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Effect of Initial Quality and Compositional Parameters on Total Polar Compounds Formation during Continuous Heating of Olive Oil

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ABSTRACT

The present study was conducted to determine the relative contribution of initial quality and compositional parameters to the rate of changes (RC_{TPC}) in the content of total polar compounds (TPC), which is considered to be one of the most reliable indicators of heated oils quality, of olive oil during continuous heating at 180 ºC. The ratio between monounsaturated and polyunsaturated fatty acids showed the highest relative contribution (44.86%) to the RC_{TPC}. The content of total phenolic compounds indicated a considerable contribution (30.76%) to the RC_{TPC} whereas the content of total tocopherols was found to be actually ineffective (0.01%). Peroxide value was another important parameter that showed a marked contribution (20.51%) in this regard. The contributions of acid value and initial TPC content to the RC_{TPC} of olive oil were 3.85 and 0.01%, respectively.

Keywords: Alteration rate, Oil quality, Relative contribution, Stability.

INTRODUCTION

Olive oil is a major source of dietary fat in the Mediterranean diet, used as salad dressing and frying oil. It accounts for less than 2% of total vegetable oil production, but its consumption is also becoming increasingly popular in non-producer countries. Several studies support a relationship between olive oil and a lower incidence of some important diseases, including cancer (Assmann *et al.,* 1997) and cardiovascular diseases (Covas, 2007). Health properties, oxidative stability, and sensory quality of olive oil are associated with prominent and well-balanced chemical composition (Bendini *et al.,* 2007). High content of oleic acid in olive oil serves to retard penetration of fatty acids into arterial walls (Charbonnier, 1982). The oils that are much higher in monounsaturated fatty acids (MUFA) and lower in saturated fatty acids (SFA) are preferred because of the proved beneficial effects of MUFA on serum cholesterol levels. The interesting properties of olive oil are also related to the presence of minor components, especially natural antioxidants such as tocopherols and, particularly, phenolic compounds (Owen *et al.,* 2000).

Fatty acid composition and significant amounts of stability- and health-promoting components make olive oil a very interesting option among heating oils and fats used for frying purposes. Numerous analytical methods have been described to measure the changes in heated oils, but determination of polar compounds is considered to be one of the most reliable indicators of heated oils quality. By definition, total polar compounds (TPC) content is the sum of the materials that are not triglycerides (Ruiz-Mendez *et al.,* 1997). Regulatory agencies in European

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countries have established a level of 24-27% TPC for the rejection of heated oils (Paul and Mittal, 1997).

The rate of changes in TPC content of olive oil may be a function of many initial quality and compositional parameters. Peroxide value (PV) and acid value (AV) are two of the most frequently determined quality indices, which indicate the levels of total hydroperoxides and hydrolytic rancidity, respectively. Therefore, they are considered to be guides to initial quality of olive oils (Lovaas, 1992). The ratio between MUFA and polyunsaturated fatty acids (PUFA) is a measure of olive oils tendency to undergo autoxidation. Phenolic compounds and tocopherols are well-known effective chain-breaking antioxidants naturally occurring in edible fats and oils (Frankel, 1998). Moreover, the initial amount of TPC in vegetable oils can have a pronounced contribution to create the off-flavour compounds as well as a marked effect on the primary oxidation (Farhoosh and Pazhouhanmehr, 2009).

The aim of this study was to determine the relative contribution of compositional parameters, including PV, AV, MUFA/PUFA ratio, total tocopherols (TT) content, total phenolics (TP) content, and TPC content, to the rate of changes in TPC content (RC_{TPC}) of olive oil during continuous heating process.

MATERIALS AND METHODS

Materials

Four deodorized olive oil samples (1, 2, 5, and 6 as shown in Table 1) and three virgin olive oil samples (3, 4, and 7 as shown in Table 1) were purchased from local shops and were stored at 4ºC until analysis. Fatty acid methyl ester (FAME) standards and all chemicals and solvents used in this study were of analytical reagent grade and supplied by Merck and Sigma Chemical Companies.

