academicJournals

Vol. 13(12), pp. 1386-1392, 19 March, 2014
DOI: 10.5897/AJB12.1763
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Characterization and quantification of phenolic compounds of extra-virgin olive oils according to their blending proportions

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Received 4 May, 2012; Accepted12 July, 2012

Chemlali olive oil was blended with oils obtained from Oueslati and Chetoui varieties to improve the quality of the former one. Parameters such as triacylglycerols and phenolic compounds were characterized for various blends of Chemlali x Oueslati and Chemlali x Chetoui. Results show that blended oils had an improved composition as compared to that of Chemlali. In fact, the highest percentage of Oueslati and Chetoui olive oils (60% of blending) could lead to 1,2,3-trioleylglycerol (OOO) of up to 30.20 and 33.44%, respectively. The amount of aldehydic form of oleuropeine aglucon was higher when Chemlali was blended using either 40 or 60% of Chetoui olive oil (from 99.17 to 299.63 and 334.16 mg kg^{-1}).

Key words: Oil blending, Chemlali x Oueslati, Chemlali x Chetoui, phenolic fraction, triacylglycerols.

INTRODUCTION

Olive oil is high-value edible oil which is prized for its flavor as well as health characteristics (Harwood and Aparicio, 2000). It is produced predominantly in Southern Europe and North Africa where it forms part of the 'Mediterranean diet' (Harwood and Yaqoob, 2002). Olive oil has a long history and is associated with a low incidence of coronary-vascular disease (Wahrburg et al., 2002). Its oxidative stability and flavour characteristics are associated with its lipid composition and the presence of minor compounds such as triacylglycerol and phenolic compounds (Baldioli et al., 1996; Servili and Montedoro, 2002). It may also have benefit in reducing obesity, anti-

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Abbreviations: VOO, Virgin olive oil; **LLL,** 1,2,3-trilinoleylglycerol; **LnLO,** 1-oleyl-2-linoleyl-3-linolenoylglycerol; **LLO,** 1,2 dilinoleyl-3-oleylglycerol; **LOO,** 1,2-dioleyl-3-linoleyglycerol; **OLnO,** 1,2-dioleyl-3-linolenoylglycerol; **LnOP,** 1-palmitoyl-2-linolenoyl-3-oleylglycerol; **LLP,** 1-palmitoyl-2,3-dilinoleyl-glycerol; **L,** 1,2-dioleyl-3-linoleyglycerol; **POL,** 1-palmitoyl-2-oleyl-3-linoleylglycerol; **OOO,** 1,2,3-trioleylglycerol; **POO,** 1-palmitoyl-2,3-dioleyl-glycerol; **SOO,** 1-stearoyl-2,3-dioleyl-glycerol; **SOP,** 1-stearoyl-2-palmitoyl-3-oleylglycerol; **AOO,** 1-arachidoyl-2,3-dioleyl-glycerol.

allergic, antimicrobial, anticarcinogenic, antiviral, cancer and inflammatory diseases (Cavaliere et al., 2008; Xia et al., 2008).

Phenolic acids have been associated with colour and sensory qualities, as well as with the health-related and antioxidant properties of foods (Cartoni et al., 2000). One impetus for analytical investigations has been the role of phenolics in the organoleptic properties (flavour, astringency and hardness) of foods (Suárez et al., 2008). Additionally, the content and profile of phenolic acids, their effect on fruit maturation, prevention of enzymatic browning, and their roles as food preservatives has been evaluated (Wu et al., 2008). Several authors have reported that flavonoids such as luteolin and apigenin are also phenolic components of VOO (Carrasco-Pancorbo et al., 2005). Luteolin may originate from rutin or luteolin-7-glucoside, and apigenin from apigenin glucosides. There are also several interesting studies in which several flavonoids have been found in olive leaves and fruits (Kalua et al., 2006).

