chrompack CP 9001 gas chromatograph equipped with a CP9050 autosampler, Maestro v.2.4 software and a flame ionisation detector (Chrompack, Middelburg, Netherlands) was used to analyze the FAME. The separation of the FAME was achieved with the BP X70 fused-silica capillary column (SGE, Austin Texas, USA) of length 50 m, i.d. 0.22 mm and film thickness 0.25 μm. One microliter of hexane containing FAME was injected in split injection mode at a split ratio of 25:1. The initial oven temperature was at 155 °C for 5 min, which was increased to 225 °C at a rate of 2 °C/min and then maintained at this temperature for 8 min. Helium was used as a carrier gas at a 200 kPa and nitrogen was used as a make-up gas at a flow rate of 30 mL/min. The injector and detector temperatures were 240 and 280 °C, respectively. Fatty acid methyl esters were identified by comparing their retention times with those of the standard FAME mixture F07. No efforts were made to identify fatty acids other than those reported in Table 1.

Analysis of Tocopherols by High-Pressure Liquid Chromatography (HPLC)

The indigenous tocopherol contents of the five different vegetable oils were analysed in triplicate with normal-phase HPLC according to the method described previously by Tabee et al. [16]. In brief, approximately 10 mg of the oil sample were dissolved in 1 mL *n*-heptane, and 10 μL were injected directly into the HPLC column. A 7725 Rheodyne injector fitted with a 20 μL loop and connected to a 510 HPLC pump (Waters, Milford, USA) was used. The column used was a LiChroCART 250-4 packed with LiChrosphere 100 NH_2, 5 μm in particle size, and coupled to a LiChroCART 4–4 guard column (Merck KGaA, Darmstadt, Germany). Tocopherols and tocotrienols were detected by a Varian 9070 fluorescence detector (Walnut Creek, CA, USA) at a wavelength of 294 nm for excitation and 320 nm for emission. The isocratic mobile phase was a mixture of *n*-heptane: *tert*-butyl methyl ether: tetrahydrofuran: methanol (79:20:0.98:0.02; v:v:v:v) at a flow rate of 1.0 mL/min. Each tocopherol and tocotrienol was quantified using an external standard method with reference samples of tocopherols. An HP 3396A integrator (Hewlett Packard, Avondale, PA, USA) was used to calculate the peak areas.

Enrichment with α-Tocopherol

Various amounts of α-tocopherol dissolved in hexane were added to the test oils containing the indigenous tocopherols (Table 2) in darkness, and the hexane was then evaporated under a stream of nitrogen. The amounts of α-tocopherol added varied within the range 50–2,000 mg/100 g oil.

Table 1 Fatty acid composition (%)[a] of vegetable oil samples

	HORO	RO	PO	ROO	SO
C14:0	0.0	0.0	1.0	0.0	0.1
C16:0	3.7	4.2	38.2	11.2	5.9
C18:0	2.3	1.7	4.3	2.8	3.6
C18:1	69.6	58.6	42.2	70.2	25.5
C18:2	11.2	19.0	11.2	9.3	61.5
C18:3	2.9	8.5	0.2	0.9	0.2
C20:0	0.9	0.6	0.4	0.4	0.3
C20:1	1.8	1.5	0.2	0.3	0.2
C22:0	0.5	0.4	0.1	0.1	0.8
P/S	1.9	4.0	0.3	0.7	5.8

HORO, high-oleic rapeseed oil; *RO*, low erucic acid rapeseed oil; *PO*, palm olein; *ROO*, refined olive oil; *SO*, sunflower oil; *P/S*, polyunsaturated fatty acid/saturated fatty acid ratio

[a] Mean of triplicate analyses (CV is <1%); figures that are <0.05% are shown as 0.0

Table 2 Contents of sterols, tocopherols and oxidative stability of the vegetable oils

	HORO	RO	PO	ROO	SO
Cholesterol (mg/100 g)[a]	3.0	2.6	1.6	1.6	ND
Brassicasterol (mg/100 g)[a]	91.3	71.8	ND	ND	ND
Campesterol (mg/100 g)[a]	219.9	275.0	14.6	13.6	28.9
Stigmasterol (mg/100 g)[a]	4.8	2.0	9.4	2.3	23.6
Sitosterol (mg/100 g)[a]	406.0	382.0	46.1	114.0	200.5
Δ^5-Avenasterol (mg/100 g)[a]	9.1	16.4	1.2	5.8	8.3
Total sterol (mg/100 g)[a]	734.1	749.8	72.9	137.3	261.3
α-Tocopherol (mg/100 g)[b]	28.5	24.6	14.2	14.2	51.3
β-Tocopherol (mg/100 g)[b]	ND	ND	ND	ND	1.7
γ-Tocopherol (mg/100 g)[b]	34.3	38.2	1.3	1.5	1.5
δ-Tocopherol (mg/100 g)[b]	0.4	ND	ND	ND	ND
Total tocopherol (mg/100 g)[b]	63.2	62.8	15.5	15.7	54.5
α-Tocotrienol (mg/100 g)[b]	ND	ND	15.3	ND	ND
γ-Tocotrienol (mg/100 g)[b]	ND	ND	19.4	ND	ND
δ-Tocotrienol (mg/100 g)[b]	ND	ND	3.7	ND	ND
Total tocotrienol (mg/100 g)[b]	ND	ND	38.4	ND	ND
Oxidative stability at 110 °C (h)[c]	18.8	8.5	27.6	17.5	5.1

