Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 102 (2007) 976–983
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Evaluation of a numerical method to predict the polyphenols content in monovarietal olive oils

Luis Carlos Matos [a,b], José A. Pereira [c], Paula B. Andrade [b], Rosa M. Seabra [b], M. Beatriz P.P. Oliveira [a,*]

[a] *REQUIMTE, Serviço de Bromatologia, Faculdade de Farmácia, Universidade do Porto, R. Aníbal Cunha, 164, 4099-030 Porto, Portugal*
[b] *Serviço de Farmacognosia, Faculdade de Farmácia, Universidade do Porto, R. Aníbal Cunha, 164, 4099-030 Porto, Portugal*
[c] *Escola Superior Agrária, Instituto Politécnico de Bragança, Quinta de Sta. Apolónia, Apartado 134, 5301-857 Bragança, Portugal*

Received 13 April 2005; received in revised form 18 April 2006; accepted 23 April 2006

Abstract

Three monovarietal virgin olive oils obtained from olives grown under biological agricultural system, in Portugal, with different ripening stages, were studied in what concerns the parameters usually related with oxidation status (total polyphenols, tocopherols, chlorophylls and carotenoids, peroxide values, oxidative stability, specific extinction coefficients K_{232} and K_{270} and titratable acidity). A total of 18 samples were analyzed: seven from *Cv.* Cobrançosa (maturation indices 1–7), five from *Cv.* Madural (maturation indices 3–7) and six from *Cv.* Verdeal Transmontana (maturation indices 1–6). Oxidative stability and polyphenols profile presented high correlation coefficients. Given this high correlation, a numerical method was developed and evaluated to predict total polyphenols contents in olive oil. The method is based on the kinetic equation of the oxidation process in the presence of antioxidants and on Rancimat profiles. Total polyphenols contents obtained with this method were similar to those obtained by the Folin-Ciocalteau method.

Keywords: Virgin olive oil; Ripening; Oxidation kinetics; Oxidative stability; Polyphenols

1. Introduction

Olive oil, the most characteristic fat used in the Mediterranean region, is obtained from the fruit of several cultivars of olive tree (*Olea europaea* L.). Each one of these cultivars exhibits specific physical and biochemical characteristics, providing oils with different compositions and performances (Aguilera et al., 2005; Gouveia, 1997). The fine composition of olive oil, and therefore its sensorial characteristics, besides being strongly dependent on the nature of the cultivar used for its production, is also influenced by several other factors like climatic and edaphic conditions, agricultural practices, etc. It is the recognition of the influence of these factors that leads the researchers to study the oil obtained from the same cultivar along several year crops and cultivated in different geographical origins (Aguilera et al., 2005; Gouveia, 1997; Salvador, Aranda, & Fregapane, 2001a).

Another factor decisive for the characteristics of olive oils is the degree of ripeness of the olives used in its production (Beltrán, Aguilera, Del Rio, Sanchez, & Martinez, 2005; Gutiérrez, Jímenez, Ruíz, & Albi, 1999; Koutsaftakis, Kotsifaki, Stefanoudaki, & Cert, 2000; Salvador et al., 2001a). In fact, as maturation progress, several metabolic processes take place with subsequent variations in the profiles of some compounds such as triglycerides, fatty acids, polyphenols, tocopherols, chlorophylls and carotenoids. These chemical changes are reflected on the sensorial characteristics, especially the flavor, oxidative stability and/or nutritional value of the final product and, obviously, on its quality grade.

Oxidative stability, although not considered a standard parameter of quality, is useful to provide information about

* Corresponding author. Tel.: +351 22 207 8927; fax: +351 22 200 3977.
E-mail address: beatoliv@ff.up.pt (M. Beatriz P.P. Oliveira).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.04.026

the hypothetical oil's shelf life. It is usually evaluated by the induction time, the time period until a critical point of oxidation is reached, and is developed a sensorial degradation of the oil as a consequence of a sudden acceleration of the oxidative process. The oxidative stability, usually evaluated by the Rancimat method, reveals the resistance of the product to initiate the oxidation process characterized by free radical reactions (Aparicio, Roda, Albi, & Gutiérrez, 1999).

The resistance to oxidative deterioration is usually attributed to two main reasons: (i) the fatty acid composition that, in the case of olive oil, is characterized by a high monounsaturated-to-polyunsaturated fatty acid ratio (Aparicio et al., 1999; Salvador, Aranda, & Fregapane, 1999) and (ii) the pool of minor compounds of powerful antioxidant activity which, in this case, is constituted mainly by tocopherols and polyphenols but also by chlorophylls and carotenoids. Antioxidants act scavenging free radicals and also chelating metal ions that initiate free radical reactions (Jadhav, Nimbalkar, Kulkarni, & Madhavi, 1995; Leonardis & Macciola, 2002). The mathematic treatment of the kinetic of oxidative reactions has already been established (Cheftel, 1977). According to one of these equations, if certain conditions are observed, there is a proportional relationship between the induction time and the amount of antioxidants.

