

# EFFECT OF OLIVE FRUIT FLY INFESTATION ON THE QUALITY OF OLIVE OIL FROM CULTIVARS COBRANÇOSA, MADURAL AND VERDEAL TRANSMONTANA

EFFETTO DELL'ATTACCO DI MOSCA OLEARIA SULLA QUALITÀ DI OLIO DA OLIVE DELLE CULTIVARS COBRANÇOSA, MADURAL E VERDEAL TRANSMONTANA

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## ABSTRACT

Olives (*Olea europaea* L.) from cultivars Cobrançosa, Madural and Verdeal Transmontana were collected separately and divided into two different groups according to the presence or absence of infestation by the olive fruit fly (*Bactrocera oleae* Gmel.). These two groups were then mixed in varying percentages to create five groups of olives per cultivar with infestation levels: 0, 12.5, 25, 50 and 100%. Each group was then processed to produce olive oil. The

## RIASSUNTO

Le olive provenienti dalle cultivars Cobrançosa, Madural e Verdeal Transmontana (*Olea europaea* L.) erano raccolte separatamente ed erano poi divise in due gruppi in funzione della presenza o dell'assenza di infestazione da Mosca delle olive (*Bactrocera oleae* Gmel.). Questi due gruppi erano mescolati tra di loro in modo da creare cinque gruppi con diverso grado di infestazione: 0, 12.5, 25, 50 and 100%. I cinque gruppi erano poi sottoposti al processo

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results, regarding mainly acidity, peroxide value, and stability to oxidation, suggest that olive fruit fly infestation reduces the quality of the olive oil. The effects of infestation varied according to cultivar, but in general the total tocopherol content was always lower at the 100% infestation level. The oil from cultivar Verdeal Transmontana had the lowest tocopherol content compared to oil from cultivars Cobrançosa and Madural, which could explain the lower quality of its oil.

di estrazione per ottenere olio di oliva. I risultati delle analisi effettuate (acidità, indice di perossidi e stabilità all'ossidazione) confermavano che l'infestazione dal mosca riduce la qualità dell'olio. L'effetto dell'attacco variava in funzione della cultivar, ma, generalmente, il contenuto in tocoferoli totali era il più basso quando l'infestazione è al 100%. L'olio della cv. Verdeal Transmontana aveva il contenuto in tocoferoli rispetto alle cv. Cobrançosa and Madural, giustificandone così la minore qualità dell'olio.

## INTRODUCTION

Olive oil (*Olea europaeae* L.) is a premium vegetable oil due to its health and nutritional benefits and distinctive flavor (KIRITSAKIS, 1998). It is known that the characteristics and quality of olive oil can be influenced by the cultivars, the degree of ripeness and the industrial processes used to extract the oil, as well as the environmental conditions (mineral nutrition, temperature, light, water availability) and cultural practices (TOVAR *et al.*, 2001; KIRITSAKIS, 1998). Oil quality is also strongly related to the physiological conditions of the fruit from which it is extracted. The action of parasites prior to harvest, such as happens with the olive fruit fly, *Bactrocera oleae* Gmel., or fungal attack between harvesting and oil extraction, are the main external agents responsible for unwanted metabolic processes in olives that lead to subsequent reduction in oil quality (KIRITSAKIS, 1998).

The olive fruit fly is a major olive pest in the Mediterranean countries (CIVANTOS, 1998; CROVETTI, 1996; KATSOYANNOS, 1992). The fly lays eggs singly in the mesocarp of the olive fruit; the larvae develop in the pulp thus destroying it

and then pupate in the fruit or in the soil. If control measures, usually in the form of pesticide sprays, are not made promptly, up to 40% of the production can be lost (CROVETTI, 1996; KATSOYANNOS, 1992). The losses are due to: (i) premature drop of the infested fruit, (ii) direct pulp destruction caused by the larvae developing in the fruit, and (iii) general reduction in olive oil quality. The impact of this damage caused by olive fruit fly infestation may vary considerably, depending on whether the fruit is used for oil extraction or for table olive production.

