GRASAS Y ACEITES 66 (1)
January–March 2015, e061
ISSN-L: 0017-3495
doi: http://dx.doi.org/10.3989/gya.0702142

Chemical composition and sensory evaluation of virgin olive oils from "Morisca" and "Carrasqueña" olive varieties

M. Fuentes[a], C. De Miguel[b], A. Ranalli[c], M.N. Franco[a], M. Martínez[d] and D. Martín-Vertedor[a,✉]

[a]Technological Agri-Food Institute (INTAEX), Avda. Adolfo Suárez s/n, 06071, Badajoz, Spain
[b]Vegetal Biology, Ecology and Soil Sciences Department, University of Extremadura, Spain
[c]Centro de Ricerca per l´Olivicoltura e l´Industria Olearia (CRA-OLI), Italy
[d]Agronomic and Forestry Engineering Department, University of Extremadura, Spain

✉Corresponding author: daniel.martin@gobex.es

Submitted: 23 June 2014; Accepted: 08 October 2014

SUMMARY: Two varieties of olive fruit ("Morisca" and "Carrasqueña"), in different ripening stages, have been characterized on the basis of the study of major (triglycerides and fatty acids) and minor compounds (sterols and triterpenics dialcohols) as well as of total phenols, oxidative stability and sensory characteristics. The "Carrasqueña" variety was found to be statistically steadier and more intense in sensory notes. The peroxide index, oxidative stability, sensory notes, oleic and linoleic acid, oxidative susceptibility, MUFA/PUFA, the main triacylglycerides (OOO, POO, OLO, and PLO+SLE), ΔECN42, total sterolic and triterpenic dialcohol composition were used to discriminate these varieties. The results of the regulated parameters led to the classification of the analyzed oils into the "extra virgin" category, except for sensory characteristics. These results are very interesting because of the high percentage of EVOO obtained, for both varieties in oils from the "almazara", or mill, to ensure that oils from the olive-growing area have a good level of quality.

KEYWORDS*: Fatty acids; Sensory quality; Stage of ripening; Sterols; Triacylglycerols; Virgin olive oil*

RESUMEN: ***Composición química y análisis sensorial de aceites de oliva virgen de las variedades "Morisca" y "Carrasqueña"***. Las variedades de aceituna "Morisca" y "Carrasqueña", en diferentes estados de maduración fueron caracterizados en base al estudio de los compuestos mayoritarios (triglicéridos y ácidos grasos) y de los compuestos minoritarios (esteroles y alcoholes triterpénicos). También se determinaron los compuestos fenólicos totales, la estabilidad oxidativa y el análisis sensorial de los aceites. La variedad "Carrasqueña" fue más estable y fue calificada con notas sensoriales más elevadas. El índice de peroxidos, la estabilidad oxidativa, el análisis sensorial, el ácido oleico y el linoleico, la susceptibilidad oxidativa, MUFA/PUFA, los principales triglicéridos (OOO, POO, OLO y PLO+SLE), ΔECN42, los esteroles totales y los alcoholes triterpénicos fueron usados para discriminar estas variedades. Los resultados de los parámetros reglamentados clasificaron todos los aceites en la categoría de "virgen extra" excepto para el análisis sensorial. Sin embargo, este resultado es muy interesante debido al alto porcentaje obtenido de aceites de la categoría virgen extra para ambas variedades estudiadas en aceites obtenidos en las almazaras, asegurando así la buena calidad obtenida de los aceites en el área de estudio analizada.

PALABRAS CLAVE: *Aceite de oliva virgen; Ácidos grasos; Calidad sensorial; Estado de maduración; Esteroles; Triglicéridos*

Citation/Cómo citar este artículo: Fuentes M, De Miguel C, Ranalli A, Franco MN, Martínez M, Martín-Vertedor D. 2015. Chemical composition and sensory evaluation of virgin olive oils from "Morisca" and "Carrasqueña" olive varieties. *Grasas Aceites* **66** (1): e061. doi: http://dx.doi.org/10.3989/gya.0702142.

1. INTRODUCTION

Virgin olive oil (VOOs) is characterized by a high nutritional value and sensory quality and it is among the most important agricultural products of the Mediterranean basin. According to the International Olive Oil Council (IOOC), virgin olive oil is the oil obtained from the fruit of the olive tree (*Olea europaea* L) exclusively by mechanical or other physical means under conditions that do not lead to alterations in the oil (IOOC, 2010).

The European Union dominates the world production of olive oil (>70%), and it is the largest consumer. In particular, Spain is the country with highest production of olive oil in Europe (54%) and Spanish olive oils have been recognized as being of high quality. Extremadura, one of the southwest Spanish regions, is the third olive oil producer in Spain after Andalucia and Castilla la Mancha, with about 30 million olive trees and 53.000 tonnes of annual oil production. Nowadays, there are two recognized designations of origin (PDO) in Extremadura for the extra-virgin olive oil (EVOO): "Gata-Hurdes", in the north, and "Aceite Monterrubio", in the south.

Extremadura occupies the 5th zone, or western area and is subdivided into 12 subzones, 6 for each of the provinces that it comprises (EEC, 1997). The Tierra de Barros area is zone 9 and it is located in the southwest of Extremadura. "Carrasqueña" and "Morisca" are among the main olive varieties in this subzone, both of them represent over 92% of the olive production. In this area there are about 21 industrial oil mills using the dual-phase decanter spread over the municipalities that make up the olive growing area of Tierra de Barros, with a production capacity of approximately 460.000 kg·8 h^{-1}, noted for producing high-quality oils, and being an important industrial and high settlement within the Extremadura food industry. Because of the socioeconomic importance of olive oil in this region, the milling sector has made a significant investment effort in improving its extraction technology and obtaining a PDO.

The chemical composition of EVOO is influenced by the olive variety, the climatic conditions, the geographical site and the maturity stage. In particular, several studies demonstrated that the choice of the optimal harvest period is essential for obtaining virgin olive oil with the highest quality. The stage of ripening may directly or indirectly affect olive oil quality. The fruit physiology undergoes changes directly related to the times, and these changes alter the oil quality. In fact, as ripening advances certain metabolic processes which involve changes in the profile of certain compounds such as triacylglycerides, fatty acids, polyphenols, tocopherols, chlorophylls and carotenoids take place (Matos *et al.*, 2007). These variations are reflected in the sensory characteristics, especially in the aroma, the oxidative stability and/or the nutritional value of the final product, and of course, in the quality degree.

This is the first evaluation of the chemical composition of "Morisca" and "Carrasqueña" monovarietal EVOOs in relation to three stages of ripening degree (green, spotted and ripe) from three successive crop years (from 2005/06 to 2007/08). Because of the importance of these two cultivars for the subzone Tierra de Barros (Extremadura, Spain), the aim of this work was to characterize these olive oils based on the study of major (triacyglycerides and fatty acids) and minor compounds (sterols and triterpenics dialcohols) as well as on the total phenols, oxidative stability and the sensory characteristics (fruity, bitter and pungent) in the three stages of ripening.

2. MATERIALS AND METHODS

2.1. Selection of plots and trees within the area studied

The study was carried out in the Tierra de Barros area (south-west of Spain) during three successive crop years (from 2005/06 to 2007/08). 18 plots were selected and fixed according to the two predominant olive varieties (*Olea europaea* L.) within the area studied: "Morisca" and "Carrasqueña". The olive orchards were composed of fifteen-year-old olive trees (plantation frame 6×6 m^2). All the orchards were managed under drip irrigation from 15 May to 18 November, with a linear irrigation scheduling of approximately 3000 cm^3 water/ha and no tillage conditions. Weeds were controlled with post-emergence herbicides. Within each plot, 6 typical trees from the represented varieties were selected and marked. Note that the fact of getting all the test samples from the same geographic area allowed to study the effect of variables "variety" and "ripening stage" on the minor components of fruits developed under the same agronomic and pedoclimatic conditions. The morphological characterization and identification of the trees was done in the laboratory of the Agri-Food Technological Institute (INTAEX). The agronomic design was maintained for the whole of the trial during the three years of study and formed the experimental basis.

For the three studied olive crops, annual average temperatures were 16.6, 16.8 and 16.2 °C respectively. The average rainfall was 291, 278 and 591 mm respectively.

2.2. Olive and oil samples

Olive samples were collected from nine mills. The sampling was carried out with three replicates for the spotted stage of ripening. After harvesting, the olive fruit samples were immediately transported to the laboratory in ventilated storage

trays to avoid compositional changes. The oil was extracted within 24 h.

Oil samples were obtained using the Abencor grinding system (Abengoa SA, Sevilla, Spain) (Martínez *et al*., 1975). Samples were stored away from the light in amber-colored glass bottles at 4 °C until analysis (within 1 month). The total number of olive samples was 54 (9 mills × 2 varieties × 1 stage of ripening × 3 campaigns).

At the same time, samples of olives were hand-picked by the farmers, in perfect sanitary conditions. The olive samplings were collected in the morning and transported to the mills where they were processed at an industrial level in each oil mill using a continuous centrifugation system in the two-phase mode within 24 hours. The mills were chosen according to their high production volume and continuous centrifugation system in the two-phase mode. The mills selected are located in the following municipalities: Fuente del Maestre, Villafranca de los Barros, Almendralejo, Entrín Bajo, Los Santos de Maimona, Santa Marta de los Barros, Olivenza, Almendral and Medina de las Torres. Technological variables of the oil extraction process were controlled as well as the time and temperature of paste mixing.

Samples of the two VOO varieties ("Morisca" and "Carrasqueña") were directly collected from the vertical centrifuge from each mill. Therefore, 9 mills were visited from November to January and in three indices of ripening: (0-2), (2-3) y (3-4), (green, spotted and ripe, respectively) using the subjective evaluation of color of the skin and flesh, as proposed by Uceda and Frías (1975), and two independent bottles of VOO of "Morisca" and of "Carrasqueña" were prepared. The total number of oil samples was 162 (9 mills × 2 varieties × 3 stages of ripening × 3 campaigns).

After sampling collection, the VOO samples were immediately transported to the laboratory. All the samples were stored away from the light at 4 °C until analysis (within 1 month).

2.3. Analytical methods

2.3.1. Quality indices

To determine the general indices of quality in the VOOs (degree of acidity, peroxides index and UV absorbance at 232 and 270 nm), the methodology described in Regulation EEC 2568/91 (EEC, 1991) was applied. Free acidity, given as percent of oleic acid, was determined by titration of a solution of oil dissolved in ethanol/ether (1:1, v/v) with potassium hydroxide. Peroxide value expressed as milliequivalents of active oxygen per kilogram of oil ($meqO_2 \cdot kg^{-1}$), was determined as follows: olive oil (2.5g) was dissolved in a mixture of chloroform/acetic acid (2:3, v/v) and was left to react with a solution of potassium iodine in the dark; the free iodine was then titrated with a sodium thiosulfate solution. K_{232} and K_{270} extinction coefficients were calculated from absorption at 232 and 270 nm, respectively, with a UV spectrophotometer (Agilent 8453), using a 1% solution of oil in cyclohexane and a path length of 1 cm. All parameters were determined in duplicate for all samples.

2.3.2. Determination of total polyphenol content

The total polyphenol contents in the VOOs were determined according to the Folin-Ciocalteau colorimetric method, using caffeic acid as a standard (Montedoro *et al*., 1992). Briefly, the extraction of polyphenols from the olive oil samples was achieved by dissolving 10 g of oil in 75 mL of hexane, followed by liquid-liquid extraction using a methanol/water (40:60 v/v) mixture completed to 100 mL with distilled deionized water. A 2 mL aliquot of the methanolic extract was mixed with 1 mL Folin-Ciocalteau reagent, 2 mL saturated sodium carbonate and 15 mL of distilled water. After 1 h, absorbance at 725 nm was read against a blank similarly prepared. Each sample was prepared in duplicate and quantification was carried out on the basis of the external standard calibration curve of caffeic acid whose linearity ranged from 5 to 75 $mg \cdot L^{-1}$. Results of polyphenol content in the olive virgin oil samples are expressed as caffeic acid equivalent (CAE), in $mg \cdot kg^{-1}$ oil.