In Tunisia, the second VOO exporter and producer after the European Union (Haddada et al., 2007a), the two main cultivars are Chemlali and Chetoui. Chetoui, the second main variety cultivated in Tunisia is widespread in the north of the country, occurring in plains as well as in mountain regions, and shows a high capacity of adaptation to various pedo-climatic conditions (Ben Temime et al., 2006). In addition, this oil has a very balanced fatty acid profile and significant/high amount of phenols, which make it quite astringent. This latter characteristic is not accepted by the majority of consumers, especially by kids. Chemlali variety, which is mainly cultivated in the central and southern areas of the country, contributes to 80% of the national olive oil production. It is a productive variety, well adapted to severe environmental conditions. However, its oil is characterized by relatively low levels of oleic acid (54 to 60%), triacylglycerols (Bachir et al., 2007) and phenols (Youssef et al., 2011a). Oueslati variety cultivated in Tunisia is widespread in the centre of the country known by their higher phenol content, >500 mg/kg, stability was measured at 101.6°C, >55 h, oleic acid, >70% and richest on volatile compounds (Youssef et al., 2011b).

Several studies have been published on the analysis of olive oil triacylglycerols (Paradiso et al., 2010; Haddada et al., 2007b; Sánchez et al., 2004) and phenolic compounds (Baccouri et al., 2008; Allalout et al., 2009; Ocakoglu et al., 2009; Youssef et al., 2011a; Stefanoudaki et al., 2011). Many publications reported on the composition of phenolic compounds and triacylglycerols of monovarietal oils, but there are no similar studies on the phenolic compounds and triacylglycerols of oils obtained by blending of monovarietal oils.

The aim of this study was to investigate the effect of blending Chemlali olive oil with the oil obtained from two Tunisian varieties (Oueslati and Chetoui) on phenolic compounds and triacylglycerols.

MATERIALS AND METHODS

Oil samples

Oil was extracted from 3 kg of fruits of each variety during the crop season 2009/2010 (October). Samples were prepared by blending oils of three different cultivars (Chemlali, Oueslati and Chetoui) in different pre-established proportions (20, 40 and 60%) (Youssef et al., 2011b). The olives were washed and deleafed, crushed with a hammer crusher, and the paste was mixed at 25°C for 30 min, centrifuged without addition of warm water and then transferred into dark glass bottles and stored (the olive oils were blended before storage) in the dark at 4°C (1 week) until analysis. Three replicates were prepared for all the samples.

Analysis of total phenolic content

Total phenol and o-diphenol contents were quantified colorimetrically (Ranalli et al., 1999). Phenolic compounds were isolated by triple extraction of a solution of oil (10 g) in hexane (20 ml) with 30 ml of a methanol-water mixture (60:40, v/v). The Folin–Ciocalteau reagent (Merck Schuchardt OHG, Hohenbrunn, Germany) was added to a suitable aliquot of the combined extracts, and the absorption of the solution at 725 nm was measured. Values are expressed as milligrams of caffeic acid per kilogram of oil (Gutfinger, 1981). O-Diphenols were also measured colorimetrically at 370 nm after adding 5% (w/v) sodium molybdate in 50% ethanol to the extract (Gutfinger, 1981). Results are expressed as milligrams of caffeic acid per kilogram of oil.

Analysis of the composition of TAG

Solid phase extraction

The optimized procedure was as follows. VOO (0.2 g) was weighed and dissolved in 0.5 ml *n*-hexane. The silica cartridge (Sep-Pak cartridge, Waters Corporation, USA) was conditioned with 10 ml of n-hexane before the application of oil solution. The TAG fraction was obtained with subsequent elution using mixtures of 15 ml of n-hexane/diethylic ether (90:10, v/v), and then, the solvent of the collected fractions were evaporated to dryness.