It has been reported that oil obtained from olives infested with the olive fruit fly, which contain developed larvae or pupae and/or exit holes have higher acidity and peroxide values, specific extinction coefficients at 232 and 270 nm, and lower total polyphenol content (DELRIO *et al.*, 1995; PARLATI *et al.*, 1990a;b; 1992; ZUNIN *et al.*, 1992; 1993).

The aim of the present work was to determine what effect olive fruit fly infestation has upon the oil quality of three commercially important cultivars (Cobrançosa, Madural and Verdeal Transmontana) produced in the Trás-os-Montes region (northeastern Portugal). Such

information could be extremely useful when choosing cultivars for new orchards especially with the interest in developing olive production that complies with the rules for ecological production, which must be free of pesticide residues.

Although some data have been published on this subject (PARLATI *et al.*, 1990a, KYRIAKIDIS and DOUROU, 2002), these Portuguese cultivars have not been studied. Furthermore, previous studies have only dealt with a few parameters and a very limited number of infestation levels by the olive fruit fly. To our knowledge, the effects of this pest on the tocopherol content of the oil have not been considered.

## MATERIALS AND METHODS

### Samples

Five levels of olive fruit fly infestation and three cultivars were used. The *Olea europaea* L. cultivars studied were Cobrançosa, Madural and Verdeal Transmontana. The trees were identified and carefully marked. Five trees per cultivar were sampled. The olive fruits were handpicked on the 15<sup>th</sup> of December 1998 in an orchard at "Mascarenhas-Paradela" (northeastern Portugal). Cobrançosa (80% of the trees) is the most important cultivar. Sampling was performed simultaneously at harvesting time. About 15 kg of olives from each cultivar were randomly collected from the outer branches of the trees, at the operator's height. Healthy and damaged fruit was collected together.

After harvest, the olive fruit was immediately transported to the laboratory and the olives were divided into two groups for each cultivar. One group contained olives infested by the olive fruit fly (olives with exit holes of the larvae and pupae), and the other group was made up of healthy fruit. Starting with these two groups, five lots were then made for each

cultivar: the first lot contained 1 kg of healthy fruit (0% infestation); the second contained 875 g of healthy fruit plus 125 g of infested olives (12.5% infestation); the third lot contained 750 g of healthy fruit plus 250 g of infested olives (25% infestation); the fourth lot contained 500 g of healthy fruit plus 500 g of infested olives (50% infestation); and the last lot had 1 kg of infested olives (100% infestation). These lots were processed separately for oil extraction.

An Abencor analyzer (Comercial Abengoa S.A., Sevilla, Spain) was used to process the olives in a pilot extraction plant. The unit consisted of three essential elements: the mill, the thermobeater and the pulp centrifuge. After processing, the oil was put 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. The oil samples were analyzed in duplicate.

The parameters evaluated were titratable acidity, peroxide value, specific extinction coefficients (232 and 270 nm), oxidative stability, *p*-anisidine value, fatty acid composition and tocopherol contents.

The titratable acidity, peroxide value and the coefficients of specific extinction at 232 and 270 nm ( $K_{232}$  and  $K_{270}$ ) were determined according to the European Union standard methods (E.C., 1991).

Stability was evaluated by measuring the oxidation induction time on a Rancimat apparatus Metrohm CH Série 679 (Metrohm SA, Herisau, Switzerland). An airflow (20 L/h) was bubbled through the oil (2.5 g) heated at  $110 \pm 0.2^\circ\text{C}$  and the volatile compounds were collected in water; the increasing water conductivity was measured continuously and the time taken to reach the conductivity inflection time was recorded.

The anisidine value (ANONYMOUS, 1984) is used to determine the increase in light absorbance, measured at 350

nm, of a sample solution of 0.4-4.0 g olive oil (m) in iso-octane (25 mL), before ( $A_1$ ) and after ( $A_2$ ) reaction with *p*-anisidine (0.25 g of 4-methoxy-aniline/1,000 mL of acetic acid) in the dark. The anisidine value is equal to 25 ( $1.2 A_2 - A_1$ )/m.

