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63.1 INTRODUCTION

In Portugal, olive oil production is considered an ancient activity, where old olive groves can still be observed. In the last few years monovarietal groves seem to be increasing, though some disadvantages, such as the susceptibility to insects and diseases, can result from the growth of individual olive varieties (Aguilera et al., 2005). In some typical producer countries, the olive cultivation is being improved by renewing old trees, reducing the association with other crops, selecting the olive varieties suited to local agroclimatic and planting new single variety orchards (Criado et al., 2008). This is leading to an increase in the prevalence of monovarietal olive oils.

Portugal is one of the ten largest olive oil producers in the world, although in recent years (2004–2006) the olive oil production decreased from approximately 50 000 to 26 000 hL. Trás-os-Montes, located in the northeast of Portugal, is the main region of olive oil production. In this province, agriculture is still one of the major activities, where table olives and olive oil productions are considered essential to the regional economy. Several factors comprising the region climate conditions, the soils, the existence of dominant varieties, the traditional production techniques and the high quality of the final product led to the creation of a Protected Designation of Origin (PDO) olive oil. After the approval of the EU Regulation (EC) No. 2081/92 and the transposition to national laws, PDO olive oils with the designation of ‘Azeite de Trás-os-Montes DOP’ started being produced and commercialized. Four olive varieties are authorized for the production of this PDO olive oil, namely Cobrançosa, Madural, Verdeal Transmontana (also known as Verdeal) and Cordovil. The latter has no commercial expression in the region. The other three varieties account for more than 90% of olive cultivation in the region (Matos et al., 2007).

Several factors are known to affect olive oil quality, namely: variety, stage of ripening, environmental conditions, cultural practices, method of oil extraction and storage conditions (Pereira et al., 2002; D’Imperio et al., 2007; Matos et al., 2007). Among them, the variety is considered to be of most importance, influencing olive oil quality and sensory characteristics. Its importance arises from the genetically defined enzymes involved in the lipoxygenase pathway, which constrain the production of flavor compounds and determine the inherent sensory quality of the virgin olive oil (Manai et al., 2008). Nevertheless, other authors state that environmental factors can sometimes exert a stronger influence than genetic factors regarding the chemical components that determine monovarietal virgin olive oil quality (Beltrán et al., 2005; Criado et al., 2008).

63.2 COMPOSITIONAL CHARACTERISTICS OF PORTUGUESE MONOVARIETAL OLIVE OIL FROM TRÁS-OS-MONTES

The authenticity and traceability of olive oils has been the object of numerous studies in the past few years. Generally, characterization of monovarietal olive oils requires the study of several compounds, including major components, such as fatty acids (FA), triacylglycerols (TAG), and the minor ones, such as phytosterols, polyphenols, tocopherols,
tocotrienols and pigments. FA have been successfully used for grouping olive oils according to the olive variety used. Besides, their knowledge is also important to estimate nutritional properties of oils as saturated FA are associated with a higher risk of cardiovascular diseases, contrarily to mono- and polyunsaturated FA. Several results have also proved the usefulness of TAG in grouping monovarietal olive oils, suggesting that the FA content and distribution on the glycerol moiety can contribute to the establishment of a cultivar chemical fingerprint (D’Imperio et al., 2007). Minor compounds, such as phenolics and tocopherols, important for their antioxidant activity, are also able to provide useful information for authenticity assessment.

The geographical characterization of olive oils is another important aspect of concern, especially for those certificated as PDO, since they are added-value products. Chemical characterization followed by multivariate statistical analysis has been reported in several studies to be a useful tool for the classification of olive oils regarding their geographical origin (Aguilera et al., 2005; Ollivier et al., 2006) (Table 63.1).

Known as high-quality products, the olive oils from Trás-os-Montes region achieve very high market prices, which can propitiate unfair commercial practices. It is therefore important to use analytical techniques to ensure the assessment of identity and quality of these oils, and guarantee the proper product classification. Because of the importance of Cobrançosa, Madural and Verdeal varieties in Portuguese olive oil, especially concerning the ‘Azeite de Trás-os-Montes’ PDO olive oil, several studies reporting their chemical composition have been conducted in recent years.