2.3.3. Oxidative stability

The oxidative stability of the examined oils was assessed in a 743 Rancimat (Metrohm, Herisau, Switzerland) eight-channel oxidative stability instrument at 100 °C and oxygen saturation with air flow from 10 $L \cdot h^{-1}$ (Gutiérrez, 1989). Changes in conductivity caused by loss in volatile organic acids, mainly formic acid, were measured automatically and continuously. The peroxidation curve was recorded, and the inflection point was selected as the induction time (IT, expressed in hours). The higher IT value, the higher the oxidative stability of the samples is. Analyses were performed in duplicate.

2.3.4. Sensory analysis

A sensory analysis (median of defects, median of fruity and panel classification test) of the samples was carried out by 12 selected and trained panellists, according to the method described in Regulation EEC 640/2008 (EEC, 2008). The intensities of both positive (fruity, bitter and pungent) and negative (fusty, winy, musty, muddy, rancid, metallic, and other) attributes were evaluated for each oil sample, on a non-structured, 10 cm scale. The results of the sensory evaluation were estimated by the median,

being valid when the coefficient of variation was less than 20.

2.3.5. Fatty acid composition

The fatty acid profile was determined by gas chromatography, expressing the results as percentages representing the relative areas of their corresponding methyl esters. The procedure followed was laid out in Regulation EEC 2568/91 (EEC, 1991). It consists of the hydrolysis of triacylglycerides and cold transesterification with a methanolic KOH solution. In particular, the methyl esters of the fatty acids were prepared by vigorously shaking a solution of the oil in hexane (0.2 g in 3 mL) with 0.4 mL of the methanolic KOH solution. The resulting solution containing the fatty acid methyl esters was then injected in split mode into a model HP 6890 gas chromatograph equipped with a flame ionization detector (FID). A DB-23 capillary column of 60 m × 0.25 mm i.d. × 0.25 Qm-thick stationary phase was used for the separation. The carrier gas was helium at a flow rate of 83.7 ml/min. During the assays, the injector operated at a temperature of 280 °C and the detector at 250 °C. The temperature program used was from 165 °C (maintained for 35 min), then ramped up at 10 °C·5 min^{-1} to 220 °C for a total time of 62 min. Fatty acids were identified by comparison of their retention times with those of standard referent compounds. Analyses were performed in duplicate.

2.3.6. Triacylglycerides

The analysis of triacylglycerols (TGs) was performed according to the official chromatographic method of the Regulations (EEC, 1991): 0.2 g of olive oil was dissolved in 0.5 mL of n-hexane and then the triacylglycerol fraction was purified with 15 mL of n-hexane/diethylic ether (90:10, v/v). After evaporation of the total dissolvent, 0.05 g of the purified triacylglycerols were dissolved in 1mL of acetone for HPLC analysis and injected into an HPLC apparatus. A Hewlett-Packard high-performance liquid chromatography (HPLC, HP1050, Agilent Technology) quaternary pump instrument equipped with a refractometeric detector was used with 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 (60:40, v/v) at a flow rate of 1.2 mL min-1201 . 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 for arachidic. The identification and the determination of the elution order of all triacylglycerols were made by means of calculation of the equivalent carbon number (ECN) of each triacylglycerol and its reflection in a graph of retention times against the ECN, of triacylglycerol standards, or by chromatograms of reference corresponding to soybean oil, a mixture of soybean oil and olive oil 30:70 and olive oil (EEC, 1991).

2.3.7. Sterols and triterpenic dialcohols

The qualitative and quantitative sterol contents of the samples were determined according to the European Official Methods (EEC, 1991). The lipid, after the addition of α-cholestanol and betulin as internal standards, was saponified with an ethanolic potassium hydroxide solution. The unsaponifiable matter was extracted with diethyl ether. The sterol fraction was separated by Silica gel plate chromatography. The sterols, erythrodiol and uvaol, recovered from the plate were transformed into the corresponding trimethylsilyl ethers and the mixture was analyzed by gas chromatography using an HP 6890 gas chromatograph (Hewlett-Packard, Agilent, CA), equipped with a flame ionization detector (FID), an HP-5MS capillary column (30m × 0.25 mm × 0.25 µm) and a 6890 Agilent automatic injector. The working conditions were: injector 300 °C, isothermal analysis at 260 °C, detector temperature 325 °C. Quantification was performed according to the internal standard (α-cholestanol) method and results were expressed as β-sitosterol (calculated as the sum of β-sitosterol, Δ-5,23-stigmastadienol, clerosterol, sitostanol and Δ-5,24-stigmastadienol). Relative amounts were expressed as percentages (%) of total sterols. Analyses were performed in duplicate.

2.4. Statistical analysis

The data were statistically analyzed by two-way ANOVA and Tukey's multiple range test to determine which levels of the factors influence the dependent variables considered. The factors involved in the experiment were: variety and crop year in each stage of ripening separately (green, spotted and ripe). The model included interactions between each factor for each of the dependent variables. The adequacy of the model was assessed through a standardized remainder study, to check the normality of the data and homogeneity of the variances. Statistical significance was accepted at a level of $p<0.05$. For variance analysis (ANOVA) the SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) was used. For those cases where the interaction was not significant, the results were expressed as the mean values of the three crop years. The deviation standard (SD) was calculated.

3. RESULTS AND DISCUSSION

3.1. Real quality

3.1.1. Oil quality

A two-way ANOVA analysis was computed to assess the physicochemical and sensory quality parameters for the olive oil samples from different varieties grown in the study area (Table 1). In general terms, all the oils produced and analyzed showed levels which were within established regulatory limits for the "extra virgin" category (acidity <0.8%; peroxide index <20 $meqO_2 \cdot kg^{-1}$; k_{270}<0.22; K_{232}<2.5) (EEC, 1991). Only olive oil from the "Carrasqueña" variety in the spotted stage of ripening in the 2007/08 campaign exceeded minimally this limit to acidity. These are good results because the VOOs were collected in mills and it must be noted that lower values for these parameters will mean a higher quality of oil. Besides, the olives in all mills were immediately processed and they were not exposed to serious hydrolysis or oxidative damage that could affect the quality of the oil.

It can be observed that there was a significant interaction in acidity between crop seasons in each stage of ripening. The values of acidity found ranged from 0.14–0.69% to 0.22–0.84% for "Morisca" and "Carrasqueña", respectively.

The main differences among varieties were clearly found in the peroxide index. In this way, regardless of the crop season studied, significant differences among both varieties can be found. The "Morisca" variety showed peroxide index values of approximately 20% greater than the "Carrasqueña" variety. In addition, there was a significant interaction in most stages of ripening in K_{270} and K_{232} so the main effects cannot be examined.

TABLE 1. Acidity (%), peroxide index ($meqO_2 \cdot kg^{-1}$) K_{270}, K_{232}, total phenols (mg CAE·kg^{-1}), oxidative stability (hours) and sensory quality of virgin olive oil samples[a] extracted from the "Morisca" and "Carrasqueña" varieties during the different stages of ripening. Results are expressed as mean ± SD of three sample replicates. For those cases where the interaction was not significant, the results were expressed as the mean values between three crop years ± SD. Different small letters in the same row indicate significant statistical differences (Tukey´s Test, p<0.05) among varieties in each stage of ripening. Different capital letters in the same row indicate significant statistical differences (Tukey´s Test, p<0.05) during ripening for each variety

		Green			Spotted			Ripe		
		I	Morisca	Carrasqueña	I	Morisca	Carrasqueña	I	Morisca	Carrasqueña
Acidity	**2005/06**		0.14±0.03$^{ns\ NS}$	0.22±0.05^{NS}		0.21±0.12^{ns}	0.23±0.33		0.14±0.24^{ns}	0.27±0.09
	2006/07	*	0.23±0.09$^{ns\ NS}$	0.31±0.08^{NS}	*	0.45±0.11^{ns}	0.34±0.18	*	0.60±0.08^{ns}	0.44±0.15
	2007/08		0.69±0.08$^{ns\ NS}$	0.52±0.06^{NS}		0.64±0.14^{ns}	0.84±0.08		0.69±0.09^{ns}	0.74±0.14
Peroxide index		ns	9.92±2.12$^{b\ NS}$	7.95±1.42$^{a\ NS}$	ns	10.9±1.57$^{b\ 2}$	8.07±0.95$^{a\ 2}$	ns	12.1±0.75^{b}	10.2±1.14^{a}
K_{270}	**2005/06**		0.14±0.01$^{ns\ NS}$	0.15±0.05^{NS}		0.15±0.02^{ns}	0.17±0.04			
	2006/07	*	0.15±0.04$^{ns\ NS}$	0.18±0.02^{NS}	*	0.14±0.03^{ns}	0.22±0.05	ns	0.14±0.01^{ns}	0.14±0.02
	2007/08		0.13±0.03$^{ns\ NS}$	0.13±0.03^{NS}		0.12±0.02^{ns}	0.13±0.06			
K_{232}	**2005/06**		2.39±0.28$^{ns\ B}$	2.15±0.19^{B}					1.55±0.28$^{ns\ A}$	1.62±0.32^{A}
	2006/07	*	1.98±0.28$^{ns\ NS}$	1.90±0.19^{NS}	ns	2.02±0.37$^{ns\ 2}$	1.96±0.25	*	1.72±0.28^{ns}	1.60±0.32
	2007/08		1.85±0.28$^{ns\ NS}$	1.77±0.19^{NS}					1.61±0.28^{ns}	1.52±0.32
Total phenols	**2005/06**		258.1±10.2$^{ns\ NS}$	345.6±116.0^{NS}		294.8±60.7^{ns}	351.7±146.9		270.7±72.2^{ns}	307.1±55.5
	2006/07	*	242.7±17.1$^{a\ B}$	530.4±67.4$^{b\ B}$	*	277.4±40.3$^{a\ B}$	486.4±76.1$^{b\ B}$	*	150.0±31.7$^{a\ A}$	390.3±75.6$^{b\ A}$
	2007/08		223.9±21.5$^{ns\ B}$	220.2±77.4^{NS}		182.0±68.1$^{a\ B}$	192.9±96.5^{b}		141.8±52.0$^{a\ A}$	219.3±45.0^{b}
O. stability	**2005/06**		57.1±5.9$^{a\ NS}$	64.1±10.2$^{b\ NS}$		62.7±9.6^{a}	75.2±6.3^{b}		50.5±6.6^{a}	97.2±16.9^{b}
	2006/07	*	45.5±6.4$^{a\ NS}$	84.0±9.3$^{b\ NS}$	*	45.5±9.1^{a}	89.4±6.4$^{b\ 1}$	*	60.9±7.4^{ns}	68.0±12.7
	2007/08		49.2±4.2$^{a\ NS}$	78.3±11.0$^{b\ NS}$		40.7±12.4^{a}	72.4±9.2^{b}		48.6±8.1^{a}	67.9±11.2^{b}
Fruity		ns	3.5±0.18$^{a\ NS}$	4.3±0.15$^{b\ NS}$	ns	3.5±0.15^{a}	4.3±0.40^{b}	ns	3.4±0.29^{a}	3.9±0.39^{b}
Bitter		ns	2.9±0.75$^{a\ B}$	3.5±0.84$^{b\ NS}$	ns	2.7±0.39$^{a\ B}$	3.4±1.32^{b}	ns	2.3±0.40$^{a\ A}$	4.0±0.77^{b}
Pungent		ns	3.1±0.81$^{a\ B}$	4.0±1.19$^{b\ NS}$	ns	3.1±0.62$^{a\ B}$	3.6±1.29^{b}	ns	2.7±0.40$^{a\ A}$	4.0±0.85^{b}

[a]Crops: 2005/06, 2006/07 and 2007/08.
CAE: caffeic acid equivalents, O. stability: oxidative stability, I: interaction between years and varieties in each stage of ripening. ns/NS: non significant; and *: significant interaction.

