Nutritional quality of fresh and heated Aleppo pine (*Pinus halepensis* Mill.) seed oil: *trans*-fatty acid isomers profiles and antioxidant properties

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Abstract Numerous studies have focused on *trans* fatty acids (TFA) technologically produced by partial hydrogenation of oils. However, TFA can also be present in fresh oils. For this reason, *cis* fatty acid (CFA), TFA and conjugated linoleic acid (CLA) of fresh and heated Aleppo pine seed oil (APSO) at frying temperature (180 °C) were evaluated and correlated with the antioxidant characteristics. Results showed that fresh APSO had a low oleic/linoleic ratio O/L (0.4). Total TFA in fresh APSO reached 1%. The predominant TFA was 18:2 n-6 (t9, t12) in both fresh and heated APSO. Individual TFA increased with significant differences ($p < 0.05$) with heating time. CLA occurred after 4 h and significantly increased ($p < 0.05$) accounting 10% of total TFA after 10 h. Total TFA are negatively correlated with α -tocopherol, γ -tocopherol ($p < 0.05$) and carotenoids ($p < 0.01$) and positively correlated with remaining DPPH. Oil stability index (OSI) showed significant negative correlation with TFA ($r = -0.925$; $p = 0.008$). A principal component analysis (PCA) showed a clear discrimination between fresh and heated oils. Temperature, heating time, unsaturation degree and antioxidants are combined factors which significantly affect the isomerization rate and nutritional quality of APSO.

Keywords *trans*-fatty acids · *Pinus halepensis* · Oxidative stability

Introduction

Nowadays consumers are more demanding and conscious about their rights and the benefits food nutrients should provide to human health. These subjects have generated more studies to increase the knowledge of chemistry, composition and structure of oils and fats and therefore their better characterization (Pimentel et al. 2006). Vegetable oils are mainly represented by complex mixtures of fatty acid esters in triglyceride form, usually of different degrees of unsaturation (Stanković et al. 2009). With rare exceptions, almost all edible fats and oils of plant origin contain unsaturated fatty acids in the *cis* conformation (Wolff et al. 1998). The physico-chemical, nutritional, biochemical and biological properties of the *trans* fatty acid (TFA) isomers are different from those of *cis* isomers (Ledoux et al. 2000). TFA in food has three major sources, partial hydrogenation of fat, high-temperature processing of edible oils, and the natural occurrence in ruminant meat and dairy products (Majø 2005). Because of the negative health effects of TFA, they have recently received increasing attention and are considered to be even more detrimental than the saturated ones (Semma 2002). Epidemiological studies have shown a strong positive association between TFA intake and cardiovascular disease (CVD) risk (Ghafoorunissa 2008). The multiple effects of TFA appear to be linked to biological effects on hepatocytes (e.g. lipid metabolism), monocytes/macrophages (e.g. systemic inflammation), endothelial cells (e.g. endothelial dysfunction), and adipocytes

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(e.g. adiposy, glucose-insulin homeostasis). Each of these tissues is directly related with CVD risk which represents the leading cause of death in nearly all nations (Micha and Mozaffarian 2008). Conjugated linoleic acid (CLA) are a specific group of different positional and geometric isomers derived from linoleic acid (LA) that can be also formed by heating (Juanéda et al. 2003). In addition to the fatty acid (FA) composition, there has been an increase interest in finding natural antioxidants because they protect the human body from free radicals and retard the progress of many chronic diseases as well as retard lipid oxidation (Fasoyiro and Adegoke 2006). An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may, therefore, have health-promoting effects in the prevention of degenerative diseases (Shahidi 1997). The search for new oleaginous seeds and the contributions of technological and biotechnological processes have promoted improvements in quantity and quality of fats and seeds (Pimentel et al. 2006). Aleppo pine seed oil (APSO) contains a high amount of oil (about 40%); it could represent a significant potential source of vegetable fat. As reported by Nasri et al. (2005) for APSO, linoleic acid is the major fatty acid (56.06% of total fatty acids) followed by oleic (24.03%) and palmitic (5.23%) acids. Even though that the Aleppo pine seed by-products are becoming increasingly consumed and since the consumption of fried foods has increased in recent years, causing a higher intake of fats and oils that have been subjected to high temperatures in the frying process, the processing effect on APSO at high temperature on their bioactive compounds remain scarcely explored.

In the present study, we have particularly interested to the quality control of natural APSO with emphasising the analysis of TFA, CLA and antioxidant compounds (tocopherols and carotenoids). Then, we are interested to investigate the effect of heat treatment at 180 °C on the geometry and position of fatty acid isomers and to evaluate their ability to scavenge free radicals and their oxidative stability. Finally, correlations among these parameters were studied and discussed in the hope of evaluating their possible significance for the quality and stability of APSO and predicting their capacity to resist to treatment as frying medium.

