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Abstract	Oil blending was conducted to study the effects of changes in fatty acid composition (FAC), tocopherols and total phenol content (TPC) on oxidative stability of virgin olive oil (VOO):walnut oil (WO) blends. The measurement of the antioxidant activity of bioactive components present in the parent oils and blends was achieved by their ability to scavenge the free stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·). The highest percentage of DPPH· inhibition was found for pure VOO, and the lowest one for pure WO. EC_{50} values obtained from the DPPH assay correlated significantly and inversely with TPC. The generation of volatile flavor components in VOO indicated the predominance of C_6 compounds produced through biochemical (enzymatic) pathways, whereas WO showed increased concentrations of medium chain (C_7–C_{11}) aldehydes produced through chemical (oxidative) pathways. The results obtained confirm the importance of VOO phenolics in providing protection against oxidation in VOO and VOO/WO blends. However, considering the impact of FAC and the content of endogenous antioxidant substances mentioned previously on the oxidative stability of the oils analyzed, the effect of an elevated unsaturation level (WO) prevails over a high amount of such bioactive components (VOO).

Keywords (separated by '-')	Olive oil - Walnut oil - Blends - Chemical composition - Oxidative stability
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ORIGINAL PAPER

Contribution of Compositional Parameters to the Oxidative Stability of Olive and Walnut Oil Blends

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Abstract Oil blending was conducted to study the effects of changes in fatty acid composition (FAC), tocopherols and total phenol content (TPC) on oxidative stability of virgin olive oil (VOO):walnut oil (WO) blends. The measurement of the antioxidant activity of bioactive components present in the parent oils and blends was achieved by their ability to scavenge the free stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·). The highest percentage of DPPH· inhibition was found for pure VOO, and the lowest one for pure WO. EC_{50} values obtained from the DPPH assay correlated significantly and inversely with TPC. The generation of volatile flavor components in VOO indicated the predominance of C_6 compounds produced through biochemical (enzymatic) pathways, whereas WO showed increased concentrations of medium chain (C_7–C_{11}) aldehydes produced through chemical (oxidative) pathways. The results obtained confirm the importance of VOO phenolics in providing protection against oxidation in VOO and VOO/WO blends. However, considering the impact of FAC and the content of endogenous antioxidant substances mentioned previously on the oxidative stability of the oils analyzed, the effect of an elevated unsaturation level (WO) prevails over a high amount of such bioactive components (VOO).

Keywords Olive oil · Walnut oil · Blends · Chemical composition · Oxidative stability

Abbreviations

AV	Acid value
CD	Conjugated dienes
CT	Conjugated trienes
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FA	Fatty acid(s)
FAC	Fatty acid composition
FAME	Fatty acid methyl ester(s)
FID	Flame-ionization detector
GC–MS	Gas chromatography–mass spectrometry
HPLC	High-performance liquid chromatography
I_2V	Iodine value
MUFA	Monounsaturated fatty acid(s)
OR	Oxidation rate
OSI	Oxidative stability index
PUFA	Polyunsaturated fatty acid(s)
PV	Hydroperoxide value
RSC	Radical scavenging capacity
SOT	Schaal oven test
SPME	Solid-phase micro-extraction
TBARS	Thiobarbituric acid reactive substances
TPC	Total phenol content
TTC	Total tocopherol content
VOO	Virgin olive oil
WO	Walnut oil

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Introduction

Although olives and olive oil are part of the Mediterranean diet and culture, their production and consumption have steadily increased around the world, even in countries that do not have a tradition in olive cultivation. Olive oil is usually

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sold as virgin olive oil (VOO) which is highly appreciated by consumers due to its unique aroma and taste and nutritional profile. One of the most important characteristics of VOO is the presence of a high content (>53%) of oleic acid. This fact together with an unusual quantity of phenolic compounds with strong antioxidant properties, make VOO particularly stable against oxidative degradation [1–3].

Walnut (*Juglans regia* L.) cultivation is also gaining interest owing to the increasing demand of the nut (kernel) and its by-products. The walnut kernel contains high levels (52–72%) of oil, which can be extracted by screw pressing [4]. Walnut oil (WO) composition is quite different to that of VOO. Although certain factors such as genotype, geographical origin and extraction methods may influence the fatty acid (FA) composition and some minor components—i.e. tocopherols, polyphenols, pigments, et cetera—of such oils [5–9], there can be no doubt that, among vegetable oils, WO contains one of the highest amounts of the essential C_{18} (linoleic and linolenic) polyunsaturated FA (PUFA). Even though this FA profile is nutritionally favorable, it may result in a poor oxidative stability and shelf life of the oil. Furthermore, the oxidation of PUFA may result in the generation of volatile compounds among which many have unpleasant odors and are responsible for flavor problems in the food industry.

