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Chemometric characterization of three varietal olive oils (Cvs. Cobrançosa, Madural and Verdeal Transmontana) extracted from olives with different maturation indices

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Abstract

This paper evaluates the usefulness of three chemical parameters (compositions on tocopherols, sterols and fatty acids) as a tool to discriminate three varietal olive oils (Cvs. Cobrançosa, Madural and Verdeal Transmontana), which are permitted cultivars for the production of “Trás-os-Montes olive oil”, a Portuguese protected designation of origin (PDO) product. The olives were collected during the year crop 2000/2001 from the same orchard, in order to eliminate the geographical and climatic influences. Lots with different maturation indices were prepared to allow the evaluation of the ripening stage on the characteristics of varietal olive oils produced from each cultivar. Statistical methods such as multivariate analysis of variance (MANOVA), principal components analysis (PCA) and cluster analysis were used to evaluate significant differences on the studied parameters. Regarding the results, the three cultivars were clearly discriminated.

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1. Introduction

Olive oil is obtained from the fruit of olive trees (*Olea europaea* L.) and is a genuine fruit juice with excellent nutritional, sensorial and functional quality. It represents a typical lipid source of the Mediterranean diet, which consumption has been associated with a low incidence of cardiovascular diseases, neurological disorders, breast and colon cancers, as well as with hipolipidemic and antioxidant properties (Gimeno et al., 2002; Medeiros, 2001). These benefits have been related either to its well-balanced fatty acid composition, where oleic acid is the main compo-

nent, or to the presence of minor biomolecules, such as vitamins and natural antioxidants (Medeiros, 2001). These characteristics, as a whole, make olive oil a premium food and, therefore, a product of major economical importance in the Mediterranean area.

Olive oil quality is influenced by a great number of factors among which the cultivar and the olive ripening stage are two of the most important ones (Garcia, Seller, & Pérez-Camino, 1996; Kiritsakis, 1998; Rotondi et al., 2004; Zamora, Alaiz, & Hidalgo, 2001). Along the ripening, several metabolic processes take place in olives with subsequent variations on profiles of some compounds. These changes are reflected on the quality grade, sensorial characteristics, oxidative stability and/or nutritional value of the obtained product. Polyphenols, tocopherols, chlorophyllic pigments and carotenoids are examples of

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compounds involved in this phenomenon as well as the fatty acid and sterol compositions.

There are different cultivars of *O. europaea*, each one with specific physical and biochemical characteristics, providing olive oils with typical compositions and performances. The aim of this work was to evaluate, compare and characterize the performances of varietal olive oils obtained from Cvs. Cobrançosa, Madural and Verdeal Transmontana based on some of its most important components. These three cultivars account for more than 90% of olive cultivation area in Trás-Os-Montes (Portugal) and, together with cv. Cordovil, are the only permitted cultivars in the “Trás-Os-Montes olive oil” (protected designation of origin, PDO). The trees used in this study belong to the same orchard and, consequently, are subjected to the same climate conditions and agricultural practices. The olives were collected during the year crop 2000/2001. Lots with different maturation indices were prepared to allow the evaluation of the ripening stage effect on the characteristics of olive oils produced from each cultivar. In this way, we can be sure that the only factors affecting the differences among samples are the cultivar and the ripening stage, the only influences that are aimed to study. A total of 18 samples were analyzed: 7 from Cv. Cobrançosa (maturation indices 1–7), 5 from Cv. Madural (maturation indices 3–7) and 6 from Cv. Verdeal Transmontana (maturation indices 1–6). In all samples the chemical parameters evaluated were the compositions on tocopherols, sterols and fatty acids. The results were subjected to statistical analysis in order to evaluate the influence of cultivar and ripening stage on those parameters and to check if PCA of the evaluated parameters could be a tool for cultivar and ripening stage discrimination.

2. Materials and methods

2.1. Sampling

The studied cultivars were Cvs. Cobrançosa, Madural and Verdeal Transmontana. The trees were identified and carefully marked, and five trees of each cultivar have been sampled. The olive fruits were handpicked in “Mascarenhas-Paradela”, an orchard 10 km North from Mirandela, in the Northeast of Portugal (U.T.M. 29 PG5602). The orchard has 6 ha with a planting density of 9 × 9 m; trees have more than 40 years; the prune is made each three years; it is not irrigated and the soil is mobilized 2–3 times each year. This olive grove is kept under Biological Agricultural system and did not have any crop health control treatment in the last 10 years. Cv. Cobrançosa is the more representative cultivar (80% of the trees). The olives were harvested in the crop year 2000/2001 in three different days (30/10, 22/11 and 5/12), in the four orientations of the trees, at the operator height. From each tree, only healthy fruits were picked. After harvest, olive fruits were immediately transported to the laboratory, carefully blended and, in a hundred

olives randomly taken from each cultivar and harvest date, the maturation indices (MI) were determined. This parameter is function of fruit colour in both skin and pulp and was determined according to the proposals of the Estación de Olivicultura y Elaiotecnia, Jaén, Spain (Hermoso et al., 1991). Following this procedure 18 samples were obtained: seven samples for Cv. Cobrançosa (MI 1–7), five for Cv. Madural (MI 3–7) and six for Cv. Verdeal Transmontana (MI 1–6). From each group, a 1 kg of olives were randomly taken and submitted to oil extraction.

2.2. Oil extraction

An Abencor analyzer (Comercial Abengoa S.A., Sevilla, Spain) was used to process the olives in a pilot extraction plant. The unit consists of three essential elements: the mill, the thermo beater, and the pulp centrifuge. After being processed in the mill, the oil was separated by decanting, transferred into dark glass bottles, and stored in the dark at 4 °C. Before the analytical procedures the samples were dehydrated with anhydrous sodium sulfate and subsequently filtered through filter paper.