One of the aims of this work was the behavior study of some of the more important parameters usually related with oxidative stability (total polyphenols, total tocopherols, total chlorophylls, total carotenoids, and also peroxide values, titratable acidity and specific extinction coefficients K_{232} and K_{270}), on three monovarietal virgin olive oils (*Cvs*. Cobrançosa, Madural and Verdeal Transmontana). These three cultivars account for more than 90% of olive area in Trás-os-Montes (Portugal) and, with *Cv*. Cordovil, are the only permitted cultivars for the production of "Trás-os-Montes Olive Oil" (Protected Designation of Origin). The few studies published on these cultivars had different goals (Pereira, Alves, Casal, & Oliveira, 2004; Pereira, Casal, Bento, & Oliveira, 2002). The trees used in this study belong to the same orchard and, consequently, were subjected to the same climatic conditions and agricultural practices. The study included oils obtained from olives collected during the crop year 2000/2001, with different maturation indices, in a total of 18 samples, so that the only variables are the cultivar and the maturation degree. Another aim of the work was to evaluate the use of a numerical method, based on the kinetic of oxidation reactions, to predict the total polyphenols content, the chemical parameter more usually related to oxidative stability.

2. Materials and methods

2.1. Sampling

The cultivars of *Olea europaea* L. under study were *Cvs*. Cobrançosa, Madural and Verdeal Transmontana. The olives were all collected from the same orchard, 10 km North from Mirandela, in the Northeast of Portugal (U.T.M. 29 PG5602). This olive grove is kept under Biological Agricultural system and had no crop health control treatment in the last 10 years. The trees corresponding to each cultivar were identified, carefully marked and five trees of each cultivar have been sampled. The olives were harvested by handpicking 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. The harvesting process was carried out in different days to obtain olives in different maturation indices (MI). The lowest MIs were obtained in first day of collection and the highest in the last one.

From each tree, only healthy fruits were picked. After harvest, olive fruits were immediately transported to the laboratory carefully blended and, in a 100 olives randomly taken from each cultivar, the maturation indices (MI) were determined. This parameter is a function of fruit color 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). The picked olives were grouped according to their MI and obtained a total of 18 samples: seven for *Cv*. Cobrançosa (MI from 1 to 7), five for *Cv*. Madural (MI from 3 to 7) and six for *Cv*. Verdeal Transmontana (MI from 1 to 6). From each group, a 1 kg sample was randomly taken and submitted separately 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 unit. 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 sulphate and subsequently filtered through filter paper.

2.3. Oil analysis

All parameters were determined in triplicate.

Acidity and specific extinction coefficients K_{232} and K_{270} were carried out according to the European Official Method of Analysis (Annexes II and IX, EC1991 Regulations 2568/91). Acidity value, expressed as percent of oleic acid, was determined by titration of a solution of oil in ethanol/ether 1:1 (v/v) with an ethanolic solution of potassium hydroxide.

Color was determined following the analytical method described in the NP-937 (1997). This spectrophotometric method allows the determination of the tristimulus values and the chromaticity coordinates that specify the color as established by the International Commission on Illumination (CIE). Chromatic characteristics, transparency, domi-

nant wavelength and purity were determined by evaluation of the oil solution transmittances using cyclohexane as reference solution, at 445, 495, 560 and 625 nm in a Hitachi 150-20 Spectrophotometer. The transparency is related with the luminosity represented by the transmittance. This parameter varies inversely with the color intensity of the oil. The dominant wavelength is the main spectral radiation of the light emitted from the oil. The purity is related with the amount of radiation, at the dominant wavelength, emitted from the oil.

Chlorophylls and carotenoids contents were determined according to Minguez, Rejano, Gandul, Higino, and Garrido (1991). For that purpose, 7.5 g of oil were weighed exactly, dissolved in cyclohexane and taken to a final volume of 25 ml. Chlorophylls and carotenoids were determined at 670 nm and 472 nm, respectively, using a spectrophotometer (Hitachi 150-20). Results are given as mg/kg of oil.

Peroxide value (PV), given in milliequivalents of active oxygen per kilogram of oil (mEq/kg) was determined as follows: a mixture of oil and chloroform/acetic acid 2:3 (v/v) was left to react in darkness with a saturated potassium iodine solution; the free iodine released was titrated with a sodium thiosulphate solution (European Union Regulation 2568/91).

Oxidative stability (OS) was evaluated as previously described by Pereira et al. (2002), using a Rancimat apparatus, an air flow of 20 L/h, and oil samples (2.5 g) heated at 110 °C. Results were registered as induction time (h).