To avoid the mentioned problems, one avenue that has not been thoroughly explored is the blending of vegetable oils as a way of modifying their physicochemical characteristics besides enhancement in thermal and oxidative stabilities. For instance, proper mixing of high-linoleic (and/or linolenic) with high-oleic oils may result in enhanced oxidative stability of the former, but the presence of pro-oxidant and antioxidant substances may also influence the oxidation rate of the resulting blends. Neff et al. [10] reported that the oxidative stability of soybean oil—which is similar to WO in FA composition—can be improved by blending with palm olein. Chu and Kung [11] used high-oleic sunflower and safflower oils, corn, canola, olive, peanut and sesame oils to improve the oxidative stability of soybean oil. They found that stability of the blends was mainly affected by the FA and tocopherol composition of the parent oils. In this work, mixing of VOO with WO was proposed to study the effects of changes in FA composition, tocopherol and phenol contents on some oxidative parameters of various VOO/WO blends.

Experimental Procedures

Oil Sources

Olive oil was obtained from Manzanilla variety cultivated at Cruz del Eje locality, Córdoba province, Argentina. Healthy olives were picked by hand from the trees. An aliquot of 100 fruits was taken in order to determine the ripeness index (3.3) in accordance with the method proposed by Hermoso et al. [12]. Fruits were cleaned and taken rapidly to a pilot plant for oil extraction using a traditional pressure system. Briefly, the olives were ground employing a metal hammer crusher. The olive paste was kneaded for 30 min at 27 ± 1 °C and then squeezed at 300 bar pressure. The liquid obtained (aqueous and oily) was separated in a stainless steel decanter. The oil obtained was filtered through anhydrous Na_2SO_4 and stored in dark glass bottles until analysis.

Walnut oil was obtained from healthy and mature fruits of the Chandler variety cultivated at Belén location, Catamarca Province, Argentina. After cleaning, the fruits were dried at 30 ± 2 °C for a day and then were shelled manually. Seeds containing about 72% oil (Soxhlet, *n*-hexane, dry basis) and 4% moisture (w/w) were ground and particles between 2.4 and 4.8 mm were selected using an automated screen. Oil expression was carried out with a Komet screw press (Model CA 59 G, IBG Monforts, Mönchengladbach, Germany), with a 5-mm restriction die and a screw speed of 20 rpm. The screw press was first run for 15 min without seed material but with heating via an electrical resistance-heating ring attached around the press barrel, to raise and maintain the screw-press barrel temperature to the desired temperature (25 °C) [4].

Four oil blends were prepared by mixing different ratios of the parent oils (VOO and WO) in proportions of 80:20, 60:40, 40:60, and 20:80% (w/w). The oils were thoroughly mixed to form uniform blends, and their quality evaluation was done by employing an accelerated oxidative stability test.

Oil Analyses

Thiobarbituric acid reactive substances (TBARS), acid, hydroperoxide, conjugated diene and conjugated triene values of the oil samples were determined according to standard methods of AOCS [13]. The oxidative stability indices (OSI) were determined by Rancimat analysis and corresponded to the break points in the plotted curves. Air flow rate was set at 20 L/h and temperature of the heating block was maintained at 110 °C.

To evaluate the radical scavenging capacity (RSC) of the oil samples, two sets of experiments were carried out. In the first one, 100 mg (in 1 mL toluene) of each oil sample was vortexed (20 s, ambient temperature) with 3.9 mL toluene solution (10^{-4} M) of the free stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical (DPPH). Against a blank of pure toluene, the absorption at 515 nm was measured in 1 cm quartz cells after 30 min of mixing using an UV–visible spectrophotometer (Perkin-Elmer Lambda

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25, Shelton, CT, USA). RSC toward DPPH· was estimated by mean of the following equation:

$$\mathrm{DPPH}\cdot_{\mathrm{r}} = 1 - \left[\frac{\text{absorbance of control} - \text{absorbance of test sample}}{\text{absorbance of control}} \times 100\right]$$

where $\mathrm{DPPH}\cdot_{\mathrm{r}}$ expresses the amount of the radical that remains in the medium after antioxidants depletion [14]. In the second experiment, six concentrations (25, 50, 75, 100, 125 and 150 mg of oil in 1 mL toluene) were prepared for each oil sample. The oil/toluene solutions were added separately to 3.9 mL of DPPH· solution (10^{-4} M) and the absorbance of each mixture was determined at 515 nm after 30 min of mixing. The RSC was expressed as EC_{50} which was defined as the concentration at which 50% of the initial absorbance was reduced. A lower EC_{50} value indicates a higher antiradical activity.

For FA composition, each oil sample (0.5 g) was subjected to alkaline saponification by reflux (45 min) using 30 mL 1 N KOH in methanol. Unsaponifiable matter was extracted with *n*-hexane (3 × 30 mL). The fatty acids were converted to methyl esters (FAME) by reflux (45 min) using 50 mL 1 N H_2SO_4 in methanol and analyzed by gas chromatography (GC) (Perkin-Elmer Clarus 500, Shelton, CT, USA) using a fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) CP Wax 52 CB (Varian, Walnut Creek, CA, USA); carrier gas N_2 at 1 mL/min; split ratio 100:1; column temperature programmed from 180 °C (5 min) to 220 °C at 2 °C/min; injector and detector temperatures at 250 °C, FID. The identification of FAME was carried out by comparison of their retention times with those of reference compounds (Sigma-Aldrich, St. Louis, MO, USA). FA levels were estimated on the basis of peak areas from known concentrations of the standards.