2.3. Standards

A mixture of 37 FAMES (standard 47885-U) and the individual fatty acid isomers *cis*-9-*trans*-12-octadecadienoate (C18:2*ct*), *trans*-9-*cis*-12-octadecadienoate (C18:2*tc*) were obtained from Supelco (Bellefonte, PA, USA). Cholesterol, campesterol, stigmasterol, β -sitosterol, β -sitostanol and betulin were purchased from Sigma (St. Louis, USA). α -, β -, γ - and δ -tocopherols were obtained from Calbiochem (La Jolla, CA, USA).

2.4. Tocopherol composition

Tocopherol composition was evaluated following the method described in Gama, Casal, Oliveira, and Ferreira (2000). A 0.1 g of olive oil was blended with 10 ml of *n*-hexane and homogenized by stirring. Sample preparation was conducted in dark and tubes containing the samples were always wrapped in aluminium foil. The mixture was filtered by membrane (Schleicher and Shuell 0.2 μ m; \varnothing 13 mm, pure polyamide) and analyzed by HPLC. The chromatographic separation of the compounds was achieved with a normal-phase LiChrosorb SI 60 (5 μ m; 25 × 0.4 cm) column from Merck (Darmstadt, Germany). The effluent used was a mixture of *n*-hexane and 2-propanol (99.7:0.3). Elution was performed at a solvent flow rate of 1.7 ml/min. The effluent was monitored with diode array and fluorimetric (290 and 330 nm as excitation and emission wavelengths respectively) detectors connected in series. Data were analyzed in the Borwin PDA Controller Software (JMBS, France). Tocopherols (α , β and γ) were identified by chromatographic comparisons with authentic standards, by co-elution and by their UV spectra.

2.5. Sterol composition

Sterol composition was evaluated by GLC/FID/capillary column following the NP EN ISO 12228. Briefly, after addition of 1.0 ml of internal standard solution (betulin 1.0 mg/ml) ca. 250 mg of oil sample was saponified with an ethanolic potassium hydroxide solution; the unsaponifiable fraction was isolated by solid phase extraction on an aluminum oxide column and the steroid fraction was obtained after TLC with *n*-hexane/diethyl ether 1:1 (v/v) as developing solvent and a methanol spray to visualize the band. The trimethylsilyl ethers were obtained by the addition of 1-methylimidazole and *N*-methyl-*N*-(trimethylsilyl)-hepta-fluorobutyramide (MSHFBA). The sterol profile was analyzed on the same equipment used for fatty acid analysis, with a 30 m × 0.25 mm i.d., 0.25 μm DB-5MS column (J&W Scientific, Folsom, CA, USA) with a maximum operating temperature of 325 °C. The temperature of the injector and the detector were both 320 °C. The column temperature was 250 °C and programmed to increase at a rate of 2 °C/min to 300 °C and then held for 12 min. The carrier gas used was helium at an internal pressure of 100 kPa. The split ratio was 1:50 and the injected volume was 1.5 μl. The total sterol content was determined considering all peaks of sterols eluted between cholesterol and Δ⁷-avenasterol. Identification was achieved by comparing the relative retention times from samples with those obtained with standards. Clerosterol, Δ⁵-avenasterol and Δ⁷-avenasterol were tentatively identified by comparison with references (Firestone & Reina, 1996; Kamm, Dionisi, Hischenhuber, & Engel, 2001) and with samples of olive and sunflower oils. β-Sitosterol and Δ⁵-avenasterol eluted very close and therefore they were quantified as Δ⁵-avenasterol.

2.6. Fatty acid composition

Fatty acids were determined by GLC/FID/capillary column using the same methodology described in Oliveira and Ferreira (1996).

Fatty acids were measured as their methyl esters after hydrolysis with an 11 g/l methanolic potassium hydroxide solution, methyl esterification with BF₃/MeOH and extraction with *n*-heptane. The analysis was carried out on a Chrompack CP 9001 chromatograph (Chrompack, Middelburg, The Netherlands) equipped with a split-splitless injector, a FID and a Chrompack CP-9050 auto sampler. The temperatures of the injector and detector were 230 and 270 °C respectively. Separation was achieved on a 50 m × 0.25 mm i.d. fused silica capillary column coated with a 0.19 μm film of CP-Sil 88 (Chrompack, Middelburg, The Netherlands). The column temperature was 160 °C, 1 min hold, and then programmed to increase to 239 °C at a rate of 4 °C/min and then 10 min hold. Helium was used as carrier gas at an internal pressure of 120 kPa. The split ratio was 1:50 and the injected volume was 1.2 μl. The results are expressed in relative percentage of

each fatty acid, calculated by internal normalization of the chromatographic peak area. Fatty acids were identified by comparing the relative retention times of FAME peaks from samples with those from standards.