Materials and methods

Plant materials Aleppo pine (*Pinus halepensis* Mill.) seed samples were purchased (March 2008) from Siliana (Tunisia), where they were harvested at growing conditions of 450 m of altitude. According to the 'Arrondissement des Forêts de Siliana' Aleppo pine represents

large forests in the vegetation zone of Siliana (80 thousands hectares where 20 thousands hectares are in full seed production). The seed samples were finely milled and then passed to oil extraction and preserved at −20 °C until analysis.

Oil extraction Fifty grams of ground seeds were placed in a dark flask (capacity 1 L), homogenized with 250 ml of hexane and shaken for 4 h. The mixture was centrifuged for 15 min at 1000 g at room temperature. The supernatant was then filtered through a filtering paper (Whatman no. 2). The extraction procedure was repeated twice and the collected solvent was removed using a rotary evaporator at 40 °C. The lipid extracts were collected and subsequently treated with anhydrous sodium sulphate to remove traces of water. The obtained seed oils were dried under a stream of nitrogen and stored at −20 °C until analysis.

Oxidation experiment of crude oils Samples of crude oils (60 ml) were placed in a series of Pyrex flasks having a volume of 200 ml each. The heat treatment was performed in an oven set at 180 °C for 2, 4, 6, 8 and 10 h. Then, the heated oils were sampled for each measurement in separate dark bottles. All the analyses were performed in triplicate.

Fatty acid methyl ester (FAME) analysis The process of converting free or esterified FAs into methyl esters is referred to a transesterification, with a methanolic solution of potassium. FAME analysis was carried out according to European Union Commission modified Regulation (EEC 1991). Individual FAMES were separated and quantified by gas chromatography using a model 5890 Series II instrument (Hewlett-Packard Ca Palo Alto, Calif) equipped with a flame ionisation detector, and a fused silica capillary column DB23 capillary column (60 m length, 0.32 mm i.d., and 0.25 µm film thickness; HP-Agilent Technologies, Wilmington). The oven temperature was set at 130 °C, increased to 170 °C at 6.5 °C/min, augmented again to 215 °C at 2.8 °C/min and was held there for 12 min. Finally, it was increased to 230 °C at 40 °C/min and maintained for 20 min. Injector and detector temperatures were set at 270 °C and 280 °C, respectively. Nitrogen was used as the carrier gas at 1 ml/min and the split ratio was set at 1:50. The FAMES were identified by comparing their retention times with those of *cis* and *trans* fatty acid standards containing the following methyl esters: lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), *trans*-9- elaidic acid (18:1n-9), oleic acid (18:1n-9), *trans*- 11- vaccenic acid (18:1n-7), linolelaidic acid: *trans*-18:2n-6 (t9,t12; t9,c12; c9,t12),

linoleic acid (18:2n-6), *cis*9, *trans*11-octadecadienoic acid (18:2 c9,t11 CLA), *trans*10, *cis*12-octadecadienoic acid (18:2 t10,c12 CLA), linolenic acid (18:3n-3), arachidic acid (20:0), behenic acid (22:0), erucic acid (22:1n-9), arachidonic acid (20:4n-6), lignoceric acid (24:0) were obtained from Merck (Darmstadt, Germany) and Supelco (Bellefonte, PA, USA). FAMES were identified by comparing their relative retention times to those of authentic *cis*-fatty acid (CFA) and TFAs standards. Oxidative susceptibility (OS) of oil was estimated according to Cert et al. (1996) by means of the formula:

$$\text{OS} = \text{monounsaturated fatty acids} + (45 \times \text{linoleic acid}) \\ + (100 \times \text{linolenic acid})$$

Iodine values (IV) were calculated according to their fatty acid compositions using the formula described by Maestri et al. (1998).

$$\text{IV} = (\% \text{ palmitoleic} \times 1.001) + (\% \text{ oleic} \times 0.899) \\ + (\% \text{ linoleic} \times 1.814) + (\% \text{ linolenic} \times 2.737)$$

Oxidative stability The oxidative stability was measured according to the method described by Tura et al. (2007) by using a Rancimat apparatus, model 734 (Metrohm, Herisau, Switzerland). Briefly, 3 g of oil were weighed and heated at 120 °C and air was bubbled through oil at a flow rate of 20 l/h. Results were expressed as induction time in hours of hydroperoxides decomposition.