HORO, high-oleic rapeseed oil; *RO*, low erucic acid rapeseed oil; *PO*, palm olein; *ROO*, refined olive oil; *SO*, sunflower oil; *ND*, not detected

[a] Mean of triplicate analyses (CV is generally <5%)

[b] Mean of triplicate analyses(CV is generally <3%)

[c] Mean of triplicate analyses (CV is generally <2%)

Sterol Content

The sterol content was determined by hot saponification of approximately 20 mg of the oil sample with 20 μg 5α-cholestane added as internal standard. Derivatization to trimethylsilyl (TMS) ether and details of the GC conditions have been described elsewhere [16].

Thermal Experiment

Four selected samples—high-oleic rapeseed oil, palm olein, refined olive oil and refined olive oil containing the indigenous tocopherols with an additional 0.2% α-tocopherol—were heated at 180 °C in a glycerin bath for a total time of 12 h. The temperature was checked using a thermometer during the experiment. The oils were heated at 180 ± 5 °C for 6 h on day 1, and oil samples were collected at 3 and 6 h, cooled, and stored at −20 °C for further analysis. The remaining oils were covered, allowed to cool, and left overnight for further heating. On the following day, the remaining oils were heated again at the same temperature, and the samples were collected after 9 and 12 h of total heating.

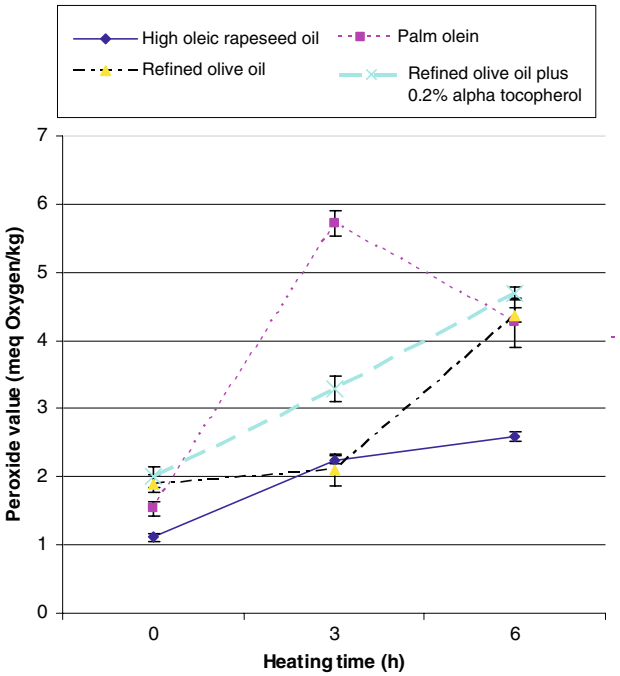

Fig. 1 Peroxide values in different vegetable oils during heating at 180 ± 5 °C for 0–6 h

Determination of Primary and Secondary Oxidation Products

Peroxide Value (PV) and P-anisidine Value (p-AV)

The method used to evaluate the PV in this study refers to International IDF Standard 74A, and the results (Fig. 1) are expressed as meq oxygen/kg oil [18]. The *p*-AV was determined following the standard method 2.504 of the International Union of Pure and Applied Chemistry (IUPAC) [19].

Transesterification for the Analysis of POPs

The transesterification of oil samples was carried out as the first step for further purification of the POPs in the oils according to the method described by Tabee et al. [16]. About 300–400 mg of each vegetable oil was weighed into a glass tube with a ground joint stopper, and 5 mL of 10% sodium methylate in dry methanol, diluted with *tert*-butyl methyl ether (4:6; v:v), were added. The sample was mixed by vortexing for 1 min and kept at room temperature for 25 min. The sample was again mixed for 1 min and kept for an additional 15 min. Thereafter, 2 mL water and 5 mL chloroform were added, and the sample was again mixed by vortexing for 1 min. The tube was centrifuged at 3,000 rpm for 3 min to facilitate phase separation and the upper phase was discarded. To neutralise excessive alkali, 3 mL of 1% citric acid in water were added and the sample was again mixed by vortexing. The tube was centrifuged at 3,000 rpm for 3 min, and again the upper phase was discarded. Finally, the residual chloroform was evaporated under nitrogen and the transesterified samples were dissolved in 1 mL of hexane:diethyl ether (9:1; v:v) prior to the SPE to enrich and purify the POPs.

