Discrimination of Varietal Olive Oils of the Portuguese Cultivars Cobrançosa, Madural and Verdeal Based on their Fatty Acids Composition

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Abstract

The fatty acid composition has been traditionally used to discriminate the different vegetable oils and confirm their authenticity. The aim of the present study was to check the possible use of the fatty acid profile to differentiate varietal olive oils of the three most important cultivars in Northeast Portugal (Cobrançosa, Madural and Verdeal). Fifteen varietal olive oils from each of the three cultivars were collected to achieve that goal.

Fatty acids were determined, as methylesters, by HRGC. Methylesters were prepared by transesterefication with BF_3/methanol after saponification with methanolic KOH. The fatty acid pattern was performed with a Chrompack CP9001 equipped with a split-splitless injector, a FID and a 50m x 0,25 mm i.d. fused silica capillary column coated with a 0.19μm film of CP-Sil88 (Chrompack). The temperatures of injector, detector and oven were 230, 250 and 185ºC, respectively.
The results as shown by two-way Anova techniques and discriminant analysis demonstrate that $C_{16:1}c$, $C_{17:1}$, C_{18}, $C_{18:1}c$, $C_{18:2}cc$ and $C_{18:3}ccc$ can be used to discriminate the origin of each of the three olive oils.

INTRODUCTION

Olive oils are premium good with an increasingly international market. They are the major crop source of farmers in the Trás-os-Montes region, in north-eastern Portugal. In this region the olive trees area is around 65.000 ha which corresponds to 30% of the Portuguese olive oil production. The most representative varieties in the region are: Cobrançosa, Madural and Verdeal (Anónimo, 1996; Manso, 1999).

The fatty acid composition together with multivariate classification methods have been reported by several researchers as important tools for the possible discrimination of geographical origin and olive cultivars (Firistone & Reina, 1996; Pereira, 2000).

Variations in the chemical composition of olive oils depend on a wide number of parameters, which include agronomical, environmental and varietal (cultivar). The cultivar factor is undoubtedly one of the most important factors but it is often ignored because commercial olive oils are usually a blend of oils from different cultivars (Perri et al., 1999).

In this work, an attempt has been made to evaluate the usefulness of fatty acid composition in distinguishing virgin olive oils from three cultivars growing in the same area of Trás-os-Montes.

MATERIALS AND METHODS

The experimental design used consisted of 45 virgin olive oils (15 virgin olive oil from each of the three cultivars in study), all analysed in triplicate, in a total of 135 sample units.

The samples were filtered through filter paper and dehydrated with anhydrous sodium sulphate. Fatty acids were determined by HRGC as methylesters by

transesterefication with BF_3/methanol, after saponication with methanolic KOH.

The fatty acids profile was analysed with a Chrompack CP9001 chromatograph equipped with a split-spiltless injector, a FID and 50m x 0,25 mm i.d. fused silica capillary column coated with a 0,19 μm film of CP-Sil88 (Chrompack). Helium was used as carrier gas at an inlet pressure of 14 kPa. The temperatures of injector, detector and oven were 230, 185 and 250ºC, respectively. The split ratio was 1:50 and the injection volume was 1 μL.

Fatty acid methylesters standards (FAME), purity > 99%, were purchased from Sigma Chemicals. These included the following FAME: dodecanoate 12:0; tetradecanoate 14:0; pentadecanate 15:0; hexadecanoate 16:0; 9-palmitoelaidate 16:1*t*; 9-hexadecenoate 16:1*c*; heptadecanoate 17:0; octadecanoate 18:0; 9-octadecenoate 18:1*t* (elaidate); 9-octadecenoate 18:1*c* (oleate); *cis*–9, *cis*-12-octadecadienoate 18:2*cc*; *cis*-9, *trans*-12-octadecadienoate 18:2*ct*; eicosanoate 20:0; 9, 12, 15 – octadecatrienoate 18:3; 11-eicosenoate 20:1; docosanoate 22:0; 13-docosenoate 22:1; and tetracosanoate 24:0.

The differences between the plots in the percentage of fatty acids on the different cultivars were analysed using the analysis of variance, after testing the homogeneity of variance, followed by a Tukey test. The discriminant analysis (Mardia et al., 1979) was carried out in the Statistica for Windows package.

RESULTS AND DISCUSSION

The fatty acids composition of the three Portuguese cultivars, Cobrançosa, Madural and Verdeal, is presented in the table 1, as mean ± standard deviation. In the same table significant differences are marked with symbol "*". It can be seen that $C_{16:1}c$, C_{18}, $C_{18:1}c$, $C_{18:2}cc$ and $C_{18:3}ccc$ can be used to discriminate between different cultivars. However, just by Anova, it is difficult to determine the quality of the discrimination achieved by each fatty acid.

The discriminant analysis (DA) was carried out with a previous definition of three groups, one for each olive cultivar. Each group had size 15, corresponding to the mean profiles of each olive oil from each cultivar. In these circumstances, DA defines only 2 dimensions and the main results are shown in Table 2. The first DA dimension is mainly separating Madural olive oils from the olive oils of the other two cultivars, based on the relative high levels of $C_{18:2}cc$ and low levels of $C_{17:1}$ and C_{20}. It is worth noting that the discrimination power of $C_{17:1}$ and C_{20}, when considered together, could not be highlighted by analysis of variance. The second DA dimension describes the separation of the olive oils from the other two cultivars (Verdeal and Cobrançosa), based on differences of relative levels of $C_{16:1}c$, C_{18} and $C_{18:1}c$. In this respect, the cultivar Madural has intermediate values.

These discriminations can be visualised in Fig. 1. To build up this figure, all 135 sample units were projected in the two DA dimensions, instead of group means as it is usual, as well as the main results.