Iodine values (I_2V) were calculated from fatty acid percentages by using the formula:

$$\begin{aligned} I_2V = {} & (\%\ \text{oleic acid} \times 0.899) \\ & + (\%\ \text{linoleic acid} \times 1.814) \\ & + (\%\ \text{linolenic acid} \times 2.737) \end{aligned}$$

Tocopherols were analyzed by HPLC according to the procedure of Pocklington and Dieffenbacher [15]. In brief, samples of 1 g oil were placed into 25-mL volumetric flasks. A quantity of *n*-hexane was added, swirling to dissolve the sample and making up to volume with the same solvent. An aliquot of 20 μL of this solution was injected on to a Lichrosorb SI 60 column (Varian, Walnut Creek, CA, USA). The mobile phase was *n*-hexane/2-propanol (98/2 v/v) with a flow rate of 0.3 mL/min. UV detection at 295 nm was performed. Individual tocopherols were identified by comparison of their retention times with those of authentic standards (ICN Biomedicals, Costa Mesa, CA). Individual tocopherols were quantified by the external standard method. The linearity of the response was verified by fitting to line results of each one tocopherol individuals of six standard solutions with known concentrations.

Total phenol content was analyzed from 20-g aliquots of oil. They were dissolved in 10 mL of *n*-hexane and extracted three times with 12.5 mL of methanol/water (60:40 v/v) by stirring over a magnetic plate for 15 min. The pooled extracts were washed twice with 10 mL of *n*-hexane, and solvents were removed in a rotating evaporator (Büchi, Flawil, Switzerland) at 30 °C under vacuum. To a suitable dilution of the extracts, Folin–Ciocalteau reagent (Fluka, Buchs, Switzerland) was added and the absorbance values of the solutions at 725 nm (total phenols, expressed as mg gallic acid/kg oil) were measured.

Chlorophyll and carotenoid compounds were determined at 670 and 470 nm, respectively, in cyclohexane via specific extinction values using the method of Mínguez-Mosquera et al. [16].

Volatile compounds were analyzed by solid-phase micro-extraction (SPME) coupled to GC–MS. Briefly, fresh oil samples (5 mL) were put in 15-mL headspace vials, fitted with silicon septa, and heated to 50 °C. Volatiles were sampled for 30 min from the headspace of the vial, with a 100-μm fiber coated with carboxen/polydimethylsiloxane, conditioned prior to use as recommended by the producer. After sampling, the fiber was immediately inserted into the injection port (250 °C) of a HP 5890 II gas chromatograph coupled to a HP 5972 A mass selective detector (Hewlett Packard, Palo Alto, CA, USA), and it was thermally desorbed for 1 min. The GC separations were performed using a HP 5 fused silica capillary column (30 m long × 0.25 mm i.d.) coated with a 0.25-μm layer of 5% phenyl methyl siloxane, and helium (flow rate 1 mL min^{-1}) as carrier gas. The GC oven temperature was initially maintained at 50 °C (2 min) and then increased to 250 °C (5 °C min^{-1}). Volatile compounds were identified by comparison of their mass spectra data with those of the Wiley 275 mass spectra search library. Identification of the components was also based on their GC retention indices on HP5 column, determined relative to the retention times of a series of C5–C30 *n*-alkanes.

Experimental Design for Oxidative Stability Test

An accelerated stability test (Schaal oven test, SOT) was performed to evaluate the oxidative stability of the parent oils and blends. It has been reported that one day of storage under Schaal oven conditions is equivalent to one month's storage at room temperature [17]. Three replicates of each oil and oil blend sample (50 g each) were

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Table 1 Compositional and oxidative parameters of virgin olive oil (VOO), walnut oil (WO) and their blends