Fatty acids were measured as their methyl esters after hydrolysis with a 11 gL<sup>-1</sup> methanolic potassium hydroxide solution, esterification with BF<sub>3</sub>/MeOH and extraction with *n*-heptane. The fatty acid profile was analyzed with a Chrompack CP 9001 Chromatograph equipped with a split-splitless injector, an FID, an autosampler (Chrompack CP-9050) and a 50 m x 0.25 mm i.d. fused silica capillary column coated with a 0.19 μ film of CP-Sil 88 (Chrompack, Middelburg, The Netherlands). Helium was used as carrier gas at an internal pressure of 12 kPa. The temperatures of the detector, injector and oven were 250°, 230° and 185°C, respectively. The split ratio was 1:50 and the injected volume was 1 μL. The results are expressed as the relative percentage of each fatty acid, calculated by internal normalization of the chromatographic peak area (OLIVEIRA *et al.*, 2001).

The tocopherol composition was evaluated according to GAMA *et al.* (2000) in which 0.1 g of olive oil was blended with 10 mL of *n*-hexane and homogenized by stirring. The samples were prepared in the dark and tubes containing the samples were always wrapped in aluminum foil. The mixture was filtered through a membrane (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; 25x0.4 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 a diode array detector and with a fluorimetric detector connected in series. The excitation and

emission wavelengths were, respectively, 290 and 330 nm.

All chemical analyses were carried out at least in duplicate.

### Statistical analysis

A simple linear regression analysis was used to evaluate the relationship between the olive oils produced with different percentages of olives infested by the olive fruit fly and each chemical parameter. Principal component analysis, cluster analysis and multiple linear regression were used to check the main differences between cultivars and to determine if there was a relationship between each chemical parameter and the level of infestation. All the statistical analyses were carried out by conventional methods (e.g., MARDIA *et al.*, 1979), as available in the Statistica for Windows statistical package (StatSoft Inc, Tulsa, USA).

## RESULTS AND CONCLUSIONS

Table 1 summarizes the main results obtained for cultivars Cobrançosa, Madural and Verdeal Transmontana, with five levels of olive fruit fly infestation, in terms of titratable acidity, peroxide value,  $K_{232}$ ,  $K_{270}$ , anisidine value, stability, total tocopherols and  $\alpha$ ,  $\beta$  and  $\gamma$ -tocopherols. The fatty acid composition of all the samples analysed is reported in Table 2. These results were analysed using two different approaches: (i) the way each parameter varied within the olive oil that came from the same cultivar with different levels of infestation; (ii) to find general structures, or regularities, in the olive oils produced, with respect to the cultivars and/or infestation levels.

Changes in acidity. All the olive oils obtained were classified as "Extra Virgin" because the acidity never exceeded 0.53%, regardless of the infestation level (Table 1). In general terms, olive oils ob-

Table 1 - Quality characteristics of cvs. Cobrançosa, Madural and Verdeal Transmontana virgin olive oil samples pressed from olives infested with the olive fruit fly, *Bactrocera oleae* (mean value).

Parameters	Cobrançosa					Madural					Verdeal Transmontana							
	Infestation (%)					Infestation (%)					Infestation (%)							
	0.0	12.5	25.0	50.0	100.0	0.0	12.5	25.0	50.0	100.0	0.0	12.5	25.0	50.0	100.0			
Acidity (% oleic acid)	0.33	0.33	0.32	0.31	0.32	n.s.	0.23	0.29	0.27	0.28	0.31	**	0.28	0.38	0.34	0.35	0.53	***
Peroxide value (mequiv. of O <sub>2</sub> /kg of oil)	11.36	12.56	13.97	14.08	16.65	***	14.70	14.78	14.83	14.70	14.92	n.s.	19.13	18.37	22.84	23.17	35.12	***
p-anisidine value	18.00	18.25	17.04	15.23	16.55	n.s.	6.29	6.88	13.74	7.01	6.31	n.s.	7.93	3.41	5.17	5.73	3.53	n.s.
Stability (hours)	35.45	35.70	33.17	29.38	29.83	n.s.	11.20	9.08	11.58	10.70	10.20	n.s.	31.00	29.10	32.90	29.60	14.60	*
K <sub>232</sub>	2.41	2.38	2.42	2.40	2.43	n.s.	2.66	2.54	2.56	2.78	2.49	n.s.	1.57	1.44	1.53	1.57	1.53	n.s.
K <sub>270</sub>	0.24	0.26	0.22	0.22	0.22	n.s.	0.18	0.23	0.24	0.20	0.21	n.s.	0.17	0.16	0.17	0.17	0.17	n.s.
Tocopherols (mg/kg)	214.5	222.2	205.2	209.4	202.5	**	185.9	162.5	185.9	178.3	144.4	**	137.2	131.5	141.6	131.4	85.6	***
α-tocopherol (mg/kg)	208.8	216.3	200.5	203.8	196.6	**	183.1	160.2	183.0	175.7	141.7	**	133.0	128.2	138.8	128.2	81.9	***
β-tocopherol (mg/kg)	1.6	1.6	1.4	1.5	1.6	n.s.	1.0	0.8	0.9	1.0	1.2	n.s.	0.7	0.6	0.6	0.5	0.5	*
γ-tocopherol (mg/kg)	4.0	4.3	3.4	4.1	4.3	n.s.	1.8	1.5	1.9	1.6	1.5	*	3.5	2.7	2.2	2.7	3.2	n.s.