2.7. Statistical analysis

Results were submitted to multivariate analysis of variance (MANOVA) with the purpose of testing the significant differences between mean values of the evaluated parameters by comparing variances. In other words we compared the variance due to the between-groups with the within-group variability. These latter were tested for statistical significance and, if significant, we rejected the null hypothesis of no differences between means, and accepted the alternative hypothesis that the means were different from each other. One-way MANOVA was used because our design had one single categorical independent variable (cultivar type) and multiple dependent variables (chemical parameters). Multivariate test of significance was performed and Wilk's lambda was observed. Alternative *t*-test for independent samples was used with the purpose of comparing differences in means between two types of cultivars. All the combinations of cultivars pairs were tested. Principal components analysis (PCA) was performed to detect structure in the relationships between variables, allowing its classification and the separation of each cultivar. Variables used in PCA were selected regarding to the MANOVA and *t*-test results. This procedure allowed the minimization of variables, and those that were selected became the principal explanation to the variability between cultivars, improving the separation and making easier the understanding of what were the stronger factors that were the base of distinction. Eigenvalues were also observed and, for each analysis, two factors were enough to explain almost all the variability. Cluster analysis was also performed in order to attempt the grouping of samples according to the similarities observed in some chemical parameters. The vertical hierarchical tree plot was performed considering the Euclidian distances of data projected in a multidimensional space. Besides, the clusters linkage was made by the Ward's method. Results are shown as figures and tables. One-way MANOVA, *t*-test for independent groups, PCA and cluster analysis were performed with Statistica for Windows release 6.0.

3. Results and discussion

Vitamin E is a term used to refer a group of minor but ubiquitous lipid-soluble compounds, comprising four tocopherols (α-, β-, γ- and δ-T) and four tocotrienols (α-, β-, γ- and δ-TTR). These compounds are believed to be involved in a diversity of physiological and biochemical functions, mainly due to its action as antioxidant, but also by its action as a membrane stabilizer (Azzi & Stocker, 2000). In olive oil the compounds usually described are those which were also detected in this work, that is:

α , β - and γ -tocopherols (Aguilera et al., 2005; Beltrán, Aguilera, Del Rio, Sanchez, & Martinez, 2005). Together with phenolic compounds they are responsible by the oxidative stability of olive oil and, therefore, for its shelf life with special emphasis for α -tocopherol (Mateos, Dominguez, Espartero, & Cert, 2003).

Table 1 reports the tocopherol composition of the studied samples. In all samples, and as expected for olive oils (Aguilera et al., 2005; Beltrán et al., 2005) α -tocopherol is by far the most abundant isoform of vitamin E. In any of the cultivars, the content of α -tocopherol decreases along the ripening. The content of β -tocopherol showed a good stability while that of γ isoform exhibited a slight increase. This behaviour has already been found for other cultivars (Aguilera et al., 2005; Beltrán et al., 2005; Gutiérrez, Jiménez, Ruíz, & Albi, 1999). The three cultivars under study can be clearly distinguished by the total amounts of tocopherols (189–139 mg/kg in Cv. Verdeal Transmontana, 226–209 mg/kg in Cv. Madural and 298–238 mg/kg in Cv. Cobrançosa) but also by the individual contents of α - and γ -tocopherols.

Tocopherol composition of the three cultivars showed highly significant differences ($p < 0.01$) and Wilk's lambda was 0.018. The three homologous were used in PCA analysis and two factors were selected justifying 96.05% of total variance (Factor 1: 75.94%; Factor 2: 20.11%). Regarding these factors, the three cultivars could be discriminated on the factor-plane (Fig. 1).

Table 1

Tocopherols composition (mg/kg)^a of Cvs. Cobrançosa, Madural and Verdeal Transmontana virgin olive oils extracted from olives with different maturation indices

	Tocopherol contents (mg/kg)			Total
	α	β	γ	
Cv. Cobrançosa				
MI 1	291.7 ± 2.4	0.9 ± 0.1	5.4 ± 0.1	298.0
MI 2	252.9 ± 0.9	0.9 ± 0.0	8.0 ± 0.1	261.8
MI 3	246.6 ± 1.6	1.0 ± 0.1	13.0 ± 0.0	260.6
MI 4	222.6 ± 1.2	0.9 ± 0.0	12.0 ± 0.0	235.5
MI 5	224.8 ± 3.6	1.0 ± 0.0	16.1 ± 0.1	241.9
MI 6	234.8 ± 0.3	1.0 ± 0.1	15.9 ± 0.1	251.6
MI 7	221.4 ± 1.7	1.0 ± 0.1	15.7 ± 0.0	238.1
Cv. Madural				
MI 3	219.9 ± 0.8	0.9 ± 0.1	5.1 ± 0.1	225.8
MI 4	212.1 ± 1.5	0.9 ± 0.1	7.6 ± 0.0	220.6
MI 5	218.8 ± 2.1	0.9 ± 0.0	7.1 ± 0.1	226.8
MI 6	209.1 ± 0.4	0.9 ± 0.1	6.3 ± 0.1	216.3
MI 7	202.2 ± 0.6	0.8 ± 0.1	5.8 ± 0.0	208.8
Cv. Verdeal Transmontana				
MI 1	188.5 ± 2.2	0.9 ± 0.1	0.0 ± 0.1	189.4
MI 2	169.4 ± 2.8	0.9 ± 0.1	1.1 ± 0.0	171.3
MI 3	135.4 ± 0.3	0.9 ± 0.1	3.1 ± 0.1	139.4
MI 4	145.5 ± 0.8	0.9 ± 0.0	2.9 ± 0.1	149.3
MI 5	146.1 ± 1.1	0.9 ± 0.1	3.7 ± 0.1	150.6
MI 6	133.6 ± 1.4	0.9 ± 0.1	4.0 ± 0.1	138.5

^a Mean and standard deviation for a confidence coefficient of 95%.