	VOO	Oil blends (VOO:WO, w/w)				WO
		80:20	60:40	40:60	20:80	
AV	0.18^{d}	0.21^{e}	0.18^{d}	0.13^{c}	0.09^{b}	0.05^{a}
PV	5.92^{f}	5.30^{e}	4.15^{d}	2.70^{c}	1.50^{b}	0.10^{a}
CD	1.95^{d}	1.73^{cd}	1.64^{bc}	1.62^{bc}	1.39^{ab}	1.27^{a}
CT	0.11^{c}	0.11^{c}	0.10^{bc}	0.10^{bc}	0.08^{a}	0.08^{a}
TBARS	0.33^{a}	0.80^{ab}	3.16^{b}	3.86^{b}	7.64^{c}	14.01^{d}
OSI	41.12^{e}	10.73^{d}	8.17^{c}	5.66^{b}	3.66^{a}	2.34^{a}
OR	0.04^{a}	0.16^{b}	0.39^{c}	0.42^{c}	0.49^{d}	0.53^{e}
EC_{50}	393.70^{a}	437.51^{b}	496.24^{c}	563.72^{d}	657.54^{e}	808.73^{f}
FA						
16:0	15.55^{f}	12.68^{e}	10.50^{d}	9.49^{c}	8.17^{b}	5.97^{a}
16:1	2.20^{c}	2.11^{bc}	1.64^{ab}	1.32^{a}	Tr	Nd
18:0	1.18^{a}	0.99^{a}	0.99^{a}	1.05^{a}	1.11^{a}	1.04^{a}
18:1	73.43^{f}	61.22^{e}	49.16^{d}	38.36^{c}	28.01^{b}	16.02^{a}
18:2	6.84^{a}	18.53^{b}	29.13^{c}	37.65^{d}	47.33^{e}	57.13^{f}
18:3	0.84^{a}	4.52^{b}	8.66^{c}	12.16^{d}	15.32^{e}	19.84^{f}
MUFA	75.65^{f}	63.39^{e}	50.66^{d}	39.70^{c}	28.14^{b}	16.05^{a}
PUFA	7.67^{a}	23.12^{b}	37.82^{c}	49.83^{d}	62.76^{e}	77.08^{f}
I_2V	82.97^{a}	103.11^{b}	122.21^{c}	137.31^{d}	153.14^{e}	172.41^{f}
TPC	255.61^{d}	158.45^{c}	77.03^{b}	46.24^{ab}	32.22^{ab}	Nd
α-Toc	246.14^{e}	180.44^{d}	115.28^{c}	61.30^{b}	30.77^{a}	Nd
γ-Toc	Nd	59.73^{a}	91.54^{b}	157.63^{c}	267.71^{d}	338.10^{e}
δ-Toc	28.71^{e}	16.50^{d}	11.32^{c}	9.15^{b}	6.05^{a}	5.48^{a}
Carotenoids	3.14^{f}	3.00^{e}	2.65^{d}	2.38^{c}	1.99^{b}	1.06^{a}
Chlorophylls	7.31^{f}	6.29^{e}	4.89^{d}	3.87^{c}	2.58^{b}	0.55^{a}

AV acid value (% oleic acid), *PV* hydroperoxide value (mequiv O_2/kg oil), *CD* conjugated dienes, *CT* conjugated trienes, *TBARS* thiobarbituric acid reactive substances (μmol MDA/g), *OSI* oxidative stability index (hours), *OR* oxidation rate, EC_{50} (mg oil/mg DPPH), *FA* fatty acids (% of total fatty acids), *MUFA* monounsaturated fatty acids; *PUFA* polyunsaturated fatty acids; I_2V iodine value; *TPC* total phenol content (μg/g oil), *Toc* tocopherol (μg/g oil), carotenoid and chlorophyll contents (μg/g oil), *Tr* trace (<0.1%), *Nd* not detected

Mean values were the averages of three independent measurements. Values in each row with different superscript letters, present significant differences ($p \leq 0.05$) among oil samples

stored in 100-mL beakers without covers at 60 °C in the dark for seven days. Every day, each individual oil sample was removed from the oven and used to measure the hydroperoxide, CD, CT and DPPH·$_r$ values as indicated previously.

Statistical Analyses

Analytical determinations reported in this study were the average of triplicate measurements from three independent oil samples for each treatment. Statistical differences among treatments were estimated from ANOVA test, at $p < 0.05$. Correlation analysis was performed employing Pearson's test. The oxidation rate (OR) of the parent oils and blends was determined as PV changes during storage time using linear regression models.

Results and Discussion

Acid (AV) and hydroperoxide (PV) values, conjugated dienes (CD) and trienes (CT), and TBARS, are indicators of hydrolytic and oxidative degradation of vegetable oils. Pure and blended oils analyzed in this study had very low AV, CD and CT values (Table 1). AV and PV from fresh VOO were significantly higher than those from WO. This may be explained by the high water content of the olive fruit which favors the hydrolysis of triglycerides during oil extraction, resulting in increased free fatty acid concentration. This fact, in turn, may enhance the PV of the oil because of the major oxidative susceptibility of free fatty acids. TBARS were present at significantly higher concentration in pure WO. These oxidation products, among which malonaldehyde is the largest, mainly arise

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from PUFA containing three or more double bonds [17], such as linolenic acid, presents in elevated concentrations in WO.

Fatty acid composition of the parent oils (VOO and WO) and their blends is presented in Table 1. Oleic acid was predominant in VOO (73.8%) followed by palmitic (15.7%) and linoleic (6.8%) acids. WO was characterized by a high content of linoleic acid (57.1%), oleic and linolenic acids at similar amounts (16.0 and 19.8%, respectively) and palmitic acid at lesser concentration (5.97%). Except stearic acid, oil blending significantly modified the concentration of FA analyzed. The major changes were observed for oleic, linoleic and linolenic FA contents. For example, adding VOO to WO at 20, 40, 60 and 80% caused a gradual increase of 12, 22.3, 33.1 and 45.2%, respectively, in oleic acid proportions of the resulting blends with respect to pure WO.