Enrichment and Purification of POPs by SPE

The separation of POPs was carried out according to the method described by Azadmard-Damirchi and Dutta [20]. In brief, the transesterified sample was applied onto the SPE cartridge (1 g silica) previously conditioned with 5 mL hexane. To separate the apolar components and unoxidised phytosterols, the column was washed with 15 mL and 10 mL hexane: diethyl ether (9:1; v:v) and (1:1; v:v), respectively. Thereafter, POPs were eluted with 10 mL acetone. The internal standard, 5α-cholestane, was added, and the solvent was evaporated to dryness using nitrogen. Prior to GC and GC–MS analysis, the mixture was derivatized to trimethylsilyl (TMS) ethers as described elsewhere [16].

Analysis of POPs by GC

Analyses were performed with a model 6890 N GC (Agilent Technologies, Wilmington, DE, USA) equipped with a GC PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) and a flame ionization detector. A combination of two fused-silica capillary columns, a DB-5MS

(length 15 m, i.d. 0.18 mm and film thickness 0.18 μm) and a DB-35MS (length 10 m, i.d. 0.2 mm, and film thickness 0.33 μm) (J&W Scientific, Folsom, CA, USA), which were joined together by a universal press-fit connector (NTK Kemi, Uppsala, Sweden) were used. Injector and detector temperatures were 260 and 310 °C, respectively. Helium was used as a carrier gas and nitrogen as a make-up gas at flow rates of 0.7 and 30 mL/min, respectively. The derivatized sample was injected in splitless mode. The initial oven temperature was 60 °C for 1 min, and was programmed to increase at a rate of 50 °C per min to 290 °C and maintained for 5 min and then increase by 1 °C per min to 305 °C, where it was kept for 15 min. The TMS ether derivatives dissolved in hexane were injected in splitless mode. The peak areas were computed with Agilent ChemStation Rev. B.02.01 (Agilent Technologies, Wilmington, DE, USA), and quantification was performed relative to the 5α-cholestane [16].

Analysis of POPs by GC–MS

To identify the POPs, GC–MS analyses were performed on a GC8000 Top Series gas chromatograph (CE Instruments, Milan, Italy) coupled to a Voyager mass spectrometer (Finnigan, Manchester, UK) operated with Xcalibur v.1.2 (ThermoQuest, Manchester, UK). The samples were injected in splitless mode. The carrier gas helium was set at 180 kPa. POPs were determined with the same column system and conditions as used for the GC analysis described above. Mass spectra were recorded at an electron energy of 70 eV and the ion source temperature was 200 °C. Spectra were scanned in the range 40–700 *m/z*, and POPs were identified on the basis of the mass spectra data reported in the literature [7, 20, 21].

Statistical Analysis

The statistical analyses in this study comprised two parts: descriptive statistics in which means of triplicate values and standard deviations (SD) were calculated; and inferential statistics in which one-way ANOVA was used to compare differences between samples. Differences were considered significant at $P < 0.05$. The statistical analysis was carried out with SPSS 15.0 for Windows software.

Results and Discussion

Composition and Oxidative Stability of Fresh Oils

The fatty acid composition, polyunsaturated/saturated fatty acid ratios (*P/S*), sterol and tocopherol contents, and OSI values of all samples are presented in Tables 1 and 2. High-oleic rapeseed oil, palm olein and refined olive oil were chosen because they are low in saturated fatty acids and are good sources of oleic acid, which has health benefits. Low erucic acid rapeseed oil and sunflower oil were also chosen to compare their oxidative stabilities with other oils. The *P/S* ratio followed the order sunflower oil > low erucic acid rapeseed oil > high-oleic rapeseed oil > refined olive oil > palm olein.

Six different 4-desmethylsterols (cholesterol, brassicasterol, campesterol, stigmasterol, sitosterol and Δ^5-avenasterol) were quantified in the different samples. The dominant sterol was sitosterol, followed by campesterol. Brassicasterol was only found in the two rapeseed oils. Levels of total sterol varied considerably, ranging from 72.9 to 749.8 mg per 100 g oil. The lowest content of total sterols was found in palm olein, whereas the highest amount of total sterols was found in low erucic acid rapeseed oil. The oil samples differed in their individual and total tocopherol contents. All of the oil samples contained α- and γ-tocopherol, whereas the α, γ and δ forms of tocotrienol were found only in palm olein. Levels of α-tocopherol ranged from 14.2 to 51.3 mg per 100 g oil, while γ-tocopherol varied from 1.5 to 38.2 mg per 100 g oil. The highest and lowest contents of total tocopherols and tocotrienols were found in high-oleic rapeseed oil and refined olive oil, respectively.