On the other hand, the acidity showed a slight increase during ripening although there were no significant differences. The authors explained this behavior by the fact that the olive quality is under degradation as the phenological stage progresses, increasing the degree of broken olives and the endogenous activity of lipases (Salvador *et al*., 2001). However, the peroxide index and the coefficient of absorption did not show any increase. On the contrary, other researchers (Vekiari *et al*., 2010) added that oxidation starts in the fruit but it increases slowly during ripening. This increase can be mainly attributed by the action of a foreign enzymatic machinery (Panzanaro *et al*., 2010) and even endogenous lipases in olives during the ripening of the fruit (Salvador *et al*., 2001).

3.1.2. Total phenolic compounds and oxidative stability

The amount of phenolic compounds in VOOs is an important factor when we assess their quality, due to the fact that natural phenolic compounds are able to improve the oxidation resistance and because the effects of these components are benefitial to human health. A two-way ANOVA analysis revealed the effects of variety and crop year on each stage of ripening, both factors are considered to have fixed effects. Both parameters considered, the total polyphenol content and oxidative stability, were useful for discriminating among the varieties evaluated. First of all, year and variety interaction in both variables indicates the great influence of the year. The lowest values found correspond to crop year 2007/08 where the range in values of total phenolic compounds obtained varied from 182.0 to 223.9 $mg \cdot kg^{-1}$ while in the other campaigns the range values were higher, from 242.7 to 530.4 $mg \cdot kg^{-1}$. In addition, the range in values of oxidative stability in the 2007/08 campaign was from 40.7 to 78.3 hours and in the other campaigns it varied from 45.5 to 97.2 hours (Table1). These low values in the last crop year are attributed mainly to the higher values of rainfall which were measured during this crop year. Our results are similar to those found by other researchers because it is well known that the total polyphenol content depends on the area of cultivation, growing conditions and, mainly, on the climatic characteristics (Aguilera *et al*., 2005).

Secondly, within each stage of ripening, the total phenolic compounds were significantly higher in the "Carrasqueña" variety than in "Morisca" in the 2006/07 and the 2007/08 campaigns in the spotted and ripe stages of ripening. Also, significant differences in the oxidative stability of the oil were observed for the different varieties in all stages of ripening except for the "Carrasqueña" variety in 2006/07 in the ripe stage of ripening. It is known that total phenolic compound and oxidative stability values are strongly dependent on each variety of olives as other researchers have indicated (Gómez-Rico *et al*., 2009; Oueslati *et al*., 2009). The highest values for both parameters were found in the oils from the "Carrasqueña" variety. Some researchers (Salvador *et al*., 2001) have confirmed a positive correlation between these parameters. However, very precise relationships between stability and the preservation of the oil cannot be set because oxidative stability is determined under the extreme conditions achieved in a Rancimat, while in the conservation of the oil, the supply of oxygen is limited and the temperature is much lower. However, this parameter has helped to have a more rigorous understanding of the oxidation capacity and the characteristics of the VOO produced.

In this study it is remarkable that there were variations depending on the stage of ripening without observing a specific trend in all the years (Table 1). It is noteworthy that the total phenolic compound in the "Morisca" variety remained constant up to the spotted stage and after that it decreased significantly up to ripe stage in the last two crops. Previous studies have described a general decrease in total phenolic compounds as fruit ripening advances, while different trends of specific compounds were observed (Oueslati *et al*., 2009). In the opposite sense, Baccouri *et al*. (2008) showed that the total amount of phenolic compounds analyzed by HPLC increased progressively throughout the ripening period until reaching a maximum in the ripening index between 3 and 4, subsequently decreasing. These results are in agreement with those observed in the "Cornicabra" variety (Salvador *et al*., 2001). However, Oueslati *et al*. (2009) did not find a clear connection between the content of phenolic compounds and the ripening index. In general terms, a significant decrease in the time of induction from the green to the ripe stage of ripening of the olives has not been found in the studied varieties. These results are in agreement with those reported by Salvador *et al*. (2001) who found that the oxidative stability decreased slightly as the olive ripening advanced, although the trend was not clear and in some cases there was a small increase with fruit maturity.

3.1.3. Sensory quality

Regarding sensory quality, crop season did not have a prominent influence in terms of the positive attributes, fruity, bitter and pungent. Therefore, regardless of the year of study, we can find significant differences between both varieties (Table 1). These results were desirable, especially for mill industries because they have managed to keep the fruity attribute regardless of the year studied. The study of quality parameters of the VOO of both varieties is considered to have an interest for the industrial sector in the oil-producing area of Tierra de Barros, both for the final consumer and international trade

in oil. In this sense, the "Morisca" variety had smaller amounts of fruity, bitter and pungent attributes. According to the Regulation EEC 640/2008 of 4th July 2008 (EEC, 2008) and modifications, both varieties are included in the medium fruity category (fruity intensities between 3 and 6). Sánchez *et al*. (2006) characterized both varieties and they showed similar values in these parameters. As for the fruity and bitter sensory notes, different authors have confirmed the influence of cultivation on the pungent attribute. Aguilera *et al*. (2005) gave values of spicy of 3 and 3.5, respectively for the Italian oils from "Frantoio" and "Leccino" varieties grown in Andalusia, Salvador *et al*. (2001) showed values of 2.1 in the "Cornicabra" variety.

The fruity attribute did not show a clear trend during ripening in both varieties. Meanwhile bitter and pungent parameters remained constant up to spotted and after that decreased significantly up to the ripe stage in the "Morisca" variety. Several authors have reported that olive ripeness has a strong impact on olive oil descriptors (Sánchez *et al*., 2006; Jimenez *et al*., 2012). These researchers found significant variations in the fruity, bitter, and pungent attributes according to the ripening stage of both studied varieties, with decreases in the studied parameters during ripening.

3.1.4. Fatty acid composition

The fatty acid composition is shown in Table 2. The most representative fatty acids in percentage are oleic, linoleic, palmitic and stearic which are the main ones representing 99% of the composition of total fatty acids in VOOs from both varieties in the Tierra de Barros area. The most abundant fatty acid is oleic, representing more than 65.8%.

Note that monounsaturated oleic acid aids in the formation of a cell membrane which is more resistant to oxidation by acting at the level of greater stability in the cell membrane (Sánchez *et al*., 2003). Therefore the highest oxidative stability discussed in previous sections for the "Carrasqueña" is corroborated by the highest amount of oleic fatty acid in this variety. Furthermore, palmitoleic, linolenic, arachidic and gadoleic acids were found in small quantities of less than 1.4% in all samples. Margaric, margaroleic, behenic, and lignoceric appeared in quantities of less than 0.2% in the monovarietal olive oils studied. It is noted that the values of all the fatty acids analyzed were found, in all the samples, within the range required by Regulation EEC 2568/91 (EEC, 1991), on the characteristics of olive oils and olive pomace oils and their methods of analysis.

On the other hand, there was no significant interaction between crop year and variety factors in each stage of ripening in most cases (Table 2). Adverse weather conditions occurred in 2007/08 but had no significant effect on the concentration in palmitic, oleic and linoleic acids studied. Furthermore, both cultivars can be differentiated in the Tierra de Barros subzone since the relative percentage of oleic and linoleic acids showed variations. The distribution of these fatty acids and the different relationships among them make up an oil acidic profile that can be used to identify and distinguish varieties among them, and determine the nutritional quality of oils (Sanchez *et al*., 2003).

Nevertheless, there are clear varietal differences, statistically significant in each stage of ripening, in the proportion of each of them, as can be observed in the oleic one, for which the level ranged from 65.8–67.5% to 70.6–72.1% in the "Morisca" and "Carrasqueña" varieties, respectively. The data obtained corroborates that the distribution of fatty acids is greatly influenced by olive variety. Furthermore, the "Carrasqueña" variety presented a significantly lower content of linoleic acid and high percentage of oleic acid. The content of linolenic acid was also high but the differences were not significant in all stages of ripening. On the contrary, the "Morisca" variety showed a high content of linoleic acid and low content of oleic acid. To corroborate this, the correlation between these parameters gave high coefficients, one of the highest corresponding to oleic and linoleic acid ($r = -0.94$). Similar results were obtained by other researchers (Vekiari *et al*., 2010). Gracia *et al*. (2009) showed values of oleic acid of 68.9% and 11.6% of linoleic acid in the "Arbequina" variety. On the other hand, Salvador *et al*. (2001) studied the "Cornicabra" variety and they obtained values of 80.8% and 4.66% of oleic and linoleic acids, respectively, which are higher than those obtained in this assay. Also, Vekiari *et al*. (2010) found a linoleic/linolenic relationship similar to that obtained in this study with a ratio of 17 approximately. Note the importance of this relationship in nutrition because nowadays it is considered that a moderate contribution of linoleic acid is suitable to the current trends of a healthy diet. In this sense, Kiritsakis (2007) pointed out that the intake of these essential fatty acids is important for preserving cardiovascular health.

We can observe that the percentage of minority fatty acids did not change significantly from the green to the ripe stage of ripening. Similar results were obtained by other researchers (Vekiari *et al*., 2010). Some researchers (Baccouri *et al*., 2008; Oueslati *et al*., 2009) showed a gradual decrease in oleic acid, while linoleic acid increased during ripening. This behavior can be explained by the activity of the oleate desaturase enzyme, which transforms oleic into linoleic acid (Baccouri *et al*., 2008).

On the other hand, significant differences were found between both varieties for palmitic acid (no significant interaction), with high values found for the "Morisca" variety in the green and ripe stages

TABLE 2. Fatty acid composition (%) of virgin olive oil samples[a] resulting from "Morisca" and "Carrasqueña" varieties during the different stages of ripening. Results are expressed as mean ± SD of three sample replicates. For those cases where the interaction was not significant, the results were expressed as the mean values between three crop years ± SD. Different small letters in the same row indicate significant statistical differences (Tukey´s Test, $p<0.05$) among varieties in each stage of ripening. Different capital letters in the same row indicate significant statistical differences (Tukey´s Test, $p<0.05$) during ripening for each variety