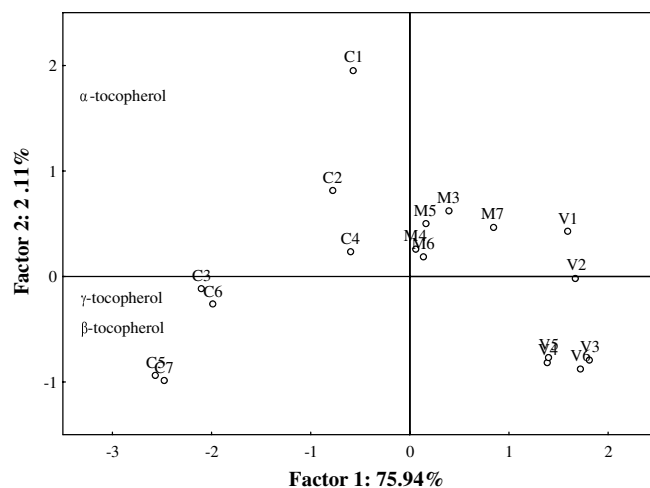


Fig. 1. Principal components analysis based on tocopherol profiles of Cvs. Cobrançosa, Madural and Verdeal Transmontana virgin olive oils extracted from olives with different maturation indices.

Sterols are major constituents of the unsaponifiable fraction and their content corresponds to around 20% of the unsaponifiable matter of olive oil. Research has shown that each oily fruit has a characteristic sterol profile which makes its determination an important tool for checking the genuineness of an oil (Gutiérrez, Varona, & Albi, 2000; Salvador, Aranda, & Fregapane, 1998). They are important components for the stability of the oil since at high temperature they act as inhibitors of polymerization reactions (Velasco & Dobarganes, 2002). In the present work, seven sterols were detected and quantified and the results obtained are displayed in Table 2. Generally, a decreasing tendency is observed along the ripeness in the values of total sterols, as already observed for Cvs. Cornicabra, Picual and Hojiblanca (Gutiérrez et al., 1999; Salvador, Aranda, & Fregapane, 2001). Contrarily to the described for these same cultivars, the values for Δ^5 -avenasterol did not show a clear tendency in any of the cultivars subjected to the present study.

All of the analyzed samples exhibited total sterol far above the 1000 mg/kg demanded by the EU legislation and all of them contain more the demanded threshold value of 93% of apparent β -sitosterol (which in this case is the sum of β -sitosterol, clerosterol and Δ^5 -avenasterol).

MANOVA results showed that cultivars presented significant differences regarding stigmasterol and Δ^7 -avenasterol contents ($p < 0.05$) and highly significant regarding Δ^5 -avenasterol ($p < 0.01$). Despite these results, the sterols chosen to PCA analysis were also selected by their abundance in the oils. These sterols stigmasterol, clerosterol, β -sitosterol and Δ^5 -avenasterol. Two factors were selected justifying 89.27% of total variance (Factor 1: 73.48%; Factor 2: 15.78%). The values were plotted and the cultivars discriminated (Fig. 2) although the separation was less evident than that observed when PCA was performed with the other chemical parameters.

Table 2
Sterol composition (mg/100 mg)^a of Cvs. Cobrançosa, Madural and Verdeal Transmontana virgin olive oils extracted from olives with different maturation indices

	Sterol parameter							Total
	Cholesterol	Campesterol	Stigmasterol	Clerosterol	β -Sitosterol	Δ^5 -Avenasterol	Δ^7 -Avenasterol	
Cv. Cobrançosa								
MI 1	0.67 ± 0.05	9.10 ± 0.06	1.83 ± 0.04	2.38 ± 0.13	237.24 ± 0.71	16.24 ± 0.12	0.30 ± 0.04	267.8
MI 2	0.29 ± 0.04	5.36 ± 0.04	1.08 ± 0.04	1.51 ± 0.01	157.76 ± 1.01	15.34 ± 0.12	0.36 ± 0.02	181.7
MI 3	1.07 ± 0.01	8.38 ± 0.19	1.48 ± 0.05	2.21 ± 0.14	206.59 ± 0.46	20.91 ± 0.12	0.43 ± 0.03	241.1
MI 4	1.05 ± 0.05	6.23 ± 0.07	1.23 ± 0.03	1.68 ± 0.04	162.27 ± 0.59	14.68 ± 0.12	0.00 ± 0.00	187.1
MI 5	0.38 ± 0.02	6.26 ± 0.02	1.45 ± 0.02	1.91 ± 0.02	174.49 ± 0.74	15.35 ± 0.02	0.00 ± 0.00	199.8
MI 6	0.90 ± 0.01	7.85 ± 0.10	2.82 ± 0.18	2.23 ± 0.03	207.84 ± 0.40	15.15 ± 0.14	0.00 ± 0.00	236.8
MI 7	0.84 ± 0.03	4.97 ± 0.07	1.01 ± 0.02	1.58 ± 0.08	143.21 ± 0.61	13.62 ± 0.08	0.19 ± 0.02	165.4
Cv. Madural								
MI 3	0.42 ± 0.02	4.56 ± 0.11	1.84 ± 0.08	1.72 ± 0.07	157.38 ± 0.35	15.22 ± 0.21	0.37 ± 0.04	181.5
MI 4	0.48 ± 0.06	7.30 ± 0.06	3.70 ± 0.01	2.83 ± 0.03	255.35 ± 1.07	23.32 ± 0.19	0.66 ± 0.04	293.6
MI 5	0.95 ± 0.05	4.87 ± 0.08	2.71 ± 0.05	1.70 ± 0.03	152.35 ± 0.69	20.39 ± 0.06	0.55 ± 0.06	183.5
MI 6	0.36 ± 0.03	4.49 ± 0.03	2.66 ± 0.03	1.70 ± 0.03	147.38 ± 1.25	20.91 ± 0.03	0.48 ± 0.01	178.0
Cv. Verdeal Transmontana								
MI 1	0.55 ± 0.02	7.34 ± 0.02	1.67 ± 0.02	1.78 ± 0.02	193.96 ± 0.36	12.58 ± 0.07	0.40 ± 0.03	218.3
MI 2	0.74 ± 0.05	6.85 ± 0.05	1.54 ± 0.11	1.71 ± 0.03	184.20 ± 1.01	13.96 ± 0.04	0.42 ± 0.01	209.4
MI 3	0.38 ± 0.02	5.28 ± 0.02	1.87 ± 0.01	1.47 ± 0.08	153.82 ± 0.11	10.26 ± 0.04	0.25 ± 0.03	173.3
MI 4	0.49 ± 0.03	4.65 ± 0.02	1.35 ± 0.00	1.36 ± 0.06	142.95 ± 0.18	11.07 ± 0.04	0.34 ± 0.01	162.2
MI 5	0.37 ± 0.03	4.88 ± 0.02	2.00 ± 0.04	1.41 ± 0.02	149.00 ± 0.42	11.21 ± 0.07	0.24 ± 0.01	169.1
MI 6	0.35 ± 0.02	4.07 ± 0.09	1.62 ± 0.18	1.24 ± 0.06	120.41 ± 0.92	8.76 ± 0.06	0.00 ± 0.00	136.5