Oil Oxidation

The oil samples (200 g) were placed in a fryer (Kenwood DF280, Havant, Hampshire, UK) and maintained at 180ºC for 16 hours with no stirring. The surface-to-volume ratio of the oil samples in the fryer was about 0.7 cm^{-1}. After intervals of 4 hours of heating, samples were removed and analyzed.

Chemical Analyses

The fatty acid composition of the oils was determined by gas-liquid chromatography. Fatty acids were transesterified into their corresponding FAMEs by vigorous shaking of a solution of oil in hexane (0.3 g in 7 mL) with 2 mL of 7N methanolic potassium hydroxide at 50ºC for 10 minutes. The FAMEs were identified using an HP-5890 chromatograph (Hewlett-Packard, CA, USA) equipped with a CP-FIL 88 (Supel Co., Inc., Bellefonte, PA) capillary column of fused silica, 60 m in length× 0.22 mm ID, 0.2 μm film thickness, and a flame ionization detector (FID). Nitrogen was used as carrier gas with a flow rate of 0.75 mL min^{-1}. The oven temperature was maintained at 198ºC, and that of the injector and the detector at 250ºC (Farhoosh *et al.,* 2008). The fatty acids in the sample were identified using standard methyl esters of fatty acids by comparing the retention times. The results were expressed as relative area percent.

The spectrophotometric method described by Shantha and Decker (1994) was used to determine the PV. The AV was determined according to the AOCS Official Method Cd 3d-63 (1993). The TT content was determined according to the colorimetric method described by Wong *et al.* (1988). The TP content was determined spectrophotometrically using Folin-Ciocalteau's reagent according to the method described by Capannesi *et al.* (2000). A calibration curve of gallic acid in methanol was performed in concentration range 0.04-0.40 mg mL^{-1}. The TPC content

was determined according to the economical micro method developed by Schulte (2004).

Statistical Analysis

All determinations were carried out in triplicate, and data were subjected to analysis of variance (ANOVA). ANOVA and regression analyses were performed according to the MSTATC and SlideWrite software. To standardize the compositional parameters, each parameter was subtracted from its mean among the olive oils, and the difference was divided by the corresponding standard deviation. Significant differences between means were determined by Duncan's multiple range tests. *P* values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The compositional parameters of the olive oil samples are shown in Table 1. As can be seen, the fatty acid compositions were statistically different. The contents of SFA, MUFA, and PUFA ranged from 15.67 to 20.19% (mainly palmitic acid, C16:0, and stearic acid, C18:0), from 59.13 to 76.10% (mainly oleic acid, C18:1), and from 7.67 to 20.21% (mainly linoleic acid, C18:2), respectively. These contents were in accordance with those reported in the literature for olive oils (Firestone *et al.* 1996). From information stated above, the MUFA/PUFA ratio varied significantly amongst the olive oils studied and widely ranged from 2.93 to 9.92.

A wide range of the PV (8.4-13.4 meq kg^{-1}) and AV (0.10-2.00 mg g^{-1}) was observed amongst the olive oil samples, indicating the olive oils had no similar initial qualities. The olive oils experimented contained statistically different contents of TT (44 - 457 ppm) and TP (12-203 ppm), leading to their different oxidative stabilities.