### 63.2.1 Fatty Acids Composition

Fatty acid composition of edible oils is generally determined by gas–liquid chromatography coupled to flame ionization detection. This methodology has been applied in the determination of FA profile of Trás-os-Montes olive oil varieties (Figure 63.1). Several works report the FA profile of olive oil varieties together with the influence of different parameters on the oil composition, such as the effect of olive storage previous to oil extraction (Pereira et al., 2002) and the influence of the maturation index of the fruits (Matos et al., 2007). Table 63.2 shows the

Table 63.1 Key features of olive oil chemical characterization.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Triacylglycerols are the major compounds in olive oil and comprise three fatty acids linked to a glycerol molecule</td>
</tr>
<tr>
<td>2.</td>
<td>According to the number of double bounds, fatty acids can be saturated (no double bond), monounsaturated (1) or polyunsaturated (&gt;1)</td>
</tr>
<tr>
<td>3.</td>
<td>Phytosterols and tocopherols are minor compounds present in the unsaponifiable fraction of olive oil; they are thought to be beneficial health compounds</td>
</tr>
<tr>
<td>4.</td>
<td>PDO olive oils are considered as added-value products</td>
</tr>
<tr>
<td>5.</td>
<td>Principal component analysis (PCA) and discriminant analysis are multivariate statistical analysis tools frequently applied to chemical data exploitation</td>
</tr>
</tbody>
</table>

**Figure 63.1** Fatty acid profile of a virgin olive oil sample of Madural variety obtained by gas-chromatography. Peak legend: 1–C16:0; 2–C16:1n7; 3–C17:0; 4–C17:1n7; 4–C18:0; 6–C18:1n9; 7–C18:2n6; 8–C20:0; 9–C20:1n9; 10–C18:3n3; 11–C22:0.
Characterization of Three Portuguese Varietal Olive Oils: Application of Chemometrics

63.2.2 Triacylglycerol Composition

Although triacylglycerol (TAG) profile can be determined by gas–liquid chromatography, non-aqueous reversed-phase high-performance liquid chromatography (RP-HPLC) is the most frequently used methodology, avoiding difficulties associated with the low volatility and the thermal stress of polyunsaturated TAG (Amaral et al., 2004). Figure 63.2 shows an RP-HPLC chromatogram of the TAG profile of an olive oil sample. Considering that in RP-HPLC TAG are mainly separated according to their equivalent carbon number (ECN = CN – 2DB; CN – total carbon number; DB – number of double bonds), those presenting the same ECN can sometimes be difficult to resolve representing a critical pair, i.e., two compounds co-eluting in the same peak. Twelve peaks were quantified based on the relative percentage peak area by Cunha et al. (2005) (Table 63.3 and Figure 63.2). Other compounds, such as, PoLO, LnOO, PoOO, PPoO, SOL and AOL can also be found as possible critical pairs, although they are in minor amounts in olive oils (Ollivier et al., 2006). Table 63.3 reports the mean values of TAG composition for Cobrançosa, Madural

### Table 63.3 Triacylglycerols composition of Cobrançosa, Madural and Verdeal monovarietal olive oils (adapted from Cunha et al. (2005)).