Polyphenols were determined according to the methodology proposed by Kiritsakis (1998). Olive oil samples were dissolved in *n*-hexane and extracted, three times, with a water/methanol mixture (60:40 v/v). Total polyphenols were determined by adding the Folin-Ciocalteau reagent and a 20% sodium carbonate solution to a suitable aliquot of the combined extracts. After centrifugation, the absorbance at 725 nm was measured. Results are given as mg/kg of caffeic acid.

Tocopherols were evaluated following the method described in Gama, Casal, Oliveira, and Ferreira (2000). Olive oil (0.1 g) 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 aluminum foil. The mixture was filtered (Schleicher & Shuell 0.2 μm; ø 13 mm, pure polyamide) and analyzed by HPLC. The chromatographic separation of the compounds was achieved with a normal-phase LiChrosorb SI 60 (5 μm; 25 × 0.4 cm) column from Merck (Darmstadt, Germany) using a mixture of *n*-hexane and 2-propanol (99.7:0.3) v/v as mobile phase. Elution was performed at a solvent flow rate of 1.7 mL/min. The effluent was monitored with a diode-array detector connected in series with a fluorescence detector programmed at the excitation and emission wavelengths of 290 nm and 330 nm, respectively. Data were analyzed in the Borwin PDA Controller Software (JMBS, France). The compounds were identified by comparing their retention time and UV spectra with authentic standards. Quantification was made by fluorescence detection based on the internal standard method.

2.4. Numerical method

The numerical method was developed using the kinetic equation of the oxidation process

$$\ln\frac{[\mathrm{ROOH}]_i}{[\mathrm{ROOH}]_0} = \frac{k}{[\mathrm{AH}]}t_\mathrm{i}$$

where t_i is the induction time determined on the Rancimat test and [AH] is the concentration of antioxidants present in the sample. To calculate the value of k (kinetic constant) for each sample, the curves of the Rancimat test were used on the following way:

- a straight line, parallel to the time axis and tangent to the curve at time zero, was drawn;
- the distances from this baseline and the Rancimat curve were measured at every hour (until time = t_i) and transposed to an Excel sheet;
- a graphic was plotted (distance *versus* time) and a linear adjustment was made;
- the slope of this straight line was taken as the kinetic constant on the above equation.

The value used for the ratio $[\mathrm{ROOH}]_0/[\mathrm{ROOH}]_i$ was the mean ($n = 18$) of the distances between the base line and the Rancimat curve at time = t_i, which, in this study, took the value 2 (see Section 3).

2.5. Statistical analysis

Results are shown as figures and tables using Microsoft Excel. Correlation analysis (Pearson's correlation) was performed with Statistica for Windows release 6.0.

3. Results and discussion

The values for acidity (%), peroxide value, K_{232} and K_{270} extinction coefficients, transparency (Y%), and purity (σ%) are displayed on Table 1.

Mean values obtained for titratable acidity were 0.33% for *Cv.* Cobrançosa, 0.37% for *Cv.* Madural and 0.36% for *Cv.* Verdeal Transmontana. When considered individually, almost all the samples showed values bellow 0.40% allowing their classification as "extra virgin" olive oils (<0.8%). In what concerns this parameter, the values obtained were similar with some slight fluctuations and no clear increasing or decreasing tendency could be noticed along the ripening.

Although it is usually admitted that, during ripening, there is a progressive activation of lipolytic enzymes (Gutiérrez et al., 1999) that originates oils with higher acidity as maturation proceeds, this is not always the case; in

Table 1
Values of acidity, peroxide value, K_{232} and K_{270} extinction coefficients, K_{232}/K_{270} ratio, transparency and purity of *Cvs.* Cobrançosa, Madural and Verdeal Transmontana virgin olive oils extracted from olives with different maturation indices

