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## Using lipid profiles and genotypes for the characterization of Corsican olive oils

In 2004, the Corsican producers of olive oils obtained a French protected designation of origin (PDO) “huile d’olive de Corse”, but up to now specifications of Corsican oil production do not clearly indicate the oil attributes related to the territory of production. That is why the fatty acid and triacylglycerol (TAG) compositions of olive oils from the nine main cultivars used to produce oils under PDO were determined and related to the olive variety. The results showed (i) that the nine cultivars covered only four olive varieties, as revealed by random-amplified polymorphic DNA markers, (ii) that the lipid composition of oils is strongly dependent on the variety, and (iii) that the lipid composition of the four varieties is completely discriminated on the basis of the proportions of four TAG (OOO, OOL, PoOO, OOL) and one fatty acid (18:0). These results clearly establish the relationships between some characteristics of oils and the area of production (Corsica) for at least three varieties that are originated from Corsica. For the fourth variety, other investigations on minor compounds and on sensory attributes of oils must be undertaken to link some oil traits to the territory of production.

**Keywords:** Olive oil, genotype, fatty acid, triacylglycerol, PDO.

### 1 Introduction

The protected designation of origin (PDO) (Council Regulation, EEC-N.2081/92) is a label of quality attractive to producers and consumers. Indeed, PDO offers to producers a protection against an unfair use of the protected designation of products covered by the registration and ensures the producers a significant added value. It also assures consumers of the geographical origin of the product. The PDO regulation compels the producers to describe in the specifications the quality attributes of the product related to the area of production and/or the practices of the producers.

In 2004, the Corsican producers of olive oils obtained a French PDO “huile d’olive de Corse” (Journal Officiel de la République Française of November 28, 2004). Up to now, specifications of Corsican oil production do not clearly indicate the oil attributes related to the territory of production (Corsica Island). However, it is not simple to establish this relationship because many parameters affect olive oil composition, such as the climate and soil [1–3], agronomic practices [3, 4], or olive tree variety [5–7]. Many works showed that fatty acid and triacylglycerol (TAG) compositions, the main components of olive oil, strongly depend on the olive variety [7–12]. In a previous

paper, we have established that many Corsican cultivars used to produce olive oils under PDO are from local varieties since they have been selected from local oleasters [13]. On the contrary, very little is known on the lipid composition of Corsican olive oils. Consequently, to determinate the fatty acid and TAG compositions of Corsican olive oils as related to their olive cultivars could be an interesting way to establish a link between some oil characteristics and the area of production.

The aim of this study was to establish a relationship between some lipid composition characteristics of oils produced from the nine main Corsican cultivars and the olive variety. The variety of the nine cultivars was identified using the random-amplified polymorphic DNA (RAPD) technique. The fatty acid and TAG compositions of oils were determined by gas chromatography and HPLC, respectively.

### 2. Materials and methods

#### 2.1 Plant material

##### 2.1.1 Cultivars

The present work was performed on the nine main olive cultivars used to produce olive oil under PDO in Corsica. Each cultivar was identified by phenotypic criteria. The name allocated to each cultivar by the producers is closely related to the sub-area of production. These cultivars are:

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Aliva bianca, Aliva nera, Biancaghja, Bonifacio, Capanacce, Ghjermana, Raspuluta, Sabina and Zinzala. The main traits of the climate (temperatures and precipitations) of each sub-area of production are shown in Tab. 1.

### 2.1.2 Oil extraction

The olives were picked by hand from nine to ten trees per cultivar. Approximately 1 kg of only healthy fruits was collected from each tree at the purple stage. Then, the olives were placed in bags and were preserved at  $-20\text{ }^{\circ}\text{C}$  prior to oil extraction carried out using a laboratory oil mill. This equipment enabled the oil extraction from small quantities of olives while respecting the various stages of the traditional process (crushing, mixing, centrifugation and decantation). The olives were defrosted, washed and then crushed using a hammer crusher. The olive paste was mixed for 30 min at  $30\text{ }^{\circ}\text{C}$  and the oil was extracted by two-phase centrifugation for 3 min to separate the liquid phase (oil and olive mill wastewaters) from the husk. The oil was separated from the olive mill wastewaters by decanting. After filtration, the oils were stored in dark glass bottles at  $4\text{ }^{\circ}\text{C}$  until the analyses were performed.

Acidity value, peroxide index and UV absorbance at 232 and 270 nm were determined following the analytical methods described in Regulation EEC/2568/91 of the European Union Commission. All sample parameters were within the levels accepted for extra-virgin olive oils (Regulations EEC/2568/91 and EEC/2472/97), which is due to the high quality of the olive fruits.