<sup>a</sup> Significance level of the model by row. n.s., not significant (p > 0.05); \* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001.

tained from infested olives had higher acidity values. The exit holes, made by the olive fruit fly favors the entrance and development of bacteria and fungi, which increase the damage and breakdown in the olive pulp (CANTERO, 1991; GARCIA-TEJERO, 1976). In these conditions, acidity can increase due to the action of the hydrolytic enzymes and the lipolytic activity of those microorganisms (STELLA and PICCHI, 1991). The maximum acidity values were recorded for oil obtained from cv. Verdeal Transmontana processed with 100% infested olives, in which the values were double those found for the oil processed with healthy fruit (0% infestation).

Changes in peroxide value (PV). Peroxide value, a measure of the primary oxidation products, increases with fruit lipooxygenase activity and in oils obtained from damaged fruit. The changes in the peroxide values in cvs. Cobrançosa and Verdeal Transmontana were different from those in cv. Madural in which no correlation was found between olive oil infestation and peroxide value. In cv. Cobrançosa the peroxide values increased significantly with olive infestation (p < 0.001) from 11 in the oil processed with healthy olives (0% infestation) to 17 in oil processed with infested olives (100% infestation) (Table 1). The highest PV was obtained in cv. Verdeal Transmontana that showed a positive correlation with the infestation level (p < 0.001); the highest PV obtained was 35 when processed with 100% infested olives. According to the results obtained, these oils could not be classified as edible and would have to be refined.

K<sub>232</sub> and K<sub>270</sub> coefficients. The specific extinction coefficients (K<sub>232</sub> and K<sub>270</sub>) did not change significantly with olive fruit fly infestation levels. Olive oils from cv. Madural had the highest values, and cv. Verdeal Transmontana had the lowest (Table 1). The values obtained in cv. Cobrançosa and Madural oils prohibited them from being classified as Extra Vir-

gin and, in many cases even as Virgin olive oils. The results in this study do not confirm the results obtained by others (e.g., DELRIO *et al.*, 1995; PARLATI *et al.*, 1992; KYRIAKIDIS and DOUROU, 2002) who reported that  $K_{232}$  values increase with increased infestation levels. For these authors, the increase in the specific coefficient values was related to the unsaturated fatty acid (UFA) levels, especially linoleic ( $C_{18:2}$ ) and linolenic ( $C_{18:3}$ ) acids. The isomerization of these acids can give rise to diene or triene conjugated double bonds coming from 1,4-pentadiene or from 1,4,7-octatriene units present in linoleic or linolenic acyl groups, respectively (GUILLÉN and CABO 2002). This fact could explain the highest values observed in cv. Madural oils, which had the highest contents of  $C_{18:2}$  and  $C_{18:3}$  (Table 2). At the same time it could probably explain the increase in oxidative processes that were also confirmed by the behavior of the PV and the decreased stability.