Tocopherols content Tocopherols were measured by the method reported by Gimeno et al. (2000). 200 µl of diluted oil in hexane (1:10) was transferred to a screw capped tube, where 600 µl of methanol and 200 µl of the internal standard solution (α -tocopherol acetate) were added. The chromatographic separation was performed using a liquid chromatography system (Agilent Technologies Ca Palo Alto, Calif) with an HP-1100 pump system and a photodiode array detection system (HP 1100). A column was Tracer Extrasil ODS-2 (150 × 4.6 mm i.d., 5 µm particle size). The mobile phase was methanol–water (96:4, v/v) and the elution was performed at a flow-rate of 2 ml/min. The analytical column was kept at room temperature. 20 µl of the diluted sample was injected. Detection was performed at 292 nm. tocopherols were identified by comparison of their retention times with those of authentic standard.

Carotenoids content A sample of oil (1 g) was placed in a Falcon tube and filled with petroleum ether until 20 ml according to the method previously described (Wolf 1968). The carotenoids fraction was measured in a UV-visible

spectrophotometer (Perkin Elmer Lambda 25) at wave lengths between 440 and 480 nm. Carotenoids content was expressed using the following equation:

$$\beta - \text{carotene} = A_{\lambda \text{ max}} \times \frac{10^5}{2650}$$

$A_{\lambda \text{ max}}$ maximum of absorption between 440 and 480 nm

DPPH radical scavenging assay (DPPH-RSA) The capacity to scavenge the “stable” free radical DPPH was monitored according to the method of Ramadan and Morsel (2003). Briefly, a toluenic solution of DPPH radicals was freshly prepared at a concentration of 10^{-4} M. For analysis, 10 mg of APSO (in 100 µL of toluene) was mixed with 390 µL of toluenic solution of DPPH radicals and the mixture was vortexed for 20 s at ambient temperature. The solution was incubated at room temperature for 60 min and the decrease in absorbance at 515 nm was determined, against a blank of pure toluene without DPPH, after 1, 30 and 60 min using a UV-visible spectrophotometer (Perkin Elmer Lambda 25). The remaining concentration of DPPH in the reaction medium was calculated from as follows:

$$\text{DPPHR}(\%) = \frac{(\text{DPPH})_T}{(\text{DPPH})_0} \times 100$$

DPPH_R remaining DPPH
 $(\text{DPPH})_T$ DPPH radical at T=1, 30 or 60 min
 $(\text{DPPH})_0$ DPPH radical at T=0 min.

Statistical analysis All analyses were carried out in triplicate. The results are reported as mean values of three replicates and standard deviation. ANOVA analysis was performed using the SPSS program, release 11.0 for Windows (SPSS, Chicago, IL, USA). Duncan test with a 95% significance level (p , 0.05) was used to discriminate among means values of individual *trans* fatty acids of the different heated APSO as well as antioxidant parameters and Student test to discriminate among fatty acids of fresh and heated APSO. Principal Component Analysis (PCA) was conducted using XLSTAT 2010 Version 3 .06. To point out the correlation between the analyzed parameters, Pearson's test was carried out.

Results and discussion

Cis and trans fatty acid profiles The three main groups of fatty acids (FA) in food lipids are saturated, *cis*-

monounsaturated and *cis*-polyunsaturated fatty acids. TFA content varied considerably among foods, reflecting differences in the type of fats and oils used in the manufacturing or preparation process. Due to the contradictor effects on human health, it remains useful to emphasis on the traces of TFA isomers. Table 1 gives the FA composition of fresh and heated APSO. The examination of individual FA shows that linoleic acid (18:2), accounting for over 56% of the total, was the major fatty acid identified, followed by oleic (18:1)

and palmitic acid (16:0), with levels of 22% and 4%, respectively. The Oleic/Linoleic ratio (O/L) of APSO was (O/L=0.4). The total *cis* monounsaturated fatty acids (MUFA) in fresh APSO were about 24.3% (Table 1). Furthermore, calculated iodine value (IV=135.2) confirmed the previous results indicating that the APSO contained high levels of oleic and linoleic acids. In fact, the FA profile of APSO was characterized by high level of polyunsaturated fraction (64.9%). These results are in agreement with those

Table 1 Fatty acid composition: *cis*, *trans* and conjugated configuration and oxidative stability of fresh and heated (180 °C for 10 h) Aleppo pine seed oil (APSO) samples