Tocopherols and other phenolic compounds (commonly named as total phenol content, TPC) in nuts and olive oils, were previously identified as the main components responsible for their free RSC and oxidative stability [14, 18]. VOO from the Manzanilla cultivar used in this work had a total tocopherol content (TTC) of 275 mg/kg, which was mainly composed of α-tocopherol. This value was in the medium range of TTC (170–400 mg/kg) reported for other olive varieties [2]. Although the pure WO presented a TTC similar to that of the pure VOO, a very different qualitative pattern was observed among them: in WO, γ-tocopherol was predominant together with minor amounts of δ-tocopherol (Table 1).

Regarding phenolic compounds, VOO analyzed here had similar a TPC to that of Manzanilla olive oil from Spain [19]. Among nuts, walnut kernels have one of the highest phenolic content [20]. Walnut phenolics are mainly polyphenolics of the non-flavonoid type and fall into the category of ellagitannins; they have been reported to display strong antioxidant and free radical-scavenging capacities [20, 21]. However, they are poorly extracted with the oil [22] probably due to their low oil solubility. Phenolic compounds were not detected in the pure WO analyzed here. Therefore, the activity of phenolics other than tocopherols appears to be negligible in providing some protection against oxidation in WOs.

Regarding the antioxidant activity of pure VOO, it is important to note that: (a) α-tocopherol accounted for 89% of the total tocopherol content, and (b) this tocopherol isomer was found to have a poor antioxidant activity in olive oil [1, 14, 23]. Therefore, the RSC of VOO should be mainly attributed to its TPC and, at a lesser extend, to the presence of tocopherols. This hypothesis is also supported by data from Baldioli et al. [1] and Ben Témine et al. [24], who showed a clear influence of TPC on olive oil stability and a much lower contribution of α-tocopherol.

Chlorophylls and carotenoids are the main pigments in olive oils ([25] and Refs. therein). There are no reports about these compounds in walnut oils. Chlorophylls content varied from 0.55 (pure WO) to 7.31 (pure VOO) μg/g oil; carotenoids were in the range 1.0–3.14 μg/g oil (WO–VOO, respectively) (Table 1). In addition to their contribution for color attributes, these pigments may play an important role in oxidative stability of vegetable oils. It has been reported that carotenoids are effective inhibitors of photosensitized oxidation by quenching singlet oxygen, whereas chlorophylls are found to have antioxidant activity in dark but pro-oxidant in light [26].

The measurement of the antioxidant activity of bioactive components present in vegetable oils may be achieved by their ability to scavenge free radicals. The DPPH· assay is widely used for determination of total antioxidant activity. By means of spectrophotometric recordings obtained in a kinetic assay, after 30 min incubation a decrease in the remaining DPPH· concentration was observed when VOO was added to WO. At that time, DPPH· radicals were quenched and the reaction reached a plateau, indicating the DPPH· concentration that remains in the medium after antioxidants present in the oils are depleted. The highest percentage of DPPH· inhibition was found for pure VOO, and the lowest one for pure WO (Fig. 1). The VOO/WO blends had intermediate values. The data also revealed an overall increase in the remaining DPPH· concentration during the storage period in the SOT indicating that a consumption (and/or degradation) of the bioactive antioxidant substances took place during storing, in a time-depending manner. The EC_{50} values from the DPPH assay using different oil concentrations (Table 1) confirm the results obtained previously: The order of effectiveness of pure oils and blends in inhibiting DPPH· was as follow: VOO > 80:20 (VOO/WO) > 60:40 > 40:60 > 20:80 > WO. EC_{50} values correlated significantly and inversely with

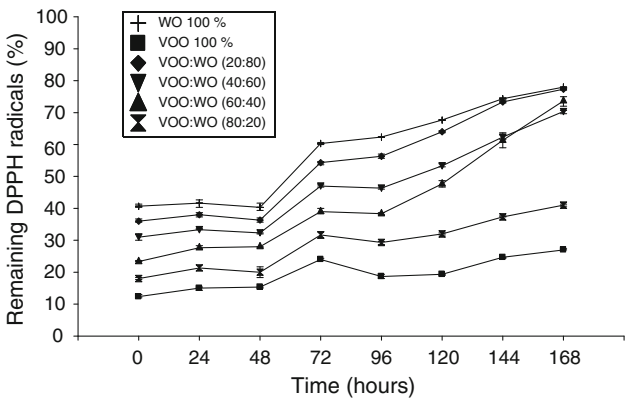

Fig. 1 Radical-scavenging activity (remaining DPPH radicals) during oxidation of VOO, WO and their blends in the Schaal oven test. Plotted values are means of three independent determinations ± standard deviation

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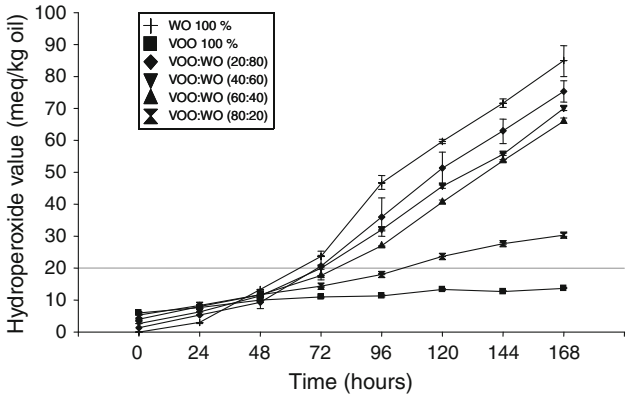

