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Olive leaf characterisation, chemical composition of olive oils from different origins, olive oil traceability

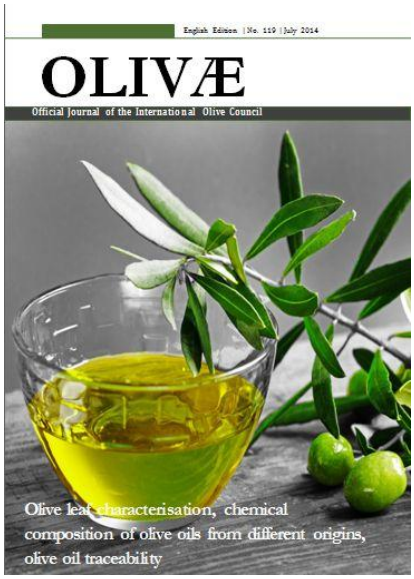

Table of Contents

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Príncipe de Vergara, 154
28002 Madrid, Spain
Tel.: 34-915 903 638
Fax: 34-915 631 263
E-mail: iooc@internationaloliveoil.org
Web: www.internationaloliveoil.org

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Major and trace element content of olive leaves

R. Alcázar Román, J. A. Amorós, C. Pérez de los Reyes, F. J. García Navarro and S. Bravo

Escuela de Ingenieros Agrónomos. Universidad de Castilla-La Mancha. Ronda de Calatrava, 7. 13071 Ciudad Real, Spain

ABSTRACT

The aim of this research was to analyse the major and trace element content of olive leaves and to study the changes in their concentration in leaves of differing ages. The resultant data were compared with data obtained for another woody plant crop (vine).
The chief novelty of this article is that it supplies data on the content of trace elements in olive leaves given that little has been published on this subject in world scientific literature.

Keywords: olive leaves, concentration, trace elements.

1. INTRODUCTION

More than 300 million olive trees are grown in Spain on almost 3,000,000 hectares of land (MAAMA, 2010). Castile-La Mancha is the second most important Autonomous Community - Andalusia is the first - in terms of total production and olive crop area. Olive research is therefore very important on account of the agricultural and economic significance of this crop.

Most known chemical elements are essential to plants and are divided into those needed in relatively large quantities (major elements or macronutrients) and those required in very small amounts (micronutrients and trace elements). These have sparked much interest in recent years because some are crucial plant micronutrients. Macronutrients tell us about the total content of structural and nutritional components in the soil (Wild, 1992; Lanyon *et al*., 2004; White, 2009). Trace elements are chemical components found in the soil at concentrations below 0.1% (1000 mg/kg) and provide information about the geochemical origin of the soil and possible toxicities (Conde *et al*., 2009). Parent rock is the main source of these elements but the way in which they are distributed in the soil profiles is influenced by several edaphogenetic processes. In addition, soil composition is reflected in the plant and in the products obtained from it, although the metabolism of the trace elements can differ in each soil–plant system. These elements are involved in key metabolic processes such as respiration, photosynthesis and fixation as well as in the assimilation of some macronutrients (for instance, N and S). Trace elements are absorbed chiefly through the roots although it has been observed that other tissue may have the capacity to absorb some nutrients and trace elements. In general, besides being influenced by the specific capacity of the plant, plant absorption of trace elements is affected by soil factors, the most significant of which are pH, redox potential, water regime, clay content, organic matter content, cation exchange capacity, nutrient balance and concentration of other trace elements and macronutrients. Climatic conditions can also influence the absorption rate of trace elements in that an increase in ambient temperature generally leads to higher absorption of these elements.

The bioavailability of trace elements from the atmosphere via the leaves can have a significant impact on plant contamination (Hg, Cd,…). It is also very important in foliar fertiliser application, especially of elements such as Fe, Mn, Zn and Cu. The trace elements absorbed by the leaves can be transported to the plant tissue, including the roots, where excess metals appear to be stored. The speed at which trace elements move between the tissues varies considerably, depending on the plant organ, plant age and the element concerned. In the case of leaves, leaf surface morphology is an important determinant of the foliar absorption of trace elements.

The literature on the macronutrient content of olive leaves includes some classic references such as the data reported by Freeman *et al*. (1994) or Barranco *et al*. (2008). However, there are few specific studies on the trace element content of olive leaves: Barranco *et al*. (2008) reported the changes in trace element content and interpreted the levels of some of those elements while the authors of this article have started to research into these elements in olive growing areas where mining is carried out (Higuera *et al*., 2012), albeit without differentiating between leaf age.

The objective of this research was to study the variation in the concentration of major and trace elements in olive leaves of differing ages in areas potentially affected by previous mining activity. The contents of these elements in olive leaves were compared with those determined in vine leaves in earlier research since vine is another woody plant crop of major importance in the same area.

2. MATERIALS AND METHODS

2.1. Research area and sampling

Samples were collected in seven different areas of the province of Ciudad Real in central Spain; lying within a 70-km radius of the Almadén mercury mine (see **Table 1**).

Table 1. Location of the sampling areas in the province of Ciudad Real (Spain) and distance from the Almadén mine

Zone	Description	Coordinates	Distance (km)
1	Almadén, Avenida de la Libertad	X: 340.054 Y: 4.293.486	0.433
2	Almadén, Carril del Norte	X: 340.075 Y: 4.293.871	0.380
3	Chillón, Polygon 1 Parcel 614	X: 337.813 Y: 4.294.755	2.052
4	Almadenejos, Calle Carretas	X: 351.507 Y: 4.289.240	12.628
5	Fontanosas (Abenojar) Polygon 37, Parcel 243	X: 366.656 Y: 4.291.811	27.012
6	Almodóvar del Campo, Polygon 71, Parcel 739	X: 390.909 Y: 4.285.415	51.870
7	Picón, Polygon 9, Parcel 69	X: 407.999 Y: 4.324.209	74.769

The data are part of a more detailed survey of mercury content and potential toxicity (Higueras *et al*., 2012). The general characteristics of the soils were as follows: acidic, salinity-free, predominantly sandy-loam texture, low carbonate and lime content and variable organic matter content.

The leaves were collected from four different olive trees in each zone. Sampling was conducted in the second week of May 2010 to take advantage of new growth and so facilitate the collection of one-year-old shoots. Between 30 and 35 shoots were taken at a height of 1.20 m from around each olive tree and it was tried to ensure that there were enough representative samples of each year. Hence, leaves of differing ages were collected: current year (Year 0), one-year-old (Year 1) and two-year-old (Year 2).