When the *P/S* ratio was low, due to a low content of polyunsaturated fatty acids, the oil was less susceptible to oxidation, resulting in higher OSI values. Palm olein had the highest OSI value (about 28 h), while high-oleic rapeseed oil and refined olive oil showed rather similar oxidative stabilities of about 19 and 18 h, respectively. Although expected, the lowest OSI value (5.1 h) was obtained for sunflower oil (Table 2). Some of the results for oxidative stability contrast with those from a previous study [22], which showed that high-oleic rapeseed oil had OSI value 7.3 h and palm olein had OSI value 12 h compared with the present 18.8 and 27.6 h, respectively. However, the experimental conditions and the samples differed between these two studies. In another study on the frying stability of regular and high-oleic sunflower oils, it was demonstrated that despite completely different fatty acid profiles, the rates of formation of free fatty acids and of total polar compounds were not different between these two oils [23]. The authors suggested that minor components may be an important factor, in addition to the fatty acid profile, for predicting the frying stability of fats and oils.

Effect of α-Tocopherol on Oxidative Stability

The antioxidative potential of α-tocopherol present at different concentrations was also tested by the Rancimat method for the five different vegetable oils (Table 3). The

Table 3 Effects of the addition of α-tocopherol to different oils on oxidative stability expressed as (OSI)*

Addition of α-tocopherol (mg/100 g)	OSI (h)				
	HORO	RO	PO	ROO	SO
0	18.8 ± 0.66^{A}	8.5 ± 0.36^{A}	27.6 ± 0.21^{A}	17.5 ± 0.36^{A}	5.1 ± 0.01^{A}
50	17.0 ± 0.17^{B}	8.2 ± 0.20^{AB}	25.9 ± 0.26^{B}	17.6 ± 0.26^{A}	5.5 ± 0.04^{B}
100	15.9 ± 0.17^{BC}	7.6 ± 0.15^{BC}	25.1 ± 0.15^{C}	17.9 ± 0.26^{AB}	5.8 ± 0.15^{C}
200	14.9 ± 0.21^{C}	7.1 ± 0.08^{C}	24.8 ± 0.15^{C}	18.5 ± 0.36^{B}	5.9 ± 0.08^{C}
600	–	–	–	21.0 ± 0.25^{C}	–
1,000	–	–	–	22.3 ± 0.30^{D}	6.4 ± 0.08^{D}
1,500	–	–	–	22.5 ± 0.1^{DE}	6.3 ± 0.12^{D}
1,750	–	–	–	–	4.3 ± 0.17^{E}
2,000	–	–	–	23.1 ± 0.21^{E}	3.8 ± 0.1^{F}

All oils contain their initial tocopherols, as presented in Table 2

HORO, high-oleic rapeseed oil; *RO*, low erucic acid rapeseed oil; *PO*, palm olein; *ROO*, refined olive oil; *SO*, sunflower oil

* Mean of triplicate analyses ± SD

$^{A–F}$ OSI values within each column with different letters differ significantly ($P < 0.05$)

results showed that, in the presence of additional amounts of α-tocopherol (50–2,000 mg/100 g oil), oil stability was increased in refined olive oil, from 17.5 to 23.1 h. The statistical analysis revealed an approximately linear relationship between the addition of α-tocopherol and OSI ($P < 0.05$) in this sample (Table 3). Similar results were observed with the addition of α-tocopherol at concentrations between 10 and 200 mg per 100 g oil to cold pressed olive oil at 120 °C [24]. In contrast to the results obtained with refined olive oil, addition of α-tocopherol had a significant ($P < 0.05$) negative antioxidative effect on all of the other vegetable oils except sunflower oil. Refined olive oil contained by far the lowest amounts (16 mg/100 g oil) of total tocopherols and tocotrienols compared with other oils (Table 2). Therefore, the addition of α-tocopherol seemed to have had a pro-oxidant effect on oxidative stability in all of the samples except refined olive oil (Tables 2, 3). Since the OSI dropped when α-tocopherol was added to high-oleic rapeseed oil, low erucic acid rapeseed oil and palm olein, the addition of α-tocopherol was not continued above 200 mg per 100 g oil. A pro-oxidant effect has been suggested at higher levels, where α-tocopherol can either abstract hydrogen from an unsaturated fatty acid or from a fatty acid hydroperoxide, producing a fatty acid free radical and a fatty acid peroxy radical, respectively, instead of its antioxidative function (to form a more stable α-tocopherol radical) [14, 25]. To summarise the results presented here (Tables 2, 3) regarding the oxidative stabilities of the oil samples, two of the original five vegetable oils with OSI < 10 h (low erucic acid rapeseed oil and sunflower oil) were dropped from the study, and high-oleic rapeseed oil, palm olein, refined olive oil and refined olive oil with an additional 0.2% α-tocopherol were selected for further heating experiments.