Fig. 2 Kinetic curve of hydroperoxide accumulation during oxidation of VOO, WO and their blends in the Schaal oven test. Plotted values are means of three independent determinations ± standard deviation

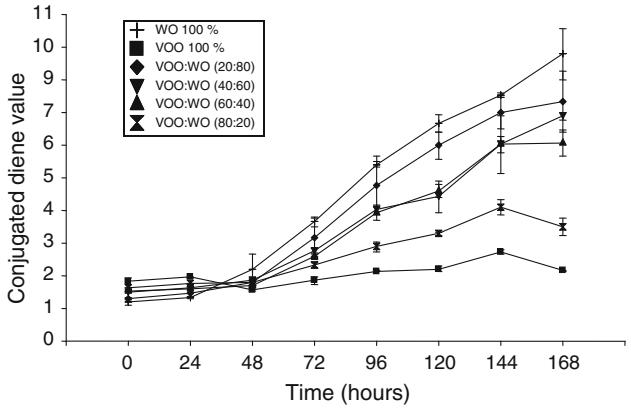

Fig. 3 Kinetic curve of conjugated diene values during oxidation of VOO, WO and their blends in the Schaal oven test. Plotted values are means of three independent determinations ± standard deviation

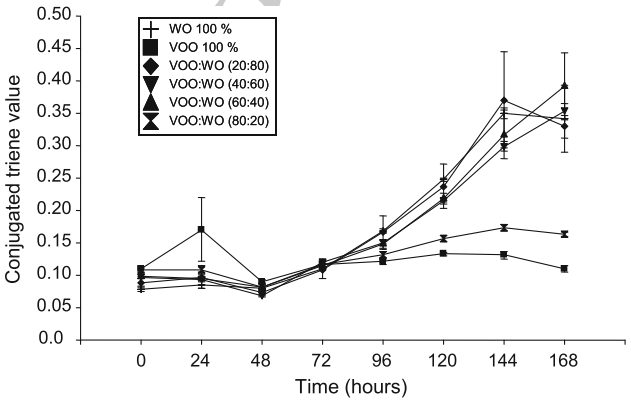

Fig. 4 Kinetic curve of conjugated triene values during oxidation of VOO, WO and their blends in the Schaal oven test. Plotted values are means of three independent determinations ± standard deviation

TPC ($r = -0.56$, $p < 0.01$). All these results indicate that antioxidants present in VOO, particularly total phenols, effectively act as free radical scavengers but their efficacy may be affected by temperature, as also suggested by Espín et al. [14].

Figures 2, 3 and 4 show the effects of blending VOO with WO on the development of PV, CD and CT during storage of the oils in the SOT. The blends 60:40, 40:60 and 20:80 (VOO/WO) and the pure WO, despite differences in their initial composition, showed similar oxidative deterioration patterns, whereas the remaining blend and the pure VOO revealed the highest stability. In each treatment, the plotted curve for hydroperoxide accumulation approximately coincided with that of CD indicating that the formation of lipid hydroperoxides matches with that of conjugated double-bond FA. After seven days of storage at 60 °C, the pure VOO did not reach the induction period (IP, the time needed for the PV of the sample to become 20 mequiv O_2/kg oil). The pure WO and the blend 20:80 (VOO/WO) had the shortest IP (about 64 h); the other blends showed IP values ranging from 72 h (40:60 VOO/WO) to 108 h (80:20 VOO/WO). The OR values (Table 1) dramatically increased in the following order: pure VOO (0.04, $R^2 = 0.90$), VOO/WO 80:20 (0.16, $R^2 = 0.99$), VOO/WO 60:40 (0.39, $R^2 = 0.95$), VOO/WO 40:60 (0.42, $R^2 = 0.97$), VOO/WO 20:80 (0.49, $R^2 = 0.93$), pure WO (0.53, $R^2 = 0.97$).

Oxidative stability indexes determined by Rancimat analysis (Table 1) confirmed that WO has very low thermal stability; the OSI value obtained (2.34 h) was in good agreement with data published previously [27]. The OSI from pure VOO was seventeen times higher than that from pure WO. The addition of VOO to WO resulted in an enhancement of the OSI from the resulting blends. The data obtained showed that every 20% addition of VOO to pure WO, the OSI values increased by factors of 1.56 (20:80 VOO/WO), 2.42 (40:60), 3.49 (60:40) and 4.57 (80:20). The results also revealed that OSI values correlated positively with both oleic and total phenol contents ($r = 0.74$ and 0.91, respectively, $p < 0.01$), and negatively with each of two PUFA (linoleic acid $r = -0.75$, linolenic acid $r = -0.71$, $p < 0.01$). No significant correlations were observed among OSI and each of the following parameters: tocopherol, chlorophyll and carotenoid contents.