<table>
<thead>
<tr>
<th>Triacylglycerol Composition</th>
<th>ECN&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Cobrançosa</th>
<th>Madural</th>
<th>Verdeal</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLL&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42</td>
<td>0.03 (0.02)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.03 (0.01)</td>
<td>0.06 (0.02)</td>
</tr>
<tr>
<td>LLO + PoLO</td>
<td>44</td>
<td>0.38 (0.10)</td>
<td>2.09 (0.31)</td>
<td>0.05 (0.03)</td>
</tr>
<tr>
<td>LLP + LnOO</td>
<td>44</td>
<td>0.56 (0.20)</td>
<td>0.40 (0.15)</td>
<td>0.42 (0.03)</td>
</tr>
<tr>
<td>LnOP</td>
<td>44</td>
<td>0.05 (0.02)</td>
<td>0.76 (0.80)</td>
<td>0.07 (0.02)</td>
</tr>
<tr>
<td>LOO + PoPP</td>
<td>46</td>
<td>9.45 (1.43)</td>
<td>18.44 (1.23)</td>
<td>3.56 (0.15)</td>
</tr>
<tr>
<td>PLO + PPoO</td>
<td>46</td>
<td>3.08 (2.59)</td>
<td>4.31 (0.23)</td>
<td>0.60 (0.10)</td>
</tr>
<tr>
<td>OO0</td>
<td>48</td>
<td>57.57 (2.26)</td>
<td>50.52 (0.92)</td>
<td>66.81 (0.79)</td>
</tr>
<tr>
<td>PO0 + SOL</td>
<td>48</td>
<td>21.01 (1.04)</td>
<td>20.20 (1.07)</td>
<td>23.44 (0.70)</td>
</tr>
<tr>
<td>PPO</td>
<td>48</td>
<td>0.68 (0.16)</td>
<td>0.83 (0.45)</td>
<td>1.08 (0.42)</td>
</tr>
<tr>
<td>SOO + AOL</td>
<td>50</td>
<td>6.54 (0.87)</td>
<td>2.15 (0.37)</td>
<td>3.65 (0.29)</td>
</tr>
<tr>
<td>SOP</td>
<td>50</td>
<td>0.53 (0.09)</td>
<td>0.17 (0.03)</td>
<td>0.25 (0.03)</td>
</tr>
<tr>
<td>OOA</td>
<td>52</td>
<td>1.13 (0.15)</td>
<td>nd&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.05 (0.01)</td>
</tr>
</tbody>
</table>

This table summarizes the TAG composition of monovarietal olive oils.

<sup>a</sup>ECN: equivalent carbon number.

<sup>b</sup>LLL: linoleoyl; LLO: linolenoyl; PO: palmitoleoyl; P: palmitoyl; S: stearoyl; O: oleoyl; L: linoleoyl; Ln: linolenoyl; A: arachidoyl;
<sup>c</sup>Mean values and standard deviation in parenthesis.

<sup>d</sup>nd: not detected.
and Verdeal varieties. All the varieties showed a similar qualitative profile with OOO as the major compound (50.5–66.8%), followed by POO (20.2–23.4%) and LOO (3.6–18.4%), reflecting the high oleic acid content in olive oils. Concerning the main triacylglycerols (OOO, POO and LOO), Verdeal variety presented the highest percentages of OOO and POO (66.8% and 23.4%, respectively, as mean values) and the lowest of LOO (3.6%). Conversely, Madural variety presented the lower proportion of OOO and POO and the highest of LOO.

**63.2.3 Phytosterol Composition**

Clinical studies have demonstrated that dietary intake of phytosterols, due to their structural similarity with cholesterol, can inhibit its intestinal absorption, thereby lowering total plasma cholesterol and low-density lipoprotein levels (Wong, 2001). Phytosterols have also been reported to present antioxidant, antibacterial and anti-inflammatory activities and may offer protection against cancers, such as breast, colon and prostate (Awad and Fink, 2000). Furthermore, they can influence the oil stability at high temperatures since some phytosterols can act as polymerization reaction inhibitors (Velasco and Dobarganes, 2002). For all these reasons, data concerning phytosterol composition are of high relevance for quality and nutritional evaluation of the oil.