α-Tocopherol and the Formation of Hydroperoxides

The PV values of the oil samples during six hours of heating at 180 °C are shown in Fig. 1. At the start (time 0) the PV varied between 1 and 2 meq oxygen/kg oil for all of the samples. Three hours after heating, the PV had increased to between 2.1 (in refined olive oil) and 5.7 meq oxygen/kg oil (in palm olein) (Fig. 1). During further heating (up to 6 h), the PV increased gradually in all of the samples except palm olein. The results showed that palm olein, which has more saturated and fewer monounsaturated fatty acids than refined olive oil and high-oleic rapeseed oil, decomposed its hydroperoxides after 3 h of heating at 180 °C, resulting in a decreased PV. It was also found that the addition of 0.2% α-tocopherol increased hydroperoxide formation after 3 h of heating in refined olive oil (Fig. 1). Based on these and some previously published results, it can be suggested that in addition to the concentrations of tocopherols and fatty acid profiles, other factors are involved in the formation of peroxides in oils during heating [25, 26].

α-Tocopherol and *p*-AV

During heating at 180 °C up to 12 h, the *p*-AV increased in all samples (Fig. 2). The rates of increase in *p*-AV up to 3 h of heating were linear and quite similar. After 6 h of heating, the increases in *p*-AV were again rather similar, except in the case of refined olive oil containing additional 0.2% α-tocopherol, which exhibited a considerable higher *p*-AV compared with the other samples. Palm olein and refined olive oil contain similar levels of polyunsaturated fatty acids (PUFA),11.4 and 10.2%, respectively (Table 1). These samples had considerably lower *p*-AV values

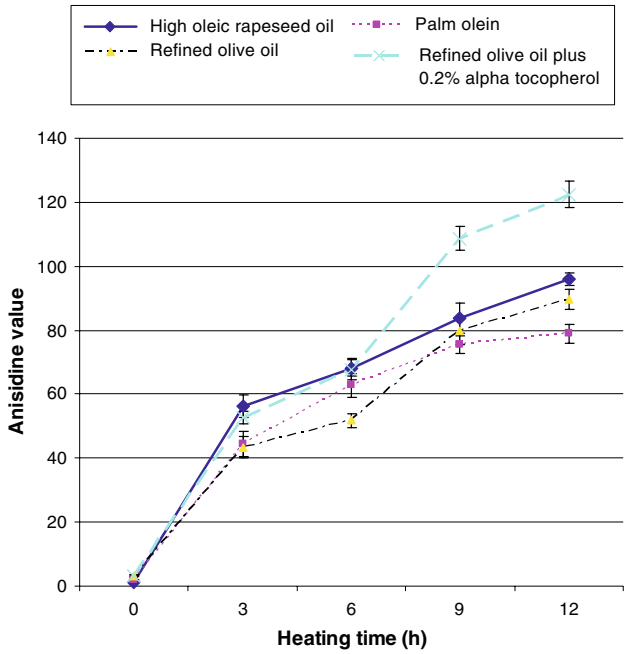

Fig. 2 *p*-Anisidine values in different vegetable oils during heating at 180 ± 5 °C for 0–12 h

compared to those seen for high-oleic rapeseed oil, which contains 14.1% of PUFA. However, it should be noted that both the fatty acids and other minor components in fats and oils contribute to their stability [3]. A previous report [22] showed lower *p*-AV values in some high-oleic vegetable oils after 12 h but at a lower temperature (175 °C) than used in our study (180 °C). Similar to the results for hydroperoxide formation, α-tocopherol at 0.2% affected the formation of secondary oxidation products and increased the *p*-AV values in refined olive oil after 6 h of heat treatment. Similarly, Nogala-Kalucka et al. [2] observed that the addition of α-tocopherol at levels of between 0.01 and 0.1% to a mix of partially hydrogenated rapeseed oil and palm oil after 2 h of heating at 160 °C increased the formation of both primary and secondary oxidation products.

α-Tocopherol and the Formation of POPs

The POPs contents in the four selected samples during 12 h of heating are shown in Tables 4, 5 and 6. The major POPs originated primarily from sitosterol, followed by

Table 4 Contents (μg/g oils)* of phytosterol oxidation products (POPs) in high-oleic rapeseed oil (HORO) during heating at 180 ± 5 °C for 0–12 h