HPLC analysis of triacylglycerol

Extracted TAG (0.05 g) was dissolved in 1 mL of acetone for HPLC analysis, and the injected volume was 0.2 µL. A Hewlett-Packard HPLC (HP 1050, Agilent Technology) quaternary pump instrument equipped with a refractometer detector was employed using a Lichrosorb RP18 column (250 × 4.6 mm, 5 µL particle size; Teknocroma, Barcelona, Spain). Settings were column oven, 45°C; elution solvent, acetone/acetonitrile (70:30, v/v) at a flow rate of 1.4 mL/min for 55 min. The standards used were trilinolein (LLL), triolein (OOO), tripalmitin (PPP), tristearin (SSS), trilinolenin (LnLnLn) and tripalmitolein (PoPoPo) (purity greater than 98%), purchased from Sigma (St. Louis, MO). The abbreviations used for the fatty acids were Po for palmitoleic, L for linoleic, Ln for linolenic, O for oleic, P for palmitic, S for stearic and A for arachidic. TAGs in olive oils were separated according to equivalent carbon number. Determination of the difference between the theoretical value of triacylglycerols (TAGs) with an equivalent carbon number of 42 (ECN 42theoretical) was calculated from the fatty acid composition (Youssef et al., 2011b), and the analytical results (ECN 42HPLC) was obtained by determination of the oil by high performance liquid chromatography.

Analysis of phenolic compounds by RP-HPLC

Analysis of phenolic compounds was carried out by reversed-phase liquid chromatography with ultraviolet detection (Mateos et al., 2001) A solution of p-hydroxyphenyl acetic (0.12 mg/ml) and o-coumaric acids (0.01 mg/ml) in methanol was used as standard. A sample of filtered virgin olive oil (2.5 ± 0.001 g) was weighed and 0.5 ml of standard solution was added. The solvent was evaporated in a rotary evaporator at 40°C under vacuum and the residue was dissolved in 6 ml of hexane. A diol-bonded phase cartridge was placed in a vacuum elution apparatus and conditioned by the consecutive addition of 6 ml of methanol and 6 ml of hexane. The vacuum was then released to prevent drying of the column. The oil solution was applied to the column, and the solvent was pulled through, leaving the sample and the standard on the solid phase. The sample container was washed with 6 ml of hexane, which was run out of the cartridge. The sample container was washed again with 4 ml of hexane/ethyl acetate (85:15, v/v), which was run out of the cartridge and discarded. Finally, the column was eluted with 10 ml of methanol and the solvent was evaporated in a rotary evaporator at room temperature and low speed under vacuum until dryness. The residue was extracted with 0.5 ml of methanol/water (1:1 v/v) at 40°C and the obtained solution was left to rest for 4 h.

Then, the aliquot of 20 µl of the final solution was injected to the HPLC system. A JASCO HPLC system was equipped with a double plunger pump and a diode array UV detector. A Lichrospher 100 RP-18 column (250 × 4 mm id, particle size 5 µm, Merck) was used. The mobile phase consisted of a mixture of water/phosphoric acid (99.5:0.5, v/v) (solvent A) and methanol/acetonitrile (50:50, v/v) (solvent B). The gradient elution program used: 95% (A) / 5% (B) in 0 min 70% (A) / 30% (B) in 25 min 62% (A) / 38% (B) in 40 min 45% (A) / 45% (B) in 45 min 52.5% (A) / 47.5% (B) in 5 min 100% (B) in 5 min 100% (B) was maintained for 5 min and run was ended. Quantification of total phenols was carried out using phydroxyphenyl acetic acid as internal standard and the quantification of flavones and ferulic acid was done using o-coumaric acid as internal standard, and the results was expressed in mg kg^{-1} oil. Triplicate determinations were made.

Statistics

All parameters were determined in triplicate for each sample. Analysis of variance (ANOVA) was conducted using SPSS statistical package (Version 12.0 for Window, SPSS Inc. Chicago, Illinois, 2003). Statistical significance was contrasted using one way ANOVA and Tukey's HSD test at 5% confidence level.