### 2.2 Fatty acid composition determination

Fatty acid compositions of the olive oils were determined by gas chromatography. The methyl esters were

prepared following the method of Morrison and Smith [14]. The gas chromatograph was equipped with a flame ionization detector and a split injector (PE 8500; Perkin Elmer). The methyl esters were separated on a capillary column (30 m long, 0.32 mm i.d., 0.25  $\mu\text{m}$  film thickness) containing a polar stationary phase (DB 225 J & W). The flow rate of the carrier gas (helium) was 2 mL/min. The detector temperature was set at  $250\text{ }^{\circ}\text{C}$ . The oven temperature was held at  $150\text{ }^{\circ}\text{C}$  for 3 min, increased to  $190\text{ }^{\circ}\text{C}$  at  $10\text{ }^{\circ}\text{C}/\text{min}$ , held at  $190\text{ }^{\circ}\text{C}$  for 10 min, increased from 190 to  $210\text{ }^{\circ}\text{C}$  at  $10\text{ }^{\circ}\text{C}/\text{min}$  and then maintained at  $210\text{ }^{\circ}\text{C}$  for 10 min. The individual methyl esters were identified by their retention times. The results were expressed as percentage of the total methyl esters.

### 2.3 TAG composition determination

TAG compositions were determined by reversed-phase HPLC using a Gilson binary system and a rheodyne valve injection system equipped with a 20- $\mu\text{L}$  loop. Two columns (25 cm long, 4.4 mm i.d.) packed with silica (5  $\mu\text{m}$ , Lichrospher 100, RP 18; Merck) and a precolumn (RP 18; Merck) were used. Of a solution of oil in acetonitrile (5 mg/mL), 20  $\mu\text{L}$  was injected. TAG were quantified with a light scattering detector (DDL, Sedex 55; Sedere) operating under the following conditions: evaporation temperature,  $43\text{ }^{\circ}\text{C}$ ; nebulizer gas pressure, 2 bar; auxiliary gas pressure, 0.5 bar. The main TAG were eluted in 60 min using a gradient mode. The eluents were acetonitrile and chloroform. The amount of chloroform increased from 30 to 45% in 60 min. The flow rate was 1 mL/min. Sixteen TAG were identified by comparison of their retention time vs. standards and by calculating the equivalent carbon numbers. The results were expressed in percentage of total TAG.

**Tab. 1.** Temperature, precipitation and altitude in the sub-areas of the production of virgin olive oil from the main Corsican cultivars.

| Cultivar     | Place                           | Altitude [m] | Mean temperature for one year [ $^{\circ}\text{C}$ ] | Mean precipitation for one year [mm] |
|--------------|---------------------------------|--------------|--|--------------------------------------|
| Aliva bianca | Coggia (South)                  | 450          | 12.2   | 83.7                                 |
| Biancaghja   | Santo Pietro di Tenda (North)   | 390          | 17.3   | 56.0                                 |
| Sabina       | Muro (North)                    | 380          | 16.3   | 44.2                                 |
| Aliva nera   | Sari d'Orcino (South)           | 360          | 15.9   | 46.5                                 |
| Ghjermana    | Sainte Lucie de Tallano (South) | 400          | 16.0   | 73.3                                 |
| Bonifacio    | Bonifacio (South)               | 60           | 16.9   | 39.3                                 |
| Zinzala      | Sollacaro (South)               | 460          | 15.9   | 54.5                                 |
| Capanacce    | Sisco (North)                   | 160          | 17.2   | 46.0                                 |
| Raspuluta    | Santo Pietro di Tenda (North)   | 390          | 17.3   | 56.0                                 |

## 2.4 Determination of the varieties of the nine cultivars

For each cultivar, branches were collected from nine or ten trees. They were stored at 4 °C no longer than 10 days. DNA was extracted from 5 g of young leaves following the CTAB method described by Saghai-Marouf *et al.* [15] modified by Bronzini de Caraffa *et al.* [16]. DNA concentration was determined using a spectrophotometer at 260 nm and then diluted to 10 ng/μL.

PCR amplifications were performed using a set of 11 deca-nucleotides [A01, A02, A09, A10, A16, A19, C09, C15, D03, O08, O15 (Eurogentec)]. The 25-μL PCR reaction mixtures contained: 75 mM Tris-HCl pH 8.8, 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.01% (vol/vol) Tween-20, 2.5 mM MgCl<sub>2</sub>, 50 μM each of dATP, dCTP, dGTP and dTTP, 40 ng primer, 30 ng DNA and 2 U Taq DNA polymerase (Eurogentec). PCR was performed using a TrioThermoblock (Biometra). The reaction mixtures were subjected to 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, 38 °C for 1 min, 72 °C for 1 min, and then a final 6 min of extension at 72 °C. Amplification products were analyzed by electrophoresis through 1.6% agarose (Eurogentec) followed by ethidium bromide staining. The molecular sizes of the fragments were estimated using a 1-kb DNA ladder (Gibco BRL). The reactions were repeated at least twice, and only reproducible, relatively intense bands were scored as present (1) or absent (0).