Oxidation product	0 h	3 h	6 h	9 h	12 h
7α-Hydroxybrassicasterol	ND	0.7 ± 0.04	1.1 ± 0.07	1.0 ± 0.06	0.9 ± 0.07
7α-Hydroxycampesterol	Tr	1.3 ± 0.09	2.4 ± 0.1	2.8 ± 0.18	3.1 ± 0.12
7α-Hydroxystigmasterol	ND	Tr	Tr	1.1 ± 0.10	1.2 ± 0.05
7β-Hydroxybrassicasterol	ND	1.1 ± 0.03	2.7 ± 0.12	3.3 ± 0.10	4.2 ± 0.17
7α-Hydroxysitosterol	Tr	2.2 ± 0.08	3.9 ± 0.09	3.8 ± 0.12	3.8 ± 0.24
6-Hydroxybrassicastanol	11.5 ± 0.3	7.8 ± 0.37	11.2 ± 0.27	10.1 ± 0.36	9.2 ± 0.40
7β-Hydroxycampesterol	Tr	2.0 ± 0.08	4.0 ± 0.24	4.3 ± 0.23	4.9 ± 0.22
6-Hydroxycampestanol	19.4 ± 0.32	12.5 ± 0.57	16.2 ± 0.92	15.4 ± 0.78	13.7 ± 0.83
7β-Hydroxysitosterol	0.2 ± 0.02	3.4 ± 0.24	6.7 ± 0.43	7.7 ± 0.31	8.3 ± 0.66
Brassicasterol-5α,6α-epoxide	Tr	0.4 ± 0.02	0.3 ± 0.03	0.3 ± 0.02	0.4 ± 0.03
Brassicastentriol	0.2 ± 0.02	0.3 ± 0.02	0.3 ± 0.01	0.3 ± 0.03	0.4 ± 0.03
6-Hydroxysitostanol	25.5 ± 0.43	17.2 ± 0.94	20.1 ± 1.06	19.6 ± 1.19	17.8 ± 1.50
Campesterol-5β,6β-epoxide	ND	ND	ND	ND	1.5 ± 0.11
Campesterol-5α,6α-epoxide	ND	ND	ND	ND	0.8 ± 0.11
Campestanetriol	ND	Tr	Tr	0.9 ± 0.07	0.8 ± 0.10
Sitosterol-5β,6β-epoxide	ND	1.0 ± 0.09	1.1 ± 0.09	1.2 ± 0.1	1.7 ± 0.04
Sitostanetriol	Tr	0.2 ± 0.02	0.5 ± 0.06	0.7 ± 0.05	0.7 ± 0.07
7-ketobrassicasterol	23.3 ± 0.8	12.4 ± 0.62	13.5 ± 0.80	13.5 ± 0.78	12.5 ± 0.8
7-Ketocampesterol	0.5 ± 0.04	2.7 ± 0.16	2.7 ± 0.2	4.3 ± 0.17	5.0 ± 0.37
7-Ketostigmasterol	Tr	0.5 ± 0.03	0.5 ± 0.04	0.6 ± 0.02	0.6 ± 0.05
7-Ketositosterol	0.5 ± 0.05	0.6 ± 0.05	0.7 ± 0.03	0.8 ± 0.07	0.8 ± 0.05
Total amount of POPs	81.1a	66.3b	87.9ac	91.7ac	92.3c

* Mean of triplicate analyses ± SD

Contents of total POPs followed by different letters differ significantly ($P < 0.05$) between different heating times

ND, not detected

Tr < 0.1 μg/g

Table 5 Contents (μg/g oils)* of POPs in palm olein (PO) during heating at 180 ± 5 °C for 0–12 h

Oxidation product	0 h	3 h	6 h	9 h	12 h
7α-Hydroxycampesterol	0.1 ± 0.01	0.2 ± 0.02	0.7 ± 0.03	0.6 ± 0.04	1.4 ± 0.15
7α-Hydroxystigmasterol	ND	ND	ND	0.7 ± 0.08	1.1 ± 0.06
7α-Hydroxysitosterol	ND	0.4 ± 0.02	0.6 ± 0.06	0.7 ± 0.03	0.9 ± 0.08
7β- Hydroxycampesterol	Tr	0.5 ± 0.03	0.3 ± 0.02	0.4 ± 0.04	0.5 ± 0.04
7β -Hydroxystigmasterol	ND	ND	ND	ND	0.6 ± 0.02
7β- Hydroxysitosterol	Tr	2.1 ± 0.10	2.1 ± 0.17	1.8 ± 0.14	2.6 ± 0.13
6-Hydroxysitostanol	4.2 ± 0.15	2.5 ± 0.12	3.0 ± 0.10	2.6 ± 0.07	3.2 ± 0.14
Campestanetriol	0.2 ± 0.01	0.5 ± 0.03	0.4 ± 0.03	0.4 ± 0.02	0.4 ± 0.03
Stigmastentriol	ND	ND	ND	ND	0.7 ± 0.04
Sitosterol-5β,6β-epoxide	ND	ND	Tr	1.3 ± 0.08	1.9 ± 0.12
Sitostanetriol	ND	0.4 ± 0.02	0.3 ± 0.02	0.1 ± 0.01	Tr
7-Ketocampesterol	ND	ND	0.4 ± 0.04	0.8 ± 0.05	2.2 ± 0.11
7-Ketostigmasterol	ND	ND	Tr	Tr	Tr
7-Ketositosterol	0.2 ± 0.02	0.2 ± 0.01	0.3 ± 0.02	0.3 ± 0.04	0.4 ± 0.03
Total amount of POPs	4.7a	6.8b	8.1c	9.7c	15.9d