**Stability.** The olive oils of cvs. Cobrançosa and Verdeal Transmontana had higher stability values than cv. Madural. The stability values changed from 35.7 to 29.4 hours in cv. Cobrançosa and from 35.3 to 14.6 hours in cv. Verdeal Transmontana oils. No relationship between the stability level and infestation levels was observed in the cv. Madural which was 60% less stable than the other oils (Table 1). This observation can be explained by the precocious maturation of this cultivar and the reduced antioxidant activity during maturation (GUTIÉRREZ *et al.*, 1999). In contrast, the stability of the cv. Verdeal Transmontana oils decreased significantly with increased infestation levels ( $p = 0.049$ ). The oil processed with olives having 100% infestation had a stability that was 52% lower than the oil processed with 0% infestation. The lower stability with increased infestation may be explained by the decrease of polyphenols in infested olives (IANNOTTA, 1990; PARLATI *et al.*, 1990a,b; ZUNIN *et al.*,

1993) and the reduction in orthodiphenols (APARICIO *et al.*, 1999; EVANGELISTI *et al.*, 1994; PSOMIADOU and TSIMIDOU, 2002). The level of tocopherols (Table 1) also influenced the stability of the olive oils. The higher stability of cv. Cobrançosa oils could be due to their higher tocopherol content. As can be seen for cv. Verdeal Transmontana oils with different degrees of olive fruit fly infestation, there are significant correlations between the stability and the total and  $\alpha$ -tocopherol contents ( $p < 0.001$  in both cases).

**Tocopherol content.** Cultivar Cobrançosa had the highest tocopherol content, whereas the cv. Verdeal Transmontana had the lowest (Table 1). Of the tocopherols detected  $\alpha$ -tocopherol occurred at the highest concentration. In general, the total tocopherol content decreased with increasing infestation levels. The decreases were statistically significant in all the cultivars: ( $p = 0.002$ ) in cv. Cobrançosa and Madural and ( $p < 0.001$ ) in cv. Verdeal Transmontana. A similar trend was observed for  $\alpha$ -tocopherol ( $p = 0.006$  in cv. Cobrançosa,  $p = 0.002$  in cv. Madural and  $p < 0.001$  in cv. Verdeal Transmontana). Tocopherols have antioxidant as well as vitamin action (RANALLI and ANGEROSA, 1996) and contribute to oil stability with respect to oxidation (APARICIO *et al.*, 1999; DEIANA *et al.*, 2002). The initial amount of  $\alpha$ -tocopherol plays a key role in preserving oil from rancidity during storage; it prolongs its shelf life, and preserves its quality (DEIANA *et al.*, 2002; PSOMIADOU and TSIMIDOU, 2002). The varying levels of tocopherols may explain the differences observed in the stability of the oils at different infestation levels.

**Anisidine values (AV).** AV is a measure of the secondary oxidation products of unsaturated fatty acids such as  $\alpha$ - and  $\beta$ -alkenals (GUILLÉN and CABO, 2002) and conjugated dienals (LABRINEA *et al.*, 2001). This parameter can be used to interpret changes in the PV,  $K_{232}$  and  $K_{270}$  values. Cvs. Cobrançosa and Verdeal

Transmontana had similar behaviour, showing a slight decrease in AV with increased infestation level. In cv. Madural this parameter followed a pattern similar to that of K<sub>270</sub>.

**Fatty acid composition.** Table 2 summarizes the fatty acid composition (%) of the three cultivars. Cv. Verdeal Transmontana had the highest oleic acid (C<sub>18:1c</sub>) content and cv. Madural had the highest linoleic acid (C<sub>18:2cc</sub>) content. The variations in fatty acid levels were not significantly related to the infestation levels. The contents in terms of *trans* isomers were minimal and seemed to be unrelated to the polyunsaturated fatty acid contents. At the higher infestation levels their percentage may surpass the limit established for edible olive oils (EEC, 1995). Cv. Madural had the lowest monounsaturated fatty acid content (MUFA) and the highest polyunsaturated fatty acid (PUFA) content. This observation concurs with the low stability values discussed above.

This study clearly shows that oils from cv. Verdeal Transmontana obtained from olives infested by the olive fruit fly, had a much lower quality than the oils from the other cultivars.