	Acidity (%)[a]	PV[a]	K_{232}[a]	K_{270}[a]	K_{232}/K_{270}	Transparency Y%	Purity σ%
Cv. Cobrançosa							
MI 1	0.33 ± 0.02	17 ± 0.0	2.12 ± 0.01	0.34 ± 0.01	6.2	62.7	79.3
MI 2	0.34 ± 0.01	19 ± 0.6	2.14 ± 0.01	0.33 ± 0.01	6.5	67.2	72.6
MI 3	0.36 ± 0.00	14 ± 0.6	2.03 ± 0.01	0.34 ± 0.01	6.0	65.3	81.7
MI 4	0.33 ± 0.02	18 ± 0.3	2.12 ± 0.01	0.32 ± 0.01	6.6	67.8	78.1
MI 5	0.32 ± 0.01	18 ± 0.5	2.10 ± 0.00	0.35 ± 0.00	6.0	71.0	74.3
MI 6	0.28 ± 0.00	15 ± 0.3	3.06 ± 0.01	0.37 ± 0.01	4.6	73.4	68.8
MI 7	0.33 ± 0.03	10 ± 0.3	2.18 ± 0.02	0.39 ± 0.00	5.6	82.9	51.0
Cv. Madural							
MI 3	0.34 ± 0.01	20 ± 0.4	2.61 ± 0.01	0.37 ± 0.01	7.1	71.7	69.1
MI 4	0.35 ± 0.00	24 ± 0.3	3.40 ± 0.01	0.47 ± 0.01	7.2	74.4	61.9
MI 5	0.34 ± 0.00	18 ± 0.0	1.95 ± 0.01	0.25 ± 0.01	7.8	77.7	49.0
MI 6	0.41 ± 0.02	12 ± 0.6	2.21 ± 0.02	0.31 ± 0.02	7.1	76.2	49.8
MI 7	0.42 ± 0.00	23 ± 0.5	2.08 ± 0.02	0.25 ± 0.02	8.3	72.8	62.8
Cv. Verdeal Transmontana							
MI 1	0.41 ± 0.01	14 ± 0.6	2.26 ± 0.01	0.31 ± 0.01	7.3	70.7	68.1
MI 2	0.34 ± 0.01	19 ± 0.5	2.24 ± 0.01	0.30 ± 0.01	7.5	68.0	75.2
MI 3	0.35 ± 0.01	19 ± 0.2	1.76 ± 0.01	0.20 ± 0.01	8.8	64.5	88.8
MI 4	0.34 ± 0.00	15 ± 0.2	1.80 ± 0.01	0.20 ± 0.01	9.0	65.8	83.6
MI 5	0.48 ± 0.01	15 ± 0.6	1.33 ± 0.00	0.13 ± 0.05	10.2	73.0	77.9
MI 6	0.27 ± 0.01	17 ± 0.3	1.66 ± 0.01	0.21 ± 0.01	7.9	75.5	64.2

[a] Mean and standard deviation.

fact some cultivars have shown a more or less pronounced increase in acidity (Garcia, Seller, & Pérez-Camino, 1996; Gutiérrez et al., 1999; Koutsaftakis et al., 2000; Rotondi et al., 2004; Salvador et al., 2001a) but others have revealed a relative stability (Garcia et al., 1996; Skevin et al., 2003) or even a decrease of free fatty acids (Garcia et al., 1996).

The values of K_{270} and K_{232} extinction coefficients showed a slight decrease in *Cvs.* Madural and Verdeal Transmontana olive oils but remained practically constant in *Cv.* Cobrançosa (Table 1). Almost all the values obtained for K_{232} were within the limits established by Reg. 1989/03 (2.50) but the values for K_{270} usually exceeded the limits established in the referred Regulation (0.20). Irregular behavior in what concerns these parameters have also been found and, according to Garcia et al. (1996), they probably arise as a result of different metabolic responses of the fatty acid oxidation pathway on different cultivars.

Besides their participation in redox reactions, chlorophyllic and carotenoid pigments are responsible for the oil color, which is considered a quality parameter and an influent factor in consumer's preference. As already stated by several authors (Beltrán et al., 2005; Gutiérrez et al., 1999; Roca & Mínguez-Mosquera, 2001), the contents of these pigments are strongly affected by the ripening stage which always leads to a decrease. According to Roca and Mínguez-Mosquera (2001) the characteristic of each cultivar is the amount of pigments and the rates of their disappearance, which implies that the catabolism of these pigments takes place at rates inherent to each variety.

In what concerns these two parameters, the three cultivars under study presented parallel behavior: they all

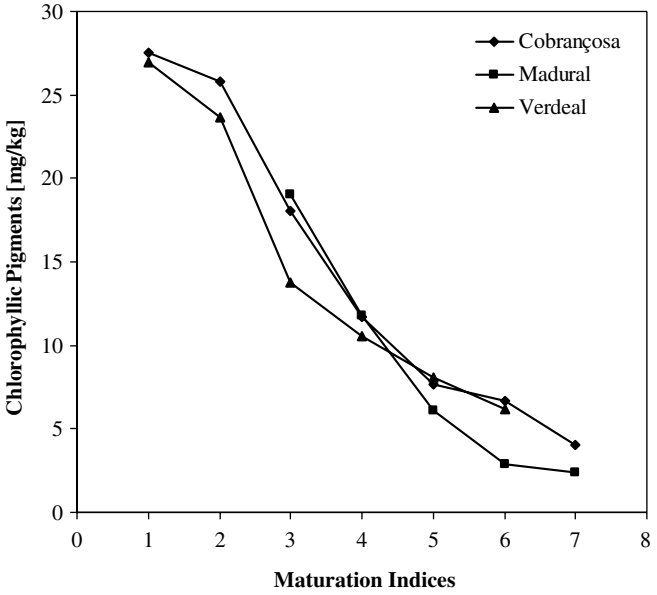

Fig. 1. Changes in chlorophyllic pigments of *Cvs.* Cobrançosa, Madural and Verdeal Transmontana virgin olive oils extracted from olives with different maturation indices.