Table 2 shows the TPC content of the olive oils during the heating process at 180ºC. Lumley (1988) showed that the TPC content of fresh oils normally ranges between 0.4 and 6.4%, although other different amounts of TPC content (up to 14%) have been reported in crude or refined vegetable oils (Ruiz-Mendez *et al.,* 1997). Depending on the fatty acid composition, initial quality, and technology used, crude oils may undergo decreases or even increases in their TPC contents during refining. Considering that the polar fraction in edible fats and oils includes essentially alteration compounds, the higher the TPC contents, the lower will be the expected quality of the oils. The olive oils tested in this study contained initial TPC contents ranging from 3.96 to 11.59% (Table 2). As can be seen, the TPC contents linearly increased with high determination coefficients ($R^2 > 0.98$) during the heating process. Assuming that the limit of acceptance for the TPC content is 24%, the time required to reach this limit was calculated as a measure of oxidative stability (t_{24}). A higher t_{24} value means greater stability over the heating process. Except for the samples 2 and 3, all the oils studied reached the discarding range of TPC content during the heating process. The olive oil samples 1-7 indicated the t_{24} values of 10.23, 19.41, 23.96, 7.75, 8.26, 10.99, and 14.49 h, respectively (Figure 1).

It is obvious that the RC_{TPC} is a function of all initial quality and compositional parameters showed in Table 1, whereas the t_{24} value is highly affected by the initial TPC content (Table 2). Therefore, the inverse of percentage increase in the TPC content after 16 h of heating ($\%TPC_{16h}^{-1}$) was considered as a quantitative measure of the RC_{TPC}. The $\%TPC_{16h}^{-1}$ was correlated with a unified variable defined as $\sum_{i=1}^{6}(C_i \times SCP_i)$, in which C_i and SCP_i were coefficients and the standardized (and inversed in the cases needed, Table 3) compositional parameters, respectively. The C_i values in the regression models were determined based on reaching the maximum possible determination coefficient. A very good correlation was found between the RC_{TPC} and the unified variable consisting

Table 1. The initial quality and compositional parameters of the olive oil samples[*].

Parameters	Olive oil samples						
	1	2	3	4	5	6	7
Fatty acids							
16:0	15.68±0.26 a	12.50±0.09 d	14.58±0.11 b	14.63±0.00 b	13.08±0.13 c	12.40±0.05 d	10.63±0.01 e
16:1	1.98±0.03 a	1.26±0.06 c	1.66±0.06 b	1.92±0.04 a	1.27±0.02 c	1.10±0.00 d	1.13±0.11 cd
18:0	3.70±0.08 d	4.56±0.12 b	3.38±0.03 e	3.52±0.10 de	4.90±0.11 a	4.82±0.06 a	4.27±0.08 c
18:1	56.70±0.51 g	68.40±0.37 b	60.91±0.10 f	63.25±0.38 e	65.03±0.13 d	67.59±0.11 c	74.62±0.37 a
18:2	19.02±0.05 a	10.34±0.10 g	16.91±0.18 b	13.87±0.08 c	12.59±0.24 e	11.01±0.15 f	6.84±0.19 h
18:3	1.20±0.05 a	0.89±0.00 d	1.13±0.04 a	1.11±0.10 ab	0.89±0.13 cd	1.00±0.04 bc	0.84±0.09 d
20:0	0.81±0.04 b	0.84±0.07 abc	0.65±0.11 c	0.76±0.05 bc	0.93±0.01 a	0.88±0.04 ab	0.77±0.06 bc
20:1	0.45±0.09 a	0.49±0.01 a	0.43±0.01 a	0.42±0.01 a	0.51±0.07 a	0.42±0.04 a	0.35±0.16 a
22:0	0.24±0.07 a	0.25±0.06 a	0.16±0.03 a	0.25±0.07 a	0.25±0.04 a	0.26±0.04 a	0.23±0.11 a
Others	0.24±0.02 c	0.48±0.10 ab	0.21±0.04 c	0.29±0.06 bc	0.57±0.04 a	0.53±0.02 c	0.32±0.11 b
SFA[a]	20.19±0.22 a	17.89±0.04 c	18.60±0.03 b	18.91±0.15 b	18.90±0.04 b	18.09±0.03 c	15.67±0.13 d
MUFA[b]	59.13±0.39 g	70.14±0.31 b	63.00±0.15 f	65.59±0.33 e	66.81±0.08 d	69.11±0.15 e	76.10±0.64 a
PUFA[c]	20.21±0.10 a	11.23±0.10 f	18.04±0.13 b	14.98±0.18 c	13.48±0.11 d	12.01±0.11 c	7.67±0.28 g
MUFA/PUFA	2.93±0.02 g	6.25±0.01 b	3.49±0.05 f	4.38±0.04 e	4.96±0.02 d	5.75±0.03 c	9.92±0.05 a
PV[d]	13.2±1.8 a	8.4±0.5 c	11.3±0.7 a	9.3±0.7 bc	9.9±0.2 b	10.4±1.2 abc	13.4±1.7 a
AV[e]	1.86±0.02 b	0.30±0.02 d	2.00±0.02 a	1.92±0.15 ab	0.10±0.02 e	0.16±0.02 e	0.66±0.02 c
TT content[f]	388±67 b	102±1 d	457±24 a	44±11 e	144±6 c	109±15 d	382±12 b
TP content[g]	165±8 b	12±1 e	203±18 a	100±12 d	13±3 e	15±2 e	138±3 c