RESULTS AND DISCUSION

Effect of the mixture of oils in its triacylglycerol composition

Table 1 shows the percentage of all compounds from the triacylglycerol for the various blends obtained from different proportions of two monovarietal oils (Chemlali x Oueslati and Chemlali x Chetoui). The main triacylglycerols detected were 1,2,3-trioleylglycerol (OOO), 2,3-dioleyl-1-palmitoylglycerol (POO) and 2,3-dioleyl-1-linoleylglycerol (LOO). Other minor triacylglycerols identified were 2,3-dioleyl-1-stearoylglycerol (SOO), 2-oleyl-3-palmitoyl-1-stearoylglycerol (SOP), 1-linolenoyl-2-oleyl-3-palmitoylglycerol (LnOP), 1,2-dilinoleyl-3-palmitoylglycerol (LLP), 1,3-dioleyl-2-linolenoylglycerol (OLnO), 1-linolenoyl-2-linoleyl-3-oleylglycerol (LnLO) and 1,2,3-trilinoleylglycerol (LLL) that remain unmentioned. These results are in agreement with the findings of other authors (Bachir et al., 2007). OOO is always the main abundant compound in olive oils, ranging from 24.95 to 38.23% of total triacylglycerols. It was observed that blending could increase OOO (Table 1). At 40% blending with Oueslati and Chetoui olive oil, OOO increased from 24.95 to 30.14 and 32.39%, respectively (Table 1). The blended oils exhibited a significant increase of LOO at the different proportions of mixing oils (from 20 to 60% of blending); in contrast, the 1-palmitoyl-2-oleyl-3-linoleylglycerol (POL) and POO decreased (Table 1).

Triacylglycerol composition can also be used as a measurement of the quality and purity of vegetable oils. Elevated levels of trilinolein in olive oil may be used as an indication of the presence of seed oils. However, some olive oils naturally have high trilinolein levels; so, it is more useful to compare the theoretical equivalent carbon number (ECN) for trilinolein, calculated from the fatty acid composition, with the ECN which is determined by analysis. The ECN is defined as CN-2n where CN is the carbon atom number of the fatty acids in the triglycerol molecule and 'n' is the number of double bonds and for trilinolein, it is 42. The difference between the empirical and theoretical ECN42 triacylglycerol content (ΔECN42) is an European Union official method since 1997. The higher mean value of ΔECN42 of Chemlali olive oil experienced a significant decrease when Oueslati and Chetoui olive oils were added.

As shown in Table 1, blending of cultivars had significant influence on the other triacylglycerols fractions. These results are consistent with other research in which mixtures of olive oil with other oils from seed sources were investigated on the basis of their triacylglycerol composition (Peter, 1993; Ali et al., 1995).

Effect of the mixture of oils on its phenolic composition

Table 2 shows the concentrations of all the phenolic compounds for the various blends obtained from different proportions of two monovarietal oils (Chemlali x Oueslati and Chemlali x Chetoui).

The analysis of phenolic compounds using reverse phase-high performance liquid chromatography (RPHPLC) with UV detection as described by Mateos et al. (2001), allowed the separation and the identification of these compounds. Results show no qualitative differences in the RPHPLC phenolic fraction profile between virgin olive oils from different proportions. However, significant quantitative differences were observed in a wide number of phenolic compounds.

Chemlali olive oil had poorest level of phenolic compounds in comparison with Chetoui andOueslati ones. The most important secoiridoids detected were:

Table 1. Triacylglycerols (%) of blended oils in different proportions (Chemlali x Oueslati and Chemlali x Chetoui).