In relation to volatile composition (Table 2), the pure VOO was characterized by elevated concentrations of C_6 compounds, mainly *trans*-2-hexanal and, to a lesser extent, 1-hexanol and hexanal. These short chain aldehydes and alcohol are produced through the lipoxygenase (LOX) pathway [28] and contribute to fruity, grassy, green-sweety and apple-like flavours (Table 2). The majority of the volatile compounds found in the pure WO were those reported previously as constituents of varietal WO using

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Table 2 Volatile composition (% normalized areas) of virgin olive oil (VOO), walnut oil (WO) and their blends

Compounds	VOO	Oil blends (VOO:WO, w/w)				WO	Sensory descriptors [3, 17, 23]
		80:20	60:40	40:60	20:80		
Hydrocarbons							
n-Pentane	Nd	Tr	Tr	3.28^{a}	15.56^{b}	20.56^{c}	ND
n-Hexane	Nd	Tr	Tr	Tr	6.80^{a}	5.22^{a}	ND
Alcohols							
1-Hexanol	31.91^{c}	10.36^{b}	5.68^{a}	Tr	Nd	Nd	Fruit, banana, soft, aromatic, rough
Aldehydes							
Pentanal	Nd	Tr	4.10^{a}	9.08^{b}	4.57^{a}	8.89^{b}	Woody, bitter, oily
Hexanal	7.53^{a}	36.12^{c}	62.81^{d}	20.34^{bc}	15.74^{b}	16.33^{b}	Green apple, grass, green-sweet
trans-2-Hexenal	60.55^{bc}	53.45^{b}	24.65^{a}	Tr	Nd	Nd	Green, apple-like, bitter, astringent
Heptanal	Nd	Nd	2.15^{a}	3.50^{ab}	2.57^{a}	Tr	Oily, fatty, woody
trans-2-Heptenal	Nd	Nd	0.59^{a}	4.89^{cd}	3.73^{bc}	5.91^{de}	Oxidized, tallowy, pungent
Octanal	Nd	Nd	Tr	4.31^{b}	2.40^{a}	2.97^{a}	Fatty, sharp
2-Octenal	Nd	Nd	Tr	3.40^{b}	2.72^{a}	Tr	Herbaceous, spicy, green
Nonanal	Nd	Tr	Tr	8.02^{c}	5.67^{a}	6.80^{ab}	Fatty, waxy, pungent
Decanal	Nd	Nd	Tr	1.41^{a}	1.93^{b}	Tr	Penetrating, sweet, waxy
trans-2-Decenal	Nd	Tr	Tr	6.35^{b}	5.90^{b}	4.09^{a}	Painty, fishy, fatty
2, 4-Decadienal	Nd	Tr	Tr	6.91^{a}	22.0^{b}	20.45^{b}	Deep-fried
2-Undecenal	Nd	Tr	Tr	5.93^{ab}	5.20^{a}	4.67^{a}	ND
Furan derivatives							
2-Pentylfuran	Nd	Nd	Tr	4.92^{b}	3.35^{a}	4.05^{ab}	ND

Mean values were the averages of three independent measurements. Values in each row with different superscript letters present significant differences ($p \leq 0.05$) among oil samples

Nd not detected, *Tr* trace (<0.3%), *ND* not determined

the SPME–GC–MS method [9, 27]. 2,4-Decadienal was quantitatively the largest carbonyl compound; *n*-pentane was also present in high amounts. These compounds are produced by oxidative breakdown of 9 and 13-hydroperoxides, respectively, arose from linoleic acid. Other carbonyl compounds found in minor amounts were saturated and unsaturated C_7–C_{11} aldehydes derived from oxidative degradation of different oleate hydroperoxide isomers [17]. In spite of the relative abundance of linolenic acid in WO, 2,4-heptadienal (one of the most important linolenate hydroperoxide derivatives) was not found. Some volatile decomposition compounds derived from linoleic acid, such as 2-octenal and 2-pentylfuran, can not be explained by the classical hydroperoxide cleavage mechanisms. They may be attributed to further oxidation of unsaturated aldehydes [17]. Addition of WO to VOO increased markedly the concentration of medium chain ($\geq C_7$) carbonyl compounds. Considering the sensory attributes characterising such volatile compounds (Table 2), these facts could affect adversely the sensory profile of the resulting VOO/WO blends.

Conclusion

The results discussed in this work provide information about the relative contribution of major and minor components present in VOO and WO to their oxidative stability. Taking into account the similar amounts of total tocopherols present in both VOO and WO, and considering the significantly higher RSC found in the former, the data obtained confirm the importance of VOO phenolics in providing protection against oxidation in VOO and VOO/WO blends. However, considering the impact of FA composition and the content of the endogenous antioxidant substances mentioned previously on the oxidative stability of the oils analyzed, the effect of an elevated unsaturation level (WO) prevails over a high amount of such bioactive components (VOO).