[*] Mean±SD (standard deviation) within a row with the same lowercase letters are not significantly different at $P< 0.05$.
[a] Saturated fatty acids (%); [b] Monounsaturated fatty acids (%); [c] Polyunsaturated fatty acid (%); [d] Peroxide value (meq O_2 per kg oil); [e] Acid value (mg KOH per g oil); [f] Total tocopherols (mg α-tocopherol per kg oil), [g] Total phenolics (mg gallic acid per kg oil).

Table 2. Total polar compounds (TPC, %) content of the olive oil samples during the heating process at 180ºC*.

Time (Hour)	Olive oil samples						
	1	2	3	4	5	6	7
0	3.98±0.19 Ec	4.74±0.28 Db	3.96±0.12 Dc	11.59±1.97 Ca	8.15±1.05 Ea	5.41±0.35 Db	11.52±1.60 Ca
4	10.27±1.50 Dc	9.76±1.12 Cc	8.03±1.10 Cc	15.98±1.27 Ca	12.95±1.16 Dabc	11.27±1.47 Cbc	14.45±1.21 BCab
8	16.98±2.31 Cab	13.63±2.10 ABCb	11.05±1.85 BCb	22.47±2.82 Ba	21.43±2.66 Ca	18.00±2.03 Bab	18.91±1.97 ABab
12	26.44±2.77 Bab	17.28±2.28 ABc	13.90±1.70 ABc	33.75±2.83 Aa	32.17±3.03 Ba	26.52±1.86 Aab	22.44±1.89 Ab
16	38.53±2.06 Aa	20.07±2.14 Acd	17.47±2.11 Ad	38.48±1.58 Aa	42.43±3.78 Aa	32.70±2.89 Aab	24.75±2.64 Abc

* Mean±SD (standard deviation) within a row with the same lowercase letters are not significantly different at P< 0.05. Means±SD within a column with the same uppercase letters are not significantly different at *P*< 0.05.
The corresponding linear equations: Sample 1, *y= 2.13x+2.19, R^2= 0.979*; Sample 2, *y= 0.96x+5.46, R^2= 0.989*; Sample 3, *y= 0.82x+4.30, R^2= 0.996*; Sample 4, *y= 1.79x+10.14, R^2= 0.976*; Sample 5, *y= 2.20x+5.87, R^2= 0.982*; Sample 6, *y= 1.75x+4.81, R^2= 0.996*, Sample 7, *y= 0.86x+11.52, R^2= 0.990*.