After the leaves were collected, they were taken to the laboratory where they were kept in the fridge until they were separated by age (**Figure 1**) from each shoot. Year 0 leaves were taken from the tip of the shoot; they were small, light green and displayed the specific, unequivocal characteristics of newly grown leaves. Year 1 leaves were taken from the central part of the shoot; they were darker green than the Year 0 leaves and medium-sized. Lastly, Year 2 leaves were taken from the part lying furthest away from the tip; they were larger and deeper green in colour than the rest of the sample.

(a) (b) (c)

Figure 1. Leaves of differing ages: (a) Year 0 leaves; (b) Year 1 leaves; (c) Year 2 leaves.

2.2. Method of analysis

All the samples were placed in an oven at a constant temperature of 36 °C for 10 days until all the moisture was removed. Then, with the aid of a grinder, they were crushed into a fine, uniform powder which was used to determine the concentration of major and trace elements on the basis of calcination losses and the X-ray fluorescence method.

The sample was calcined in a muffle furnace at 1100 °C for 5 hours at a heating ramp rate of 10 °C/min. Calcination losses indicate the percentage weight of the volatile elements that have to be taken into account when determining the contents of major and trace elements obtained by the fluorescence method.

A commercial spectrometer (Philips Magix Pro with a rhodium anode in the X-ray tube) capable of operating at a maximum power of 4kW was used for the X-ray fluorescence method. Quality control was evaluated by duplicate analysis of certified reference materials (BCR-62).

Microsoft Office Excel 2007 was used for statistical processing and graphs.

3. RESULTS AND DISCUSSION

3.1. Major elements

Table 2 reports the mean contents of major elements in the leaves. Comparison with the values cited in various bibliographical references (Freeman *et al*., 1994; Barranco *et al*., 2008) shows that most were present in suitable amounts.

Table 2. Mean content of major elements in leaves (g/kg)

Element	$\overline{X}_{year0}$	$\overline{X}_{year1}$	$\overline{X}_{year2}$	$\overline{X}_{leaf}$
Na	0.030	0.034	0.013	0.026
Mg	1.503	2.489	2.346	2.112
Al	0.546	0.449	0.444	0.480
Si	1.149	1.313	1.133	1.198
P	3.594	2.406	2.123	2.708
S	3,827	4.213	4.191	4.077
K	11.781	7.521	7.724	9.009
Ca	10.180	23.850	23.843	19.291
Mn	0.007	0.029	0.039	0.025
Fe	0.110	0.160	0.166	0.145
Cl	0.947	0.367	0.299	0.538

Comparison of these mean values with the values reported in the literature for the leaves of other woody plant crops such as vine (Amorós *et al*., 2011) helps to give a general perspective on the levels of these elements in olive leaves. The values for vine leaves are reported in **Table 3**.

As can be seen, Na, Mg, Si, Ca, Mn and Fe levels are higher in vine (0.075 g/kg, 4.420 g/kg, 8.200 g/kg, 27.502 g/kg, 0.102 g/kg and 7.336 g/kg, respectively) than in olive. Conversely, the mean values of P, S and K are lower in vine (1.793 g/kg, 2.215 g/kg and 0.555 g/kg) than in olive.

Table 3. Mean values of major and trace elements in vine leaves (Amorós *et al*., 2011)

Major element	$\overline{X}_{leaf}$ (g/kg)	Trace element	$\overline{X}_{leaf}$ (mg/kg)
Na	0.075	**V**	6.933
Mg	4.420	**Cr**	5.183
Al	0.553	**Co**	2.783
Si	8.200	**Ni**	2.050
P	1.793	**Zn**	15.617
S	2.215	**Rb**	3.917
K	0.555	**Sr**	133.550
Ca	27.502	**Nb**	4.250
Mn	0.102	**Cs**	5.450
Fe	7.336	**Ba**	39.483
		Ce	10.767
		Pb	3.550
		Nd	4.300

The values obtained for macronutrient accumulation in leaves per year (**Figure 2**) are similar to those reported in the specialist literature (Barranco *et al*., 2008). A higher concentration of Cl, P and K was observed in the younger leaves because these elements are mobile and move to the points of greatest metabolic intensity; conversely, while Mg is also a mobile element, its concentration is higher in old leaves.

Despite the fact that Mn, Fe, Ca and S are not very mobile (although the mobility of S does vary) they behave in the same way as magnesium. Calcium, for its part, is essential for cell wall formation. The concentrations of the rest of the elements remain practically constant in the leaves of differing ages.