Phytosterols are a major portion of the unsaponifiable fraction of olive oils and present a more or less characteristic profile, making it an important tool for assessing the genuineness of the oil. According to Aparicio and Aparicio-Ruiz (2000), the qualitative and quantitative differences in phytosterol composition among several vegetable oils make them a suitable parameter to verify the botanical origin of vegetable oils and, thus, to detect eventual adulterations/contaminations. Other authors have suggested that sterol composition can be useful in virgin oil characterization, especially in detecting the adulteration with hazelnut oil and, more recently, in the classification of virgin olive oil according to its variety (Lazzez et al., 2008). Although the dominant phytosterols in olive oils are reported to be β-sitosterol, Δ3-avenasterol and campesterol, several other minor compounds, such as cholesterol, stigmasterol, clerosterol, sitosterol, Δ7-stigmastenol and Δ7-avenasterol have also been reported to occur in olive oils (Matos et al., 2007; Lazzez et al., 2008). To prevent olive oil adulteration several international organizations,
such as the Codex Alimentarius of the FAO/WHO, the EU and the International Olive Oil Council have established characteristic values for some of these compounds. Table 63.4 presents the sterol composition of olive oils produced with the varieties Cobrançosa, Madural and Verdeal. Considering the aforementioned regulations, all samples studied by Matos et al. (2007) presented a total sterol content far above the threshold of 1000 mg kg\(^{-1}\) established for virgin olive oil, presenting also a higher value than that demanded (\(\geq 93\%\)) for apparent \(\beta\)-sitosterol (comprising the sum of clerosterol, \(\beta\)-sitosterol, \(5\)-avenasterol, \(\beta\)-sitosterol and \(5,24\)-stigmastenol). The results reported by Matos et al. (2007) and Alves et al. (2005) were under the upper limit of 4% established by the EU (Regulation No. 2568/91/EEC and later amendments) for olive oil campesterol content and were in general below the established upper limits of cholesterol (0.5%). Regarding the study of the effect of ripening in sterol composition, Matos et al. (2007) reported a decreasing tendency, although with some variations along the maturation.

### 63.2.4 Tocopherols and Tocotrienols Composition

Vitamin E is the term used to designate a group of lipid-soluble compounds, comprising four tocopherols (\(\alpha\), \(\beta\), \(\gamma\) and \(\delta\)) and four tocotrienols (\(\alpha\), \(\beta\), \(\gamma\) and \(\delta\)). This family of compounds is particularly important in preventing lipid oxidation processes in olive oils, mainly due to their antioxidant activity. Moreover, a large range of biological activities have been ascribed for these compounds since vitamin E vitamers have been associated with a preventive action against reactive oxygen species in biological systems (Woollard and Indyk, 2003). Although in the past \(\alpha\)-tocopherol was probably the most studied vitamer since it was considered the most active vitamin E isoform, nowadays several studies have shown that the other vitamers also have important roles in the human organism, and thus, are considered to contribute to the total bioactivity in foods. For example, \(\gamma\)-tocopherol and tocotrienols are correlated with the reduction of blood cholesterol levels (Mishima et al., 2003) and may have a chemopreventive action (Campbell et al., 2003).

Vitamin E determination has also been used for the authenticity and quality assessment of oils, based on qualitative and quantitative profiles. Tocopherol contents in monovarietal olive oils of Cobrançosa, Madural and Verdeal varieties (Table 63.5) were reported by Matos et al. (2007) and the influence of olive storage on their contents in olive oils of the same varieties was reported by Pereira et al. (2002). In both studies, only the three major tocopherols were quantified, possibly due to the low values of the other vitamers. \(\alpha\)-Tocopherol was the major compound, followed by \(\gamma\) and \(\delta\)-tocopherols, respectively, which is in accordance with other authors (Cunha et al., 2006), although they reported also the presence of \(\beta\)-tocopherol and two tocotrienols (\(\alpha\) and \(\gamma\)) in ‘Azeite de Trás-os-Montes’ PDO olive oils. The results reported by Matos et al.
TABLE 63.5 Tocopherol composition of Cobrançosa, Madural and Verdeal monovarietal olive oilsa.