		Green			Spotted			Ripe		
		I	**Morisca**	**Carrasqueña**	**I**	**Morisca**	**Carrasqueña**	**I**	**Morisca**	**Carrasqueña**
$C_{16:0}$		ns	13.3±0.13$^{b\ NS}$	12.6±0.46$^{a\ NS}$	ns	13.0±0.23^{ns}	13.0±0.30^{ns}	ns	13.3±0.15^{b}	12.8±0.26^{a}
$C_{16:1}$	**2005/06**		1.09±0.11$^{ns\ NS}$	1.12±0.06^{NS}		1.10±0.12^{ns}	1.22±0.11^{ns}			
	2006/07	*	1.21±0.10$^{ns\ NS}$	1.10±0.09^{A}	*	1.34±0.19^{ns}	1.42±0.16$^{ns\ B}$	ns	1.15±0.16^{ns}	1.22±0.12^{ns}
	2007/08		1.12±0.13$^{ns\ NS}$	1.15±0.03^{NS}		1.21±0.16^{ns}	1.23±0.13^{ns}			
$C_{17:0}$									0.05±0.02^{a}	0.11±0.01^{b}
		ns	0.07±0.01$^{a\ NS}$	0.11±0.02$^{b\ NS}$	ns	0.07±0.00^{a}	0.12±0.04^{b}	*	0.06±0.03^{a}	0.12±0.04^{b}
									0.06±0.05^{a}	0.10±0.03^{b}
$C_{17:1}$	**2005/06**		0.07±0.02$^{a\ NS}$	0.15±0.03^{b}						
	2006/07	*	0.07±0.01$^{a\ NS}$	0.13±0.03^{b}	ns	0.07±0.00^{a}	0.13±0.03^{b}	ns	0.07±0.02^{a}	0.12±0.02^{b}
	2007/08		0.06±0.01$^{a\ NS}$	0.12±0.03^{b}						
$C_{18:0}$	**2005/06**					3.23±0.10$^{ns\ B}$	3.42±0.16$^{ns\ B}$			
	2006/07	ns	3.22±0.05$^{ns\ A}$	3.34±0.12^{A}	*	3.43±0.16$^{ns\ B}$	3.64±0.12$^{ns\ B}$	ns	3.53±0.55$^{ns\ B}$	3.43±0.09$^{ns\ B}$
	2007/08					3.43±0.12$^{ns\ B}$	3.31±0.18$^{ns\ B}$			
$C_{18:1}$		ns	65.8±1.17$^{a\ A}$	72.1±1.52$^{b\ NS}$	ns	67.5±1.41$^{a\ B}$	70.6±0.56^{b}	ns	66.4±2.00$^{a\ AB}$	71.2±0.93^{b}
$C_{18:2}$		ns	14.9±1.58$^{b\ NS}$	8.96±1.57$^{a\ NS}$	ns	13.3±1.13^{b}	9.94±1.13^{a}	ns	14.0±1.25^{b}	9.64±0.79^{a}
$C_{18:3}$		ns	0.76±0.04$^{ns\ NS}$	0.74±0.14^{NS}	ns	0.73±0.05^{ns}	0.65±0.01^{ns}	ns	0.73±0.01^{b}	0.64±0.01^{a}
$C_{20:0}$	**2005/06**		0.46±0.08$^{ns\ NS}$	0.45±0.07^{NS}					0.45±0.07^{ns}	0.46±0.09^{ns}
	2006/07	*	0.33±0.09$^{ns\ NS}$	0.37±0.06^{NS}	ns	0.37±0.07^{ns}	0.39±0.08^{ns}	*	0.33±0.09^{ns}	0.35±0.04^{ns}
	2007/08		0.33±0.08$^{ns\ NS}$	0.32±0.05^{NS}					0.32±0.05^{ns}	0.32±0.07^{ns}
$C_{20:1}$		ns	0.21±0.03$^{ns\ NS}$	0.21±0.04^{NS}	ns	0.21±0.03^{ns}	0.22±0.04^{ns}	ns	0.21±0.04^{ns}	0.21±0.04^{ns}
$C_{22:0}$	**2005/06**		0.13±0.01$^{ns\ NS}$	0.13±0.02^{NS}					0.13±0.00^{ns}	0.13±0.01^{ns}
	2006/07	*	0.13±0.04$^{ns\ NS}$	0.15±0.03^{NS}	ns	0.13±0.00^{ns}	0.14±0.02^{ns}	*	0.13±0.00^{ns}	0.14±0.02^{ns}
	2007/08		0.12±0.02$^{ns\ NS}$	0.12±0.02^{NS}					0.13±0.00^{ns}	0.12±0.01^{ns}
$C_{24:0}$	**2005/06**		0.07±0.01$^{ns\ NS}$	0.06±0.01^{NS}		0.06±0.02^{ns}	0.06±0.02^{ns}			
	2006/07	*	0.06±0.03$^{ns\ NS}$	0.08±0.04^{NS}	*	0.06±0.03^{ns}	0.09±0.03^{ns}	ns	0.06±0.01^{ns}	0.06±0.01^{ns}
	2007/08		0.06±0.02$^{ns\ NS}$	0.06±0.01^{NS}		0.06±0.00^{ns}	0.06±0.02^{ns}			
$C_{18:1}/C_{18:2}$		ns	4.4±0.74$^{a\ NS}$	8.05±0.99$^{b\ NS}$	ns	5.08±1.08^{a}	7.1±0.50^{b}	ns	4.74±1.6^{a}	7.39±1.18^{b}
Ox.Suscep		ns	814.4±71.5$^{b\ B}$	550.6±57.1$^{a\ NS}$	ns	740.1±52.3$^{b\ A}$	584.7±51.2^{a}	ns	769.7±54.4$^{b\ A}$	569.9±34.3^{a}
MUFA/SFA		ns	3.9±0.17$^{a\ NS}$	4.4±0.18$^{b\ NS}$	ns	4.1±0.11^{ns}	4.2±0.10^{ns}	ns	3.9±0.25^{a}	4.3±0.13^{b}
MUFA/PUFA		ns	4.3±0.54$^{a\ A}$	7.7±1.34$^{b\ B}$	ns	4.9±0.52$^{a\ B}$	6.9±0.77$^{b\ A}$	ns	4.6±0.50$^{a\ AB}$	7.1±0.64$^{b\ A}$

[a]Crops: 2005/06, 2006/07 and 2007/08. Values were calculated as the % of the total fatty acids.
Ox.Suscep: oxidative susceptibility, MUFA: monounsaturated fatty acids sum, SFA: saturated fatty acids sum, PUFA: polyunsaturated fatty acids sum, I: interaction between years and varieties in each stage of ripening, ns/NS: non significant; and *: significant interaction.

of ripening. Salvador *et al*. (2001) and Pardo *et al*. (2011) showed that the "Cornicabra" variety presented values for palmitic acid of 9.12±0.67%, which are lower than in our study. However, Aguilera *et al*. (2005) observed that in the "Frantoio" and "Leccino" varieties, the saturated fatty acid in the highest amount was palmitic acid with average values of around 12.9 and 14.1%, respectively.

The oleic/linoleic ratio is also a parameter frequently used to evaluate the stability of the oil and in the characterization of olive oil cultivar to detect mixtures of virgin olive oil with 5–10% refined seed oils (Oueslati *et al*., 2009). In this sense, oils from the "Carrasqueña" variety with higher ratios are those which showed higher values in oxidative stability (Table 1), while oils from the "Morisca" variety which had less oxidative stability, presented ratios of oleic/linoleic of about 40% lower than the "Carrasqueña" variety. Some researchers (Aguilera *et al*., 2005; Gracia *et al*., 2009; Vekiari *et al*., 2010) demonstrated the importance of that relationship in the nutritional quality of the oil and its oxidative stability.

This fact was corroborated with the oxidative susceptibility parameter which was assessed by the formula (m + (45 × L) + (100 × Ln), where m = % monounsaturated fatty acids, L = % linoleic acids and Ln = % linolenic acids) (Gracia *et al*., 2009). The "Carrasqueña" variety presented significantly lower values, and therefore showed less susceptibility to oxidation than the "Morisca" variety which had values twice as low in some cases (Table 2). Gracia *et al*. (2009) presented significant differences to oxidative susceptibility between "Arbequina" and "Empeltre" varieties, with the latter one being more susceptible to oxidation, maintaining these results over three campaigns. It was observed that data obtained by these researchers were similar to those obtained for the oxidative susceptibility in the "Morisca" variety. Salvador *et al*. (2001) obtained values of around 350 for the oxidative susceptibility in the "Cornicabra" variety, which is lower than those obtained in this study. They indicated that this variety had less oxidation risk than ours. In fact, it was concluded that the polyunsaturated fatty acid content is an indicative of the increase in oxidative susceptibility (Cert *et al*., 1996). Vekiari *et al*. (2010) showed higher oxidative susceptibility values for the "Throumbolia" variety than the "Koroneiki" variety with values from the latter variety similar to those obtained for the "Carrasqueña" variety in our study. The highest oxidative susceptibility value obtained in "Throumbolia" with values of 1040 could be due to the lower phenolic content presented in this variety and therefore this variety would be more sensitive to oxidation.

In the ratio MUFA/SFA we can appreciate that the "Carrasqueña" variety showed ratios significantly higher than the "Morisca" variety in the green and ripe stages of ripening. Pereira *et al*. (2002) indicated that these considerable differences in monounsaturated and saturated fatty acid contents between varieties may explain the variation in the measurement of stability of oils measured with Rancimat equipment.

On the other hand, the MUFA/PUFA ratio was 30% higher in the "Carrasqueña" than in the "Morisca" variety, so theoretically the "Carrasqueña" variety had good characteristics in terms of stability (Oueslati *et al*., 2009). Gracia *et al*. (2009) showed a significantly higher ratio. Pardo *et al*. (2011) indicated that the low content in oleic acid and the high content in linoleic acid obtained in "Manzanilla de Centro", "Manzanilla Local" and "Onil de Bovedilla" varieties can be attributed to a low oxidative stability and therefore, as a result, to a low monounsaturated/polyunsaturated ratio.

3.1.5. Triacylglycerides

The TG composition has also been established as an index of the quality and purity of vegetable oils. The main TG profiles are shown in Table 3. Both types of oils were characterized by three main TGs: OOO, POO and OLO and eight secondary TGs: OLL, OLnO, PLO+SLL, PPO, SOO and SLS+POS. Furthermore, small amounts (<1.5%) of OLLn, PLLn, PLL, PPL, LLLn, LLL and PPP were also identified in all the analyzed samples. According to the bibliography examined, triacylglycerides (OOO, POO and OLO) were the main ones in most VOOs from Spain, Italy and Greece. Note that the percentage of the three main TGs make up an average of 70% for the "Morisca" and a 78% for the "Carrasqueña" varieties.

OOO was the major TGs found in both varieties in all the campaigns and during the three stages of ripening. The crop year and variety interaction in this variable indicate the influence of the year compared to treatments. In this sense, note the high content in triolein (OOO) during the 2005/06 campaign in the "Carrasqueña" variety (40.1–45.1%). In the "Morisca" case, in the same campaign, the percentage was a little lower (30.1–33.9%). Similar values were found by Osorio *et al*. (2003), at 41.7–45.3% for "Carrasqueña" and 27.1–30.8% for "Morisca" in the crop of years 2001/02 and 2002/2003. Elevated values of this triacyglyceride were also shown by other authors from 24.6 to 50.4% in "Chétoui" and "Chemlali" varieties (Baccouri *et al*., 2008), and 50.9% in oils from the "Picual" variety in Jaen, Extremadura and the south of Portugal (Osorio *et al*., 2003).

POO was the second triacylglyceride on the basis of quantitative importance, which presented a range from 22.3 to 24.1% and from 25.1 to 25.9% in "Morisca" and "Carrasqueña" varieties, respectively, with no significant interactions in any stage of ripening. Baccouri *et al*. (2008) obtained results above 30% in "Chétoui" and "Chemlali" varieties. At the same time, OLO was the following in percentual importance presenting a range from 14.4 to 15.5% and from 11.4 to 12.7% in "Morisca" and "Carrasqueña" varieties, respectively. It is interesting to observe, as has been noted by other researchers (Osorio *et al*., 2003), that the variety with the highest contents in OOO and POO was the one that gave a lower content of OLO, regardless of ripening stage. Therefore, we can see that there are significant differences among varieties in our study in terms of TG contents and moreover, these results are in accordance with those found in the fatty acid composition.

Within the secondary TGs, PLO+SLL is relevant because its importance is much greater in oils of varieties with lower OOO contents, such as the "Morisca" variety in this study. In this variety the content ranged from 5.00–8.78 and 6.50–6.99% for "Morisca" and "Carrasqueña" varieties, respectively. On the other hand, the "Carrasqueña" variety, which presented higher values of OOO at almost 40%, had low values of PLO+SLL (Table3). This relationship has been found in the bibliography, in

TABLE 3. Triglycerides composition (%) of virgin olive oil samples[a] extracted from "Morisca" and "Carrasqueña" varieties during the different stages of ripening. Results are expressed as mean ± SD of three sample replicates. For those cases where the interaction was not significant, the results were expressed as the mean values between three crop years ± SD. Different small letters in the same row indicate significant statistical differences (Tukey´s Test, $p<0.05$) among varieties in each stage of ripening. Different capital letters in the same row indicate significant statistical differences (Tukey´s Test, $p<0.05$) during ripening for each variety