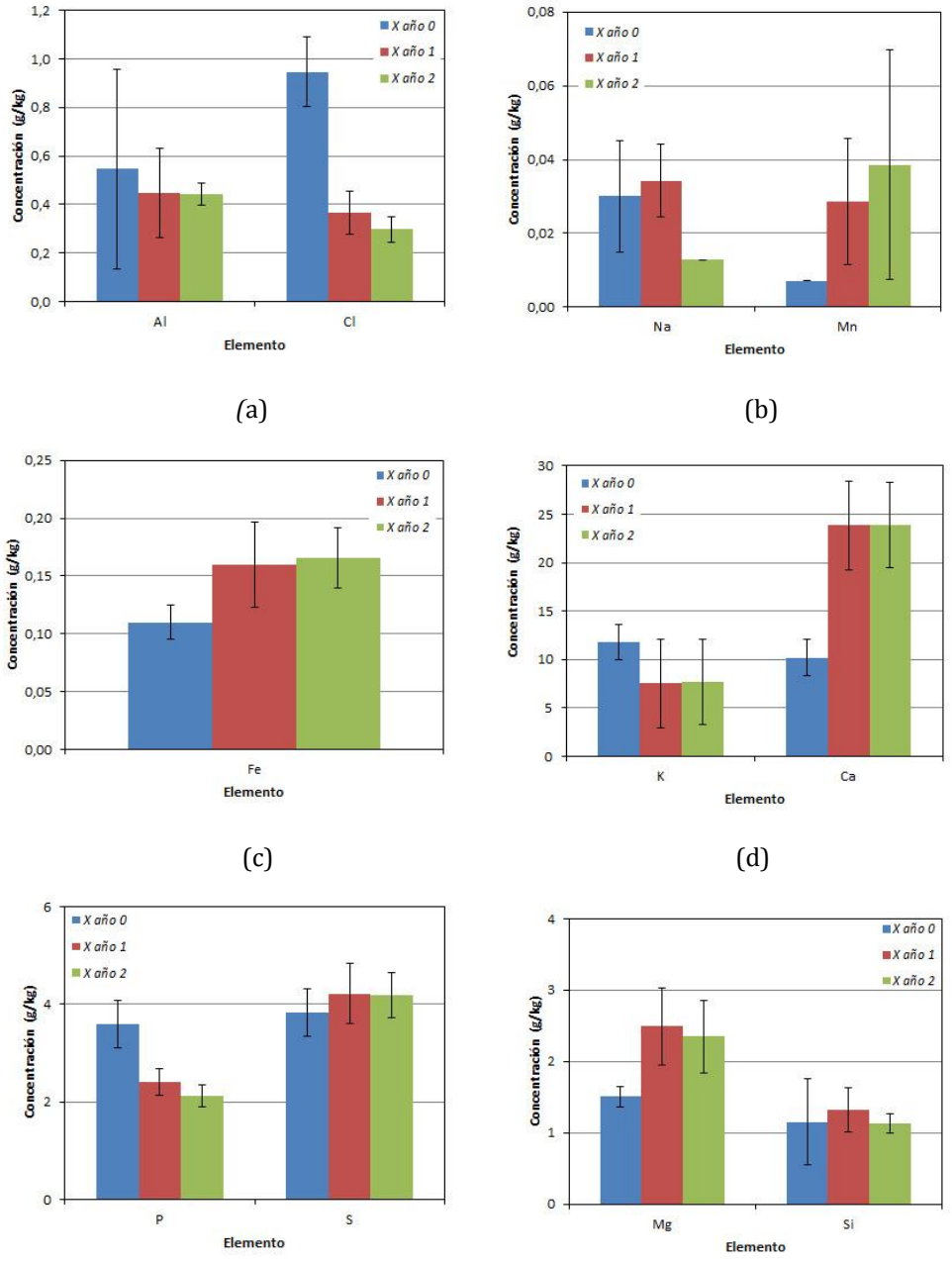

Figure 2. Mean content of major elements, expressed in g/kg, of leaves aged 0, 1 and 2 years: (a) Al and Cl, (b) Na and Mn, (c) Fe, (d) K and Ca, (e) P and S, (f) Mg and Si.

3.2. Trace elements

The mean content of trace elements in leaves is reported in Table 4 with the exception of Sc, Zr, W, Hf, Ga, Y, U and Hg, which lie outside the detection limits of the method employed. Since little has been published on trace element concentrations, the results were compared with those obtained for trace elements in vine leaves (**Table 3**). It can be seen that the levels of V, Cr, Nb and Ba are higher in vine (6.933 mg/kg, 5.183 mg/kg, 4.250 mg/kg and 39.483 mg/kg, respectively) than in olive and considerably higher in the case of Sr (133.550 mg/kg in vine versus 48.014 mg/kg in olive). In contrast, mean Ni and Zn values (2.050 mg/kg and 15.617 mg/kg, respectively) are lower in vine than in olive (3.205 mg/kg and 22.657 mg/kg, respectively). The rest of the elements lie at similar levels in both crops.

Table 4. Mean content of trace elements in leaves (mg/kg)

Element	$\overline{X}_{year\ 0}$	$\overline{X}_{year\ 1}$	$\overline{X}_{year\ 2}$	$\overline{X}_{leaf}$
V	3.829	3.686	4.029	3.848
Cr	5.514	4.571	4.486	4.857
Co	3.057	2.729	2.914	2.900
Ni	2.929	2.971	3.714	3.205
Cu	19.400	18.571	22.914	20.295
Zn	26.400	21.157	20.414	22.657
Rb	3.886	2.700	2.843	3.143
Sr	31.086	56.700	56.257	48.014
Nb	.3.629	3.300	3.357	3.429
Cs	6.371	5.414	6.157	5.981
Ba	32.000	42.443	41.357	38.600
La	1.486	0.757	1.214	1.152
Ce	11.314	10.386	11.086	10.929
Pb	3.686	3.800	3.800	3.762
Th	1.700	1.614	1.414	1.576
Nd	4.600	4.629	4.500	4.576

Figure 3 shows the results obtained for trace elements (mg/kg) by olive leaf age as well as the standard deviation except in the case of Cu, which is not reported because of the large variation caused by the plant health treatments applied in olive growing.

These results reflect a higher concentration of Cr, Zn, Rb and Th in young leaves; hence, it can be deduced that these elements are connected with plant metabolism. The concentration of Ni, Cu, Sr and Ba is higher in old leaves because they are difficult for the plant to excrete. The mobility of La and Ce varies in the leaves while the behaviour of the rest of the elements is practically constant over the three years.

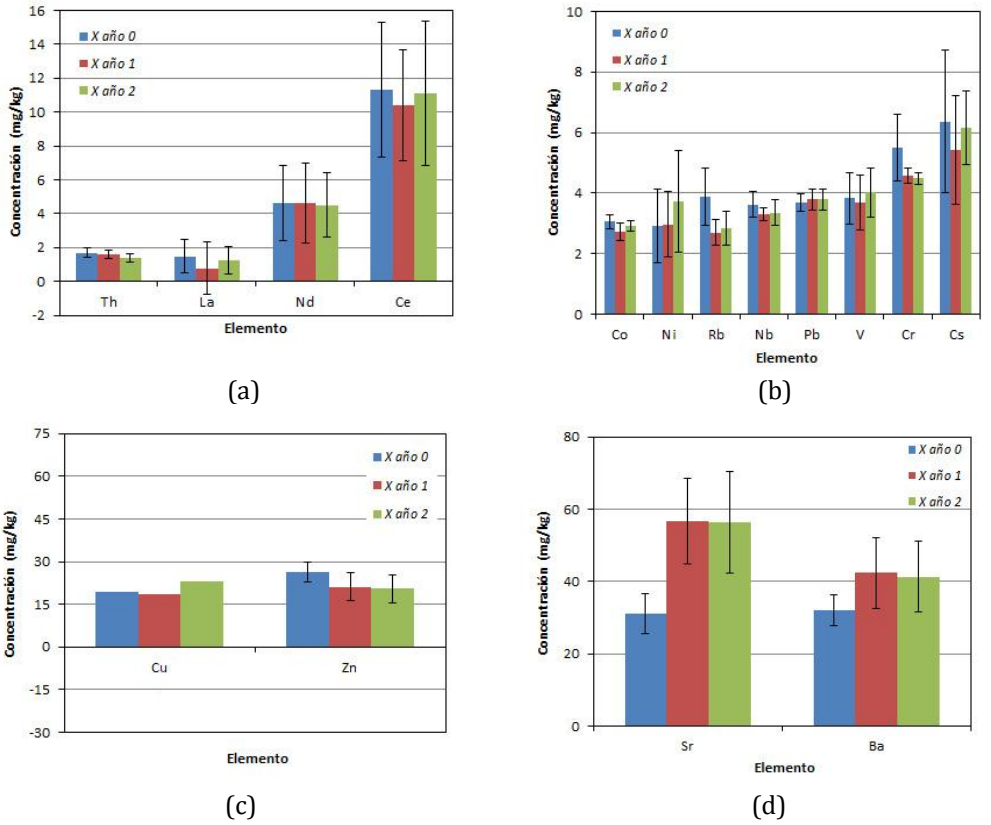

Figure 3. Trace element content, expressed in g/kg, of leaves aged 0, 1 and 2 years: (a) Th, La, Nd and Ce, (b) Co, Ni, Rb, Nb, Pb, V, Cr and Cs, (c) Cu and Zn, (d) Sr and Ba.