Nomenclature	Shorter form	RT (min)	Fatty acids (%)		
			Fresh APSO	Heated APSO	P
Tetradecanoic acid	14:0	19.0	0.05±0.02	0.06±0.012	ns
<i>cis</i> -Tetradecenoic acid	14:1	20.7	0.02±0.009	0.03±0.006	ns
Palmitic acid	16:0	22.9	4.9±0.006	5.2±0.072	**
<i>trans</i> -Hexadecenoic acid	<i>trans</i> - 16:1 n-7	23.2	0.05±0.003	0.08±0.00	***
<i>cis</i> -Hexadecenoic acid	<i>cis</i> -16:1 n-7	23.6	0.08±0.001	0.10±0.011	ns
Margaric acid	17:0	24.4	0.13±0.004	0.15±0.001	ns
<i>cis</i> -Heptadecenoic acid	17:1	25.0	0.06±0.009	0.12±0.016	*
Stearic acid	18:0	27.7	3.3±0.034	3.4±0.004	*
Elaidic acid	<i>trans</i> -18:1 n-9	27.7	nd	0.10±0.00	***
Trans-Vaccenic acid	<i>trans</i> -18:1 n-7	27.9	nd	0.07±0.003	***
Oleic acid	<i>cis</i> -18:1 n-9	28.3	22.9±0.155	22.2±0.115	**
Vaccenic acid	<i>cis</i> -18:1 n-7	28.4	0.39±0.055	0.46±0.005	ns
<i>trans,trans</i> -Linoleic acid	18:2 n-6 (t9. t12)	28.7	0.95±0.002	1.06±0.001	**
<i>cis,trans</i> -Linoleic acid	18:2 n-6 (c9. t12)	29.0	nd	0.012±0.002	**
<i>trans,cis</i> -Linoleic acid	18:2 n-6 (t9. c12)	29.4	nd	0.13±0.030	ns
<i>cis,cis</i> -Linoleic acid	18:2 n-6 (c9. c12)	29.8	56.9±0.117	55.5±0.128	**
<i>cis,cis,cis</i> - γ -Linolenic acid	<i>cis</i> -18:3 n-6	30.3	3.4±0.022	3.5±0.027	**
<i>trans,cis,cis</i> - α -linolenic acid	<i>trans</i> -18:3 n-3	30.8	nd	0.05±0.00	***
<i>cis,trans,cis</i> - α -linolenic acid	<i>trans</i> -18:3 n-3	31.3	nd	0.02±0.001	***
<i>cis,cis,cis</i> - α -linolenic acid	<i>cis</i> -18:3 n-3	31.6	0.72±0.017	0.70±0.057	ns
Rumenic acid	18:2 (c9. t11)	32.5	nd	0.03±0.00	***
Alternate CLA	18:2 (t10. c12)	32.7	nd	0.15±0.001	***
Eicosanoic acid	20:0	33.3	0.57±0.014	0.58±0.084	ns
<i>trans</i> -Eicosanoic acid	<i>trans</i> -20:1 n-9	34.2	0.06±0.008	0.15±0.060	***
<i>cis</i> -Eicosamonoenoic acid	20:1 n-9	34.3	0.62±0.029	0.71±0.080	ns
Eicosadienoic	20 :2 n-9	35.1	0.46±0.004	0.48±0.027	ns
di-homo- γ -linolenic acid	20 :3 n-6	36.2	0.48±0.032	0.43±0.022	*
<i>cis</i> -Arachidonic acid	20 :4 n-6	36.7	3.0±0.035	3.1±0.004	*
Behenic acid	22 :0	39.7	0.16±0.065	0.22±0.009	**
Erucic acid	22 :1	40.9	0.13±0.026	0.21±0.018	**
Σ SFA			9.2±0.143	9.1±0.182	
Σ <i>cis</i> MUFA			24.2±0.275	23.7±0.253	
Σ <i>cis</i> PUFA			64.9±0.227	62.9±0.238	
MUFA/PUFA ratio			0.37	0.37	
Oxidative stability (h)			5.4	0.86	
Oxidative susceptibility			2993.9	2941.5	
Iodine value			135.5	132.7	

* $P<0.05$; ** $P<0.01$; *** $P<0.001$. ($n=3$)

RT: retention time; Elution by high polar capillary column: DB23 (60 m)

nd: not detected. ns: not significant

SFA: Saturated Fatty Acids; **MUFAs**: Monounsaturated Fatty Acids; **PUFAs**: Polyunsaturated Fatty Acids. **CLA**: Conjugated Linoleic Acid