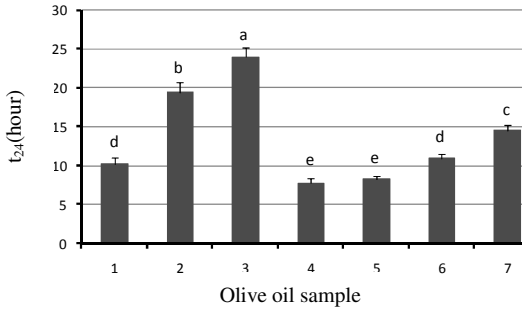

Figure 1. The time required to reach total polar compounds (TPC) content of 24% (t_{24}) for the olive oil samples (1–7) during the heating process at 180ºC. Means with the same lowercase letters are not significantly different at *P*< 0.05.

of the compositional parameters (Figure 2).

The relative contributions of compositional parameters to the RC_{TPC} of olive oils were calculated from the C_i values obtained from the regression models in Figure 2 (Table 3). Amongst the parameters studied, the MUFA/PUFA ratio showed the highest contribution (44.86%) to the RC_{TPC} of the olive oils. This clearly means that less monounsaturated and/or more polyunsaturated olive oils are more prone to produce polar compounds during heating processes. In other words, the MUFA/PUFA ratio can be considered a good indicator of olive oils stability.

The TP content, as Table 3 shows, played an important role in the RC_{TPC} of olive oils, so that its relative contribution to the production of polar compounds was 30.76%. The contribution of phenolic compounds to the olive oils stability and antioxidant activity has been estimated to be higher than that of other compounds (Gutierrez *et al.,* 2001; Pellegrini *et al.,* 2001), and a number of researchers have demonstrated a positive linear relationship between oil stability and the TP content (Gutfinger, 1981; Baldioli *et al.,* 1996). It has been shown that the major phenolic compounds in olive oils are oleuropein, hydroxytyrosol (3-dihydroxyphenylethanol), and tyrosol (4-

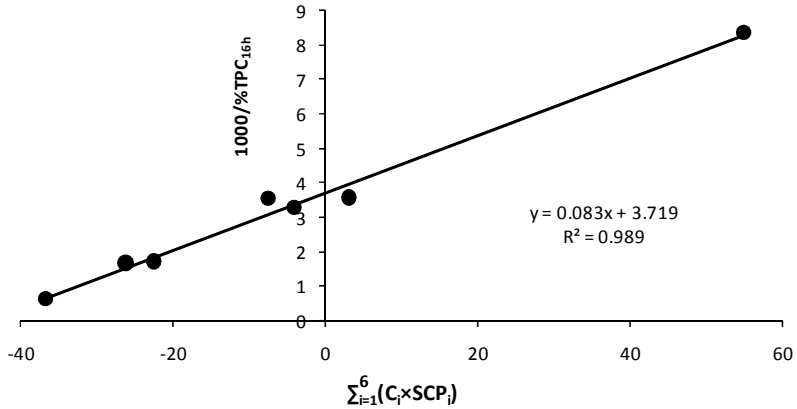

Figure 2. Linear relationship between the inverse of percentage increase in total polar compounds (TPC) content after 16 h of heating at 180ºC ($\%TPC_{16\,h}^{-1}$) and the unified variable consisting of the compositional parameters ($\sum_{i=1}^{6}(C_i \times SCP_i)$).

hydroxyphenylethanol). The phenolic compounds presented in olive oils are strong metal chelators (Chimi *et al.,* 1991) and radical scavengers (Tuck and Hayball, 2002). Typically, hydroxytyrosol is a superior antioxidant and radical scavenger to oleuropein and tyrosol (Tuck and Hayball 2002).

In addition to phenolic compounds, tocopherols are a group of the most important natural antioxidants in edible fats and oils. Autoxidation studies have demonstrated that tocopherols not only inhibit the formation of hydroperoxides (Lampi *et al.,* 1999) in many substrates, but also reduce the total amount of decomposition products from hydroperoxides (Lampi *et al.,* 1999; Kulas *et al.,* 2001; Makinen *et al.,* 2001; Kulas *et al.,* 2002). Nevertheless, it was interesting to find that these antioxidative compounds were actually ineffective on the production rate of polar compounds (0.01%) in our study. It should be noted that research on the antioxidant activity of tocopherols is conflicting sometimes, and this may be due to different experimental conditions and antioxidants concentrations (Schieberle and Grosch, 1981; Koskas *et al.,* 1984).