		Green			Spotted			Ripe		
		I	**Morisca**	**Carrasqueña**	**I**	**Morisca**	**Carrasqueña**	**I**	**Morisca**	**Carrasqueña**
LLLn		ns	$0.01\pm0.01^{ns\ NS}$	$0.03\pm0.02^{ns\ NS}$	ns	0.01 ± 0.01^{ns}	0.02 ± 0.01^{ns}	ns	0.02 ± 0.01^{ns}	0.22 ± 0.01^{ns}
LLL	**2005/06**							*	0.18 ± 0.05^{b}	0.05 ± 0.03^{a}
	2006/07	ns	0.23 ± 0.06^{ns}	0.16 ± 0.05^{ns}	ns	0.27 ± 0.07^{b}	0.16 ± 0.03^{a}		0.23 ± 0.07^{b}	0.14 ± 0.03^{a}
	2007/08								0.27 ± 0.06^{b}	0.15 ± 0.03^{a}
OLLn	**2005/06**		0.29 ± 0.04^{b}	0.24 ± 0.03^{a}						
	2006/07	*	0.36 ± 0.05^{b}	0.25 ± 0.07^{a}	ns	0.36 ± 0.07^{b}	0.26 ± 0.02^{a}	ns	0.34 ± 0.04^{b}	0.25 ± 0.02^{a}
	2007/08		0.36 ± 0.04^{b}	0.35 ± 0.06^{a}						
PLLn		ns	$0.12\pm0.03^{b\ NS}$	$0.07\pm0.00^{a\ NS}$	ns	0.12 ± 0.02^{b}	0.09 ± 0.02^{a}	ns	0.12 ± 0.02^{b}	0.08 ± 0.01^{a}
OLL		ns	$3.28\pm0.73^{b\ NS}$	$2.56\pm0.83^{a\ NS}$	ns	3.67 ± 0.61^{b}	2.31 ± 0.45^{a}	ns	3.40 ± 0.35^{b}	2.28 ± 0.28^{a}
OLnO		ns	$2.52\pm0.15^{b\ B}$	$1.85\pm0.04^{a\ B}$	ns	$2.42\pm0.28^{b\ AB}$	$1.89\pm0.15^{a\ B}$	ns	$2.27\pm0.27^{b\ A}$	$1.75\pm0.04^{a\ A}$
PLL	**2005/06**		$0.41\pm0.13^{a\ A}$	$0.87\pm0.18^{b\ NS}$					$1.06\pm0.02^{ns\ B}$	0.73 ± 0.04^{ns}
	2006/07	*	$0.65\pm0.11^{ns\ A}$	$0.52\pm0.12^{ns\ NS}$	ns	0.61 ± 0.06^{ns}	0.55 ± 0.00^{ns}	*	$0.84\pm0.03^{ns\ B}$	0.63 ± 0.03^{ns}
	2007/08		$0.62\pm0.16^{ns\ A}$	$0.64\pm0.17^{ns\ NS}$					$0.92\pm0.05^{ns\ B}$	0.59 ± 0.06^{ns}
OLO		ns	$14.8\pm1.16^{b\ NS}$	$11.4\pm1.54^{a\ NS}$	ns	15.5 ± 0.81^{b}	12.7 ± 1.19^{a}	ns	14.4 ± 1.61^{b}	12.4 ± 0.65^{a}
PLO+SLL		ns	$8.78\pm1.22^{b\ NS}$	$6.50\pm1.37^{a\ NS}$	ns	$5.00\pm0.86^{b\ 1}$	6.99 ± 0.87^{a}	ns	8.66 ± 1.33^{b}	6.72 ± 0.44^{a}
PPL	**2005/06**		$0.95\pm0.06^{b\ A}$	$0.64\pm0.05^{a\ A}$				*	$1.46\pm0.34^{b\ B}$	$0.73\pm0.07^{a\ B}$
	2006/07	*	$1.01\pm0.05^{b\ A}$	$0.63\pm0.06^{a\ NS}$	ns	0.96 ± 0.09^{b}	0.72 ± 0.08^{a}		$1.84\pm0.24^{b\ B}$	0.63 ± 0.05^{a}
	2007/08		$1.06\pm0.06^{b\ A}$	$0.71\pm0.04^{a\ NS}$					$1.92\pm0.34^{b\ B}$	0.59 ± 0.08^{a}
OOO	**2005/06**		$30.9\pm0.93^{a\ NS}$	$44.2\pm4.41^{b\ NS}$		33.9 ± 1.97^{a}	45.1 ± 5.02^{b}		30.1 ± 1.94^{a}	40.1 ± 2.47^{b}
	2006/07	*	$32.3\pm0.63^{a\ NS}$	$38.3\pm4.73^{b\ NS}$	*	30.3 ± 2.33^{a}	38.7 ± 4.12^{b}	*	33.9 ± 1.14^{a}	39.8 ± 1.27^{b}
	2007/08		$30.5\pm0.79^{a\ NS}$	$36.4\pm4.53^{b\ NS}$		31.0 ± 2.14^{ns}	35.2 ± 4.82^{b}		31.9 ± 2.34^{a}	37.6 ± 1.67^{b}
POO		ns	$22.3\pm2.45^{a\ NS}$	$25.9\pm0.96^{b\ NS}$	ns	23.5 ± 0.55^{a}	25.1 ± 0.79^{b}	ns	24.1 ± 0.93^{a}	25.8 ± 1.11^{b}
PPO		ns	$3.65\pm0.45^{a\ NS}$	$4.00\pm0.13^{b\ NS}$	ns	3.81 ± 0.12^{ns}	3.87 ± 0.17^{ns}	ns	3.68 ± 0.14^{ns}	3.81 ± 0.05^{ns}
PPP	**2005/06**		$0.50\pm0.06^{ns\ B}$	$0.60\pm0.12^{ns\ NS}$				*	$0.35\pm0.11^{ns\ A}$	0.45 ± 0.02^{ns}
	2006/07	*	$0.45\pm0.07^{ns\ NS}$	$0.49\pm0.15^{ns\ NS}$	ns	0.38 ± 0.03^{a}	0.51 ± 0.13^{b}		0.56 ± 0.12^{ns}	0.41 ± 0.03^{ns}
	2007/08		$0.39\pm0.09^{ns\ NS}$	$0.30\pm0.16^{ns\ NS}$					0.40 ± 0.16^{ns}	0.44 ± 0.05^{ns}
SOO		ns	$4.84\pm0.35^{a\ NS}$	$5.76\pm0.21^{b\ NS}$	ns	5.12 ± 0.27^{ns}	5.98 ± 0.46^{ns}	ns	5.58 ± 0.45^{ns}	6.20 ± 0.41^{ns}
SLS+POS	**2005/06**					1.54 ± 0.04^{a}	1.71 ± 0.03^{b}			
	2006/07	ns	1.54 ± 0.04^{a}	1.63 ± 0.05^{b}	*	1.46 ± 0.06^{a}	1.70 ± 0.05^{b}	ns	1.55 ± 0.13^{a}	1.63 ± 0.08^{b}
	2007/08					1.50 ± 0.09^{a}	1.66 ± 0.08^{b}			
ΔECN42	**2005/06**					0.14 ± 0.04^{a}	0.04 ± 0.03^{b}			
	2006/07	ns	$0.29\pm0.03^{a\ NS}$	$0.04\pm0.01^{b\ NS}$	*	0.11 ± 0.03^{a}	0.02 ± 0.02^{b}	ns	0.17 ± 0.03^{a}	0.04 ± 0.01^{b}
	2007/08					0.12 ± 0.02^{ns}	0.05 ± 0.04			

[a] Crops: 2005/06, 2006/07 and 2007/08. Values were calculated as the % of the total triacylglicerols.
I: interaction between years and varieties in each stage of ripening, ns/NS: non significant; and *: significant interaction.

addition to the "Morisca" variety for "Cornezuelo" and "Verdial de Badajoz" (Osorio *et al*., 2003). Foreign varieties such as "Carolea" and "Koroneiki" also presented a POL+SLL content that exceeded 6% (Stefanoudaki *et al*., 1999).

Finally, the main triacylglycerides did not change during ripening. Similar results were obtained by other researchers (Pereira *et al*., 2002). However, Baccouri *et al*. (2008) showed variations in some TGs depending on the stage of ripening. These researchers indicated that the biggest changes were observed in OOO and POO triacylglycerides that decreased during ripening. They showed that this reduction could be explained by the decrease in the lipophosphatase acyltransferase activities and glycerol-3-phosphate acyltransferase.

Another quality parameter, the ΔECN42 is also shown in Table 3. According to the framework of

Community Regulation, the ΔECN42 maximum difference admitted is 0.2 for extra virgin oils. The difference between theoretic and experimental ECN42 values allowed genuine and adulterated olive oil to be distinguished with a significance level >99.5%. It is indeed a valid method to detect the presence of seed oil in olive oil. ΔECN42 has values in the range of those of olive oil in other varieties. This variable shows a significant increase with the increase in linoleic acid percentage, and it is always higher in the "Morisca" variety than in "Carrasqueña" olive oil. It seems important especially in the case of the EVOOs coming from the "Morisca" variety which have a high content in linoleic acid. In fact, the result of ΔECN42 in this variety was near the maximum legal value for VOOs, except in the green stage of maturation in the "Morisca" variety which exceeded the maximum allowed (Table 3). That is why it is important to show confidence towards "Morisca" VOOs and consider them a special variety in relation to the ΔECN42 parameter. Other researchers (Aranda *et al*., 2004; Oueslati *et al*., 2009) obtained very low values of ΔECN42 in the VOO from "Cornicabra", "Tataouine", "Fakhari Douirat" and "Zarrazi Douirat", while Baccouri *et al*. (2008) and Manai *et al*., (2007) experienced the same for VOOs from the "Chemlali" variety which were characterized by a higher mean value of ΔECN42, which greatly exceeds the established limit.

3.1.6. Sterols

The main sterols and triterpenic alcohol are shown in Table 4. The apparent β-Sitosterol was higher than the minimum limit required by the regulation of the European Union for EVOO (93%). The most representative percentage of sterols are β-Sitosterol, Δ-5-Avenasterol and Campesterol, which represented 95% of the sterol contents in the VOO studied in the area of Tierra de Barros, similar to that found by other researchers (Sánchez *et al*., 2004) for the same varieties. The levels of sterols obtained in different oils are within the limits established by the regulations.

β-Sitosterol represented more than 75% of the sterols, followed by Δ-5-Avenasterol. In addition, both varieties did not present a significant interaction between crop years, presenting a varietal differentiation between "Morisca" and "Carrasqueña" varieties but there was only a significant difference in the green stage. The average content of β-Sitosterol ranged from 79.8–81.5% for "Morisca" and from 82.7–83.4% for "Carrasqueña". Several researchers noted that these sterols have a high negative correlation with Δ-5-Avenasterol (Sánchez *et al*., 2004). Our varieties presented the same trend. This trend was proven by other researchers (Haddada *et al*., 2007) who observed that the "Jarboui" variety showed the highest values of β-Sitosterol (85.2%) and the lowest to Δ-5-Avenasterol, while "Chétoui" was characterized by just the contrary.

The apparent β-Sitosterol was expressed as the sum of the contents of β-Sitosterol and four other sterols: Sitostanol, Δ-5,24-Stigmasterol, Clerostanol and Δ-5-Avenasterol, which were higher than the minimum established by the regulation of the European Union for VOOs (93%).

The main sterols did not present a defined trend throughout ripening, however, Δ-5-Avenasterol maintained steady from the green to the spotted stage in "Morisca", while it decreased in "Carrasqueña". The pattern observed coincided with that noted by Sakouihi *et al*. (2009) and Lukic *et al*. (2013) in which the levels of sterols in olives decreased during ripening. The authors explained this behavior by the fact that in later ripening stages the enzymatic activity of sterol biosynthesis was stopped, and the rate conversion of sterols to other sterol forms, hydrogenation and dehydrogenation reactions, increased.

The percentages of Cholesterol, Campesterol, Campestenol, Clerostenol and Sitostanol were relatively stable in the oils from both varieties and at all ripening stages. Regarding Δ-5-Avenasterol, there were not significant differences between varieties in the green and ripe stages of ripening. Other significant sterols are Campesterol and Stigmasterol. The Campesterol never exceeded the maximum limit allowed by the rules of procedure of the EC (4%), although, in general, the regulation has established very narrow tolerance margins in the composition of sterols in olive oils. In our study, the Campesterol did not present significant interactions and this sterol presented similar levels between "Carrasqueña" and "Morisca" varieties (Table 4). This sterol could have an important differentiating power for being insensitive to variation factors such as water stress, geographical location and conservation (Sánchez *et al*., 2004).

Due to the fact that it is related to the different parameters on the quality of VOO, Stigmasterol was determined. High levels of this sterol are related to high acidity and low sensory quality. (Termine *et al*., 2008). In the studied samples, low levels of this sterol were found, which is indicative of oils from healthy fruits. In addition, the data showed that the levels of Stigmasterol were only slightly discriminated between the varieties studied, although the differences were not significant in the green and spotted and ripe stages in the last two crop years. Giacometti and Milin (2001) found values of Stigmasterol from 0.06 to 2.81% and of Campesterol from 1.26 to 3.32% in VOO. Note the significant interaction between the factors in the spotted and ripe stages of ripening. The highest values for Stigmasterol found in the crop year 2007/08 in the spotted and ripe stages of ripening ranged from 0.72 to 1.09% for the "Morisca" and from 1.27 to 1.47% for the "Carrasqueña" varieties.