previously reported by Cheikh-Rouhou et al. (2006). The saturated fraction represents about (9.2%). The *trans*, *trans* linoleic 18:2n-6 (t9, t12) isomer was identified in fresh APSO. Before applying the heat processing, 18:2n-6 (t9, t12) represented 1.66% of *cis* form of linoleic acid and then increased significantly ($p < 0.05$) after 2 h reaching 1% of the total FA (Table 2). With applying heating at 180 °C to APSO, an individual separation of linoleic acid 18:2 *cis*-9, *cis*-12 and of its 3 geometrical isomers *trans*-9/*trans*-12, *cis*-9/*trans*-12 and *trans*-9/*cis*-12 can be clearly detected by the 60 m DB23 capillary column (Fig. 1). As reported by Ledoux et al. (2000) gas chromatography using these very polar phases resulted in suitable resolution for linoleic acid geometrical isomers. These peaks correspond to an important number of methylene interrupted octadecadienoic acid isomers. The total *trans* polyunsaturated isomers were significantly correlated with heating time ($p < 0.05$). Significant differences in TFA composition were noted between oil samples with regard to the different times of heat treatment ($p < 0.05$) (Fig. 2a). However, the main heating effects, at frying temperature, on the relative proportions of FA were observed in the monounsaturated fraction and especially on oleic acid. Additionally, significant differences were observed in the *trans* 20:1 isomer between fresh and heated APSO accounting for more than 10% of *cis* eicosanoic acid in fresh oil then increased with heating time exceeding the three fold of its initial amount ($p < 0.05$) Table 2.

The FA composition of APSO was found to be sensitive to heat treatment. It was interesting to note that the longer exposure to high temperature, in which the visible damage was equally severe, can induce significant changes in the overall FA composition. Because of the high level of PUFA, APSO appears to be susceptible to oxidation at frying conditions and therefore to isomerization reactions. *Trans*

MUFA, which represent most of the intake of TFA, were found in processed fats as hydrogenated oils. However, *trans* isomers of PUFA can also formed during oil processing (Ackman et al. 1974). Positive evidence for the occurrence of *cis*–*trans* and positional isomerization is important from the theoretical standpoint, since it can be useful for supporting the assumption of a radical index of lipid oxidation.

Conjugated linoleic acid (CLA) Conjugated linoleic acid (CLA) is a collective term for different positional and geometric isomers of octadecadienoic acid which contain a pair of double bonds in a conjugated configuration (Jesper and Götsche 2006). CLA are mainly present in milk and its products, but also to a lesser extent in meat products from ruminants (Ledoux et al. 2005). Then, diverse beneficial effects are attributed to this compound as metabolic regulator, hypocholesterolemic, antiatherogenic, anticarcinogenic and antioxidant, as well as favorable effects in the prevention and treatment of certain nourishing allergies (Pariza 2004). Juanéda et al. (2003) reported that at a temperature of 180 °C, the main mono *trans* positional isomers found were the *cis*-9, *trans*-11-CLA and the *trans*-10, *cis*-12-CLA. Natural vegetable oils are not a source of methylene non-interrupted octadecadienoic acid. Fresh APSO contained the methylene interrupted octadecadienoic acid form with the *cis* and *trans* isomers (Table 1). The positional isomerization reaction was subsequently occurred after 4 h of heating and total CLA significantly increased with increasing heating time ($p < 0.05$). In addition, as illustrated in (Fig. 2b), the total CLA/total isomerized fatty acids (TIFA) ratio increased and total CLA, in heated APSO, accounted after 4, 6, 8 and 10 h, respectively 2.1, 5.6, 6.8 and 10% of their total TFA (Fig. 2b). Consequently, the present results suggested that heating could induce conjugation reactions in the linoleic

Table 2 Individual *trans*-fatty acids (%) of Aleppo pine seed oil (APSO) samples before and during heating at 180 °C

	Heating time, h					
	0	2	4	6	8	10
<i>trans</i> -16:1 n7	0.04 ^b ±0.003	0.06 ^b ±0.015	0.07 ^{ab} ±0.02	0.08 ^a ±0.032	0.08 ^a ±0.006	0.08 ^a ±0.00
<i>trans</i> -18:1 n7	nd	nd	nd	0.03 ^b ±0.002	0.04 ^a ±0.00	0.10 ^c ±0.00
<i>trans</i> -18:1 n9	nd	nd	nd	0.02 ^a ±0.003	0.02 ^a ±0.008	0.07 ^a ±0.003
18:2 n6 (t9, t12)	0.95 ^c ±0.002	1.01 ^b ±0.012	1.05 ^a ±0.02	1.06 ^a ±0.02	1.05 ^a ±0.002	1.06 ^a ±0.001
18:2 n6 (c9, t12)	nd	0.08 ^b ±0.009	0.02 ^b ±0.012	0.01 ^c ±0.019	0.05 ^b ±0.018	0.012 ^c ±0.002
18:2 n6 (t9, c12)	nd	0.06 ^b ±0.024	0.09 ^a ±0.04	0.08 ^a ±0.003	0.13 ^a ±0.01	0.13 ^a ±0.03
<i>trans</i> -18:3 n3	nd	nd	0.04 ^b ±0.01	0.07 ^a ±0.00	0.06 ^a ±0.004	0.07 ^a ±0.000
<i>trans</i> -20:1	0.06 ^b ±0.008	0.07 ^b ±0.024	0.07 ^b ±0.004	0.10 ^b ±0.012	0.12 ^b ±0.05	0.15 ^a ±0.004

nd: not detected, (n=3)

Different superscripts letters indicated significant difference between heated oils ($p < 0.05$)

Fig. 1 Fatty acid Chromatograms (GC) of fresh (panel A) and heated Aleppo pine seed oil (panel B) for 10 h at 180 °C. The retention times (min) of individual fatty acids were indicated in Table 1

acid chain. However, the positional isomerization level was significantly ($p < 0.05$) correlated to the initial level of *cis* form of linoleic acid (56.9%) and geometric isomerization was more vulnerable to high temperature than positional conjugation reactions.