## 2.5 Statistical analysis

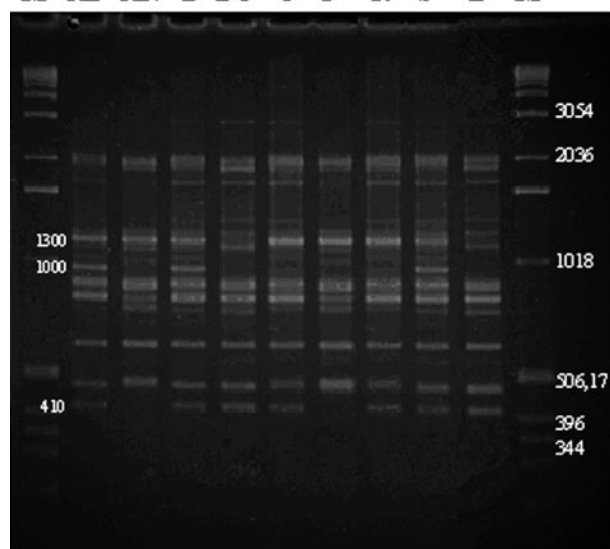
Fatty acid and TAG compositions were compared by a one-way variance analysis (factor: cultivar, nine modalities). Newman-Keuls's test was used to establish differences between means at 1% significance level ( $p = 0.01$ ), for all variables studied. A stepwise factorial discriminant analysis (FDA) was performed to identify the most discriminant variables to differentiate either cultivars or varieties. Calculations were performed using the Statgraphics software (version 3.0).

## 3 Results

### 3.1 Variety determination

The RAPD analysis revealed that the nine cultivars corresponded to only four varieties. Indeed, Capanacce and Raspuluta exhibited identical band patterns as well as Ghjermana and Aliva nera, Sabina, Aliva bianca and Biancaghja, Zinzala and Bonifacio. The amplification patterns obtained with the primer A2 are shown in Fig. 1. Hence, it is concluded that they are synonymous because

M AB AN B BO C G R S Z M



**Fig. 1.** RAPD products from the nine Corsican cultivars using primer A2. AB = Aliva bianca; AN = Aliva nera; B = Biancaghja; BO = Bonifacio; C = Capanacce; G = Ghjermana; R = Raspuluta; S = Sabina; Z = Zinzala; M = 1-kb DNA ladder. AB, B and S generated a 1000-bp specific band; AN and G are characterized by the absence of the 410-bp band; BO and Z are characterized by the absence of the 1300-bp band; C and R patterns show simultaneously the 1300 and 410-bp bands and the absence of the 1000-bp band.

the probability is very low that two different varieties give identical amplification profiles [16]. In the rest of the text, the synonymous cultivars are named by the more common appellation: Capanacce (synonymous to Raspuluta), Ghjermana (synonymous to Aliva nera), Sabina (synonymous to Aliva bianca, Biancaghja), and Zinzala (synonymous to Bonifacio).

### 3.2 Fatty acid composition

The nine olive oils showed a typical fatty acid composition with a high proportion of monounsaturated fatty acids (MUFA) (70.9–80.7%) and a low proportion of polyunsaturated fatty acids (PUFA) (4.5–12.4%) (Tab. 2). The major fatty acids are oleic acid (18:1) (69.9–78.9%), palmitic acid (16:0) (8.7–16.1%) and linoleic acid (18:2 *n*-6) (4.0–11.5%).

The fatty acid compositions of the oils strongly depended on the cultivar. Thus, this factor explained between 61.3 and 87.6% of the total variance of the samples. Our results showed that the fatty acid compositions of Capanacce and Raspuluta were similar. They differed from the other oils by their higher proportion of oleic acid (18:1)