presented similar amounts of pigments and their decrease, as can be observed in Figs. 1 and 2, follow the same pattern. In all cases there is a continuous decreasing in the amounts of pigments but a more pronounced decay is noticed between MI 2 and MI 4. The referred decreasing tendency in pigments was clearly confirmed by the visual analysis of oils and by the values related with the color evaluation (Table 1): the oils showed a deep green color in the initial ripening stages and a golden yellow in the final

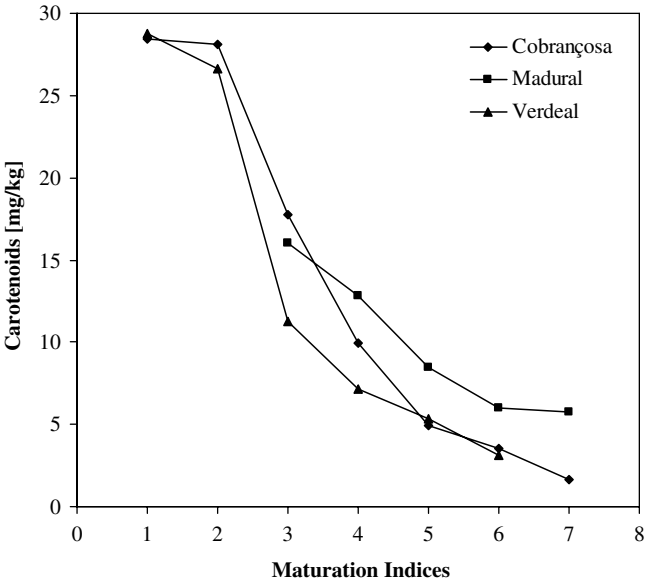

Fig. 2. Changes in carotenoids contents of *Cvs.* Cobrançosa, Madural and Verdeal Transmontana virgin olive oils extracted from olives with different maturation indices.

ones. Simultaneously it was verified an increase on the transparency values (*Y*%) with special emphasis for *Cv.* Cobrançosa (from 62.7 to 82.9).

Contrarily to the pigments behavior, the evolution of peroxide values along the maturation presented an irregular performance (Table 1). The three cultivars showed different behaviors among them, with fluctuations along the ripening and, in neither of them, a clear decreasing tendency was observed as reported by Gutiérrez et al. (1999) and by works cited by them. However, this decreasing tendency in peroxide values is not a rule (Rotondi et al., 2004) and performances similar to the one found for cultivars under these study were also described for *Cv.* Cornicabra by Salvador et al. (2001a). A particular behavior was already observed in these same cultivars, when olives from another crop year were subjected to different storage periods (Pereira et al., 2002).

Polyphenols and tocopherols are recognized as antioxidant compounds and their presence in olive oils has been related to their general quality, improving stability, nutritional value and sensorial properties (Kiritsakis, 1998).

Mean values for total polyphenols content of the cultivars Cobrançosa, Madural and Verdeal Transmontana were 203 mg/kg, 124 mg/kg and 192 mg/kg, respectively, while the mean values for total tocopherols were 255 mg/kg, 220 mg/kg and 156 mg/kg, respectively. Total tocopherols content express the sum of partial contributions of the three homologous α, β and γ. These contributions were, in mean values, 96.3%, 1.0% and 3.3% for α, β and γ-tocopherols, respectively. Although tocopherols contents are widely distinct according to the variety, ripening stage, climate and agronomic factors, the ones found in this particular work were in accordance with previous work related with this kind of cultivars. Just as with peroxide values, the amounts of these bioactive components varied in an irregular way along the ripening (Fig. 3). Although a decreasing tendency was verified (more easily noticed if linear adjustments were made), the sinusoidal behavior exhibited by the three cultivars under study is more similar to

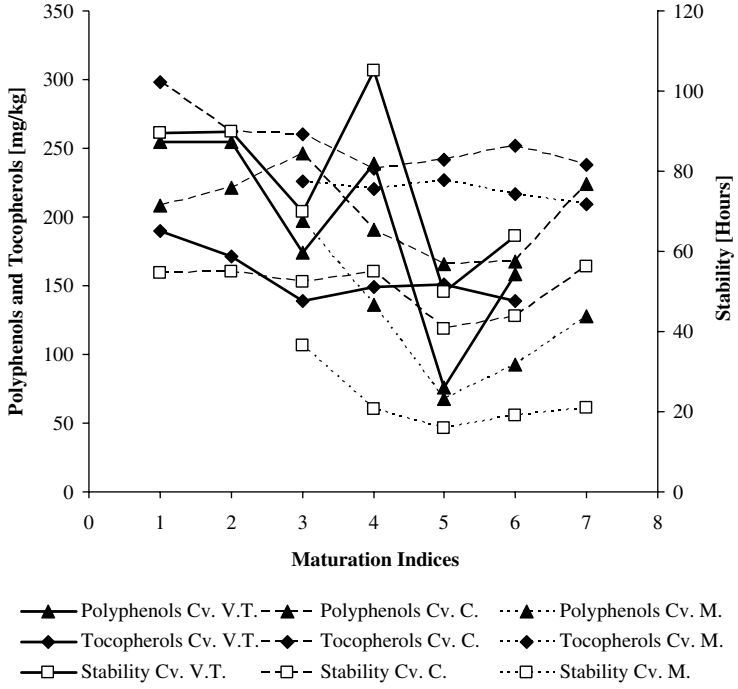

Fig. 3. Changes in oxidative stability, polyphenols and tocopherols contents of *Cvs.* Cobrançosa, Madural and Verdeal Transmontana virgin olive oils extracted from olives with different maturation indices.