Antioxidant compounds Adding to FA isomers evaluation and knowing that these compounds are biologically very important, the determination of antioxidant compounds is needed to extend the studied oil quality. The distribution of individual tocopherols and carotenoids content in fresh and

Fig. 2 Changes in fatty acid fractions of Aleppo pine seed oil (APSO) during heating at 180 °C. (a): *trans* unsaturated fatty acid fractions in APSO; (b): Total CLA (%) and total CLA/total isomerised FA ratio in APSO. *trans*-MUFAs: *trans* monounsaturated fatty acids; *trans*-PUFAs: *trans* polyunsaturated fatty acids; **Total TFA**: total *trans* fatty acids; ***trans* FA**: *trans* fatty acids. **TCLA**: total conjugated linoleic acid; **TIFA**: total isomerised fatty acids. Values are given as mean $\pm$ SD ($n=3$). Different superscripts letters (a–e) indicated significant difference between heated oils ($p<0.05$)

heated oils are reported in Table 3. It appears clearly that, in both fresh and treated APSO, γ -tocopherol isomer dominated

the α -tocopherol one. In a general way, α -tocopherol seemed to be only slightly affected at the beginning of the heat treatment. Then, tocopherols concentrations varied during heating by declining γ -tocopherol from about 155 mg/kg in fresh APSO to 24 mg/kg after 10 h. However, we noted an entire disappearance of the α -tocopherol isomer at the end of the heat treatment (Fig. 3b). As reported by Diego et al. (2004), tocopherols are easily oxidized and oxidation losses can be incremented by heat, light, alkaline pH and by the presence of free radicals. Native antioxidants including tocopherols present in the oil during oxidation undergo chemical processes decreasing its activity and concentration (Gómaš et al. 2006). During the heating, the more the antioxidant compounds were present in oils, the more the process of lipid peroxidation was retarded and the isomerization reactions were weakly produced (Table 3). Total TFA are significantly negative correlated with α -tocopherol, γ -tocopherol ($p<0.05$) and carotenoids ($p<0.01$). These expected results can be explained by the presence antioxidant compounds as tocopherols and carotenoids that exert a protective effect during autooxidative process. Moreover, the degree of unsaturation of FA also affects the stability of oils, since the more double bonds there are in the FA, the more easily the attack by free radicals takes place (Arranz et al. 2008).

Radical scavenging activity DPPH-RSA Lipid oxidation is one of the problems associated with food losses and human diseases due to generation of free radicals. Free radical scavenging capacity is usually measured with the aim of determining possible biological activities of a sample rich in antioxidants since the latter have been

Table 3 Antioxidant characteristics of APSO before and after heat treatment at frying temperature.

	Heating (180 °C) time, h					
	0	2	4	6	8	10
Carotenoids (mg/kg)	9.3 ^a $\pm$ 0.19	8.6 ^a $\pm$ 0.08	8.5 ^a $\pm$ 0.26	8.3 ^a $\pm$ 0.18	7.9 ^a $\pm$ 0.38	7.5 ^a $\pm$ 0.2
α -tocopherol (mg/kg)	23.2 ^a $\pm$ 2.3	23.2 ^a $\pm$ 1.2	19.0 ^b $\pm$ 0.89	17.9 ^b $\pm$ 1.3	7.5 ^c $\pm$ 0.53	0.00 ^d $\pm$ 0.00
γ -tocopherol (mg/kg)	155.7 ^a $\pm$ 3.1	152.2 ^a $\pm$ 2.2	144.1 ^b $\pm$ 2.7	123.6 ^c $\pm$ 4.8	97.4 ^d $\pm$ 1.1	24.1 ^e $\pm$ 0.43
DPPH _R 1	47.9 ^a $\pm$ 2.1	48.0 ^a $\pm$ 0.8	51.1 ^b $\pm$ 1.00	52.8 ^b $\pm$ 0.62	59.5 ^c $\pm$ 1.9	72.9 ^d $\pm$ 2.5
DPPH _R 30	27.9 ^a $\pm$ 2.1	30.9 ^b $\pm$ 0.81	33.8 ^c $\pm$ 0.04	38.2 ^d $\pm$ 0.86	46.5 ^e $\pm$ 0.8	59.5 ^f $\pm$ 0.04
DPPH _R 60	25.4 ^a $\pm$ 3.1	26.2 ^a $\pm$ 0.3	32.3 ^b $\pm$ 3.9	34.3 ^b $\pm$ 0.85	43.3 ^c $\pm$ 0.43	56.1 ^d $\pm$ 1.8
OSI	5.36	3.19	2.92	2.55	2.29	0.86