Cv. Madural oils were less stable which can lead to autoxidation processes, which in turn reduce its shelf life during storage. Cv. Cobrançosa oils had a high stability and high tocopherol contents and the losses caused by olive fruit fly infestation were less than those found in the other cultivars.

In the second approach used to analyse the results, i.e., the multivariate approach, three data sets were considered: fatty acid composition, tocopherol composition and overall chemical composition. Fig. 1 shows the main features of the fatty acid composition as depicted by principal component analysis. The first two principal components show that all cultivars were well distinguished by their fatty acid patterns: cv. Cobrançosa had the highest levels of saturated fatty

Table 2 - Fatty acid composition (in relative percentages) of cvs. Cobrançosa, Madural and Verdeal Transmontana olive oil samples pressed from olives infested with olive fruit fly, *Bactrocera oleae* (mean value).

Fatty acid	Cobrançosa					Madural					Verdeal Transmontana				
	Infestation (%)					Infestation (%)					Infestation (%)				
	0.0	12.5	25.0	50.0	100.0	0.0	12.5	25.0	50.0	100.0	0.0	12.5	25.0	50.0	100.0
C <sub>16</sub>	9.64	9.61	9.55	9.50	9.54	10.10	10.29	10.24	10.29	10.46	9.71	9.50	9.43	9.56	10.04
C <sub>18:1c</sub>	0.41	0.41	0.41	0.39	0.4	0.25	0.26	0.25	0.25	0.26	0.33	0.31	0.30	0.35	0.36
C <sub>18</sub>	4.94	4.99	5.09	5.21	5.02	2.29	2.34	2.39	2.32	2.30	3.40	3.42	3.21	3.05	2.92
C <sub>18:1c</sub>	76.71	76.81	76.70	76.63	76.55	71.56	71.85	71.94	71.52	71.40	80.20	80.87	81.58	81.60	81.01
C <sub>18:2cc</sub>	5.91	5.86	5.82	5.79	6.10	12.96	12.97	12.68	13.01	13.17	3.89	3.30	2.86	2.94	3.16
C <sub>20</sub>	0.46	0.46	0.50	0.47	0.47	0.26	0.28	0.29	0.28	0.28	0.42	0.46	0.48	0.44	0.43
C <sub>18:3c</sub>	0.67	0.64	0.65	0.60	0.63	0.79	0.76	0.76	0.78	0.82	0.63	0.55	0.52	0.60	0.65
Saturated	15.85	15.82	16.02	16.17	15.84	14.29	13.78	14.00	14.16	13.96	14.45	14.42	14.22	13.92	14.23
Unsaturated	84.14	84.15	84.02	83.85	84.10	85.88	86.20	86.00	85.93	86.02	85.51	85.50	85.77	86.07	85.72
Polyunsaturated	6.58	6.49	6.47	6.39	6.73	13.74	13.73	13.44	13.80	14.00	4.52	3.86	3.38	3.54	3.81
Monounsaturated	77.56	77.66	77.55	77.46	77.37	72.14	72.47	72.56	72.14	72.03	80.99	81.64	82.39	82.53	81.91
Trans isomers	0.05	0.04	0.04	0.04	0.03	0.03	0.04	0.05	0.05	0.04	0.04	0.04	0.03	0.08	0.06

<sup>a</sup> Significance level of the model by row. n.s., not significant ( $p > 0.05$ ); \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