that described by Beltrán et al. (2005) than that described by Gutiérrez et al. (1999). Besides, the slope of the descending curves of these chemicals are not similar in the three cultivars: while in *Cv.* Cobrançosa polyphenols and tocopherols presented a weak and parallel decrease, in *Cvs.* Madural and Verdeal Transmontana the total polyphenols content presented a more pronounced decrease than total tocopherols. The less strong effect of ripeness on tocopherols content has already been noticed for other cultivars (Garcia et al., 1996; Gutiérrez et al., 1999).

Using the described methodology, the mean oxidative stability for *Cvs.* Cobrançosa, Madural and Verdeal Transmontana olive oils were 51, 23 and 78 h, respectively. Just as with the values of tocopherols and polyphenols, a decreasing tendency was noticed on the individual values of oxidative stability as ripening occurred in all the cultivars (Fig. 3). This decreasing tendency of stability seems also to be a characteristic of olive oils (Beltrán et al., 2005) and is usually explained by the decrease of natural antioxidants like polyphenols, tocopherols, chlorophylls and carotenoids.

Given this scenery, several researchers have tried to find out which of the mentioned constituents are more correlated with the oil stability. The relationship between stability and chemical constituents is usually appreciated by statistical methods (Pearson's correlation) although other methods have also been used (Mateos, Dominguez, Espartero, & Cert, 2003). Although the nature of the fatty acids present in olives is one of the responsible for the high stability of olive oil (Aparicio et al., 1999), the polyphenols are, among the minor constituents, the group to which the stability is more correlated (Aparicio et al., 1999; Salvador et al., 1999; Salvador, Aranda, Gómez-Alonso, & Fregapane, 2001b; Tsimidou, Papadopoulus, & Boskow, 1992). A high correlation between polyphenols content and oxidative stability was also verified with the three cultivars under study, as can be stated in Fig. 3 and Table 2. Pearson's correlation analysis provided high correlation coefficients between oxidative stability and polyphenols contents ($p = 0.051$, 0.019 and 0.009 for *Cvs.* Cobrançosa, Madural and Verdeal Transmontana, respectively) but no significant correlation between oxidative stability and tocopherol amounts ($p = 0.647$, 0.648 and 0.370) for *Cvs.* Cobrançosa, Madural and Verdeal Transmontana, respectively. Significance (p value) was established in 0.05 and, in the case of *Cv.* Verdeal Transmontana, it was smaller than 0.01 showing that oxidative stability and polyphenols contents were strictly related.

Table 2
Correlation between polyphenols and tocopherols contents and oxidative stability of *Cvs.* Cobrançosa, Madural and Verdeal Transmontana virgin olive oils extracted from olives with different maturation indices [display n, r(X:Y) and p]

Variable	Stability		
	Cv. Cobrançosa	*Cv.* Madural	*Cv.* Verdeal T.
Polyphenols content	$r = 0.7531$	$r = 0.9375$	$r = 0.9213$
	$p = 0.051$	$p = 0.019$	$p = 0.009$
Tocopherols content	$r = 0.2126$	$r = 0.2805$	$r = 0.4508$
	$p = 0.647$	$p = 0.648$	$p = 0.370$

According to Cheftel (1977), if certain conditions are presumed [(i) almost all the finalization reactions are due to the interaction with the antioxidant, (ii) the oxygen content is not a restrictive factor, (iii) the initiation and finalization velocities are the same, and (iv) supposing that [RH] is constant, which means a low level of oxidation] the oxidation is ruled by the following equation:

$$\ln \frac{[\mathrm{ROOH}]_i}{[\mathrm{ROOH}]_0} = \frac{k}{[\mathrm{AH}]} t_\mathrm{i}$$

where $[\mathrm{ROOH}]_0$ is the content of hydroperoxides at time zero, $[\mathrm{ROOH}]_i$ is the content of hydroperoxides at the beginning of rancidity, t_i is the induction time and [AH] is the content of antioxidants (admitting that the antioxidants destroyed during the induction time was negligible, so antioxidants concentration remained constant). Using this equation and the curves registered in the Rancimat test, the amount of antioxidants in each sample was calculated (using the methodology described).