4. CONCLUSIONS

It can be concluded from the results obtained in this research that most of the major elements were at normal levels in the olive leaf samples collected from the seven areas studied, irrespective of age, and that high levels of Ca and K were observed. The most mobile elements (Cl, P and K) were concentrated in the younger leaves, except for Mn which is concentrated in older leaves as are the less mobile elements (Mn, Fe, Ca and S).

Comparison of the mean contents of these elements with those in vine reveals that the levels of Na, Mg, Si, Ca, Mn and Fe are higher in vine than in olive; however, the mean values of P, S and K are lower in vine while Al lies at practically the same level in both crops.

The changes in the concentration of trace elements reveal that Ni, Cu, Sr and Ba content increase over time whereas the content of Cr, Zn, Rb, Zr, Hf and Th decreases. When mean trace element content is compared in olive and vine, it emerges that Sr, Ba, V, Cr and Nb lie at higher levels in vine than in olive. In contrast, Ni and Zn values are lower in vine. The rest of the elements lie at similar levels in both crops.

In the future, a deeper insight is needed into the content of these elements in representative crops such as olive in order to understand their dynamics and their potential use as a geochemical soil fingerprint of olive oils, so guaranteeing their authenticity.

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Contribution to the study of the typical characteristics of the virgin olive oils produced in the region of Sais (Morocco)

M. Essiari[1], R. Zouhair[1] and H. Chimi[2]

[1]. Département de Biologie, Faculté des sciences, Université Moulay Ismail –Meknès, Morocco.
[2]. Département des Sciences Alimentaires et Nutritionnelles, Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco.

ABSTRACT

The aim of this study was to analyse the effect of growing area on the quality of the olive oil produced from four varieties ('Picholine marocaine', 'Arbequina', 'Haouzia' and 'Menara') cultivated in two different soil and climatic zones. Carpometric characteristics, maturity index and physico–chemical characteristics (oil content, free acidity, peroxide value, absorbance in ultra violet, fatty acids and sterols) were the variables monitored.
The results show that growing area influences harvest date and the quantity and quality of oil obtained. The oils that were analysed were graded as extra virgin. The weight of the olives belonging to the four varieties in Area 1 (**Ain Taoujdate, El Hajeb province**) was higher than that of those from Area 2 (**Ait Oualal, Meknès province**). Carpometric characteristics were affected by variety and growing area.
'Menara' and 'Haouzia' recorded the highest oil content in the **2010/11 and 2011/12** crop years, respectively.
The free acidity of the oils from Area 2 differed slightly from that of the oils from Area 1 in the 2010/11 crop year. The same occurred in 2011/12, except in the case of the 'Arbequina' variety.
The peroxide value, which provides a snapshot of oil oxidative status, was higher in the oils produced from the 'Arbequina' and 'Haouzia' varieties in Area 1 than from the same varieties in Area 2 while the opposite was the case of the 'Picholine marocaine' and 'Menara' oils.
The results obtained for the absorbance in ultra violet show that the values recorded for the oils complied with the limits recommended in the IOC standard.
The fatty acid composition of the oils varied and was clearly influenced by variety. Oleic acid content in the two crop years was low for 'Arbequina' but quite high for 'Picholine marocaine' and intermediate for 'Haouzia' and 'Menara'.
On comparison with the sterol limits fixed in the IOC standard, the results show that the campesterol content of the 'Arbequina' oils was slightly higher.
Overall, geographical characterisation can be used to select top quality oils that meet national and international market standards.

Keywords: Carpometric characteristics, physico-chemical parameters, soil/climatic areas.

1. INTRODUCTION

Olive is the chief fruit crop in Morocco where it is cultivated on 920,000 ha. Accounting for over 57% of tree crop area, it is found nationwide, except along the Atlantic coastal strip, due to its ability to adapt to all the bioclimatic zones.

In 2008/09 and 2009/10 Morocco produced 1,500,000 t and 1,200,000 t of raw olives, respectively. Sixty-five per cent of this tonnage went for olive oil production and the rest (35%) for table olive processing and self-consumption by producer households.

The olive oil industry is made up of modern and semi-modern processing plants and traditional mills called 'maâsra'. Half of the olive oil facilities are concentrated in the regions of Fez, Meknes and Marrakech. Between 2004 and 2008 olive oil production averaged around 65,000 t compared with 160,000 t in 2009 and 2010.

Olive oil is targeted mainly at the home market. It accounts for almost 75% of production and covers 17% of Morocco's shortfall in edible oils. Olive oil exports continue to be small because of the volume and quality required. The swings in production mean that it is not possible to sign lasting contracts with foreign clients seeking stable, regular product volume and quality.

The implementation of the *Maroc Vert* plan has led to the rapid development of the olive sector both at the production stage and further along the supply chain.

In this context, the Regional Agricultural Plan for Meknès-Tafilalet, which takes into account the soil/climatic conditions and olive oil processing facilities available in the Sais region, has played an important role in the expansion of olive crop area and value creation. Besides being affected by soil/climatic conditions and varietal diversification, fruit and oil yields also differ from one area to the next according to the microclimate, soil type (Çavusoglu *et al*., 1994) and altitude (Mouawad, 2005).

The varieties chosen for this research were 'Picholine marocaine', 'Haouzia', 'Menara' and 'Arbequina'. These account for a large percentage of the varieties grown in olive orchards in the Sais region and are in great demand from olive growers.

Quality plays an essential part in the marketing of virgin olive oils and is affected by several factors, in particular variety (Fontanazza, 1988; Nurhayat, 1989), good agricultural practices, harvest date (Rahmani *et al*., 1997), growing area (Ranalli *et al*., 1999), olive quality, extraction method (Di Giovacchino, 1996; Rahmani, 1996 ; Chimi, 2006) and oil storage (Montedoro, 1989; Cimato, 1990; Rahmani, 1993, Inglese, 1994; Koutsfaiks, 2000; Chimi, 2006).

In this context, the oils produced from four oil varieties grown in two different areas of the region of Sais, namely the Ain Taoujdate and Ait Ouallal areas in the provinces of Meknès and El Hajeb, underwent evaluation of their distinctive physico–chemical characteristics. Over 30,000 ha of olives are cultivated in this region, 40% of which are under irrigation. The sector is characterised by its productive capital and the rapid expansion of its agro-processing infrastructure. The objective of the Regional Agricultural Plan is precisely to increase olive acreage.