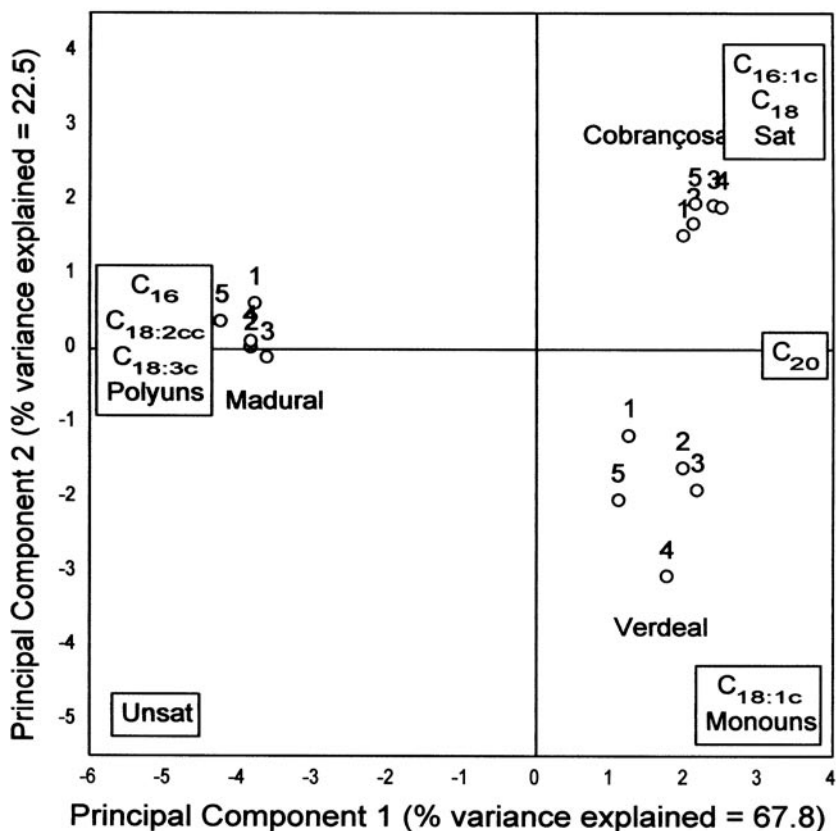


Fig. 1 - Plot of the first two principal components, showing average values for C = Cobrançosa, M = Madural and V = Verdeal Transmontana; Fatty acid labels over principal component edges indicate their meaning, and the corners indicate the meaning of the principal components plane. Sat = Saturated, Uns = Unsaturated, Polyuns = polyunsaturated and Monouns = monounsaturated. Numbers 1 to 5 refer to the level of fly infestation.

acids, mainly C<sub>18</sub>, and also the highest levels of C<sub>16:1c</sub>. Cv. Verdeal Transmontana was characterized by the highest percentages of monounsaturated fatty acids, specially C<sub>18:1c</sub>, while cv. Madural had the highest levels of polyunsaturated fatty acids, mainly C<sub>18:2cc</sub> and C<sub>18:3c</sub>, and low levels of C<sub>20</sub> (in contrast to the other two cultivars). In Fig. 1, all samples are numbered according to the infestation level; it is clear that fatty acid levels did not change in relation to infestation levels.

A PCA applied to the tocopherol composition of olive oils is shown in Fig. 2. It is important to emphasize that the

second component represents only 1.4% of the total available information (which corresponds to a non significant eigen value). As illustrated by projecting all samples onto the first component, the cultivars can be distinguished from each other. An ordering from left to right with respect to the infestation level is apparent (with the exception of C5). In this case, the principal components were not easy to explain. Therefore, to try to explain the observed ordering of the samples, a multiple regression was carried out. The results are presented in Table 3. It can be seen that there is a relationship between infestation and  $\alpha$ -tocopherol levels and