The fact that all sample points cluster together in the respective groups means that differences between replicates are very small, corresponding to a high precision of the analytical methodology. This would correspond to a very small residual error in an analysis of variance (results not shown in this paper). Also, the fact that sample points of each group (cultivar) tend to form one homogenous cluster shows that differences between different olive oils within each cultivar are also very small. As a matter of fact, the homogeneity within each group is so marked that the existence of fifteen different olive oils within each group is not apparent in the graph of Fig. 1.

Finally, it is important to note that this analysis, like any other multivariate analysis, is based on relative differences. Therefore, as the differences, or distances, between group means is very large, when compares to the differences within groups, it becomes obvious that fatty acid profiles constitute a good discrimination parameter and can be used with no ambiguity to discriminate the olive oils arising from each of the cultivars under study.

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Tables

Table 1. Fatty acids profile of the studied samples

	Cultivar		
Fatty acids	Cobrançosa	Madural	Verdeal
C_{14}	0,005±0,005	0,008±0,015	0,002±0,002
C_{16}	9,629±0,088	10,282±0,148∗	9,666±0,191
$C_{16:1t}$	0,029±0,008	0,027±0,008	0,029±0,010
$C_{16:1c}$	0,413±0,024∗	0,248±0,018∗	0,298±0,031∗
C_{17}	0,097±0,014	0,024±0,008	0,091±0,025
$C_{17:1}$	0,219±0,010	0,073±0,006	0,212±0,031
C_{18}	4,988±0,095∗	2,378±0,067∗	3,211±0,157∗
$C_{18:1t}$	0,006±0,005	0,004±0,004	0,005±0,015
$C_{18:1c}$	76,377±0,342∗	71,595±0,386∗	80,982±0,418∗
$C_{18:2ct}$	0,003±0,002	0,007±0,006	0,001±0,001
$C_{18:2cc}$	6,192±0,553∗	13,102±0,235∗	3,450±0,281∗
C_{20}	0,460±0,017	0,275±0,009∗	0,448±0,021
$C_{18:3c}$	0,676±0,041∗	0,780±0,044∗	0,580±0,045∗
$C_{20:1}$	0,155±0,020	0,218±0,030	0,178±0,029
C_{22}	0,095±0,017	0,080±0,010	0,116±0,017
C_{24}	0,561±0,113	0,851±0,256	0,703±0,112

∗ Means differ significantly at 1% level based on Tukey test.

Table 2. The seven most important fatty acids for discrimination between cultivar groups

	Wilks` Lambda	Partial Lambda	F-remove (2,115)	p-level	Toler.	1-Toler. (R-Sqr.)
$C_{18:2cc}$	0,000026	0,465370	66,05768	0,000000	0,085994	0,914006
C_{18}	0,000016	0,758064	18,35114	0,000000	0,540942	0,459058
$C_{16:1c}$	0,000013	0,896745	6,62080	0,001899	0,318693	0,681307
C_{17}	0,000014	0,880784	7,78273	0,000676	0,076550	0,923450
$C_{18:3}$	0,000014	0,854550	9,78688	0,000119	0,256878	0,743122
$C_{17:1}$	0,000012	0,977094	1,34796	0,263843	0,165534	0,834466
C_{181c}	0,000015	0,777173	16,48607	0,000001	0,087045	0,912955

Figures

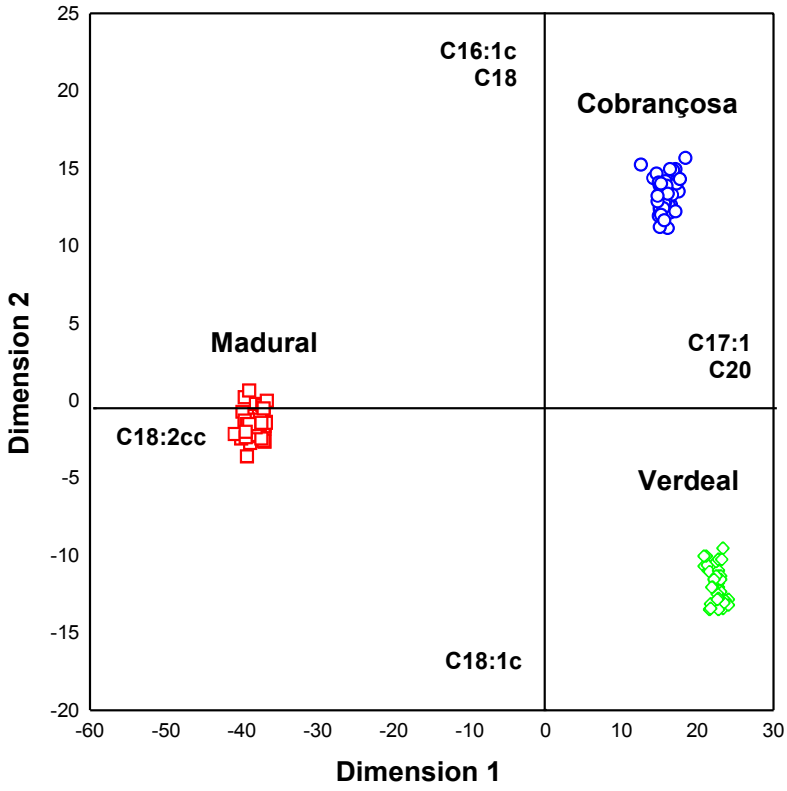

Fig. 1. Discriminant analysis of olive oils by Cvs. Cobrançosa, Madural and Verdeal based on the fatty acid profile. Main fatty acids for discrimination are indicated at the edges of each dimension.