The quality parameters studied were free acidity, peroxide value, specific extinctions, fatty acids and sterols and differed from season to season according to the geographical area of each variety studied.

2. MATERIALS AND METHODS

The research was conducted during the **2010/11** and **2011/12** crop years in the Sais region of Morocco, which is known for its wealth and diversity of olive resources.

The plant material used was from three Moroccan varieties, specifically 'Picholine marocaine' **(Variety 1)**, 'Haouzia' **(Variety 3)** and 'Menara' **(Variety 4)** and one foreign variety, 'Arbequina' **(Variety 2)**. Through the implementation of the *Maroc Vert* plan, these varieties have seen rapid development in terms of production and value creation.

2.1. Samples

Olive samples

The samples for assessing fruit maturity index and carpometric characteristics were collected weekly from **10 November through to 12 January** (2010/11 and 2011/12 crop years). The areas chosen for the study were olive orchards in the Ain Taoujdate area in the province of El Hajeb **(Area 1)** and orchards in the Ait Ouallal district of the province of Meknès **(Area 2)**.

Each sample comprised approximately 1 kg of olives collected at shoulder height from around the tree canopies.

Olive oil samples

The physico–chemical characteristics of the oils were determined from samples collected from two-phase processing facilities in the study areas.

2.2. Fruit testing

Maturity index (MI)

A method developed by the *Institut National de Recherche Agronomique* (INRA) and the *Estación de Jaén* (Spain) was used to evaluate the maturity index. This entails assessing the colour of 100 olives taken at random from a 1 kg sample and assigning them to one of eight colour classes ranging from olives with a deep green skin to olives with a black skin and violet flesh.

The index is calculated as follows:

$$MI= (0xA+1xB+2xC+3xD+4xE+5xF+6xG+7xH)/100$$

where **A**, **B**, **C**, **D**, **E**, **F**, **G** and **H** are the numbers of olives belonging to classes **0**, **1**, **2**, **3**, **4**, **5**, **6**, **7** and **8**.

The characteristics of each class are listed below:

A: deep green skin

B: yellow or yellow-green skin

C: green skin with reddish spots over half of the fruit (start of colour change)

D: red to purple skin over more than half of the fruit (end of colour change)

E: black skin and white flesh

F: black skin and violet coloured flesh halfway to the stone

G: black skin and violet coloured flesh almost to the stone

H: black skin and dark flesh all the way to the stone

Carpometric characteristics of the fruit

A precision balance was used to determine the weight of the fruit, stone and flesh of 100 olives (Atouati, 1991; Chimi *et al*. 1993).

Fruit oil content

Samples of olive oil were collected every week from two-phase processing facilities located in both areas during the period from 10 November to 12 January in each of the two crop years.

2.3. Olive oil testing

Free adicity

Free acidity is expressed as the percentage oleic acid content of the oil. It is a simple and effective determination for the quality evaluation of olive oils and their classification in commercial categories. This parameter was determined according to ISO method 660:2009, the principle of which is to disperse a known quantity of fat in heated ethanol and to titrate the mixture with an aqueous solution of potassium hydroxide KOH to neutralise the free fatty acids present in 1 g of fat.

Peroxide value

The peroxide value is expressed as milliequivalents of active oxygen per kilogram of oil. This index evaluates the keeping properties of a fat during storage and must not exceed 20 meq O_2/kg for all the categories of olive oil.

The amount of iodine liberated by the sample after adding potassium iodide according to the conditions described in ISO method 960:2007 gives a measure of the active oxygen present.

The principle of the ISO method is to dissolve a test portion in an acetic acid/iso-octane solution to which potassium iodide is added. The iodine liberated by the peroxides is titrated visually with a standard solution of sodium thiosulfate using starch as indicator.

Absorbance in the ultraviolet region

Oxidative stability is an important parameter for evaluating the quality of olive oil. It is defined as the time necessary for an olive oil to start showing symptoms of rancidity after accelerated oxidation of the unsaturated fatty acids.

The absorbance was determined according to ISO method 3656:2011. The principle of this method is to determine the absorbance of an oil sample dilution by spectrophotometry at a specific wavelength in the ultraviolet. The absorbance was calculated at a concentration of 1 g/100 ml using a 10 mm cuvette.

Fatty acids (FAs)

The fatty acids present in the oil were analysed with the aid of a gas chromatograph on the basis of the retention time of each fatty acid in reference samples according to the IOC method (COI/T.20/Doc. No 24).

Sterols

The composition of the sterol fraction was determined by capillary column gas chromatography. The method entails saponification of a test portion, extraction of the unsaponifiable matter, isolation of the sterols by thin-layer chromatography and analysis of the isolated sterols or derivatives by gas chromatography (COI/T.20/Doc. No 30).

Statistical analysis

The measurement data underwent descriptive statistical processing using Statistica 10.0 software. The aim was to summarise all the information and results in the form of standardised curves permitting easy interpretation and visualisation of the large differences between the qualitative and quantitative variables studied.

3. Results and Discussion

3.1. Maturity index

Fruit ripening increases as the harvest season advances and varies from area to area depending on the soil/climatic conditions (Chimi *et al*., 2007). **Figures 1 and 2** show the changes in the maturity

index of the four varieties in the two different areas during the 2010/11 and 2011/12 crop years. According to the results, all the varieties studied ripened early in Area 1 compared with Area 2.

During the 2010/11 crop year, the maturity index went from 2.68 to 5.35 in Area 1 and from 2.25 to 5.25 in Area 2. In the 2011/12 season, it varied from 2.78 to 5.38 in Area 1 and from 1.95 to 5.30 in Area 2.

From the varietal standpoint, 'Arbequina' was the earliest ripener in both areas and both crop years.