* Mean of triplicate analyses ± SD

Contents of total POPs followed by different letters differ significantly ($P < 0.05$) between different heating times

ND, not detected

Tr < 0.1 μg/g

campesterol and stigmasterol. This pattern seemed to follow the phytosterol contents shown in Table 2. The dominant POP was 6-hydroxysitostanol in all oil samples. Azadmard-Damirchi and Dutta [20] reported higher amounts of 6-hydroxysitostanol and 6-hydroxycampestanol compared with other POPs in a blend of interesterified refined olive oil and palm stearin at different temperatures. They concluded that 6-hydroxysitostanol and 6-hydroxycampestanol cannot be generated from the oxidation of sitostanol and campestanol, because vegetable oils such as rapeseed oil, refined olive oil and palm stearin contain only small amounts of these saturated sterols. Instead, they suggested that the formation of saturated dihydroxyphytostanols may be due to the reductive cleavage of 5,6-epoxyphytosterols during the oil refining process. More research is needed to confirm this proposed pathway for the formation of 6-dihydroxystanols.

Although unoxidised brassicasterol was present in much lower amounts in high-oleic rapeseed oil compared with campesterol and sitosterol (Table 2), a remarkably high amount of 7-ketobrassicasterol (23.3 μg/g oil) was observed at 0 h in this sample. After 3 h of heating at 180 °C, 7-ketobrassicasterol degraded to about half the original amount and remained at this level up to 12 h. Similarly, Lambelet et al. [7] observed the highest amounts of brassicasterol oxides (49–54.7% of total POPs) in semi-refined and fully refined low erucic acid rapeseed oil after deodorisation at different temperatures, although unoxidised brassicasterol was present in much lower concentrations compared with unoxidised sitosterol and campesterol. Those authors also reported that 7-ketobrassicasterol was the most abundant POP in all of their oil samples and concluded that brassicasterol is more easily oxidised than other phytosterols. However, the mechanism needs to be studied further.

The total POPs contents for all of our samples generally increased during the heating time, except for high-oleic rapeseed oil after heating for 3 h at 180 °C. The decreasing amounts of some POPs might be a consequence of simultaneous reactions in the oil, such as isomerization and decomposition of sterol structure. The formation of phytosterol oxides in different matrices depends on reactions such as reduction or dehydration of hydroperoxides and dehydrogenation of hydroxysterols. However, the mechanisms of their formation and the kinetics of some reactions are not yet well understood [10, 27, 28]. The highest level of total POPs was found in high-oleic rapeseed oil, ranging from 66.3 to 92.3 μg/g oil. The corresponding figures for palm olein and refined olive oil were 4.7–15.9 and 8.7–24.9 μg/g oil, respectively (Tables 4, 5 and 6). The POPs content in refined olive oil was in line with previous results reported by Johnsson and Dutta [10] during 2 h of heating at 180 °C. The results for palm olein are in line with those of our previous study, where we measured the POPs content in commercial potato crisps prepared in palm oil [17]. The

Table 6 Contents (μg/g oils)* of POPs in refined olive oil (ROO) and ROO plus 0.2% α-tocopherol during heating at 180 ± 5 °C for 0–12 h