**Tab. 2.** Fatty acid compositions of olive oils from the main Corsican cultivars.

| Fatty acids            | Cultivars                     |                             |                         |                            |                            |                            |                          |                            |                            | <i>p</i> value | Part of the variance explained by cultivars [%] |
|------------------------|-------------------------------|-----------------------------|-------------------------|----------------------------|----------------------------|----------------------------|--------------------------|----------------------------|----------------------------|----------------|---|
|                        | Aliva bianca<br><i>n</i> = 10 | Biancaghja<br><i>n</i> = 10 | Sabina<br><i>n</i> = 10 | Aliva nera<br><i>n</i> = 9 | Ghjermana<br><i>n</i> = 10 | Bonifacio<br><i>n</i> = 10 | Zinzala<br><i>n</i> = 10 | Capanacce<br><i>n</i> = 10 | Raspuluta<br><i>n</i> = 10 |                |   |
| 16:0                   | 11.5 <sup>de</sup>            | 10.7 <sup>e</sup>           | 8.7 <sup>f</sup>        | 14.7 <sup>ab</sup>         | 12.7 <sup>cd</sup>         | 16.1 <sup>a</sup>          | 14.0 <sup>bc</sup>       | 12.9 <sup>cd</sup>         | 12.6 <sup>cd</sup>         | ***            | 64.2  |
| 18:0                   | 3.2 <sup>a</sup>              | 3.2 <sup>a</sup>            | 3.4 <sup>a</sup>        | 1.7 <sup>c</sup>           | 1.9 <sup>c</sup>           | 2.8 <sup>b</sup>           | 3.4 <sup>a</sup>         | 1.6 <sup>c</sup>           | 1.9 <sup>c</sup>           | ***            | 84.3  |
| 20:0                   | 0.4                           | 0.3                         | 0.4                     | 0.3                        | 0.3                        | 0.4                        | 0.4                      | 0.3                        | 0.3                        | ns             | 50.0  |
| <b>Saturated</b>       | <b>15.1<sup>cd</sup></b>      | <b>14.2<sup>d</sup></b>     | <b>12.5<sup>e</sup></b> | <b>16.7<sup>bc</sup></b>   | <b>14.9<sup>cd</sup></b>   | <b>19.3<sup>a</sup></b>    | <b>17.8<sup>b</sup></b>  | <b>14.8<sup>cd</sup></b>   | <b>14.8<sup>cd</sup></b>   | <b>***</b>     | <b>62.3</b>                                     |
| 16:1                   | 0.6 <sup>cd</sup>             | 0.6 <sup>cd</sup>           | 0.6 <sup>cd</sup>       | 0.8 <sup>c</sup>           | 0.5 <sup>d</sup>           | 1.1 <sup>b</sup>           | 0.8 <sup>c</sup>         | 1.5 <sup>a</sup>           | 1.5 <sup>a</sup>           | ***            | 84.2  |
| 18:1                   | 72.8 <sup>c</sup>             | 74.0 <sup>c</sup>           | 74.3 <sup>c</sup>       | 74.1 <sup>c</sup>          | 77.0 <sup>b</sup>          | 71.1 <sup>d</sup>          | 69.9 <sup>d</sup>        | 78.9 <sup>a</sup>          | 78.8 <sup>a</sup>          | ***            | 80.0  |
| 20:1                   | 0.2                           | 0.3                         | 0.2                     | 0.3                        | 0.3                        | 0.2                        | 0.2                      | 0.3                        | 0.2                        | ns             | 47.1  |
| <b>Monounsaturated</b> | <b>73.6<sup>cd</sup></b>      | <b>74.9<sup>c</sup></b>     | <b>75.1<sup>c</sup></b> | <b>75.1<sup>c</sup></b>    | <b>77.8<sup>b</sup></b>    | <b>72.4<sup>d</sup></b>    | <b>70.9<sup>e</sup></b>  | <b>80.7<sup>a</sup></b>    | <b>80.5<sup>a</sup></b>    | <b>***</b>     | <b>81.9</b>                                     |
| 18:2 <i>n</i> -6       | 10.4 <sup>ab</sup>            | 10.1 <sup>b</sup>           | 11.5 <sup>a</sup>       | 7.5 <sup>c</sup>           | 6.7 <sup>c</sup>           | 7.6 <sup>c</sup>           | 10.6 <sup>ab</sup>       | 4.0 <sup>d</sup>           | 4.2 <sup>d</sup>           | ***            | 87.6  |
| 18:3 <i>n</i> -3       | 0.9 <sup>a</sup>              | 0.8 <sup>a</sup>            | 0.9 <sup>a</sup>        | 0.7 <sup>b</sup>           | 0.6 <sup>bc</sup>          | 0.7 <sup>b</sup>           | 0.7 <sup>b</sup>         | 0.5 <sup>c</sup>           | 0.5 <sup>c</sup>           | ***            | 61.3  |
| <b>Polyunsaturated</b> | <b>11.3<sup>ab</sup></b>      | <b>10.9<sup>b</sup></b>     | <b>12.4<sup>a</sup></b> | <b>8.2<sup>c</sup></b>     | <b>7.3<sup>c</sup></b>     | <b>8.3<sup>c</sup></b>     | <b>11.3<sup>ab</sup></b> | <b>4.5<sup>d</sup></b>     | <b>4.7<sup>d</sup></b>     | <b>***</b>     | <b>87.4</b>                                     |

In the same row, means with different letters differ significantly (\*\*\*)  $p < 0.001$ .  
ns: not significant.

(78.8–78.9%) and their lower proportion of PUFA (4.5–4.7%), mainly linoleic acid (18:2 *n*-6) (4.0–4.2%). Aliva bianca, Biancaghja and Sabina oils showed similar fatty acid compositions characterized by the highest proportion of PUFA (10.9–12.4%). In contrast, Bonifacio and Zinzala oils exhibited the highest proportion of saturated fatty acids (17.8–19.3%) and the lowest proportion of MUFA (70.9–72.4%), especially oleic acid (18:1) (69.9–71.1%).

However, some differences were observed between the oils of cultivars from the same variety. Thus, Sabina exhibited a lower palmitic acid proportion than Aliva bianca and Biancaghja (12.5% vs. 15.1 and 14.2%) although the olive cultivars belonged to the same variety. Similarly, the PUFA proportion in Zinzala oil was higher than in Bonifacio oil (11.3% vs. 8.3%), and close to Aliva bianca, Biancaghja and Sabina oils.

### 3.3 TAG composition

TAG compositions of the nine oils showed a high proportion of triunsaturated TAG (51.6–64.7%) and diunsaturated TAG (20.5–33.3%) and large variation in the proportions of polyunsaturated TAG (5.7–20.9%) (Tab. 3). The major TAG were triolein (OOO) (46–62.2%), dioleo-palmitin (POO) (16–29.3%) and dioleo-linolein (OOL) (4.9–18.1%). These data are consistent with the high proportion of oleic acid in olive oils.