^a Mean and standard deviation for a confidence coefficient of 95%.

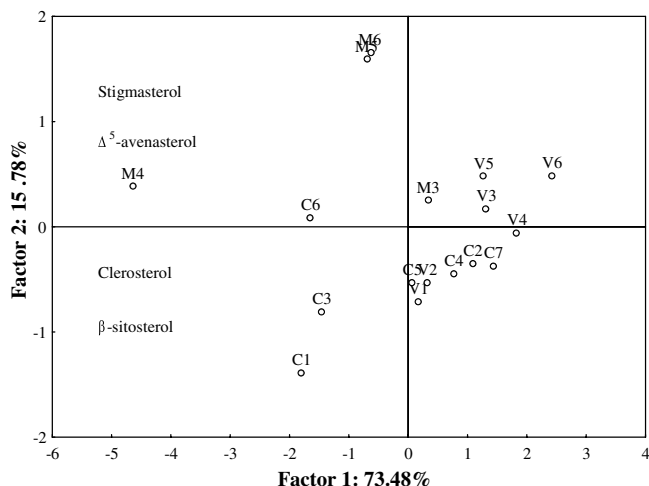


Fig. 2. Principal components analysis based on sterols profiles of Cvs. Cobrançosa, Madural and Verdeal Transmontana virgin olive oils extracted from olives with different maturation indices.

The fatty acid composition of these cultivars was studied previously (Pereira, Oliveira, Casal, & Alves, 2002) and the results subjected to statistical analysis in order to differentiate the cultivars. The samples analyzed at that time correspond to another year crop and were collected at the same ripening stage. In this work, the oils under study were also subjected to a fatty acid analysis in order to evaluate the effect of the ripening stage on the fatty acid composition.

Tables 3–5 report the results obtained for each fatty acid identified, total saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), total *trans* isomers of unsaturated fatty acids

and the ratios oleic/linoleic acids and MUFA/PUFA for the three cultivars under evaluation. The now obtained values are, in general terms, in agreement with those already reported by Pereira et al. (2002) for the same cultivars in another crop year. Cv. Cobrançosa showed the highest mean value for SFA (14.65%) while Cv. Madural showed the highest mean value for PUFA (13.06%) and Cv. Verdeal Transmontana the highest mean value for MUFA (82.00%). The ratios oleic to linoleic acid were also different among the cultivars and varied along the ripening; however, MANOVA results showed that differences on individual fatty acid compositions were not significant ($p > 0.05$) when maturation index was considered the independent variable. Cv. Madural presented always the lowest value (mean value of 6.0) and Cv. Verdeal Transmontana the highest (mean value of 28.2). In Cv. Cobrançosa the values were intermediate (mean value of 12.2). The oleic to linoleic acids ratio is frequently used as a stability parameter (Velasco & Dobarganes, 2002) and, in previous studies, the cultivars with higher ratios were those with higher oxidative stability (data not shown).

Fatty acids results were submitted to one-way MANOVA with the purpose of testing for significant differences due to cultivar. Differences on C_{16} contents were not considered significant when the three cultivars were compared ($p > 0.05$). Differences on the others fatty acids were highly significant ($p < 0.01$). Wilk's lambda is the most commonly used multivariate measure and in this case was near zero ($5.20E-5$) indicating a perfect relationship of cultivars and responses in fatty acid composition. With the purpose of reducing variables but maximizing the variability between cultivars three fatty acids were selected to PCA

Table 3
Fatty acid composition (percent)^a of Cv. Cobrançosa virgin olive oils extracted from olives with different maturation indices