Given the high correlation found, by the statistical method, between oxidative stability and polyphenols content, the results obtained for the concentration of antioxidants [AH] by this numerical method were displayed in Table 3 and compared with those obtained for polyphenols (colorimetric method). As can be observed, the values were

Table 3
Polyphenols contents of *Cvs.* Cobrançosa, Madural and Verdeal Transmontana virgin olive oils extracted from olives with different maturation indices

	Polyphenol content (mg/kg)		
	Colorimetric method mean ± SD	Numerical method mean ± SD	Error (%)
Cobrançosa			
MI 1	209 ± 0	218 ± 2	4
MI 2	221 ± 3	213 ± 33	4
MI 3	246 ± 1	255 ± 7	3
MI 4	190 ± 1	221 ± 16	16
MI 5	166 ± 0	222 ± 18	34
MI 6	168 ± 0	177 ± 9	5
MI 7	224 ± 1	204 ± 5	9
Madural			
MI 3	198 ± 0	234 ± 24	18
MI 4	136 ± 1	165 ± 2	22
MI 5	67 ± 1	80 ± 0	19
MI 6	93 ± 2	106 ± 10	14
MI 7	128 ± 1	139 ± 3	9
Verdeal Transmontana			
MI 1	255 ± 7	269 ± 14	5
MI 2	255 ± 1	263 ± 9	3
MI 3	175 ± 1	180 ± 21	3
MI 4	239 ± 5	223 ± 15	7
MI 5	76 ± 2	103 ± 18	36
MI 6	158 ± 1	165 ± 10	4

Results obtained by the colorimetric and numerical methods.

similar and the error found was, in most cases, less than 10% (mean values of 11% for *Cv.* Cobrançosa, 16% for *Cv.* Madural and 10% for *Cv.* Verdeal Transmontana). Nominal high error values can be due to the printing system coupled with the Rancimat apparatus and the measuring methodology used. These factors add, in some cases, an inevitable error to the main data decreasing the slope. However the final values presented a similar magnitude and profile compared to the colorimetric method. Therefore this can be considered as another approach to evaluate the role of polyphenols on oxidative stability of olive oil.

Since the Rancimat test is obligatory in any quality control of olive oil, it seems possible, by the numerical method here proposed, to use the data obtained in this test to predict, with an assumed error, the polyphenols content of olive oil samples. The error can be minimized using a data acquisition system coupled to the Rancimat apparatus, providing directly the files to an Excel sheet, making the analysis easier and more precise (the procedure used in this work was based on the measurement, on paper, of each point, which added an error to the final values).

In what concerns the quality parameters determined under this study, it is possible to say in short that although it is noticed that, in general, ripening entails a decreasing in quality, the cultivars Cobrançosa, Madural and Verdeal Transmontana, grown in Trás-os-Montes, showed a particular behavior in some of the parameters evaluated. Some of the values registered exceed those demanded by the European legislation for Virgin Olive Oil but, as Aparicio et al. (1999) observed, it is difficult to obtain monovarietal olive oil samples covering all the requirements. The *Cv.* Verdeal Transmontana revealed an excellent shelf life having in mind the drastic conditions of the Rancimat test (110 °C). Best maturation indices for collecting the olives were MI 4 for *Cv.* Cobrançosa, MI 5 for *Cv.* Madural and MI 3-4 for *Cv.* Verdeal Transmontana.

In conclusion, the proposed method is cheap, non-pollutant and requiring no reagent consumption or extra special equipment being a suitable approach to implement in quality control of olive oils.

References

Aguilera, M. P., Beltrán, G., Ortega, D., Fernández, A., Jiménez, A., & Uceda, M. (2005). Characterization of virgin olive oil of Italian olive cultivars "Frantoio" and "Leccino" grown in Andalusia. *Food Chemistry, 89*, 387–391.

Aparicio, R., Roda, L., Albi, M. A., & Gutiérrez, F. (1999). Effect of various compounds on virgin olive oil stability measured by rancimat. *Journal of Agricultural and Food Chemistry, 47*, 4150–4155.

Beltrán, G., Aguilera, M. P., Del Rio, C., Sanchez, S., & Martinez, L. (2005). Influence of fruit ripening process on the natural antioxidant content of Hojiblanca virgin olive oils. *Food Chemistry, 89*, 207–215.

Cheftel, J. C. (1977). *Introduction a la biochimie et a la technologie des aliments* (vol. 2). Paris, France: Technique et Documentation, pp. 278–280.

European Union Commission. (1991). Regulation EEC 2568/91 on the characteristics of olive oil and olive pomace and their analytical methods. *Official Journal of European Communities* L248.

Gama, P., Casal, S., Oliveira, B., & Ferreira, M. (2000). Development of an HPLC/diodoarray/fluorimetric detector method for monitoring tocopherols and tocotrienols in edible oils. *Journal of Liquid Chromatography & Related Technologies, 23*, 3011–3022.

Garcia, J. M., Seller, S., & Pérez-Camino, M. C. (1996). Influence of fruit ripening on olive oil quality. *Journal of Agriculture and Food Chemistry, 44*, 3516–3520.