The TAG composition was strongly related to the cultivar, which explained from 70.8% up to 92.4% of the variability of the proportion of individual TAG. Capanacce and Raspuluta had a similar TAG composition characterized by the highest proportion of triunsaturated TAG (64.7%), particularly of triolein (OOO) (62.1–62.2%). In contrast, they exhibited the lowest proportion of dioleo-linolein (OOL) (4.9–5.3%) and of dilinoleo-olein (OLL) (0.2%). Aliva bianca, Biancaghja and Sabina showed very similar TAG compositions with the highest proportion of dioleo-linolein (OOL) (15.5–18.1%) and of dilinoleo-olein (OLL) (1.2–1.7%) and the lowest proportions of monounsaturated and diunsaturated TAG (1.4–1.7% and 20.5–23.2%, respectively). Bonifacio and Zinzala showed the lowest proportion of triunsaturated TAG (51.6–51.7%), particularly of triolein (OOO) (46.0–47.1%). However, some differences were revealed in their TAG profile. Thus, Bonifacio had higher proportions of monounsaturated and diunsaturated TAG (3.6 and 33.3%, respectively) while it had lower proportions of palmito-oleo-linolein (POL) (3.9%), dioleo-linolein (OOL) (14.9%) and dilinoleo-olein (OLL) (1.6%) than Zinzala, although they are from the same variety.

### 3.4 Discrimination of oils according to the cultivar and the variety

Stepwise FDA was carried out from the six main fatty acids (16:0, 18:0, 16:1, 18:1, 18:2 and 18:3) and the ten main TAG (PPO, PStO, StOO, POO, OOO, POL, PoOO,