	MI 1	MI 2	MI 3	MI 4	MI 5	MI 6	MI 7
C ₁₄	0.01 ± 0.00	n.d. ^a	n.d.	0.01 ± 0.00	n.d.	0.01 ± 0.00	0.01 ± 0.00
C ₁₆	10.43 ± 0.27	9.92 ± 0.05	9.93 ± 0.03	10.12 ± 0.07	9.01 ± 0.10	9.78 ± 0.02	9.17 ± 0.02
C _{16:1^t}	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.07 ± 0.00	0.09 ± 0.00	0.10 ± 0.00
C _{16:1^c}	0.34 ± 0.01	0.32 ± 0.00	0.30 ± 0.00	0.34 ± 0.00	0.26 ± 0.00	0.44 ± 0.00	0.39 ± 0.00
C ₁₇	0.18 ± 0.01	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.15 ± 0.00	0.20 ± 0.00	0.19 ± 0.00
C _{17:1^c}	0.26 ± 0.01	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.28 ± 0.00	0.27 ± 0.00
C ₁₈	3.91 ± 0.21	3.21 ± 0.02	3.95 ± 0.04	4.13 ± 0.10	4.31 ± 0.05	4.78 ± 0.01	4.84 ± 0.00
C _{18:1^t}	0.01 ± 0.00	n.d.	n.d.	n.d.	n.d.	0.01 ± 0.00	0.01 ± 0.00
C _{18:1^c}	76.46 ± 1.31	78.81 ± 0.08	78.00 ± 0.15	76.16 ± 0.64	77.79 ± 0.50	73.72 ± 0.01	75.35 ± 0.02
C _{18:2^{ct}}	n.d.	n.d.	n.d.	n.d.	n.d.	0.01 ± 0.00	n.d.
C _{18:2^{cc}}	5.28 ± 0.01	5.62 ± 0.07	5.60 ± 0.10	6.99 ± 0.35	6.35 ± 0.28	8.14 ± 0.01	7.02 ± 0.01
C _{18:3^{ccc}}	0.63 ± 0.01	0.68 ± 0.00	0.55 ± 0.00	0.59 ± 0.02	0.61 ± 0.01	0.74 ± 0.00	0.73 ± 0.00
C ₁₉	0.01 ± 0.00	n.d.	n.d.	n.d.	n.d.	0.01 ± 0.00	0.01 ± 0.00
C ₂₀	0.44 ± 0.02	0.39 ± 0.01	0.41 ± 0.00	0.39 ± 0.00	0.40 ± 0.00	0.44 ± 0.00	0.47 ± 0.00
C _{20:1}	0.13 ± 0.01	0.16 ± 0.00	0.16 ± 0.00	0.15 ± 0.01	0.15 ± 0.00	0.22 ± 0.00	0.23 ± 0.00
C ₂₁	0.02 ± 0.00	n.d.	n.d.	0.02 ± 0.00	n.d.	0.02 ± 0.00	0.02 ± 0.01
C ₂₂	0.08 ± 0.00	0.07 ± 0.01	0.08 ± 0.00	0.08 ± 0.01	0.08 ± 0.00	0.10 ± 0.00	0.11 ± 0.00
C ₂₄	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
∑SFAs	15.11	13.75	14.54	14.92	13.98	15.39	14.87
∑PUFAs	5.91	6.30	6.15	7.58	6.96	8.89	7.75
∑MUFAs	77.25	79.54	78.71	76.90	78.47	74.76	76.35
∑transFA	0.06	0.05	0.05	0.05	0.07	0.11	0.11
C _{18:1} /C _{18:2}	14.48	14.02	13.93	10.90	12.25	9.06	10.73
MUFA/PUFAs	13.07	12.63	12.80	10.15	11.27	8.41	9.85

n.d. – not detected, ∑ – sum.

^a Mean and standard deviation for a confidence coefficient of 95%.

Table 4
Fatty acid composition (percent)^a of Cv. Madural virgin olive oils extracted from olives with different maturation indices

	MI 3	MI 4	MI 5	MI 6	MI 7
C ₁₄	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
C ₁₆	10.92 ± 0.02	10.26 ± 0.03	10.39 ± 0.02	9.98 ± 0.05	9.83 ± 0.02
C _{16:1^t}	0.07 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00
C _{16:1^c}	0.35 ± 0.00	0.31 ± 0.00	0.33 ± 0.00	0.32 ± 0.01	0.31 ± 0.00
C ₁₇	0.06 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00
C _{17:1^c}	0.07 ± 0.00	0.08 ± 0.00	0.07 ± 0.00	0.08 ± 0.00	0.07 ± 0.00
C ₁₈	2.33 ± 0.02	2.22 ± 0.01	2.27 ± 0.00	2.19 ± 0.00	2.22 ± 0.00
C _{18:1^t}	0.02 ± 0.01	0.01 ± 0.00	n.d.	0.01 ± 0.00	0.02 ± 0.00
C _{18:1^c}	71.77 ± 0.02	72.56 ± 0.05	71.23 ± 0.04	71.95 ± 0.03	71.55 ± 0.02
C _{18:2^{ct}}	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
C _{18:2^{cc}}	11.29 ± 0.02	11.43 ± 0.02	12.67 ± 0.00	12.27 ± 0.01	12.85 ± 0.01
C _{18:3^{ccc}}	0.89 ± 0.00	0.93 ± 0.00	0.99 ± 0.02	0.98 ± 0.02	0.94 ± 0.01
C ₁₉	n.d.	n.d.	n.d.	0.01 ± 0.00	0.01 ± 0.00
C ₂₀	0.33 ± 0.00	0.33 ± 0.00	0.32 ± 0.00	0.32 ± 0.00	0.31 ± 0.00
C _{20:1}	0.32 ± 0.00	0.33 ± 0.01	0.32 ± 0.00	0.34 ± 0.01	0.34 ± 0.00
C ₂₁	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
C ₂₂	0.09 ± 0.00	0.10 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.00
C ₂₄	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
∑SFAs	13.79	13.05	13.20	12.71	12.58
∑PUFAs	12.19	12.37	13.67	13.26	13.80
∑MUFAs	72.60	73.38	72.04	72.79	72.38
∑transFA	0.10	0.11	0.10	0.11	0.12
C _{18:1} /C _{18:2}	6.36	6.35	5.62	5.86	5.57
MUFA/PUFAs	5.96	5.93	5.27	5.49	5.24

n.d. – not detected, ∑ – sum.