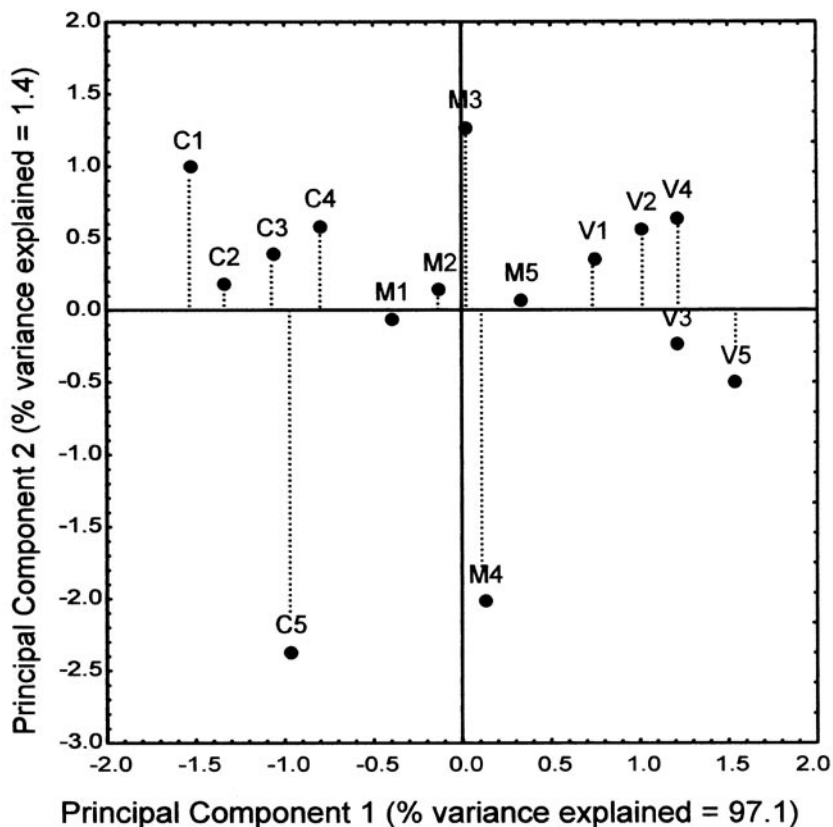


Fig. 2 - Plot of the first two principal components derived from a PCA applied to the tocopherol contents of the olive oils. All indications are the same as in Fig. 1.

Table 3 - Summary of multiple regression analysis with the infestation level as the dependent variable and tocopherols as independent variables.

	$\beta$	St. Error. of $\beta$	B	St. Error. of B	t (d.f. = 11)	p-level
Intercept			309.8640	312.5748	0.99133	0.342831
$\alpha$ -tocoph.	2.95201	0.954907	27.7383	8.9727	3.09141	0.010260
$\beta$ -tocoph.	-0.84246	0.780418	-9.9066	9.1770	-1.07950	0.303465
$\gamma$ -tocoph.	-1.87958	0.913812	-20.9209	10.1713	-2.05686	0.064213

R = 0.69589631; R<sup>2</sup> = 0.48427168; F(d.f. = 3;11) = 3.4430 p < 0.05542.  
d.f. = degrees of freedom.

possibly  $\gamma$ -tocopherol levels since both levels were higher in infested fruits.

A cluster analysis was applied to the results of the overall chemical analysis

of the oils, and the corresponding dendrogram is shown in Fig. 3. All the cultivars can be distinguished according to these parameters. With the aid of PCA

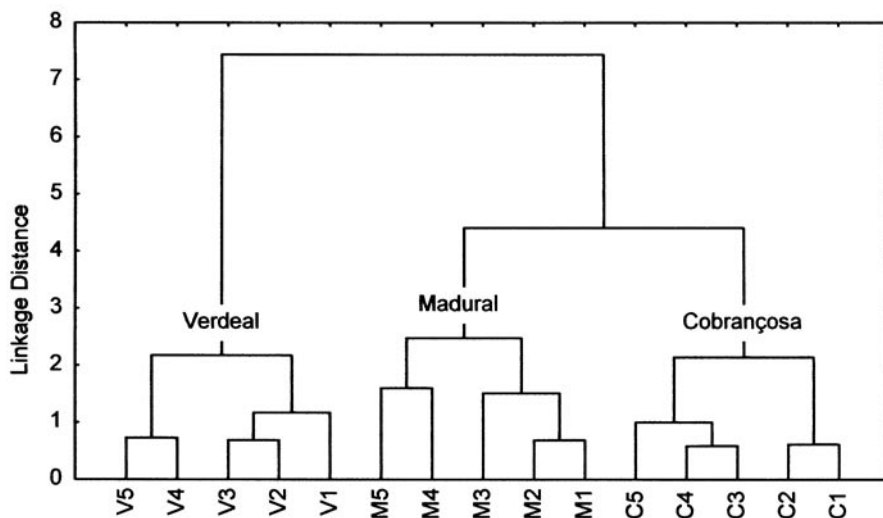


Fig. 3 - Results from a cluster analysis based on the overall chemical parameters. The letters and numbers are the same as in Fig. 1.

it was shown that acidity and peroxide values were responsible for the observed clustering. These values increase at the two or three highest infestation levels in all the cultivars. It is concluded that cv. Cobrançosa performed the best with respect to the parameters analysed.

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