**Tab. 3.** TAG compositions of olive oils from the main Corsican cultivars.

| TAG                    | Cultivars                |                         |                         |                         |                         |                         |                         |                         |                         |            | p value     | Part of the variance explained by cultivars [%] |
|------------------------|--------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|------------|-------------|---|
|                        | Aliva bianca<br>n = 10   | Biancaghja<br>n = 10    | Sabina<br>n = 10        | Aliva nera<br>n = 9     | Ghjermana<br>n = 10     | Bonifacio<br>n = 10     | Zinzala<br>n = 10       | Capanacce<br>n = 10     | Raspuluta<br>n = 10     |            |             |   |
| PPSt                   | 0.2                      | 0.2                     | 0.3                     | 0.2                     | 0.2                     | 0.3                     | 0.3                     | 0.3                     | 0.3                     | ns         | 37.2        |   |
| <b>Trisaturated</b>    | <b>0.2</b>               | <b>0.2</b>              | <b>0.3</b>              | <b>0.2</b>              | <b>0.2</b>              | <b>0.3</b>              | <b>0.3</b>              | <b>0.3</b>              | <b>0.3</b>              | <b>ns</b>  | <b>37.2</b> |   |
| PPO                    | 1.2 <sup>e</sup>         | 1.2 <sup>e</sup>        | 0.9 <sup>f</sup>        | 2.5 <sup>b</sup>        | 2.0 <sup>c</sup>        | 2.8 <sup>a</sup>        | 1.8 <sup>d</sup>        | 1.8 <sup>d</sup>        | 1.7 <sup>d</sup>        | ***        | 91.0        |   |
| PStO                   | 0.4 <sup>c</sup>         | 0.4 <sup>c</sup>        | 0.4 <sup>cd</sup>       | 0.3 <sup>d</sup>        | 0.3 <sup>cd</sup>       | 0.7 <sup>a</sup>        | 0.6 <sup>b</sup>        | 0.2 <sup>e</sup>        | 0.3 <sup>d</sup>        | ***        | 80.1        |   |
| StStO                  | 0.1                      | 0.1                     | 0.1                     | 0.1                     | 0.1                     | 0.1                     | 0.1                     | 0.1                     | 0.1                     | ns         | 45.2        |   |
| <b>Monounsaturated</b> | <b>1.7<sup>e</sup></b>   | <b>1.7<sup>e</sup></b>  | <b>1.4<sup>f</sup></b>  | <b>2.9<sup>b</sup></b>  | <b>2.4<sup>c</sup></b>  | <b>3.6<sup>a</sup></b>  | <b>2.5<sup>c</sup></b>  | <b>2.1<sup>d</sup></b>  | <b>2.1<sup>d</sup></b>  | <b>***</b> | <b>89.9</b> |   |
| StOO                   | 3.9 <sup>ab</sup>        | 4.1 <sup>ab</sup>       | 4.4 <sup>a</sup>        | 1.9 <sup>c</sup>        | 2.3 <sup>c</sup>        | 3.6 <sup>b</sup>        | 4.2 <sup>ab</sup>       | 2.0 <sup>c</sup>        | 2.5 <sup>c</sup>        | ***        | 73.7        |   |
| POO                    | 19.0 <sup>d</sup>        | 18.9 <sup>d</sup>       | 16.0 <sup>e</sup>       | 27.0 <sup>b</sup>       | 24.9 <sup>c</sup>       | 29.3 <sup>a</sup>       | 23.5 <sup>c</sup>       | 24.9 <sup>c</sup>       | 24.1 <sup>c</sup>       | ***        | 92.4        |   |
| PPoO                   | 0.1                      | 0.1                     | Traces                  | 0.2                     | 0.1                     | 0.2                     | 0.1                     | 0.3                     | 0.3                     | ns         | 81.5        |   |
| PPL                    | 0.2                      | 0.1                     | 0.1                     | 0.2                     | 0.1                     | 0.2                     | 0.2                     | traces                  | 0.1                     | ns         | 69.1        |   |
| <b>Diunsaturated</b>   | <b>23.2<sup>d</sup></b>  | <b>23.2<sup>d</sup></b> | <b>20.5<sup>e</sup></b> | <b>29.3<sup>b</sup></b> | <b>27.4<sup>c</sup></b> | <b>33.3<sup>a</sup></b> | <b>28.0<sup>c</sup></b> | <b>27.2<sup>c</sup></b> | <b>27.0<sup>c</sup></b> | <b>***</b> | <b>90.3</b> |   |
| OOO                    | 51.4 <sup>c</sup>        | 53.1 <sup>c</sup>       | 52.9 <sup>c</sup>       | 51.2 <sup>c</sup>       | 56.8 <sup>b</sup>       | 47.1 <sup>d</sup>       | 46.0 <sup>d</sup>       | 62.1 <sup>a</sup>       | 62.2 <sup>a</sup>       | ***        | 90.3        |   |
| POL                    | 4.2 <sup>b</sup>         | 3.8 <sup>b</sup>        | 3.8 <sup>b</sup>        | 4.0 <sup>b</sup>        | 2.8 <sup>c</sup>        | 3.9 <sup>b</sup>        | 5.3 <sup>a</sup>        | 1.5 <sup>d</sup>        | 1.5 <sup>d</sup>        | ***        | 84.0        |   |
| PoOO                   | 0.2 <sup>d</sup>         | 0.3 <sup>d</sup>        | 0.2 <sup>d</sup>        | 0.5 <sup>c</sup>        | 0.2 <sup>d</sup>        | 0.6 <sup>b</sup>        | 0.4 <sup>c</sup>        | 1.1 <sup>a</sup>        | 1.0 <sup>a</sup>        | ***        | 84.5        |   |
| <b>Triunsaturated</b>  | <b>55.8<sup>c</sup></b>  | <b>57.2<sup>c</sup></b> | <b>56.9<sup>c</sup></b> | <b>55.7<sup>c</sup></b> | <b>59.8<sup>b</sup></b> | <b>51.6<sup>e</sup></b> | <b>51.7<sup>e</sup></b> | <b>64.7<sup>a</sup></b> | <b>64.7<sup>a</sup></b> | <b>***</b> | <b>89.9</b> |   |
| OOL                    | 16.5 <sup>b</sup>        | 15.5 <sup>b</sup>       | 18.1 <sup>a</sup>       | 10.5 <sup>c</sup>       | 9.3 <sup>c</sup>        | 9.8 <sup>c</sup>        | 14.9 <sup>b</sup>       | 4.9 <sup>d</sup>        | 5.3 <sup>d</sup>        | ***        | 89.0        |   |
| OLL                    | 1.6 <sup>ab</sup>        | 1.2 <sup>b</sup>        | 1.7 <sup>a</sup>        | 0.6 <sup>c</sup>        | 0.4 <sup>c</sup>        | 0.6 <sup>c</sup>        | 1.6 <sup>ab</sup>       | 0.2 <sup>c</sup>        | 0.2 <sup>c</sup>        | ***        | 72.9        |   |
| OOLn                   | 0.6 <sup>b</sup>         | 0.6 <sup>b</sup>        | 0.7 <sup>a</sup>        | 0.5 <sup>c</sup>        | 0.3 <sup>d</sup>        | 0.5 <sup>c</sup>        | 0.5 <sup>c</sup>        | 0.4 <sup>d</sup>        | 0.3 <sup>d</sup>        | ***        | 70.8        |   |
| PLL                    | 0.2                      | 0.2                     | 0.2                     | 0.1                     | 0.1                     | 0.1                     | 0.3                     | 0.1                     | traces                  | ns         | 66.1        |   |
| POLn                   | 0.2                      | 0.2                     | 0.2                     | 0.2                     | 0.1                     | 0.2                     | 0.2                     | 0.1                     | 0.1                     | ns         | 59.5        |   |
| <b>Polyunsaturated</b> | <b>19.1<sup>ab</sup></b> | <b>17.7<sup>b</sup></b> | <b>20.9<sup>a</sup></b> | <b>11.9<sup>c</sup></b> | <b>10.2<sup>c</sup></b> | <b>11.2<sup>c</sup></b> | <b>17.5<sup>b</sup></b> | <b>5.7<sup>d</sup></b>  | <b>5.9<sup>d</sup></b>  | <b>***</b> | <b>87.3</b> |   |

In the same row, means with different letters differ significantly (\*\**p* < 0.001).

ns: not significant.