<table>
<thead>
<tr>
<th>Tocopherols (mg kg⁻¹)</th>
<th>Cobrançosa</th>
<th>Madural</th>
<th>Verdeal</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>221.4</td>
<td>291.7</td>
<td>222.6</td>
</tr>
<tr>
<td>β</td>
<td>0.9</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>γ</td>
<td>5.4</td>
<td>16.1</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>235.5</td>
<td>298</td>
<td>235.5</td>
</tr>
</tbody>
</table>

aMinimum and maximum values concerning the evaluation of samples with different maturation indexes: Cobrançosa-MI1 to MI7, Madural-MI3 to MI7, Verdeal-MI1 to MI6; MI- maturation index. Adapted from
bMatos et al. (2007) and
bPereira et al. (2002); nd: not determined.

(2007) showed, in general, higher α- and γ-tocopherol contents. However, a more accurate observation of results by comparing values for the same olive maturation index used by Pereira et al. (2002), shows that the differences among α-tocopherol contents in both studies were not so large (Table 63.5). Beltrán et al. (2005) referred that tocopherol profile can change depending on the variety, fruit ripening stage, climatic conditions and olive growing techniques. Since samples studied by Pereira et al. (2002) were collected in the 1998/1999 crop year and those of Matos et al. (2007) in 2000/2001, this can explain the differences among results. Furthermore, in the latter only healthy fruits were collected, while in the former 30% of fruits were attacked by the olive fly.

63.3 CHEMOMETRICS APPLIED TO MONOVARIETAL OLIVE OIL CHARACTERIZATION

For authenticity assessment of monovarietal olive oils the chemical data frequently need to be further explored by using statistical tools. Multivariate statistical analysis has been used to recognize the parameters able to discriminate the olive varieties. Among multivariate statistical analysis, principal component analysis (PCA) and discriminant analysis occupy a very important position. Nowadays, powerful statistical software packages are available, facilitating the application of complex and sophisticated algorithms, even by individuals with no special mathematical background (Alves et al., 2005).

Several examples of statistical analysis applied to olive oil compositional data are available in the literature (Diaz et al., 2005; Cerretani et al., 2006; Ollivier et al., 2006; D’Imperio et al., 2007), sometimes coupling several statistical techniques to obtain high discrimination levels and reliable models (Alves et al., 2005). Generally, the multivariate statistical approach applied to olive oil analytical data allows recognizing the most significant descriptive variables among many, and to cluster olive oil samples, which can lead to a first classification of olive variety (Cichelli and Pertesana, 2004). Recently, statistical tools have been applied to explore several data obtained from the chemical analysis of Trás-os-Montes monovarietal olive oils, namely FA and tocopherols (Matos et al., 2007), sterols (Alves et al., 2005; Matos et al., 2007) and TAG (Cunha et al., 2005).

63.3.1 Application to TAG Profile

Cunha et al. (2005) compared the values for TAG by PCA after using a one-way analysis of variance to determine the differences among mean scores of each TAG profile of the three varieties. The PCA plot obtained (Figure 63.3) was able to explain approximately 61.5% of total variance, with the first dimension (PC1) representing 35.7% and the second dimension (PC2) representing 25.8%. The most important TAG defined by these two components were LLO, LOO and PLO for PC1 and SOO, SOP and OOA for PC2. In this plot, the three varieties could be perfectly distinguished, forming three groups. Madural and Verdeal were separated on PC1: the samples from Madural were located on the right side of the plot since they presented higher contents of LOO, LLO and PLO. The Verdeal olive oils presented lower values of the mentioned TAG and were, consequently, located on the left side of the plot. Cobrançosa differed from the other counterparts since it presented higher contents of SOP, SOO and OOA, thus was separated on PC2, being located at the top of the plot. The results reported by Cunha et al. (2005) are in accordance with other works, which showed that TAG composition could be useful to discriminate the varieties used in olive oil production (Cerretani et al., 2006).