Parameter	Chemlali x Oueslati					Chemlali x Chetoui			
	Chemlali (100%)	Oueslati (20%)	Oueslati (40%)	Oueslati (60%)	Oueslati (100%)	Chetoui (20%)	Chetoui (40%)	Chetoui (60%)	Chetoui (100%)
LLL (%)	0.51^{a} ±0.01	0.52^{a} ±0.01	0.50^{b} ±0.00	0.49^{b} ±0.03	0.46^{c} ±0.01	0.4 8^{b} ±0.05	0.45^{c} ±0.02	0.41^{d} ±0.03	0.38^{e} ±0.08
LnLO (%)	0.30^{a} ±0.02	0.32^{b} ±0.02	0.36^{c} ±0.03	0.37^{d} ±0.01	0.48^{e} ±0.06	0.31^{b} ±0.02	0.30^{c} ±0.02	0.32^{d} ±0.03	0.36^{e} ±0.02
LLO (%)	5.99^{a} ±0.11	5.97^{a} ±0.12	6.14^{ab} ±0.08	6.29^{b} ±0.05	6.97^{c} ±0.62	5.66^{b} ±0.09	5.17^{c} ±0.05	5.04^{d} ±0.04	4.59^{e} ±0.41
OLnO (%)	1.56^{a} ±0.02	1.42^{b} ±0.04	1.21^{c} ±0.03	1.07^{d} ±0.02	0.84^{e} ± 0.03	1.49^{b} ±0.01	1.39^{c} ±0.03	1.16^{d} ±0.06	0.95^{e} ±0.22
LnOP (%)	2.6 1^{a} ±0.03	2.70^{a} ±0.01	2.06^{b} ±0.01	1.98^{b} ±0.07	1.35 ^{c} ±0.11	2.33^{b} ±0.04	2.10^{c} ±0.01	1.93^{d} ±0.07	1.10^{e} ±0.02
LLP (%)	1.34^{a} ±0.04	1.29^{b} ±0.02	1.10^{c} ±0.02	0.93^{d} ±0.01	0.38^{e} ±0.06	1.29^{b} ±0.01	1.13^{c} ±0.03	0.89^{d} ±0.01	0.40^{e} ±0.09
LOO (%)	17.10^{a} ±0.14	17.94^{b} ±0.22	18.62^{c} ±0.19	18.99^{d} ±0.44	21.00^{e} ±0.17	17.32^{b} ±0.21	18.89^{c} ±0.17	19.95^{d} ±0.33	21.00^{e} ±0.62
PO L (%)	13. 83^{a} ±0.21	12.87^{b} ±0.05	11.11^{c} ±0.02	10.86^{d} ±0.51	8.73^{e} ±0.12	12.95^{b} ±0.11	12.06^{c} ±0.11	10.15^{d} ±0.12	8.02^{e} ± 0.11
OOO (%)	24.95^{a} ±0.45	25.26^{b} ±0.02	30.14^{c} ±0.03	30.20^{cd} ±0.74	34.23^{e} ±1.11	25.69^{b} ±0.53	32.39^{c} ±0.92	33.44^{d} ±0.31	38.23^{e} ±0.19
POO (%)	21.13^{a} ±0.76	21.15^{a} ±0.06	20.06^{b} ±0.55	19.93^{bc} ±0.35	17.19^{d} ±0.59	20.77^{b} ±0.77	19.03^{c} ±0.22	18.12^{d} ±0.13	15.10^{e} ±1.02
SOO (%)	5.09^{a} ±0.01	4.89^{b} ±0.03	4.30^{c} ±0.12	3.49^{d} ±0.11	2.90^{e} ±0.01	4.97^{b} ±0.10	3.79^{c} ±0.00	3.32^{d} ±0.03	2.91^{e} ±0.34
SOP (%)	4. 61^{a} ±0.02	4.65^{b} ±0.03	4.58^{c} ±0.03	4.51^{c} ±0.10	4.40^{d} ±0.41	4.72^{b} ±0.14	4.44^{c} ±0.04	4.35^{d} ±0.03	3. 91^{e} ±0.03
AOO (%)	0.98^{a} ±0.01	1.02^{a} ±0.02	0.92^{b} ±0.01	0.89^{c} ±0.00	0.81^{d} ±0.03	0.97^{a} ±0.00	0.96^{b} ±0.03	0.97^{b} ±0.02	1.00^{c} ±0.05
ECN_{42}	0.93^{a} ±0.03	0.77^{b} ±0.01	0.049^{c} ±0.00	0.037^{d} ±0.01	0.14^{e} ±0.04	0.64^{b} ±0.02	0.41^{c} ±0.02	0.31^{d} ±0.04	0.20^{e} ±0.01

$^{a-e}$Mean ± SD, significant differences within the same row are shown by different letters ($P < 0.05$). Data values expressed in %. LLL, 1,2,3-Trilinoleylglycerol; LnLO, 1-oleyl-2-linoleyl-3-linolenoylglycerol; LLO, 1,2 dilinoleyl-3-oleylglycerol; LOO, 1,2-dioleyl-3-linoleyglycerol; OLnO, 1,2-dioleyl-3-linolenoylglycerol; LnOP, 1-palmitoyl-2-linolenoyl-3-oleylglycerol; LLP, 1-palmitoyl-2,3-dilinoleyl-glycerol; L, 1,2-dioleyl-3-linoleyglycerol; POL, 1-palmitoyl-2-oleyl-3-linoleylglycerol; OOO, 1,2,3-trioleylglycerol; POO, 1-palmitoyl-2,3-dioleyl-glycerol; SOO, 1-stearoyl-2,3-dioleyl-glycerol; SOP, 1-stearoyl-2-palmitoyl-3-oleylglycerol; AOO, 1-arachidoyl-2,3-dioleyl-glycerol.