The varieties studied ripened in the following order in the two areas and two crop years:

2010/11crop year:

- Area 1 'Arbequina', 'Menara', 'Haouzia' and 'Picholine marocaine'
- Area 2 'Arbequina', 'Menara', 'Picholine marocaine' and 'Haouzia'

2011/12 crop year:

- Areas 1 and 2: 'Arbequina', 'Picholine marocaine', 'Haouzia 'and 'Menara'

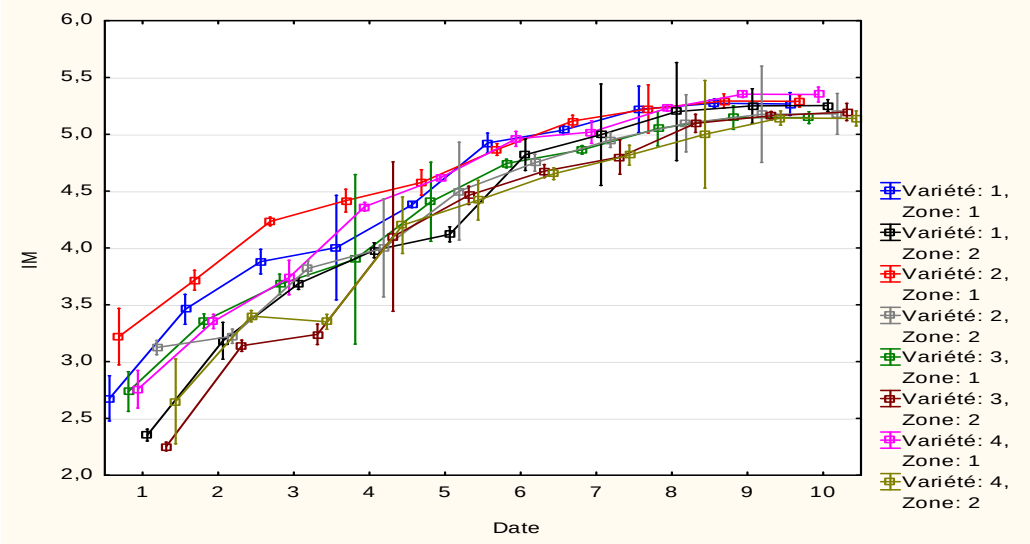


Figure 1. Changes in the fruit maturity index of four varieties of olive cultivated in two areas of the Sais region in Morocco (Area 1: Ain Taoujdate and Area 2: Ait Ouallal) (2010/11 crop year).

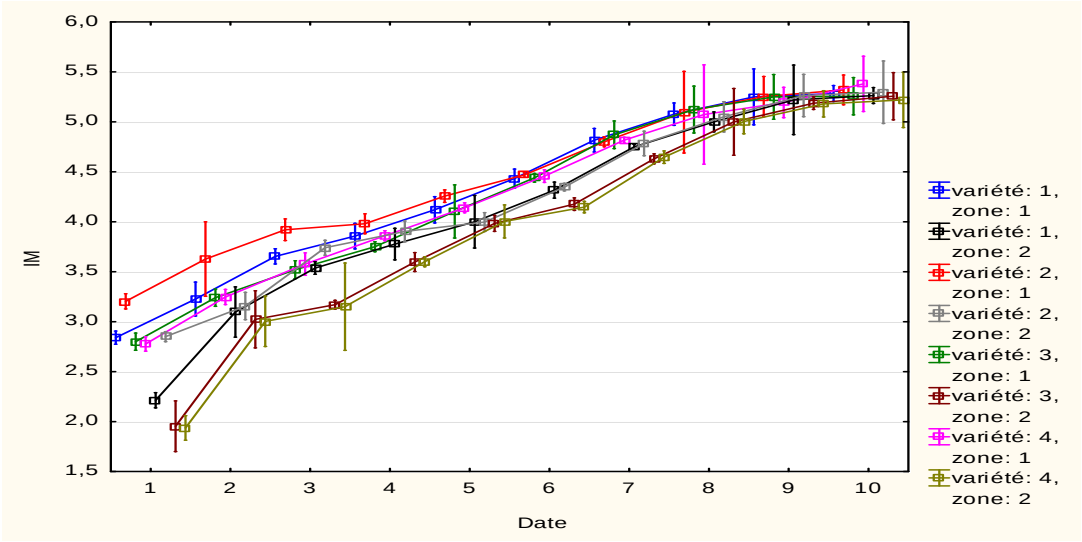


Figure 2. Changes in the fruit maturity index of four varieties of olive cultivated in two areas of the Sais region in Morocco (Area 1: Ain Taoujdate and Area 2: Ait Ouallal) (2011/12 crop year).

3.2. Carpometric characteristics

The data entered in **Figure 3 (a,b,c,d,e,f)** plot the changes in the carpometric traits of the four varieties ('Picholine marocaine', 'Arbequina', 'Haouzia' and 'Menara') .

The results show that the fresh weight of the olives increases as the fruit ripens until it peaks at full maturity. This trend has also been reported by Atouati (1991). Fruit, flesh and stone weight on a fresh weight basis were higher for the olives from Area 2 compared with Area 1.

From the varietal point of view, in both areas and both crop years 'Picholine marocaine' recorded the highest fruit weight in terms of fresh matter (4.23 g and 4.36 g; 4.26 g and 4.43 g), followed by 'Haouzia' (2.83 g and 3.42 g; 2.59 g and 2.653 g), 'Menara' (2.56 g and 2.57 g; 2.59 g and 2.65 g) and 'Arbequina' (1.85 g and 1.85 g; 1.85 g and 1.94 g). Sweeney (2005) reported the same results for 'Arbequina'.

The same results were obtained for the weight of the fruit flesh, with 'Picholine marocaine' recording the highest value and 'Arbequina' the lowest. The other varieties had an intermediate flesh weight close to that of 'Picholine marocaine'.

According to research carried out by Lachir and Sidi Baba (1994) taking 100 olives as the basis of measurement, fruit and flesh weight tends to decrease after the olives reach an advanced stage of maturity (MI>5) due to heavy fruit moisture loss. However, this tendency was not observed in this research in either of the crop years reviewed. This might be explained by the fact that the olive orchards were irrigated (drip irrigation) and climatic conditions were favourable, thus leading to lower intensity of transpiration.

According to the categories defined by Del Río and Caballero (1994) (very low: < 0.2; low: 0.2-0.4; average: 0.4-0.6; high: 0.6-0.8; very high: > 0.8), the fresh weight of the olive stones of 'Arbequina', 'Haouzia' and 'Menara' was low while that of 'Picholine marocaine' was intermediate. During the two crop years concerned and in both areas, an intermediate flesh-to-stone ratio of between 4.08 and 7.93 was recorded for all four varieties. Del Río and Caballero (1994) established the following categories for this criterion: (i) low (< 5.0), (ii) average (5.0-7.5), (iii) high (7.5-10.0) and (iv) very high (> 10.0). According to this criterion, the flesh-to-stone ratio was low for 'Arbequina', average for 'Picholine marocaine' and 'Menara' and high for 'Haouzia'.

The olives of the 'Picholine marocaine' variety have a high weight, are quite large and have a free stone (International Olive Council, 2000). The fruit of the 'Haouzia' and 'Menara' varieties, which are selections of 'Picholine marocaine'; is average in size and therefore dual purpose (for oil and table olives), whereas 'Arbequina' is an oil variety. It is hardy and resistant to cold and salinity but susceptible to iron chlorosis on calcareous soils. It also has a high rooting ability and starts bearing early. Its flowering period is intermediate and it is self-compatible. This variety is particularly appreciated for its high, constant yields (International Olive Council, 2000).