^a Mean and standard deviation for a confidence coefficient of 95%.

analysis. The selection of C₁₈, C_{18:1} and C_{18:2} was justified by their significant differences ($p < 0.01$) and by their abundance in the oils. By the observation of eigenvalues we con-

clude that two factors were sufficient to justify 99.58% of the total variance (Factor 1: 68.16%; Factor 2: 31.42%). With the factors coordinates of the variables, based on

Table 5
Fatty acid composition (percent)^a of Cv. Verdeal Transmontana virgin olive oils extracted from olives with different maturation indices

	MI 1	MI 2	MI 3	MI 4	MI 5	MI 6
C ₁₄	0.01 ± 0.00	0.01 ± 0.00	n.d. ^a	0.01 ± 0.00	n.d.	n.d.
C ₁₆	10.18 ± 0.01	9.73 ± 0.02	9.54 ± 0.01	9.56 ± 0.01	9.55 ± 0.00	9.81 ± 0.00
C _{16:1t}	0.08 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.09 ± 0.00	0.08 ± 0.00	0.07 ± 0.00
C _{16:1c}	0.43 ± 0.00	0.39 ± 0.00	0.41 ± 0.00	0.38 ± 0.00	0.43 ± 0.00	0.39 ± 0.01
C ₁₇	0.26 ± 0.00	0.21 ± 0.00	0.24 ± 0.00	0.21 ± 0.00	0.19 ± 0.00	0.21 ± 0.00
C _{17:1c}	0.39 ± 0.00	0.35 ± 0.00	0.37 ± 0.00	0.34 ± 0.00	0.31 ± 0.00	0.34 ± 0.00
C ₁₈	2.94 ± 0.01	2.74 ± 0.01	2.97 ± 0.01	2.75 ± 0.01	2.69 ± 0.01	2.78 ± 0.00
C _{18:1t}	0.01 ± 0.00	0.01 ± 0.00	n.d.	0.01 ± 0.00	0.01 ± 0.00	n.d.
C _{18:1c}	79.74 ± 0.00	80.85 ± 0.00	81.05 ± 0.00	80.90 ± 0.00	81.56 ± 0.00	81.09 ± 0.00
C _{18:2ct}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C _{18:2cc}	3.15 ± 0.08	2.89 ± 0.02	2.89 ± 0.01	2.89 ± 0.04	2.54 ± 0.01	2.93 ± 0.00
C _{18:3ccc}	0.72 ± 0.03	0.65 ± 0.01	0.63 ± 0.01	0.65 ± 0.02	0.58 ± 0.01	0.61 ± 0.01
C ₁₉	0.02 ± 0.00	0.02 ± 0.00	n.d.	0.01 ± 0.00	n.d.	n.d.
C ₂₀	0.49 ± 0.01	0.46 ± 0.00	0.44 ± 0.01	0.47 ± 0.00	0.44 ± 0.02	0.47 ± 0.08
C _{20:1}	0.30 ± 0.00	0.31 ± 0.00	0.27 ± 0.02	0.31 ± 0.00	0.29 ± 0.00	0.27 ± 0.00
C ₂₁	0.03 ± 0.00	0.02 ± 0.00	n.d.	0.02 ± 0.00	0.03 ± 0.00	n.d.
C ₂₂	0.15 ± 0.00	0.13 ± 0.00	0.12 ± 0.01	0.14 ± 0.00	0.13 ± 0.01	0.12 ± 0.01
C ₂₄	0.07 ± 0.01	0.07 ± 0.00	0.05 ± 0.00	0.07 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
∑SFAs	14.15	13.39	13.36	13.24	13.08	13.44
∑PUFAs	3.87	3.54	3.52	3.54	3.12	3.54
∑MUFAs	80.95	81.99	82.18	82.03	82.68	82.16
∑transFA	0.09	0.09	0.08	0.10	0.07	0.07
C _{18:1} /C _{18:2}	25.31	27.98	28.04	27.99	32.11	27.68
MUFA/PUFAs	20.92	23.16	23.35	23.17	26.50	23.21

n.d. – not detected, ∑ – sum.

^a Mean and standard deviation for a confidence coefficient of 95%.

correlations, a projection on the factor-plane was made, leading us to understand that the cultivars near those specific locations were strongly influenced by those variables. For example, Cv. Cobrançosa is the richer in C₁₈ while Cv. Madural presented the lowest C_{18:1} content. Actually Cv. Verdeal Transmontana was the richer in C_{18:1} and presented the highest values of stability (measured by the Rancimat method) against the lower ones presented by Cv. Madural (data not shown). The now obtained results are in good accordance with those obtained previously, confirming the fact that the three cultivars are perfectly discrim-

inated by its fatty acid composition. However, the results obtained with samples in different ripening stages allow further conclusions. As can be observed (Fig. 3) the Cvs. Verdeal Transmontana and Madural, appear as a very homogeneous group where the MI has almost no influence, while cv. Cobrançosa presents an evident dispersion of values. In this last cultivar, with the exception of sample with MI 1, there is an increase of stearic acid as ripening proceeds.