($n=3$)

DPPH_R: Remaining DPPH radical activity (%) in fresh and heat treated APSO for (0. 2. 4. 6. 8 and 10 h) incubated with DPPH radical for 1, 30 and 60 min

OSI: oxidative stability index

Different superscripts letters indicated significant difference ($p<0.05$) between heated oils

Fig. 3 Tocopherol Chromatograms (HPLC) of fresh (panel A) and heated Aleppo pine seed oil (panel B) for 10 h at 180 °C. Retention time (min) of alpha-tocopherol (1); alpha-tocopherol acetate (2); gamma-tocopherol (3)

related to the prevention of several diseases (Stanner et al. 2004). The DPPH assay measures the ability of the antioxidants present in the sample to scavenge free radicals. After 1, 30 and 60 min of heated oils incubation with the DPPH radical, the scavenging activities were well proved. The highest scavenging ability was exhibited by fresh APSO followed by oils heated for 2 h and incubated for 1 min (Table 3). Furthermore, remaining DPPH increased with increasing treatment

time showing a significant negative correlation with tocopherol and carotenoid levels ($p < 0.01$). A decrease in percentage of inhibition, as regard to heat treatment, suggest higher effectiveness in quenching the DPPH radicals for fresh oils than after completion of thermooxidation. This finding may be explained by the removal of antioxidant compounds in the oxidized oils during heating in the air stream. Although the total removal of α -tocopherol isomer after 10 h of heating,

Table 4 Correlation between Oxidative stability TFA composition. Tocopherols. Carotenoids and DPPH activity in Aleppo Pine seed oil (APSO)

	OSI	<i>t</i> MUFA	<i>t</i> PUFA	TotalTFA	DPPH _{R1}	DPPH _{R30}	DPPH _{R60}	α -tocopherol	γ -tocopherol	carotenoids
OSI	1									
<i>t</i> MUFA	−0.869*	1								
<i>t</i> PUFA	−0.823*	0.626	1							
Total TFA	−0.925**	0.857*	0.880*	1						
DPPH _{R1}	−0.823*	0.973**	0.575	0.838*	1					
DPPH _{R30}	−0.878*	0.987**	0.680	0.911*	0.988**	1				
DPPH _{R60}	−0.858*	0.972**	0.669	0.891*	0.990**	0.995**	1			
α -tocopherol	0.826*	−0.955**	−0.692	−0.901*	−0.979**	−0.987**	−0.966**	1		
γ -tocopherol	0.830*	−0.986**	−0.563	−0.843*	−0.997**	−0.988**	−0.984**	0.969**	1	
carotenoids	0.977*	−0.913*	−0.867*	−0.971**	−0.867*	−0.927**	−0.906*	0.895*	−0.872*	1

* $P < 0.05$ ** $P < 0.01$

OSI: Oxidative Stability Index

*t*MUFAs: *trans* monounsaturated fatty acids*t*PUFAs: *trans* polyunsaturated fatty acidsTFA: *Trans* fatty acidsDPPH_R: remaining DPPH

the capacity to scavenge free radicals was still maintained in APSO. These results suggest the great ability of the γ -tocopherol isomer as potent natural antioxidant. As reported by Christen et al. (1997), α -tocopherol exhibits the greatest activity in the prevention of vitamin E deficiency abnormalities, whereas γ -tocopherol was found to be potent reactive nitrogen oxide species (NOx) radical scavenger.

Oxidative stability Storage of oil samples at high temperatures (oven test) was employed for monitoring oxidative stability (OS) of oils (Ramadan and Morsel 2004). Oil stability is usually determined by objective methods and under accelerated conditions because ambient temperatures may demand an excessively long period (Abou-gharbia et al. 2000). Using rancimat test, fresh APSO showed a low oil stability index OSI (5.4 h) which, dramatically, decreased to 0.86 h at the end of the heating process. In fact, high levels of linoleic and linolenic acids may result in a lower oxidative stability and a shorter shelf life of the oils as declared by Martínez et al. (2006). Having a very low O/L ratio (0.4), APSO showed detectable *trans* isomerization level for palmitoleic, linoleic and eicosenoic acids before and after applying the heat treatment. The highest levels were observed for *trans*, *trans*-linoleic isomers 18:2 n6 (t9.t12) with 89.6% of total TFA. After 2 h of heating, two linoleic isomers containing a *trans* double bond