Generally, olive growing is traditionally interested in varieties displaying several agronomic and processing traits. Cultivars are favoured for their large fruit and, if intended for table olives, must have a high flesh-to-stone ratio (Hannachi *et al.*, 2006).

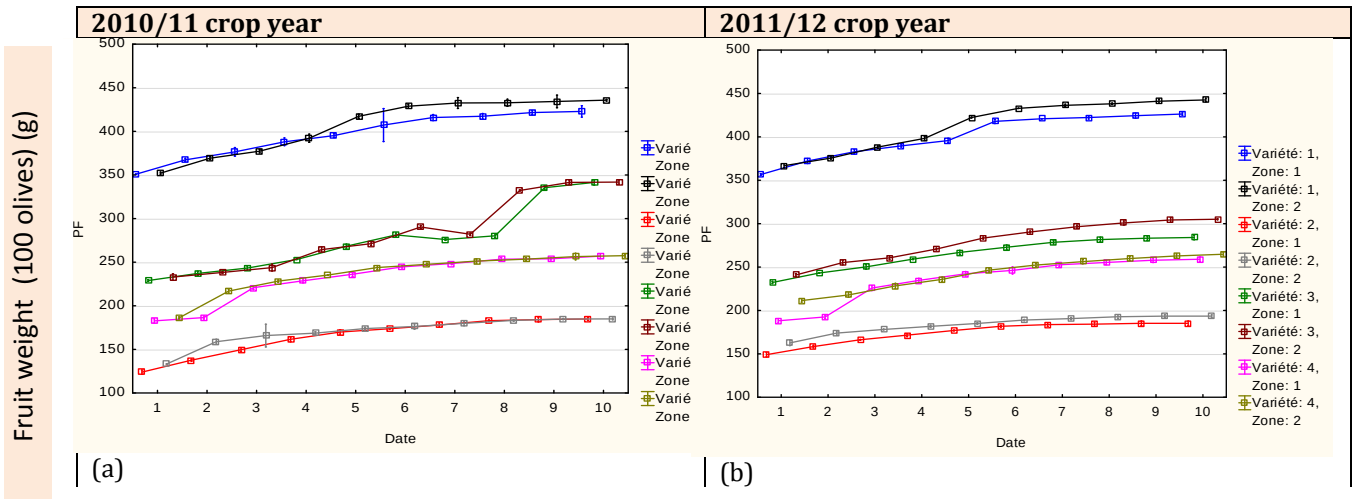


Figure 3 (a, b, c, d, e, f). Changes in the carpometric characteristics of the olives of four varieties cultivated in the Sais region of Morocco.

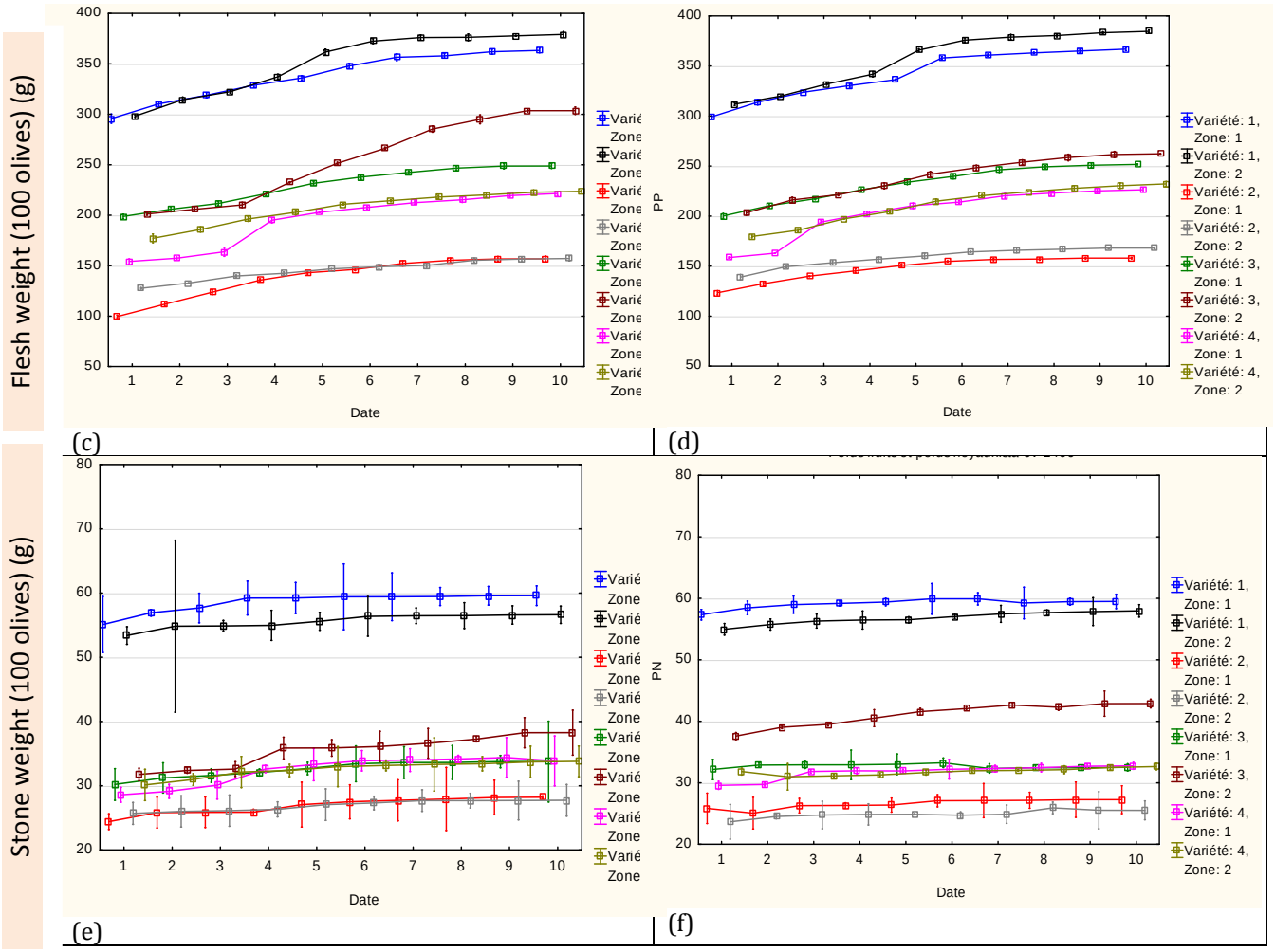


Figure 3 (contd). Changes in the carpometric characteristics of the olives of four varieties cultivated in the Sais region of Morocco.