TABLE 4. Sterol composition (%), total sterols (mg·kg^{-1}) and alcohol triterpenic (%) of virgin olive oil samples[a] extracted from "Morisca" and "Carrasqueña" varieties during the different stages f ripening. Results are expressed as mean ± SD of three sample replicates. For those cases where the interaction was not significant, the results were expressed as the mean values between three crop years ± SD. Different small letters in the same row indicate significant statistical differences (Tukey´s Test, $p<0.05$) among varieties in each stage of ripening. Different capital letters in the same row indicate significant statistical differences (Tukey´s Test, $p<0.05$) during ripening for each variety

		Green			Spotted			Ripe		
		I	Morisca	Carrasqueña	I	Morisca	Carrasqueña	I	Morisca	Carrasqueña
Cholesterol	**2005/06**		0.11±0.01$^{ns\ NS}$	0.14±0.02$^{ns\ NS}$					0.11±0.02^{ns}	0.12±0.01^{ns}
	2006/07	*	0.12±0.03$^{ns\ NS}$	0.15±0.04$^{ns\ NS}$	ns	0.13±0.02^{ns}	0.13±0.01^{ns}	*	0.15±0.04^{ns}	0.14±0.05^{ns}
	2007/08		0.14±0.04$^{ns\ NS}$	0.11±0.01$^{ns\ NS}$					0.13±0.05^{ns}	0.15±0.03^{ns}
24-M-Cholesterol	**2005/06**					0.24±0.03^{b}	0.18±0.04^{a}			
	2006/07	ns	0.27±0.05$^{b\ NS}$	0.20±0.04$^{a\ NS}$	*	0.24±0.05^{b}	0.15±0.06^{a}	ns	0.22±0.04^{b}	0.19±0.02^{a}
	2007/08					0.30±0.06^{b}	0.22±0.07^{a}			
Campesterol		ns	2.26±0.15$^{ns\ NS}$	2.39±0.06$^{ns\ NS}$	ns	2.37±0.08^{ns}	2.36±0.07^{ns}	ns	2.33±0.09^{ns}	2.47±0.19^{ns}
Campestanol		ns	0.10±0.01$^{ns\ NS}$	0.13±0.06$^{ns\ NS}$	ns	0.09±0.00^{ns}	0.10±0.02^{ns}	ns	0.11±0.00^{ns}	0.11±0.02^{ns}
Stigmasterol	**2005/06**					0.79±0.18$^{ns\ NS}$	0.80±0.29$^{ns\ NS}$		0.68±0.21$^{ns\ B}$	0.70±0.39$^{ns\ B}$
	2006/07	ns	0.64±0.09$^{ns\ A}$	0.76±0.16$^{ns\ A}$	*	0.69±0.19$^{a\ A}$	0.84±0.17$^{b\ A}$	*	0.83±0.25$^{a\ B}$	1.03±0.32$^{b\ B}$
	2007/08					0.72±0.28$^{a\ A}$	1.27±0.36$^{b\ A}$		1.09±0.11$^{a\ B}$	1.47±0.49$^{b\ B}$
Δ-7-Campesterol	**2005/06**		0.27±0.50^{b}	0.09±0.22^{a}						
	2006/07	*	0.25±0.53^{ns}	0.18±0.67^{a}	ns	0.61±0.62^{b}	0.47±0.34^{a}	ns	0.47±0.48^{ns}	0.45±0.44^{ns}
	2007/08		1.12±0.43^{ns}	1.04±0.62^{a}						
Clerosterol		ns	1.09±0.11$^{ns\ NS}$	0.96±0.09$^{ns\ NS}$	ns	1.02±0.04^{ns}	0.99±0.05^{ns}	ns	0.99±0.02^{ns}	1.04±0.11^{ns}
β-Sitosterol		ns	79.8±0.30$^{a\ NS}$	82.7±1.63$^{b\ NS}$	ns	81.1±1.19^{ns}	83.4±1.42^{ns}	ns	81.5±0.93^{ns}	83.2±1.99^{ns}
Sitostanol	**2005/06**					0.49±0.08^{ns}	0.48±0.02^{ns}			
	2006/07	ns	0.54±0.09$^{ns\ NS}$	0.51±0.08$^{ns\ NS}$	*	0.52±0.02^{ns}	0.47±0.04^{ns}	ns	0.51±0.09^{ns}	0.50±0.03^{ns}
	2007/08					0.42±0.05^{ns}	0.45±0.06^{ns}			
Δ-5-Avenasterol		ns	11.7±1.04$^{ns\ B}$	10.6±1.60$^{ns\ B}$	ns	11.6±1.01$^{b\ B}$	9.34±1.06$^{a\ A}$	ns	10.8±1.84$^{ns\ A}$	9.15±1.56$^{ns\ A}$
Δ-5-24-Stigmastadienol	**2005/06**		0.72±0.09^{ns}	0.71±0.09^{ns}						
	2006/07	*	0.73±0.04^{ns}	0.68±0.15^{ns}	ns	0.69±0.02^{ns}	0.68±0.03^{ns}	ns	0.66±0.07^{ns}	0.63±0.04^{ns}
	2007/08		0.79±0.11^{ns}	0.78±0.05^{ns}						
Δ-7-Stigmastenol	**2005/06**		0.22±0.09$^{ns\ NS}$	0.19±0.21$^{ns\ NS}$		0.25±0.13^{ns}	0.20±0.05^{ns}		0.18±0.02^{ns}	0.24±0.03^{ns}
	2006/07	*	0.22±0.05$^{ns\ NS}$	0.27±0.31$^{ns\ NS}$	*	0.20±0.08^{a}	0.30±0.09^{b}	*	0.21±0.05^{ns}	0.27±0.06^{ns}
	2007/08		0.13±0.07$^{a\ NS}$	0.58±0.29$^{b\ NS}$		0.19±0.03^{ns}	0.22±0.15^{ns}		0.17±0.09^{ns}	0.22±0.05^{ns}
Δ-7-Avenasterol	**2005/06**					0.69±0.09^{ns}	0.61±0.09^{ns}			
	2006/07	ns	0.68±0.04$^{b\ NS}$	0.58±0.06$^{a\ NS}$	*	0.54±0.19^{ns}	0.61±0.13^{ns}	ns	0.63±0.09^{b}	0.54±0.13^{a}
	2007/08					0.54±0.07^{ns}	0.55±0.14^{ns}			
Total sterols		ns	1680.6±43.7$^{b\ NS}$	1537.3±40.2$^{a\ NS}$	ns	1684.0±117.8^{b}	1551.1±49.4^{a}	ns	1688.5±78.6^{b}	1491.5±50.6^{a}
Apararent Sitosterol		ns	95.0±0.35$^{ns\ NS}$	95.0±0.53$^{ns\ NS}$	ns	94.9±0.76^{ns}	94.9±0.59^{ns}	ns	94.7±1.04^{ns}	94.5±0.61^{ns}
Erythrodiol + Uvaol		ns	3.04±0.35$^{a\ B}$	3.86±0.45$^{b\ B}$	ns	2.76±0.13$^{a\ A}$	3.43±0.15$^{b\ AB}$	ns	2.74±0.48$^{a\ A}$	3.23±0.67$^{b\ A}$

[a]Crops: 2005/06, 2006/07 and 2007/08. Values were calculated as the % of the total sterols.
I: interaction between years and varieties in each stage of ripening, ns/NS: non significant; and *: significant interaction.

The content of total sterols (non significant interaction) in all cases presented levels that exceed the threshold established in the regulation, but with differences between varieties. The "Morisca" variety presented contents significantly greater than The "Carrasqueña" variety in each stage of ripening. These results are in agreement with those obtained by Sánchez *et al.* (2004). It is well known that the composition of total sterols could be used to identify the tampering of olive oil, and it has been recently suggested that it can be used to classify oils according to the variety of its fruit (Sánchez *et al.*, 2004). In our study the values of total sterols are below those observed by other researchers, who indicated values of 2.017 mg·kg^{-1} (Termine *et al.*, 2008) and 2.682 mg·kg^{-1} in a study on Portuguese varieties (Alves *et al.*, 2005).

Triterpenic alcohols (erythrodiol and uvaol) are generally located in the exocarp of the olive.

According to EU regulations, their VOO content should not exceed 4.5% of total sterols, since higher values may indicate blends with olive oil from pomace. In all the analyzed samples, the sum of erythrodiol and uvaol was within the limits for the categories of EVOO and VOO. Furthermore, there is a clear difference between both varieties. These results are in accordance with those found by other researchers (Sánchez *et al*., 2004; Pardo *et al*., 2011). These compounds have been proposed along with the sterol profile for the characterization of varieties (Stefanoudaki *et al*., 1999; Sánchez *et al*., 2003; Aguilera *et al*., 2005).

3.2. Potential quality

The physicochemical, sensory quality, main fatty acids, triacylglycerides, sterol profiles and erythrodiol and uvaol parameters for the olive oil samples from the different varieties grown in the studied area are showed in Table 5. In general, and in all the samples studied, the regulated physicochemical parameters evaluated were much lower than the upper limit established for the best commercial quality olive oil, designated as "extra virgin" (EEC, 1991). This is not surprising since the raw material was carefully selected, picked and processed immediately through the Abencor system. Therefore, the olives were not exposed to serious hydrolysis or oxidative damage that could affect the quality of the oil. In addition the amount of processed olives was obviously lower than the one used in the industry. It should be noted that lower values for these parameters will be translated into a higher quality oil.

Main differences among varieties were clearly found in the peroxide index. In this way, we can find significant differences between both varieties but both were within the limit established by the legislation for the "extra virgin" category. The "Morisca" variety only showed 1 $meqO_2 \cdot kg^{-1}$ greater than the "Carrasqueña" variety, and therefore the real values obtained by the Abencor system were similar and well suited for VOOs extracted in excellent conditions.

The "Carrasqueña" variety presented a high stability in the oils analyzed. This is corroborated by the oxidative susceptibility parameter which presented lower values and therefore showed less susceptibility to oxidation than the "Morisca" variety. The low values for the polyunsaturated fatty acid content in the "Carrasqueña" variety is an indication of the lower oxidative susceptibility obtained which also presented a greater phenolic content in this variety and therefore this variety would be less sensitive to oxidation. In this sense, Gómez-Rico *et al*. (2009) presented much higher potential oxidative susceptibility in "Morisca" VOO (lower oxidative stability) than other VOOs, such as the "Cornicabra" or "Picual" varieties.