Gouveia, J. M. B. (1997). Comparación de los aceites de oliva de los cultivares Cobrançosa, Blanqueta, Azeiteira y Picual con los del cv. Galega vulgar, producidos en el norte del Alentejo. Principales características químicas y sensoriales. *OLIVAE, 66*, 34–45.

Gutiérrez, F., Jímenez, B., Ruíz, A., & Albi, M. A. (1999). Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties Picual and Hojiblanca and on the different components involved. *Journal of Agricultural and Food Chemistry, 47*, 121–127.

Hermoso, M., Uceda, M., Garcia, A., Morales, B., Frias, M.L. & Fernández, A. (1991). Elaboracion de aceite de calidad. Sevilla, Spain: Consejeria de Agricultura y Pesca, Serie Apuntes 5/92.

Jadhav, S. J., Nimbalkar, S. S., Kulkarni, A. D., & Madhavi, D. L. (1995). Lipid oxidation in biological and food systems. In D. L. Madhavi, S. S. Deshpande, & D. K. Salunkhe (Eds.), *Food antioxidants, technological, toxicological and health perspectives* (pp. 5–63). New York, USA: Marcel Dekker, Inc..

Kiritsakis, A. K. (1998). *Olive oil from the tree to the table*. Trumbull, Connecticut, USA: Food & Nutrition Press, Inc., p. 269.

Koutsaftakis, A., Kotsifaki, F., Stefanoudaki, E., & Cert, A. (2000). Estudio sobre las variaciones de determinados parámetros químicos y de componentes menores de los aceites de oliva virgen obtenidos de aceitunas recolectadas en distintas fases de maduración. *OLIVAE, 80*, 22–27.

Leonardis, A., & Macciola, V. (2002). Catalytic effect of the Cu(II)- and Fe(III)-cyclohexanebutyrates on olive oil oxidation measured by Rancimat. *European Journal of Lipid Science and Technology, 104*, 156–160.

Mateos, R., Dominguez, M. M., Espartero, J. L., & Cert, A. (2003). Antioxidant effect of phenolic compounds, alpha-tocopherol and other minor components in virgin olive oil. *Journal of Agricultural and Food Chemistry, 51*, 7170–7175.

Minguez, M. I., Rejano, L., Gandul, B., Higino, A., & Garrido, J. (1991). Color pigment correlation in virgin olive oil. *Journal of the American Oil Chemistry Society, 68*, 332–336.

NP (Portuguese Rule) 937 (1997). Edible fats and oils. Determination of oils color and its characteristics.

Pereira, J. A., Alves, M. R., Casal, S., & Oliveira, M. B. P. P. (2004). Effect of olive fruit fly infestation on the quality of olive oil from cultivars Cobrançosa, Madural and Verdeal Transmontana. *Italian Journal of Food Science, 16*, 355–365.

Pereira, J. A., Casal, S., Bento, A., & Oliveira, M. B. P. P. (2002). Influence of olive storage period on quality of three portuguese cultivars of *Olea europea*, Cobrançosa, Madural and Verdeal Transmontana. *Journal of Agriculture and Food Chemistry, 50*, 6335–6340.

Roca, M., & Mínguez-Mosquera, M. I. (2001). Changes in chloroplast pigments of olive varieties during fruit ripening. *Journal of Agricultural and Food Chemistry, 49*, 832–839.

Rotondi, A., Bendini, A., Cerretani, L., Mari, M., Lercker, G., & Toschi, T. G. (2004). Effect of olive ripening degree on the oxidative stability and organoleptic properties of cv. Nostrana di Brisighella extra virgin olive oil. *Journal Agricultural Food Chemistry, 52*, 3649–3654.

Salvador, M. D., Aranda, F., & Fregapane, G. (1999). Contribution of chemical components of Cornicabra virgin oils to oxidative stability. A study of three successive crop seasons. *JAOCS, 76*, 427–432.

Salvador, M. D., Aranda, F., & Fregapane, G. (2001a). Influence of fruit ripening on "Cornicabra" virgin olive oil quality. A study of four successive crop seasons. *Food Chemistry, 73*, 45–53.

Salvador, M. D., Aranda, F., Gómez-Alonso, S., & Fregapane, G. (2001b). Cornicabra virgin oil: a study of five crop seasons. Composition, quality and oxidative stability. *Food Chemistry, 74*, 267–274.

Skevin, D., Rade, D., Strucelj, D., Mokrovcak, Z., Nederal, S., & Bencic, D. (2003). The influence of variety and harvest time on the bitterness and phenolic compounds of olive oil. *European Journal of Lipid Science and Technology, 105*, 536–541.

Tsimidou, M. G., Papadopoulus & Boskow, D. (1992). Phenolic compounds and stability of virgin olive oil. Part I. *Food Chemistry, 45*, 141–144.