Dialde-hydic form of ligostroside aglucon, dialdehydic form of oleuropeine aglucon and aldehydic form of oleuropeine aglucon.

The low dialdehydic form of ligostroside aglucon con-tent (<47.11 mg kg^{-1}) of Chemlali olive oil, experienced a significant increase when Oueslati and Chetoui olive oils were added (Table 2). The amount of dialdehydic form of ligostroside aglucon content slowly increased with the percentage of blending. It is also remarkable that Chetoui olive oil is the best one for dialdehydic form of ligostroside aglucon fortification in Chemlali olive oil (123.27 mg kg^{-1}). For the aldehydic form of oleuropeine aglucon, virgin olive oil from Oueslati showed the lowest value (25.63 $mgkg^{-1}$) and the highest value was observed from Chetoui (578.18 mg kg^{-1}). At 60% blending with Chetoui olive oil, aldehydic form of oleuro-peine aglucon increased from 99.17 to 334.16 mg kg^{-1}, and oleuropeine aglucon tyrosol acetate decreased from 5.36 to 2.12 mg kg^{-1} (Table 2). Using 60% Oueslati olive oil, aldehydic form of oleuropeine aglucon underwent a significant de-crease to 54.88 mg kg^{-1} and, at the same time, a decrease of oleuropeine aglucon tyrosol acetate to 3.12 mg kg^{-1} was observed.

With regards to phenolic acids, the concentration of vanillic acid, vanillin, p-coumaric acid and ferulic acid were dissimilar in different blends of olive oil. All the oils produced and analysed (Table 2) showed very low values for the phenolic acid (vanillic, p-coumaric and ferulic acids). These results are similar to those reported by several authors for other olive oil varieties (Krichene et al., 2009; Youssef et al., 2011a). There were only minor changes in the amount of phenolic compounds when olive oil from both cultivars were blended (Table 2).

The main simple phenols found in the Chemlali, Oueslati and Chetoui virgin olive oil were hydroxytyrosol and tyrosol. The concentration of hydroxytyrosol, was generally higher than that of tyrosol. The blending process improved phenols by increasing the hydroxytyrosol and tyrosol content of Chemlali oil. At 60% blending, hydroxytyrosol

Table 2. Phenolic composition (mg.kg^{-1}) of blended oils in different proportions (Chemlali x Oueslati and Chemlali x Chetoui).