Oxidation product	0 h		3 h		6 h		9 h		12 h	
	ROO	ROO plus 0.2% α-tocopherol	ROO	ROO plus 0.2% α-tocopherol	ROO	ROO plus 0.2% α-tocopherol	ROO	ROO plus 0.2% α-tocopherol	ROO	ROO plus 0.2% α-tocopherol
7α-Hydroxycampesterol	Tr	Tr	0.3 ± 0.03	Tr	1.1 ± 0.1	0.2 ± 0.03	0.8 ± 0.06	0.2 ± 0.01	1.2 ± 0.08	0.7 ± 0.05
7α-Hydroxystigmasterol	ND	ND	0.6 ± 0.07	0.7 ± 0.05	1.3 ± 0.1	0.9 ± 0.06	2.0 ± 0.15	0.9 ± 0.10	2.3 ± 0.15	1.3 ± 0.08
7α-Hydroxysitosterol	ND	ND	1.8 ± 0.23	0.9 ± 0.16	3.2 ± 0.22	1.3 ± 0.15	3.2 ± 0.09	1.3 ± 0.16	3.4 ± 0.16	1.5 ± 0.09
7β-Hydroxycampesterol	Tr	Tr	Tr	Tr	0.7 ± 0.04	0.4 ± 0.06	0.8 ± 0.06	0.5 ± 0.04	1.4 ± 0.15	0.9 ± 0.10
7β-Hydroxystigmasterol	Tr	0.3 ± 0.03	0.8 ± 0.06	0.9 ± 0.12	1.4 ± 0.03	0.7 ± 0.02	0.9 ± 0.05	0.8 ± 0.07	0.8 ± 0.09	0.8 ± 0.06
7β-Hydroxysitosterol	ND	ND	2.0 ± 0.07	1.0 ± 0.1	2.9 ± 0.17	1.6 ± 0.05	3.3 ± 0.30	1.4 ± 0.12	3.7 ± 0.19	2.5 ± 0.17
6-Hydroxysitostanol	8.3 ± 0.5	7.5 ± 0.65	6.8 ± 0.1	6.0 ± 0.5	6.3 ± 0.38	5.3 ± 0.45	5.2 ± 0.43	3.7 ± 0.18	5.3 ± 0.24	4.7 ± 0.18
Campestanetriol	0.1 ± 0.02	0.2 ± 0.01	0.2 ± 0.04	0.3 ± 0.03	0.4 ± 0.04	0.3 ± 0.01	0.4 ± 0.03	0.2 ± 0.02	0.4 ± 0.03	0.3 ± 0.03
Stigmastentriol	ND	Tr	1.2 ± 0.05	0.7 ± 0.03	1.1 ± 0.06	0.6 ± 0.06	1.7 ± 0.12	0.4 ± 0.04	1.7 ± 0.1	0.9 ± 0.04
Sitosterol-5β,6β-epoxide	ND	ND	0.7 ± 0.02	0.6 ± 0.04	0.6 ± 0.03	0.5 ± 0.03	1.0 ± 0.13	0.5 ± 0.03	1.1 ± 0.05	0.9 ± 0.1
Sitosterol-5α,6α-epoxide	ND	ND	ND	ND	0.6 ± 0.12	0.8 ± 0.06	0.7 ± 0.04	0.8 ± 0.05	0.9 ± 0.1	0.9 ± 0.07
Sitostanetriol	Tr	Tr	Tr	Tr	0.4 ± 0.04	0.3 ± 0.01	0.5 ± 0.08	0.5 ± 0.06	0.5 ± 0.02	0.7 ± 0.06
7-Ketocampesterol	ND	ND	Tr	Tr	0.8 ± 0.09	Tr	1.2 ± 0.08	2.3 ± 0.16	1.8 ± 0.13	1.3 ± 0.10
7-Ketostigmasterol	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
7-Ketositosterol	0.3 ± 0.03	0.2 ± 0.01	0.5 ± 0.03	0.5 ± 0.03	0.5 ± 0.02	0.3 ± 0.03	0.3 ± 0.04	0.3 ± 0.02	0.4 ± 0.02	0.3 ± 0.02
Total amount of POPs	8.7^{A}_{a}	8.2^{A}_{d}	14.9^{B}_{b}	11.6^{B}_{de}	21.3^{C}_{c}	13.2^{D}_{e}	22.0^{E}_{c}	13.8^{F}_{ef}	24.9^{G}_{c}	17.7^{H}_{f}

ROO, refined olive oil; *ND*, not detected

* Mean of triplicate analyses ± SD

Contents of total POPs followed by different subscript letters differ significantly ($P < 0.05$) at different heating times for each oil

Tr < 0.1 μg/g

$^{A–H}$ Values followed by different superscript letters differ significantly ($P < 0.05$) between total POPs in refined olive oil with and without 0.2% α-tocopherol for the same heating time

low content of total POPs in palm olein may be due, among other factors, to the lower amounts of total unoxidised sterols (Table 2) in this sample [5].

To the best of our knowledge, our results are the first evaluation of such a large number of individual POPs with α-tocopherol antioxidant implications at frying temperature for different times. These results showed that addition of α-tocopherol can significantly ($P < 0.05$) inhibit the formation of total POPs in refined olive oil after 6, 9 and 12 h of heating (Table 5). Rudzinska et al. [15] showed that the total increase in stigmasterol oxidation products in purified triacylglycerols from sunflower oil was lowest in the presence of 0.02% α-tocopherol during incubation at 60 °C for 0–9 days. These authors concluded that α-tocopherol is a strong inhibitor of the formation of stigmasterol oxidation products.

The present study showed that in the three monounsaturated vegetable oils investigated, refined olive oil and high-oleic rapeseed oil had rather similar oxidative stabilities which were lower than that of palm olein. The addition of α-tocopherol at relatively high concentrations increased the OSI value only in refined olive oil. In addition, α-tocopherol was able to improve some established quality criteria, e.g. by inhibiting the formation of POPs at high temperatures in refined olive oil. Refined olive oil is a potential alternative to palm olein for industrial frying to achieve healthier food products; however, additional studies are needed to minimise the formation of PV and *p*-AV in this oil in real frying operations.

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