TABLE 5. Acidity (%), peroxide index ($meqO_2\ kg^{-1}$); K_{270}, K_{232}, total phenols (mg CAE/kg), oxidative stability (hours) and sensory quality, Fatty acid composition (%), Triglyceride composition (%), Sterol composition (%), total sterols (mg/kg) and alcohol triterpenic (%) of virgin olive oil samplesa from the Abencor system extracted from "Morisca" and "Carrasquena" varieties during the spotted stage of ripening. Results are expressed as mean + SD of three sample replicates. For those cases where the interaction was not significant, the results were expressed as the mean values between three crop years + SD. Different small letters in the same row indicate significant statistical differences (Tukey's Test, $p<0.05$) among varieties in the spotted stage of ripening. Different capital letters and numbers in the same row indicate significant statistical differences (Tukey's Test, $p<0.05$) among extraction systems for each variety

			Spotted	
ABENCOR SYSTEM		I	**Morisca**	**Carrasqueña**
Acidity		ns	$0.33\pm0.05^{ns\ NS}$	0.31 ± 0.06^{NS}
Peroxide index		ns	$6.55\pm0.75^{b\ 1}$	$5.35\pm1.37^{a\ 1}$
K_{270}		ns	0.11 ± 0.04^{ns}	0.14 ± 0.05
K_{232}	**2005/06**		$1.60\pm0.28^{ns\ 1}$	1.67 ± 0.18^{NS}
	2006/07	*	$1.64\pm0.15^{ns\ 1}$	1.73 ± 0.17^{NS}
	2007/08		$1.54\pm0.21^{ns\ 1}$	1.59 ± 0.23^{NS}
Total phenols	**2005/06**		$309.1\pm55.7^{a\ NS}$	$428.5\pm79.2^{b\ NS}$
	2006/07	*	221.9 ± 40.5^{aNS}	$536.5\pm60.7^{b\ NS}$
	2007/08		$194.2\pm51.1^{ns\ NS}$	240.2 ± 59.5^{NS}
Ox. stability	**2005/06**		$60.6\pm6.5^{a\ NS}$	$88.3\pm8.7^{b\ NS}$
	2006/07	*	$45.8\pm7.7^{a\ NS}$	$113.8\pm10.2^{b\ 2}$
	2007/08		39.1 ± 10.5^{aNS}	$89.6\pm6.14^{b\ NS}$
Fruity		ns	3.5 ± 0.17^{aNS}	$4.3\pm0.25^{b\ NS}$
Bitter		ns	2.8 ± 1.41^{aNS}	$4.0\pm1.76^{b\ NS}$
Pungent		ns	3.1 ± 0.45^{aNS}	$3.8\pm0.31^{b\ NS}$
$C_{16:0}$		ns	$13.0\pm0.17^{ns\ NS}$	12.5 ± 0.15^{NS}
$C_{18:1}$		ns	$66.8\pm0.75^{a\ NS}$	$71.9\pm0.46^{b\ NS}$
$C_{18:2}$		ns	$14.0\pm1.24^{b\ NS}$	$9.31\pm1.05^{b\ NS}$
Ox.Suscep		ns	$773.5\pm50.2^{b\ NS}$	$559.6\pm0.75^{a\ NS}$
OLO		ns	$14.7\pm0.87^{b\ NS}$	11.8 ± 0.95^{aNS}
PLO+SLL	**2005/06**		$8.08\pm0.55^{ns\ 2}$	6.78 ± 0.85^{NS}
	2006/07	*	$9.46\pm0.95^{b\ 2}$	5.51 ± 0.61^{aNS}
	2007/08		$8.87\pm0.35^{b\ 2}$	$7.04\pm0.25^{a\ NS}$
OOO		ns	32.4 ± 1.99^{aNS}	$41.0\pm1.46^{b\ NS}$
POO		ns	$23.8\pm0.95^{ns\ NS}$	25.9 ± 0.45^{NS}
ΔECN42	**2005/06**		$0.09\pm0.04^{a\ NS}$	$0.01\pm0.01^{b\ NS}$
	2006/07	*	0.12 ± 0.03^{aNS}	$0.01\pm0.01^{b\ NS}$
	2007/08		$0.18\pm0.02^{a\ NS}$	$0.04\pm0.02^{b\ NS}$
Campesterol		*	$2.36\pm0.25^{ns\ NS}$	2.43 ± 0.31^{NS}
Stigmasterol		ns	$0.53\pm0.15^{ns\ NS}$	0.74 ± 0.17^{NS}
β-Sitosterol		ns	81.4 ± 1.75^{aNS}	$84.0\pm0.73^{b\ NS}$
Δ-5-Avenasterol	**2005/06**		$12.2\pm0.95^{a\ NS}$	$10.5\pm0.84^{b\ NS}$
	2006/07	*	$9.5\pm1.17^{b\ NS}$	$7.0\pm0.84^{a\ NS}$
	2007/08		$11.7\pm1.27^{b\ NS}$	$8.3\pm0.75^{a\ NS}$
Total sterols		ns	$1685.0\pm125.5^{a\ NS}$	$1509.6\pm69.7^{b\ NS}$
Erythrodiol + Uvaol		ns	$2.97\pm0.15^{a\ NS}$	$3.65\pm0.24^{b\ NS}$

[a]Crops: 2005/06, 2006/07 and 2007/08.
I: interaction between years and varieties in each stage of ripening, ns/NS: non significant; and *: significant interaction.

In addition, the fatty acid, sterol and triterpenic dialcohol compositions comply with all the requirements of Regulation (EEC, 1991). The ΔECN42 parameter was within the limit regulated and it was always higher in the "Morisca" variety than in "Carrasqueña" olive oil.

3.3. Real versus potential quality

The study of the quality parameters of oils of both varieties is considered to be of high interest for the industrial sector in the oil-producing area of Tierra de Barros, both for the final consumer and the international oil trade. The lowest values found correspond to acidity and peroxide index in the oils from the Abencor system. However, the rest of the variables analyzed did not present any significant differences between extraction systems. This is very interesting for industrial mills due to the fact that the elaboration process did not affect the sensory attributes of VOOs. Regarding the extraction system (Abencor vs Industrial mills), it was observed that the main fatty acids, TGs and sterols were very similar between both systems. Our results were similar to those obtained by other researchers (Inarejos, 2007) who observed that these parameters mainly depend on variety, ripening stage and geographic area. Also, they indicated that technological parameters such as time and mixing temperature did not significantly affect these parameters. However when oils are produced at higher temperatures than 40 °C, the fatty acid composition is affected due to an increase in the enzymatic activity of lipase, which accelerates the hydrolysis of triacylglycerides, diacylglycerides and free fatty acids (Ranalli *et al*., 2001; Di Giovacchino *et al*., 2002).

In addition, there was significant interaction in some studied parameters between extraction system and crop season in each variety analyzed. The 2005/06 campaign showed higher levels of quality with 96% of EVOO from the Abencor system and

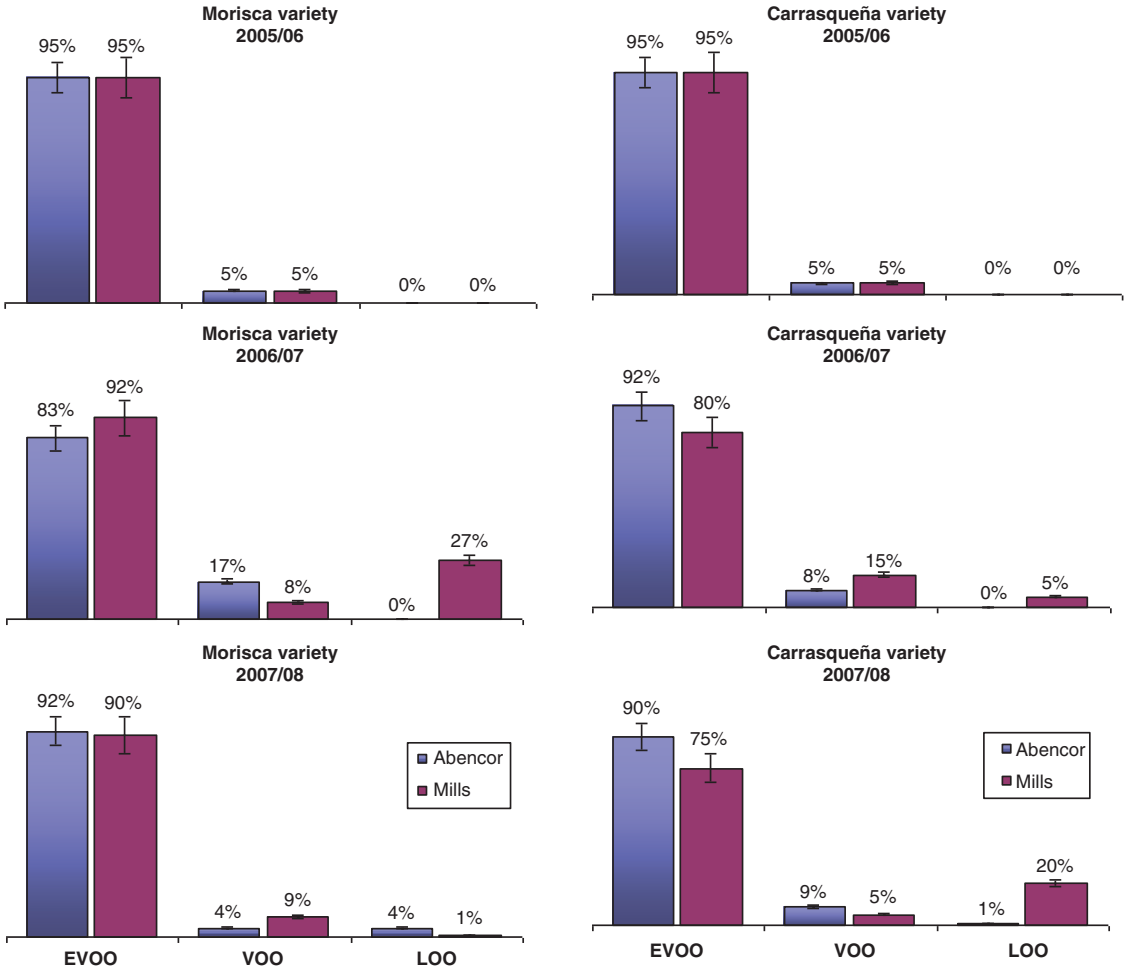

FIGURE 1. Organoleptic classification of VOO samples[a] from Abencor and industrial samples extracted from "Morisca" and "Carrasqueña" varieties during the spotted stage of ripening.
[A]CROPS: 2005/06, 2006/07 and 2007/08.
EVOO: extra virgin olive oil, VOO: virgin olive oil and LOO: lampante olive oil.

Mills (Fig. 1). On the other hand, the 2007/08 campaign was characterized by 24 and 20% of oils from both varieties that belong to the LOO category. This could be attributed to the fact that this campaign was more rainy (591 mm precipitation). Among the most frequent sensory defects which the olive oils presented was "fusty", typical defect in oils from olives that have been stored for several days before their processing, or oils that have long remained in contact with sediments; and the default "winy", because of the aerobic fermentation of olives, producing mainly acetic acid and resulting in a harsh taste.

These differences between the two systems of extraction can be attributed, as it has already been mentioned by other researchers (Luaces *et al*., 2005; Sánchez *et al*., 2006) to the so-called technological factors ranging from the input and storage of olives in olive Mills to the final preservation of extracted oils. In addition, the intrinsic characteristics of olives, like harvesting campaigns, where variables such as cleanliness, work load, olive pulp and oil areas of contact (batch filters versus stainless steel containers), etc. should be considered. Several authors have studied the sensory quality of industrial olive oils and have obtained percentages of "extra virgin" quality of 100% (Ollivier *et al*., 2006; Fuentes, 2013) in oils from Catalan, French, and Extremadura varieties or up to 80% as found by Tamendjari *et al*. (2009) in oils from Tunisian varieties.

4. CONCLUSIONS

The chemical data discussed in this study can be considered useful in providing information about the presence of major and minor compounds in the oils produced in mills from the Tierra de Barros area. Depending on crop season, a high percentage of VOO from "Carrasqueña" and "Morisca" varieties were "extra virgin" quality oils according to the examined characteristics which provide information about the quality of olives and virgin olive oil during olive ripening. Furthermore, the results obtained point out the differentiation of both varieties especially in the content of peroxide index, oxidative stability, sensory notes, oleic and linoleic acid, oxidative susceptibility, MUFA/PUFA, the main TGs (OOO, POO, OLO, and PLO+SLE), ΔECN42, total sterols and triterpenic dialcohol composition. In this sense these compounds could be used as markers to characterize and differentiate these VOOs obtained by different extraction systems. This fact is very important to mills because there is an increase in the competitiveness and quality protection of the companies in the oleic sector.

ACKNOWLEDGEMENTS

The present study has been carried out thanks to the financial support of the Consejería de Educación, Ciencia y Tecnología of the Junta de Extremadura (2PR04B004). M.N. Franco wishes to thank the FSE and the Government of Extremadura for her grant (TEC09072). Thanks go out to the nine mills which supplied us with the olives and olive oil and to M. García Serrano for corrections and translation into English and J. Hernández Carretero, J.M. Garcia Ballesteros, and J. Barahona Nogales for their help in the performance of this study.