18:2 n6 (t9.c12) and 18:2 n6 (c9.t12) were identified. The low oxidative stability of APSO (Table 4), even before any treatment, and the high oxidative susceptibility of fresh APSO (OS=2993.9) can be an excellent index of the oxidation state of the oil. Moreover, oil stability index (OSI) of APSO showed significant negative correlation with total TFA ($r = -0.925$; $p = 0.008$) indicating that the intensity of isomerization reactions from *cis* into *trans* configuration of unsaturated fatty acid is well associated with lipid oxidation and oil quality deterioration (Table 4). In addition, oxidative stability is closely related to tocopherols and β -carotene contents and therefore associated with the ability to scavenge free radicals.

Chemometric analysis Principal component analyses (PCA) of Aleppo pine seed oil (APSO) was conducted to determine the effect of frying conditions on FA profiles (*trans* and conjugated linoleic acid isomers), oxidative stability, DPPH-RSA, tocopherols and carotenoids. The levels of the variance explained by the PCA were 69.8 and 11.7% for PC1 and PC2, respectively (Fig. 4) which explained 81.5% of the total variance. The PCA separated the samples into two distinct groups. The first one contained the fresh APSO and the treated APSO for 2 and 4 h while the second group contained the excessively heated APSO (8 and 10 h). Figure 4 showed that the first group was positively

Fig. 4 Effect of heating process on Aleppo pine seed oil (APSO) nutritional value. **(a)** Ordination plots of the principal component analyses (PCA) based on FA isomers profile, Oxidative stability (OSI), antioxidant compounds and DPPH Radical Scavenging Activity (DPPH-RSA). **(b)** PCA shows loading values for APSO. Quality parameters to the right in the plot indicate those that are more represented in fresh APSO and heat treated APSO for 2, 4 h. *Trans* FA and conjugated linoleic acid (CLA) isomers to the left in the plot indicate those that are more present in excessively heated APSO (after 8 and 10 h of heating). The variance explained by each principal component axis (PC1 and PC2) is shown in parentheses. (Black diamond suit: fresh APSO, Black square: APSO 2h, Black up-pointing triangle: APSO 4h, *: APSO 6 h, Asterisk: APSO 8h, Bullet: APSO 10 h)

associated with PC1 whereas the second was negatively associated. These results indicated clear discrimination between fresh and different heated APSO. In fact, heating at frying process increases the relative proportion of total *trans* fatty acid (TFA) isomers when compared to fresh APSO and decreased oxidative stability, ability to

scavenge radicals and, certainly, antioxidant compounds. This finding was also illustrated by the fact that *trans* MUFA (*trans*-C16:1 n7, *trans*-18:1 n9, *trans*-18:1 n7 and *trans* 20:1 n-9, *trans* PUFA [18:2 n6 (t9,t12), 18:2 n6 (t9,c12)] and 18:2 n6 (c9,t12) and *trans*-18:3 n3] and conjugated linoleic acid CLA [18:2 (c9,t11)-CLA and 18:2 (t10,c12)-CLA] are located more on the left hand side of PC1. However, OSI, DPPH-RSA, tocopherols and carotenoids were more common to the right of PC1, which indicated a clear chemical and nutritional discrimination between fresh and treated APSO.

Conclusion

Because food authenticity is an important topic in a commercial and a health point of view, the present study was undertaken to explore the variation of TFA profiles isomers and antioxidant capacity of Aleppo pine seed oil (APSO) under thermooxidative treatment. Natural APSO contained considerable amount of TFA isomers which can be considered an exceptional level for a crude vegetable oil. In concluding, this investigation on TFA composition, antioxidant profiles of APSO, it is clearly observed their variation in fresh state and during all the heating process. Oxidative stability has dramatically decreased under thermooxidation. Present data is emerging to show that fatty acids in APSO isomerize under high temperature with remarkable differences between monounsaturated and polyunsaturated fractions and also between geometric and positional configurations. Temperature, heating time and degree of unsaturation and also the initial amount of the *cis* form of fatty acid are factors which significantly affect the rate of isomerization. Natural antioxidants are the main factors influencing the oxidative stability, the isomerization rate and scavenging activity of APSO by exerting a protective effect to oil autooxidation. Moreover, potentially toxic compounds (*trans* fatty acids) found in APSO, whether naturally occurring or having been induced by heating, their levels had not exceeded the 2% of the total fat for edible oils limited by the European Food Safety Authority (EFSA). Viewing that APSO is being largely used in food industry, it will be important to evaluate the quality of their by-products in order to involve a safe and edible supply of food to the consumer.

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