O, oleic acid; P, palmitic acid; St, stearic acid; L, linoleic acid; Ln, linolenic acid.

OOL, OLL and OOLn). The discrimination of the nine cultivars was based on the proportions of five components, which are from the most to the least discriminating variables: POO (*F* = 121.3), OOO (*F* = 72.4), 16:1 (*F* = 34.5), 18:0 (*F* = 26.3), POL (*F* = 10.4). So, 75 of the oil samples (84.3%) were correctly classified in their cultivar of origin (Fig. 2). Fourteen samples were not classified in their cultivar but in another cultivar of the same variety ■ pls check■. Thus, two samples of Aliva bianca were classified in Biancaghja and one in Sabina, two samples of Biancaghja were classified in Aliva bianca and two in Sabina, one Sabina was classified in Biancaghja, one Ghjermana was classified in Aliva nera, two samples of Capanacce were classified in Raspuluta and two Raspuluta in Capanacce, and one Zinzala was classified in Bonifacio.

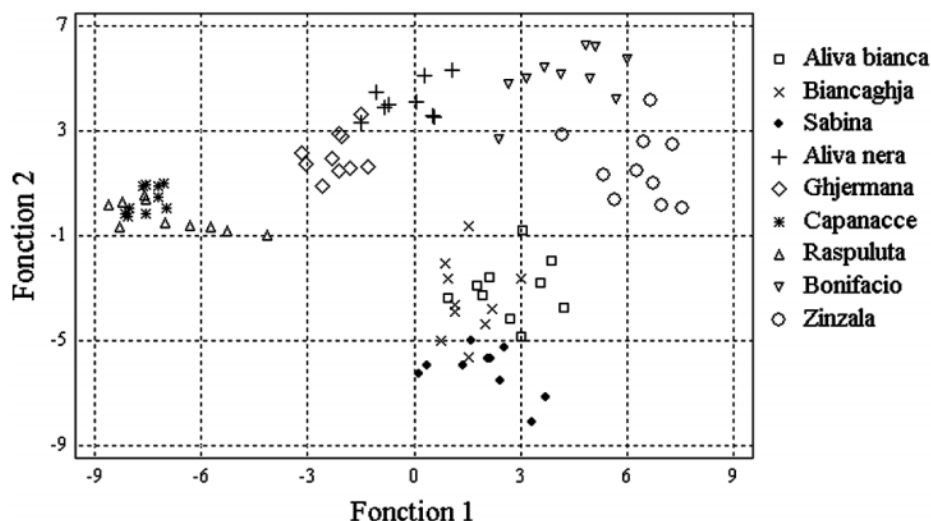
These results led us to carry out a new discriminating factorial analysis on the basis of the four varieties. This analysis showed that 100% of the samples were classified in the group corresponding to their variety (Fig. 3). The discrimination of the four varieties was based on the

proportions of five components, which are from the most to the least discriminating variables: OOO (*F* = 131.6), OOL (*F* = 104.5), PoOO (*F* = 76.1), 18:0 (*F* = 62.1) and OLL (*F* = 25.9). In order to validate the model, the same discriminating functions were used to classify 24 commercial oils produced in Corsica (six from each variety). The results showed that the model had a good predictive ability to classify Corsican oils in the four varieties since the classification was correct for 21 samples out of the 24 (87.5%). The misclassified oils were two samples of Zinzala classified in Sabina and one sample of Ghjermana classified in Zinzala.

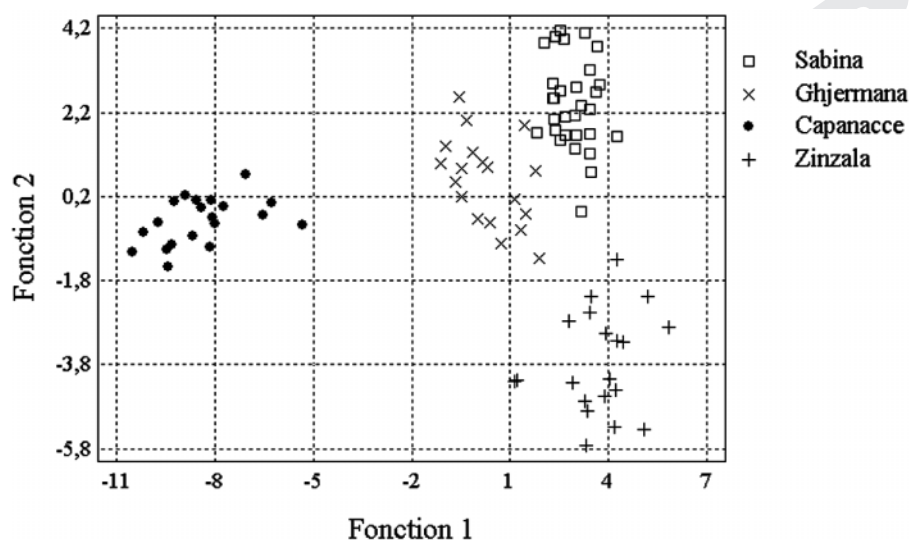
## 4 Discussion

### 4.1 The nine cultivars of olive trees cover only four varieties

The nine cultivars used to produce the Corsican olive oils corresponded to only four varieties, as supported by



**Fig. 2.** Representation of oil samples from the nine cultivars in the plan of the first discriminant functions 1 and 2.