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References

1. Baldioli M, Servili M, Perretti G, Montedoro GF (1996) Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. J Am Oil Chem Soc 73:1589–1593
2. Uceda M, Hermoso M (1999) La Calidad del Aceite de Oliva. In: Barranco D, Fernández-Escobar L, Rallo L (eds) El Cultivo del Olivo. Ediciones Mundi-Prensa, Andalucía, pp 571–596
3. Kalua CM, Allen MS, Bedgood DR Jr, Bishop AG, Prenzler PD, Robards K (2007) Olive oil volatile compounds, flavour development and quality: a critical review. Food Chem 100:273–286
4. Martínez ML, Mattea M, Maestri DM (2008) Pressing and supercritical carbon dioxide extraction of walnut oil. J Food Eng 88:399–404
5. Amaral JS, Rui Alvez M, Seabra RM, Oliveira BPP (2005) Vitamin E composition of walnuts (*Juglans regia* L): a 3-year comparative study of different cultivars. J Agric Food Chem 53:5467–5472
6. Crews C, Hough P, Godward J, Brereton P, Lees M, Guiet S, Winkelmann W (2005) Study of the main constituents of some authentic walnut oils. J Agric Food Chem 53:4853–4860
7. Torres MM, Martínez ML, Maestri DM (2005) A multivariate study of the relationship between fatty acids and volatile flavor components in olive and walnut oils. J Am Oil Chem Soc 82:105–110
8. Torres MM, Maestri DM (2006) Chemical composition of arbequina virgin olive oil in relation to extraction and storage conditions. J Sci Food Agric 86:2311–2317
9. Martínez ML, Mattea M, Maestri DM (2006) Varietal and crop year effects on lipid composition of walnut (*Juglans regia*) genotypes. J Am Oil Chem Soc 83:791–796
10. Neff WE, El-Agaimy MA, Mounts TL (1994) Oxidative stability of blends and interesterified blends of soybean oil and palm olein. J Am Oil Chem Soc 71:1111–1116
11. Chu Y, Kung Y (1998) A study on vegetable oil blends. Food Chem 62:191–195
12. Hermoso M, Uceda M, Frías L, Beltrán G (1999) Maduración. In: Barranco D, Fernández-Escobar L, Rallo L (eds) El Cultivo del Olivo. Ediciones Mundi-Prensa, Andalucía, pp 151–167
13. CS AO (1998) Official methods and recommended practices of the American Oil Chemists' Society, 5th edn. AOCS Press, Champaign
14. Espín JC, Soler-Rivas C, Wichers H (2000) Characterization of the total free radical scavenger capacity of vegetable oils and oils fractions using 2,2-diphenyl-1-picrylhydrazyl radical. J Agric Food Chem 48:648–656
15. Pocklington WD, Dieffenbacher A (1988) Determination of tocopherols and tocotrienols in vegetable oils and fats by high performance liquid chromatography. Pure Appl Chem 60: 877–892
16. Mínguez-Mosquera MI, Rejano L, Gandul B, Sánchez AH, Garrido J (1991) Colour pigment correlation in virgin olive oil. J Am Oil Chem Soc 68:322–337
17. Frankel EN (2005) Lipid oxidation, 2nd edn. The Oily Press, Bridgwater
18. Ramadan MF, Moersel J (2006) Screening of the antiradical action of vegetable oils. J Food Comp Anal 19:838–842
19. Pardo JE, Cuesta MA, Alvarruiz A (2007) Evaluation of potential and real quality of virgin olive oil from the designation of origin "Aceite Campo de Montiel" (Ciudad Real, España). Food Chem 100:977–984
20. Yang J, Liu RH, Halim L (2009) Antioxidant and antiproliferative activities of common edible nut seeds. LWT-Food Sci Technol 42:1–8
21. Fukuda T, Ito H, Yoshida T (2003) Antioxidative polyphenols from walnut (*Juglans regia* L.). Phytochemistry 63:795–801
22. Arranz S, Pérez-Jiménez J, Saura-Calixto F (2008) Antioxidant capacity of walnut (*Juglans regia* L.): contribution of oil and defatted matter. Eur Food Res Technol 227:425–431
23. Wagner KH, Elmadfa I (2000) Effect of tocopherols and their mixtures on the oxidative stability of olive oil and linseed oil under heating. Eur J Lipid Sci Technol 102:624–629
24. Ben Témine S, Baccouri B, Taamalli B, Abaza L, Ben Miled D, Zarrouk M (2006) Location effects on oxidative stability of chétoui virgin olive oil. J Food Biochem 30:659–670
25. Criado MN, Morelló JR, Motilva MJ, Romero MP (2004) Effect of growing area on pigment and phenolic fractions of virgin olive oils of the arbequina variety in Spain. J Am Oil Chem Soc 81:633–640
26. Beltrán G, Aguilera MP, Del Río C, Sánchez S, Martínez L (2005) Influence of fruit ripening process on the natural antioxidant content of hojiblanca virgin olive oils. Food Chem 89:207–215
27. Martínez ML, Maestri DM (2008) Oil chemical variation in walnut (*Juglans regia* L) genotypes grown in Argentina. Eur J Lipid Sci Technol 110:1183–1189
28. Angerosa F, Servili M, Selvaggini R, Taticchi A, Esposto S, Montedoro G (2004) Volatile compounds in virgin olive oil: occurrence and their relationship with the quality. J Chrom A 1054:17–31

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