63.3.2 Application to FA and Sterol Profiles

Several authors have reported the usefulness of FA composition (Ollivier et al., 2006; D’Imperio et al., 2007) and sterol composition (Diaz et al., 2005) associated with
statistical tools in olive oil characterization. A model for the characterization of monovarietal olive oils produced from Cobrançosa, Madural and Verdeal varieties, based on their main phytosterol composition, was presented by Alves et al. (2005). The developed model was considered suitable to classify commercial PDO olive oils produced from those varieties. A wider approach exploiting several chemical parameters (FA, tocopherols and phytosterols), was proposed by Matos et al. (2007). In this work, data obtained from Trás-os-Montes monovarietal olive oils were subjected to statistical analysis to evaluate the ability of single or multiple parameters as tools for variety discrimination. First, the authors used a multivariate analysis of variance for testing the significant differences between mean values of the evaluated parameters. The authors also performed the Student \( t \)-test for independent groups, and a PCA to detect structures in the relationship between variables (Matos et al., 2007). Regarding the individual exploitation of data obtained from FA analysis, the authors reported significant differences \( (p > 0.01) \) among the three varieties for all the quantified FA with the exception of C16:0. The analysis of PCA explained 99.6% of total variance, with PC1 and PC2 representing 68.2% and 31.4%, respectively. To reduce the number of variables, yet keeping the maximum information possible, three FA were chosen by the definition of these two components, namely C18:0, C18:1 and C18:2. In the PCA plot presented by Matos et al. (2007) the three varieties could be perfectly discriminated by their FA composition (Figure 63.4). In general, Madural variety was discriminated by its higher linoleic acid content and lower oleic acid. Verdeal variety presented higher values of oleic acid and lower values of linoleic acid. Cobrançosa variety was the one showing higher stearic acid content. In the same study, Madural and Verdeal varieties appeared as very homogeneous groups independent of the stage of ripening considered, while Cobrançosa presented a considerable dispersion of values, attributed mainly to the increasing stearic acid content along the stage of ripening.

The discrimination achieved with statistical analysis of tocopherol data was less evident than the one performed with FA data. Nevertheless, Cobrançosa was differentiated from the others by the higher \( \alpha \)- and \( \gamma \)-tocopherol contents, while Verdeal presented much lower values for these vitamins.

Data obtained from phytosterol analysis were also evaluated using statistical tools (Matos et al., 2007). The phytosterols chosen to perform a PCA, namely stigmasterol, cleroстерol, \( \beta \)-sitosterol and \( \Delta^5 \)-avenasterol, were selected by their statistical significance and also by their abundance in olive oil samples. Considering the multivariate analysis applied to the individual parameters, namely FA, tocopherols and phytosterols, the best level of discrimination for the three monovarietal olive oils was achieved using the FA data. Identical results were obtained by Cerretani et al. (2006), who stated that the use of minor components for the differentiation of olive oil variety is not reliable, since other external factors can influence minor compound composition beyond the genetic factor.

### 63.3.3 Global PCA Using Fatty Acids, Phytosterols and Tocopherols

Matos et al. (2007) performed a global PCA considering simultaneously all data from FA, phytosterols and tocopherols. The most informative variables that represent the main data structures were selected avoiding co-linearity problems.
Six variables, namely C18:0, C18:1, α-tocopherol, γ-tocopherol, β-sitosterol and Δ2-avenasterol, were extracted and the information condensed and simplified in a single graph. Two principal components were enough to describe the main features of the data, with PC1 and PC2 representing 55.6% and 23.1% of the information, respectively (Figure 63.5).

In general, better results were achieved considering the individual statistical analysis performed with the FA data as well as with the TAG data, although samples could be differentiated when all data parameters are used. Considering the results of the several studies performed on monovarietal ‘Trás-os-Montes’ olive oil, one can conclude that it is possible to apply analytical methods, which in this case are relatively inexpensive and easy to implement (such as FA or TAG analysis), coupled with statistical analysis to characterize the oils obtained from the varieties used in the production of extra virgin ‘Trás-os-Montes’ olive oil.

**SUMMARY POINTS**

- For authenticity assessment, the chemical data are further explored by using multivariate statistical analysis, such as principal component analysis and discriminant analysis.
- In general, better results were obtained using individual statistical analysis coupled to fatty acid or triacylglycerol data, although differentiation could also be achieved with several parameters (fatty acids, phyto-sterols and tocopherols) simultaneously.

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