**Fig. 3.** Representation of oil samples from the four varieties in the plan of the first two discriminant functions 1 and 2.

RAPD analysis. This technique is an efficient method to identify the olive varieties since the probability that two varieties show the same RAPD profile is very low [16]. The existence of several synonymous cultivars within each variety is explained by the attribution of cultivar names by producers several decades ago, in the sub-areas of production, only on the basis of phenotypic traits.

#### 4.2 The lipid characteristics of oils are strongly related to the olive variety

The fatty acid and TAG compositions of oils are strongly dependent on the variety. This conclusion is supported by two results:

(1) Oils of cultivars of the same variety had very close compositions in fatty acids and TAG, even when they had been produced in different geographical areas (Tabs. 2, 3).

(2) Oils are completely discriminated on the basis of variety, as is shown by discriminating analysis (Fig. 3).

This close relationship between the variety and the composition of oils is in accordance with other studies [5, 10, 17, 18]. It was based mainly on the TAG since four of the five discriminating variables are TAG (OOO, OOL, PoOO, and OLL) of which two are very discriminating (OOO, OOL). These results are in agreement with those of previous studies [7, 8, 11, 12]. They are the consequence of the greatest variability in TAG composition compared to

that of the fatty acid composition. Stearic acid was the only discriminating fatty acid. The discriminating character of this fatty acid was already shown with Greek olive oils [6]. On the basis of these results, it appears that the lipid composition could be a good tool to determine the genetic origin of an olive oil.

The close relationship between some TAG and fatty acid proportions in olive oils and the olive variety strongly suggested that these differences result from the level of expression of the genes coding for the enzymes involved in the synthesis of these molecules, and also from the enzymatic activity levels. Many enzymes are implied in the synthesis of the fatty acids and TAG [19, 20]. To date, there exists very little information on the genetic variability of these enzymes in olives. However, it can be expected that the differences in oil compositions between varieties are related to differences in the activity of acyl desaturases. These enzymes introduce double bonds into fatty acids in the ACP-bound form or when they are bound to glycerol [21]. It was shown that the composition in TAG is strongly correlated to that of fatty acids. Thus, the proportion of a given TAG in olive oil shows a strong correlation with the proportion of the fatty acid that is in the lowest proportion in the fatty acid composition of the oil. For instance, an  $R^2$  equal to 0.98 is found between the proportions of OOL and linoleic acid, which is in a lower proportion than oleic acid in olive oils (results not shown). This suggests that the difference in TAG compositions is a direct consequence of differences in fatty acid composition.

### 4.3 A weak influence of the environment

Even if the variety is the main factor of variation of the composition of Corsican olive oils, some differences were observed between oils extracted from cultivars of the same variety. The most significant difference was in the content of linoleic acid and the corresponding TAG (POL, OOL, OLL), which were significantly higher in Zinzala than in Bonifacio oils. Similar results were described previously [10]. These differences in fatty acid and TAG compositions within the same variety should be related to environmental factors that affect the lipid metabolism in plants. So, many works showed that the reduction in the average temperature of the climate, related or not to an increase in altitude, induces an increase in the unsaturation of fatty acids of oils, mainly in linoleic acid proportions [22–24]. This is generally explained by the fact that low temperatures promote the activity of acyl-desaturases [25–27]. However, in our study, oils from Zinzala contained more linoleic acid than those from Bonifacio while the average temperatures of the two areas of production

were similar (Tab. 1). Other environmental parameters could explain these differences in oil composition, such as the altitude (460 m vs. 60 m), the nature of the soil, or the proximity of the sea.

## 5 Conclusion

This study demonstrated that the nine main Corsican olive cultivars correspond to only four varieties. Three of them (Capanacce, Sabina and Zinzala) are local varieties, probably directly selected from local oleasters [13]. In contrast, the Ghjermana variety is synonymous to the Italian variety, Moraiolo. It was probably introduced into Corsica several centuries ago, during the Genoa domination [16]. For the first three varieties, the close correlation that exists between the lipid composition of the oils and the varieties makes it possible to establish an objective relationship between the area of production (Corsica) and some characteristics of the oils, as recommended by PDO regulation. For Ghjermana, the link between the area of production and the oil composition traits remains to be established because this variety is of Italian origin. Other investigations are required to settle this link. A possible way is to quantify minor compounds of oil (phenols, sterols, tocopherols) because several studies indicate that their contents are strongly correlated to environmental factors [22, 28, 29]. Another possibility would be to carry out sensory analyses of the oils, which are strongly related to the process used by the producers [30]. Thus, in Corsica, oils are produced from very ripe olives that lead to oils with a less pronounced bitter taste than that of other European oils.

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