Parameter	Chemlali x Oueslati					Chemlali x Chetoui			
	Chemlali (100%)	Oueslati (20%)	Oueslati (40%)	Oueslati (60%)	Oueslati (100%)	Chetoui (20%)	Chetoui (40%)	Chetoui (60%)	Chetoui (100%)
Hydroxytyrosol (mg.kg^{-1})	1.74^{a} ±0.02	1.66^{b} ±0.03	1.42^{c} ±0.02	4.04^{d} ±0.00	7.43^{e} ±0.03	3.17^{b} ±0.00	6.87^{c} ±0.03	10.11^{d} ±0.02	19.79^{e} ±0.53
Tyrosol (mg.kg^{-1})	1.13^{a} ±0.04	1.31^{b} ±0.05	1.30^{b} ±0.03	1.61^{c} ±0.02	1.94^{d} ±0.03	5.12^{b} ±0.12	8.43^{c} ±0.02	14.17^{d} ±0.13	25.22^{e} ±0.66
Vanillic acid (mg.kg^{-1})	tr	0.11^{b} ±0.00	0.11^{b} ±0.01	0.12^{b} ±0.04	0.29^{c} ±0.05	0.09^{b} ±0.00	0.11^{c} ±0.00	0.14^{d} ±0.02	0.28^{e} ±0.02
Vanillin (mg.kg^{-1})	0.66^{a} ±0.01	0.65^{a} ±0.12	0.64^{a} ±0.00	0.61^{b} ±0.03	0.50^{c} ±0.01	0.63^{b} ±0.01	0.59^{c} ±0.02	0.48^{d} ±0.01	tr
p-coumaric acid (mg.kg^{-1})	tr	0.10^{b} ±0.01	0.19^{c} ±0.01	0.29^{d} ±0.01	0.54 ^{e} ±0.09	0.14^{b} ±0.02	0.22^{c} ±0.01	0.34^{d} ±0.03	0.85^{e} ±0.02
Ferulic acid (mg.kg^{-1})	tr	0.12^{b} ±0.01	0.18^{c} ±0.02	0.27^{d} ±0.02	0.52^{e} ±0.03	0.08^{b} ±0.00	0.11^{c} ±0.02	0.15^{d} ±0.02	0.41^{e} ±0.03
Hydroxytyrosol acetate (mg.kg^{-1})	0.03^{a} ±0.00	0.14^{b} ±0.02	0.32^{c} ±0.07	0.63^{d} ±0.02	1.07^{e} ±0.02	0.10^{a} ±0.01	0.16^{b} ±0.00	0.19^{c} ±0.00	0.32^{d} ±0.03
Dialdehydic form of oleuropeine aglucon (mg.kg^{-1})	16.32^{a} ±0.52	21.17^{b} ±0.33	28.44^{c} ±0.04	34.17^{d} ±0.54	46.22^{e} ±1.00	20.35^{a} ±0.11	26.18^{b} ±0.23	37.12^{c} ±0.02	53.11^{d} ±0.34
Oleuropeine aglucon tyrosol acetate (mg.kg^{-1})	5.36 ^{a} ±0.12	5.03^{b} ±0.14	4.66^{c} ±0.07	3.12^{d} ±0.21	tr	5.29^{b} ±0.07	4.89^{c} ±0.12	2.12^{d} ±0.01	2.04^{e} ±0.02
Dialdehydic form of ligostroside aglucon (mg.kg^{-1})	47.11^{a} ±1.11	60.17^{b} ±1.41	81.28^{c} ±1.37	99.15^{d} ±1.34	128.74^{e} ±1.15	68.68^{b} ±0.89	98.14^{c} ±0.04	123.27^{d} ±0.77	199.22^{e} ±0.98
Aldehydic form of oleuropeine aglucon (mg.kg^{-1})	99.17^{a} ±1.46	90.27^{b} ±1.01	70.19^{c} ±1.14	54.88^{d} ±1.21	25.21^{e} ±0.46	133.69^{f} ±1.11	299.63^{a} ±1.23	334.16^{b} ±1.44	578.18^{c} ±1.11
Aldehydic form of ligostroside aglucon (mg.kg^{-1})	37.55^{a} ±0.52	33.75^{b} ±0.78	29.28^{c} ±0.67	25.63^{d} ±0.34	14.77^{e} ±0.12	42.55^{b} ±0.56	53.18^{c} ±0.61	71.03^{d} ±0.81	122.16^{e} ±0.13
Pinoresinol (mg.kg^{-1})	3.66^{a} ±0.02	3.32^{a} ±0.22	3.10^{a} ±0.03	2.47^{b} ±0.08	tr	3.98^{b} ±0.02	4.14^{c} ±0.06	5.22^{d} ±0.22	7.22^{e} ±0.12
Acetoxypinoresinol (mg.kg^{-1})	1.03^{a} ±0.03	4.18^{b} ±0.03	7.98^{c} ±0.11	11.16^{d} ±0.04	19.53^{e} ±0.52	3.13^{b} ±0.04	4.99^{c} ±0.01	6.87^{d} ±0.31	11.54^{e} ±0.21
Luteolin (mg.kg^{-1})	1.13^{a} ±0.01	1.13^{a} ±0.02	1.29^{b} ±0.03	1.36^{c} ±0.03	1.66^{d} ±0.01	1.29^{b} ±0.05	1.45^{c} ±0.01	1.53^{d} ±0.01	1.96^{e} ±0.05
Apigenin (mg.kg^{-1})	0.43^{a} ±0.03	0.44^{a} ±0.03	0.45^{b} ±0.05	0.41^{c} ±0.02	0.46^{d} ±0.02	0.51^{b} ±0.01	0.62^{c} ±0.02	0.73^{d} ±0.02	1.39^{e} ±0.04
Phenols (mg.kg^{-1})	190.09^{a} ±0.4	215.17^{b} ±2.40	283.17^{c} ±1.27	401.2^{d} ±2±1.01	510.42^{e} ±0.40	254.12^{b} ±0.6	406.14^{c} ±0.32	736.89^{d} ±4.66	928.88^{e} ±13.2
o-Diphenols (mg.kg^{-1})	91.13^{a} ±0.20	100.65^{b} ±0.16	120.31^{c} ±2.06	141.42^{d} ±0.12	185.62^{e} ±0.20	93.08^{b} ±1.27	142.14^{c} ±2.22	202.19^{d} ±3.47	282.82^{e} ±40.95