REFERENCES

Aguilera MP, Beltrán G, Ortega D, Fernández A, Jiménez A, Uceda M. 2005. Characterisation of virgin olive oil of Italian olive cultivars: "Frantoio" and "Leccino", grown in Andalusia. *Food Chem*. **89**, 387–391. http://dx.doi.org/10.1016/j.foodchem.2004.02.046.

Alves MR, Cunha SC, Amaral JS, Pererira JA, Oliverira MB. 2005. Classification of PDO olive oils on the basis of their sterol composition by multivariate analysis. *Anal. Chim. Acta*. **549**, 166–178. http://dx.doi.org/10.1016/j.aca.2005.06.033.

Aranda F, Gómez-Alonso S, Rivera del Álamo RM, Salvador MD, Fregapane G. 2004. Triglyceride, total and 2-position fatty acid composition of Cornicabra virgin olive oil: Comparison with other Spanish cultivars. *Food Chem*. **86**, 485–492. http://dx.doi.org/10.1016/j.foodchem.2003.09.021.

Baccouri O, Guerfel M, Baccouri B, Cerretani L, Bendini A, Lercker G, Zarrouk M, Daoud D, Miled, B. 2008. Chemical composition and oxidative stability of Tunisian monovarietal virgin olive oils with regard to fruit ripening. *Food Chem*. **109**, 743–754. http://dx.doi.org/10.1016/j.foodchem.2008.01.034.

Cert A, Alba J, León-Camacho M, Moreda W, Pérez-Camino MC. 1996. Effects of talc addition and operating mode on the quality and oxidative stability of virgin oils obtained by centrifugation. *J. Agric. Food Chem*. **44**, 3930–3934. http://dx.doi.org/10.1021/jf9603386.

Di Giovacchino L, Costantini N, Ferrante ML, Serraiocco A. 2002. Influence of malaxation time of olive paste on oil extraction yields and Chemicals and organoleptic characteristics of virgin olive oil obtained by a centrifugal decanter at water saving. *Grasas Aceites* **53**, 179–186. http://dx.doi.org/10.3989/gya.2002.v53.i2.302.

EEC. 1991.Characteristic of olive and olive romance oils and their analytical methods. Regulation EEC/2568/1991 and later modification. *O. J. Eur. Comm*. **L248**, 1–82.

EEC. 1997. Regulation EEC 2138/1997. Whereby the homogeneous olive oil production areas are outlined. *O. J. Eur. Comm*. L297, 1–61.

EEC. 2008. Characteristic of olive oil and olive-residue oil and on the relevant methods of analysis. Regulation EEC/640/2008. *O. J. Eur. Comm*. **L178**, 11–16.

Fuentes M. 2013. Caracterización de los aceites de oliva virgen producidos en la zona oleícola de Tierra de Barros. *Tesis doctoral*, Universidad de Extremadura.

Giacometti J, Milin C. 2001. Composition and qualitative characteristics of virgin olive oils produced in northern Adriatic region, Republic of Croatia. *Grasas Aceites* **52**, 397–402. http://dx.doi.org/10.3989/gya.2001.v52.i6.350.

Gómez-Rico A, Salvador MD, Fregapane G. 2009. Virgin olive oil and olive fruit minor constituents as affected by irrigation management based on SWP and TDF as compared to ETc in medium-density young olive orchards (*Olea europaea* L. cv. Cornicabra and Morisca). *Food Res. Int*. **42**, 1067–1076. http://dx.doi.org/10.1016/j.foodres.2009.05.003.

Gracia MS, Royo A, Guillén M. 2009. Composición química de aceites de las variedades Arbequina y Empeltre cultivadas en regadío. *Grasas Aceites* **60**, 321–329. http://dx.doi.org/10.3989/gya.097008.

Gutiérrez F. 1989. Determinación de la estabilidad oxidativa de aceites de oliva vírgenes: comparación entre el método del oxígeno activo (A.O.M) y el método Rancimat. *Grasas Aceites* **40**, 1–5.

Haddada FM, Manai H, Oueslati I, Daoud D, Sánchez J, Osorio E, Zarrouk M. 2007. Fatty acid, triacylglycerol and phytosterol composition in six Tunisian Olive Varieties. *J. Agric. Food Chem*. **55**, 10941–10946. http://dx.doi.org/10.1021/jf071727a.

Inarejos AM. 2007. Efecto de las condiciones de tiempo-temperatura durante la operación de batido que inciden en la calidad del aceite de oliva virgen elaborado en almazara experimental. *Tesis doctoral*. Universidad de Castilla-La Mancha.

IOOC. 2010. International Olive Oil Council. Trade standard applying to olive oils and olive pomace oils. COI/T. 15/NC No3/Rev. **5**, 1–17.

Jimenez B, Rivas A, Sánchez-Ortiz A, Lorenzo ML, Úbeda M, Callejón RM, Ortega E. 2012. Influence of fruit maturation process on the sensory quality of virgin olive oils from Picual, Hojiblanca and Picudo cultivars. *Grasas Aceites* **63**, 403–410. http://dx.doi.org/10.3989/gya.058212.

Kiritsakis A. 2007. Olive oil as functional food in *Kiritsakis A*. (Ed.) Olive oil: Conventional and biological/table olives, olive paste. Thesniki, ISBN 978-960-631-714-9, 621–644.

Luaces P, Pérez AG, García JM, Sanz C. 2005. Effect of heat treatments of olive fruit on pigment composition of virgin olive oil. *Food Chem*. **90**, 169–174. http://dx.doi.org/10.1016/j.foodchem.2004.03.035.

Lukic M, Lukic J, Krapac M, Sladouja B, Pilirota V. 2013. Sterols and triterpene diols in olive oil as indicators of variety and degree of ripening. *Food Chem*. **136**, 251–258. http://dx.doi.org/10.1016/j.foodchem.2012.08.005.

Manai H, Mahjoub–Haddada F, Trigui A, Daoud D, Zarrouk M. 2007. Compositional quality of virgin olive oil from two new Tunisian cultivars obtained through controlled crossings on Chemlali variety. *J. Sci. Food Agric*. **87**, 600–606. http://dx.doi.org/10.1002/jsfa.2732.

Martínez JM, Muñoz E, Alba J, Lanzón A. 1975. Informe sobre la utilización del analizador de rendimientos "Abencor". *Grasas Aceites* **26**, 379–385.

Matos LC, Cunha SC, Amaral JS, Pereira JA, Andrade PB, Seabra RM, Oliveira BP. 2007. Chemometric characterization of three varietal olive oils (cvs. Cobrançosa, Madural and Verdeal Transmontana) extracted from olives with different maturation indices. *Food Chem*. **102**, 406–414. http://dx.doi.org/10.1016/j.foodchem.2005.12.031.

Montedoro G, Servili M, Baldioli M, Miniati E. 1992. Simple and hydrolysable phenolic compounds in virgin olive oil. 1. their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. *J. Agric. Food Chem*. **40**, 1571–1576. http://dx.doi.org/10.1021/jf00021a019.

Ollivier D, Artaud J, Pinatel C, Durbec J-P, Guérère M. 2006. Differentiation of French virgin olive oil RDOs by sensory characteristics, fatty acid and triacylglycerol compositions and chemometrics. *Food Chem*. **97**, 382–393. http://dx.doi.org/10.1016/j.foodchem.2005.04.024.

Osorio E, Sánchez J, Martínez M, Montaño A. 2003. Estudio del contenido en triglicéridos de aceites monovarietales elaborados a partir de aceitunas producidas en la región extremeña. *Grasas Aceites* **54**, 1–6.

Oueslati I, Manai H, Haddada FM, Daoud D, Sánchez J, Osorio E, Zarrouk M. 2009. Sterol, Triterpenic Dialcohol, and triacylglycerol compounds of extra virgin olive oils from some Tunisian varieties grown in the region of Tataouine. *Food Sci. Technol. Int*. **15**, 5–13. http://dx.doi.org/10.1177/1082013208101024.

Panzanaro S, Nutricati E, Miceli A, De Bellis L. 2010. Biochemical characterization of a lipase from olive fruit (*Olea europaea* L.). *Plant Physiol. Biochem*. **48**, 741–745. http://dx.doi.org/10.1016/j.plaphy.2010.05.004.

Pardo JE, Cuesta MA, Alvarruiz A, Granell JD, Álvarez-Ortí M. 2011. Evaluation of potencial and real qualities of virgin olive oil from the designation of origin (DO) "Aceite Montes de Alcazaz" (Albacete, Spain). *Food Chem*. **124**, 1684–1690. http://dx.doi.org/10.1016/j.foodchem.2010.07.068.

Pereira JA, Casal S, Bento A, Oliveira M. 2002. Influence of olive storage period on oil quality of three Portuguese cultivars of *Olea europaea*, Cobrancosa, Madural and Verdeal Transmontana. *J. Agric. Food Chem*. **50**, 6335–6340. http://dx.doi.org/10.1021/jf011661y.

Ranalli A, Contento S, Schiavone C, Simone N. 2001. Malaxing temperature affects volatile and phenol composition as well as other analytical features of virgin olive oil. *Eur. J. Lipid Sci. Technol*. **103**, 228–238. http://dx.doi.org/10.1002/1438-9312(200104)103:4<228::AID-EJLT228>3.0.CO;2-7.

Sakouihi F, Absalou C, Harrabi S, Yitry C, Sebel K, Boukhchina S, Fouquet E, Kallet H. 2009. Dynamic accumulation of 4-desmethylsterols and phytostanols during ripening of Tunisian Meski olives (*Olea europaea*, L). *Food Chem*. **112**, 897–902. http://dx.doi.org/10.1016/j.foodchem.2008.06.068.

Salvador MD, Aranda F, Fregapane G. 2001. Influence of fruit ripening on "Cornicabra" virgin olive oil quality: a study of four successive crop seasons. *Food Chem*. **73**, 45–53. http://dx.doi.org/10.1016/S0308-8146(00)00276-4.

Sánchez J, De Miguel C, Osorio E, Marín J, Gallardo L, Martínez M. 2006. Calidad sensorial de aceites de oliva virgen procedentes de variedades de aceitunas producidas en Extremadura. *Grasas Aceites* **57**, 313–318.

Sánchez J, Osorio E, Montaño AM, Martínez M. 2003. Estudio del contenido en ácidos grasos de aceites monovarietales elaborados a partir de aceitunas producidas en la región extremeña. *Grasas Aceites* **54**, 371–377.

Sánchez J, Osorio E, Montaño AM, Martínez M. 2004. Sterol and erythrodiol+uvaol content of virgin olive oils from cultivars of Extremadura (Spain). *Food Chem*. **87**, 225–230. http://dx.doi.org/10.1016/j.foodchem.2003.11.012.

Stefanoudaki E, Kotsifaki F, Koutsaftakis, A. 1999. Classification of virgin olive oils of the two major cretan cultivars based on their fatty acid composition. *J. Am. Oil Chem. Soc*. **76**, 623–626. http://dx.doi.org/10.1007/s11746-999-0013-7.

Tamendjari A, Angerosa F, Mettouchi S, Bellal MM. 2009. The effect of fly attack (*Bactrocera oleae*) on the quality and phenolic content of Chemlali olive oil. *Grasas Aceites*. **60**, 507–513. http://dx.doi.org/10.3989/gya.032209.

Termime SB, Manai H, Methenni K, Baccouri B, Abaza L, Daoud D, Sánchez J, Osorio E, Zarrouk M. 2008. Sterolic composition of Chétoui virgin olive oil: Influence of geographical origin. *Food Chem*. **110**, 368–374. http://dx.doi.org/10.1016/j.foodchem.2008.02.012.

Uceda M, Frías, L. 1975. Harvest dates: evolution of the fruit oil content, oil composition and oil quality. In: *Proceedings II Seminario Oleícola Internacional*. International Olive Council. Córdoba, Spain, **1975**, 125–128.

Vekiari SA, Oreopoulou V, Kourkoutas Y, Kamoun N, Msallem M, Psimouli V, Arapoglou D. 2010. Characterization and seasonal variation of the quality of virgin olive oil of the Throumbolia and Koroneiki varieties from Southern Greece. *Grasas Aceites* **61**, 221–231. http://dx.doi.org/10.3989/gya.108709.