Global PCA and cluster analysis were performed with all the chemical parameters considered statistical relevant in

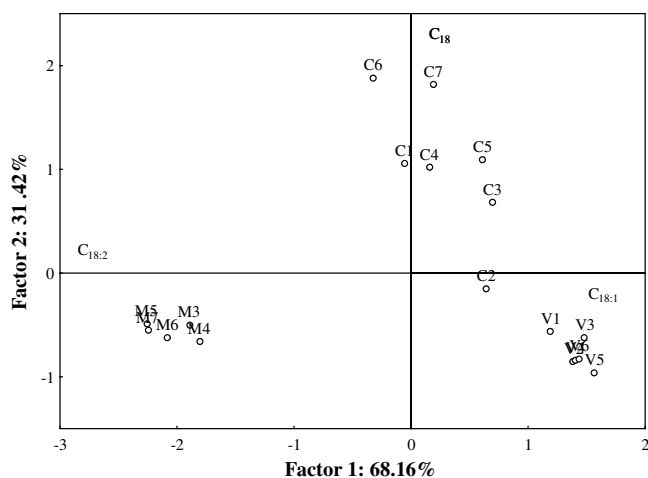


Fig. 3. Principal components analysis based on fatty acid profiles of Cvs. Cobrançosa, Madural and Verdeal Transmontana virgin olive oils extracted from olives with different maturation indices.

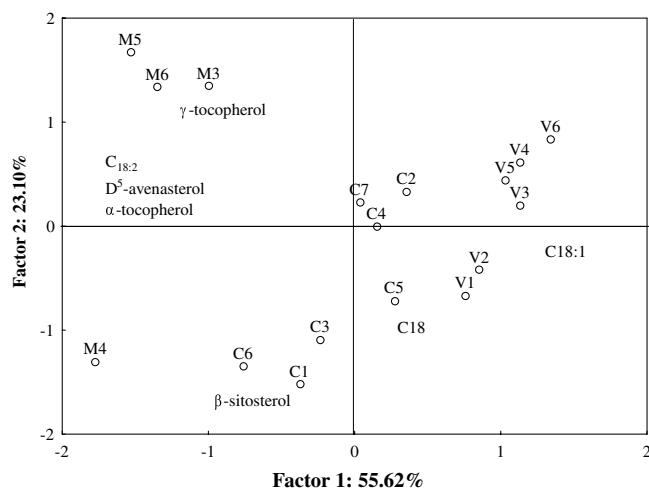


Fig. 4. Principal components analysis based on all relevant chemical parameters of Cvs. Cobrançosa, Madural and Verdeal Transmontana virgin olive oils extracted from olives with different maturation indices.

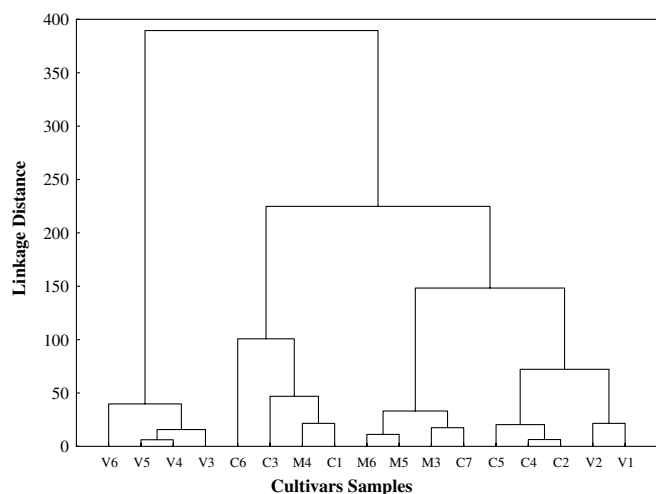


Fig. 5. Cluster analysis based on all relevant chemical parameters of Cvs. Cobrançosa, Madural and Verdeal Transmontana virgin olive oils extracted from olives with different maturation indices.

order to observe similarities between the samples and also to compare both methods. As can be seen in Fig. 4, the discrimination of cultivars was accomplished, although some elements were distant from the formed groups. In cluster analysis (Fig. 5) we also observed the formation of groups based on the similarities between the samples. In this case, the formation of clusters and its linkage could be compared and related with the groups formed in PCA. For example, the cluster composed by Cvs. V6, V5, V4, V3, could be related with the group plotted in Fig. 4, with samples Cvs. V5 and V4 being very similar in what concerns the chemical parameters evaluated and used in these analysis.

Table 6 summarizes the discriminate variable factors of principal components based in the parameters evaluated.

Table 6
Discriminate variables factors of principal components analysis based on fatty acids, sterol and tocopherols compositions

Variable	Factor 1	Factor 2
C ₁₆	0.538	-0.459
C _{16:1c}	-0.616	-0.578
C ₁₇	-0.961	-0.189
C _{17:1c}	-0.958	-0.259
C ₁₈	-0.447	0.747
C _{18:1c}	-0.908	-0.131
C _{18:2cc}	0.96	0.129
C _{18:3ccc}	0.874	-0.324
C ₂₀	-0.957	-0.148
C _{20:1c}	0.348	-0.877
Cholesterol	0.389	-0.580
Campesterol	0.773	-0.550
Stigmasterol	0.630	0.534
Clerosterol	0.961	-0.092
β-Sitosterol	0.924	-0.195
Δ ⁵ -Avenasterol	0.803	0.316
Δ ⁷ -Avenasterol	0.502	0.656
α-Tocopherol	-0.739	0.673
β-Tocopherol	-0.945	-0.209
γ-Tocopherol	-0.916	-0.327

Factors entry (relevant entries |Factor| > 0.70).

In conclusion, it can be said that the olive oils subject of this study show good nutritional characteristics in what the analyzed parameters are concerned. Besides, it was possible to conclude that the results obtained for such parameters allow the differentiation of the cultivars. The variables chosen to differentiate the cultivars also permitted, in some cases, to appreciate the influence of the maturation index. Probably each varietal olive oil characteristics contribute to the unique characteristics of Trás-Os-Montes olive oils, a PDO Portuguese product.

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