$^{a-e}$Mean ± SD, significant differences within the same row are shown by different letters ($P < 0.05$). tr: < 0.1%; Data values are expressed in mg.kg^{-1}.

increased from 1.74 to 4.04 and 10.11 mg kg^{-1}, while tyrosol increased from 1.13 to 1.61 and 14.17 mg kg^{-1} when Oueslati and Chetoui olive oil was used respectively.

Lignans are present also in considerable amount, particularly acetoxypinoresinol which is found in all analysed samples at concentrations ranging from 1.03 mg kg^{-1} in virgin olive oils from Chemlali to 11.54 and 19.53 mg kg^{-1} in virgin olive oils from Chetoui and Oueslati, moreover, significant differences were observed in its contents with different proportions (Table 2). The process was observed only when the blending was carried out at important percentages, such as 60% (from 1.03 to 11.16 mg kg^{-1} with Oueslati and from 1.03 to 6.87 mg kg^{-1} with Chetoui olive oils), while, pinoresinol is detected in low concentration not more than 7.22 mg kg^{-1} (Table 2).

As seen in simple phenols and secoiridoids, a considerable variation in lignans concentrations between olive oils of various proportions also occurs in this case, the reasons probably being related when some cultivars are contemporary milled separately and then obtained oils were blended, some interactions and/or synergisms could occur among the enzymes involved in the lipoxygenase cascade.

Low flavonoid levels represented by luteolin and apigenin were observed in all the olive oils analysed, with concentrations that varied from 1.13 to 1.96 mg kg^{-1} and 0.43 to 1.39 mg kg^{-1}, respectively.

The low phenol content (<200 mg kg^{-1}) of Chemlali olive oil showed a significant increase when Oueslati and Chetoui olive oils were added (Table 2). The amount of phenols slowly increased with the percentage of blending. At 20%, the amount of phenols increased from 190.09 to 215.17 and 254.12 mg kg^{-1} with Oueslati and Chetoui olive oils, respectively. At the highest percentages (60%) with Oueslati, o-diphenols in Chemlali olive oil underwent about twofold increase. Further, 60% of blending with Chetoui oil improved the amount of total phenols in Chemlali from 91.13 to 202.19 mg/kg (threefold). The concentrations of total phenols and o-diphenols appeared to proportionally vary according to the relative proportion of each monovarietal oils in the mixtures.

Conclusion

The changes of quality of monovarietal VOOs play an important role in blending application. The blending process using different percentages of other olive oils improved the fatty acid compo-sition and volatile compounds (Youssef et al., 2011b). The lowest proportions to obtain blends endowed with equilibrated triacylglycerols and phenolic compound were about 40%. In addition, these results confirmed that the accumulation of each triacylglycerols and phenolic compound in monovarietal oils was different and closely dependent on the genetic store of each variety. The understanding of the pathway that produces the phenolic compounds is also important in enhancing the quality of olive oils (Youssef et al., 2011a). Therefore, blending of cultivars had significant influence on the triacylglycerols fractions and phenolic compounds.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work was done as part of a National Research Project. We thank the Ministry of Higher Education, Scientific Research and Technology for financially supporting this program. This work was supported by The Tunisian Ministry of High Education, Scientific Research and Technology. The authors gratefully acknowledge the personnel of the laboratories.

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