The PV was another important parameter that showed considerable contribution (20.51%) to the RC_{TPC} of olive oils. This means that olive oils with higher initial PVs are more prone to produce the polar compounds. Measurement of AV is important to determine the degree of hydrolytic

Table 3. The coefficients of compositional parameters (C_i values) obtained from the regression model and the relative contribution (%) of each parameter to the rate of changes in total polar compounds (TPC) content (RC_{TPC}) of olive oil samples.

Parameters	RC_{TPC}: $[1000\times(\%TPC_{16h}^{-1})]$	
	C_i value	Relative contribution (%)
$(PV^{-1})_{Std}$ [a]	16	20.51
$(AV^{-1})_{Std}$ [b]	3	3.85
$MUFA/PUFA_{Std}$ [e]	35	44.86
TT_{Std} [d]	0.01	0.01
TP_{Std} [e]	24	30.76
$(TPC^{-1})_{Std}$	0.01	0.01

[a] Peroxide value; [b] Acid value; [c] Monounsaturated/Polyunsaturated fatty acids ratio; [d] Total tocopherols, [e] Total phenolic compounds.

deterioration (Cecchi, 2003) and its level is a valuable determinant of oils quality. Surprisingly, however, the AV had a partial role in the RC_{TPC} of olive oils, so that its relative contribution to the production of polar compounds was 3.85%. A previous study indicated that the initial TPC content has a pronounced contribution to create the off-flavor compounds as well as a marked effect on the primary oxidation (Farhoosh and Pazhouhanmehr, 2009). However, our findings in the present study revealed that the initial TPC content had almost no influence on the production rate of polar compounds (0.01%, Table 3).

CONCLUSIONS

In the present study, the effect of two groups of initial quality (PV, AV, and TPC content) and compositional parameters (TT content, TP content, and MUFA/PUFA ratio) on the rate of changes in the TPC content of olive oil was investigated during heating process. The parameters in the first group are function of handling, storage, and/or processing conditions, while the parameters in the second group are indigenous properties of edible fats and oils. Our study showed that the most effective initial quality and compositional parameters during heating process of olive oil were MUFA/PUFA ratio (44.86%), TP content (30.76%), and PV (20.51%), respectively.

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اثر پارامترهای ساختاری و کیفی اولیه بر شکل‌گیری کل ترکیبات قطبی طی حرارت دهی مداوم روغن زیتون

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چکیده

پژوهش حاضر به قصد تعیین سهم نسبی پارامترهای ساختاری و کیفی اولیه در سرعت تغییرات میزان کل ترکیبات قطبی به عنوان یکی از مطمئن‌ترین شاخصهای کیفیت روغنهای حرارت دیده طی حرارت دهی پیوسته روغن زیتون در دمای ۱۸۰ درجه سانتیگراد به اجرا در آمد. نسبت اسیدهای چرب تک غیراشباع به چند غیراشباع حائز بالاترین سهم نسبی (۴۴/۸۶ درصد) در این خصوص بود. سرعت تغییرات میزان کل ترکیبات قطبی به طور قابل ملاحظه‌ای (۳۰/۷۶ درصد) تحت تاثیر میزان کل ترکیبات فنلی قرار گرفت اما میزان کل ترکیبات توکوفرولی در این خصوص بواقع بی-تاثیر بود (۰/۰۱ درصد). عدد پراکسید، پارامتر حائز اهمیت دیگری بود که توانست سهم مشخصی از سرعت تغییرات میزان کل ترکیبات قطبی را به خود اختصاص دهد (۲۰/۵۱ درصد). سهم عدد اسیدی و میزان کل ترکیبات قطبی اولیه در این خصوص به ترتیب ۳/۸۵ و ۰/۰۱ درصد تعیین شد.