3.3. Olive composition

Oil content

Fruit oil content varies according to variety, harvest date and growing area. This parameter is not a quality criterion but above all permits determination of the optimal harvest date.

From the varietal standpoint, the maximum oil content of the Moroccan varieties did not differ greatly in the two areas, oscillating between 20.10% and 21.20%, while it ranged from 17.56% to 18.75% for 'Arbequina'.

In terms of harvest date, the results show that maximum oil contents were recorded as of 1 December in Area 1 (Ain Taoujdate) and 7 December in Area 2 (Ait Ouallal) in both crop years (**Figures 4 and 5**). On these dates, all the varieties had a maturity index of close to 4. This confirms the results reported in earlier research revealing a difference in oil content according to harvest date (Walali *et al.*, 1984) in five clones of the 'Picholine marocaine' variety. El Antari (2006) reported a similar difference between 'Menara' and 'Haouzia' for harvest dates between October and November.

Olive biosynthesis proceeds rapidly when the olives are at the green stage until they turn fully black, after which oil content levels off (Uceda *et al.*, 1975; Suarez, 1984; Civantos, 1999) and even records a small decrease at the advanced stages of maturity (Lachir *et al.*, 1994; El Cadi *et al.*, 1998; Faqih *et al.*, 1999). This drop in oil content can be attributed to the accumulation of dry matter in the olives at advanced ripening, as well as to endogenous lipases (active at the black stage) which hydrolyse the triacylglycerols and fatty acids (Harrar, 2007).

These changes help to identify when oil yield is at its height and so to determine the optimal harvest date.

From the point of view of growing area, the results reveal that the varieties had a higher oil content when grown in Area 2 compared with Area 1 in both of the crop years studied (**Figures 4 and 5**). Civantos (1999) attributed such differences to the intensity of oil formation which, while a genetic trait, is also dependent on soil/climatic conditions and orchard management.

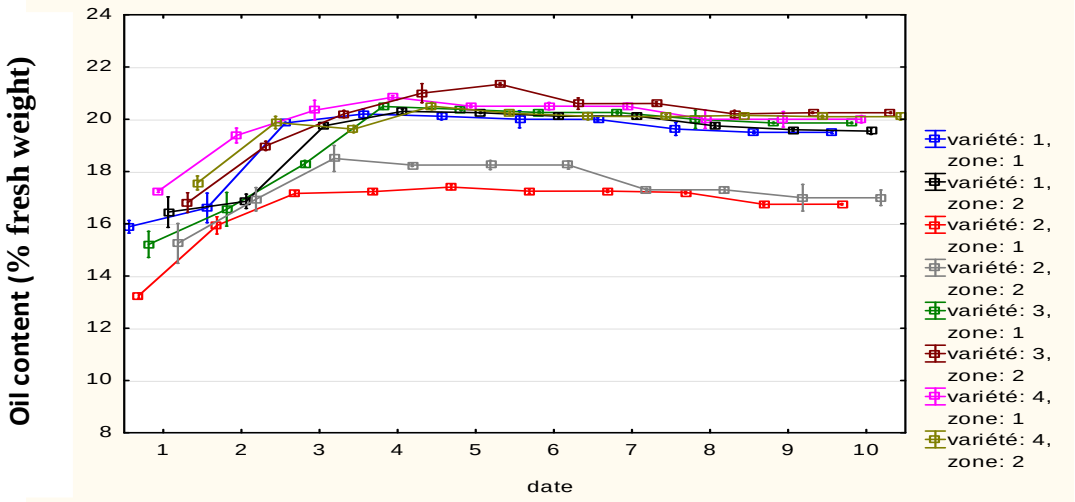


Figure 4. Changes in the oil content of olives belonging to four varieties cultivated in two areas of the Sais region in Morocco (2010/11 crop year).

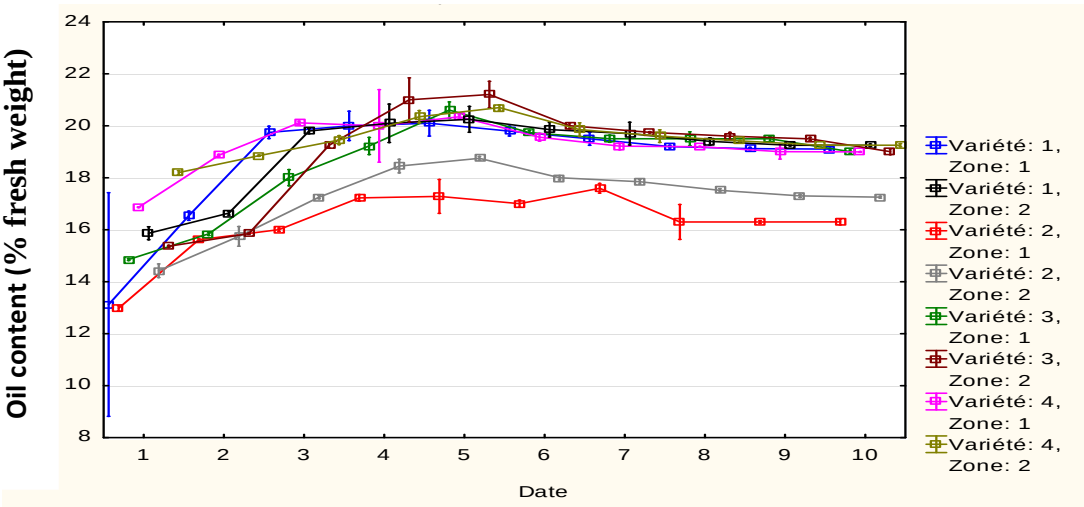


Figure 5. Changes in the oil content of olives belonging to four varieties cultivated in two areas of the Sais region in Morocco (2011/12 crop year).

3.4. Free acidity

Figures 6 and 7 plot the free acidity (expressed as a % of oleic acid) of the olive oils obtained from the varieties when harvested at fruit maturity. According to this parameter, all the oils analysed, irrespective of growing area, belonged to the virgin grade because their free fatty acid content was below 0.8%.

In the 2010/11 crop year a slight difference was noted in the free acidity of the oils produced from the varieties cultivated in Area 1, which was higher than in Area 2. The same tendency was recorded in 2011/12, except for 'Arbequina' for which the free acidity level was considerably higher in Ait Ouallal than in Ain Taoujdate. This can be ascribed to oil processing practices and the length of time the fruit was stored prior to crushing.

The results also show that the 'Arbequina' oils had a high acidity in both crop years and both areas. In the majority of cases, acidity levels were higher in the oils from Area 1 than in those from Area 2.

Variety continues to play quite an important role in that it affects the levels of polyphenols and tocopherols in the oil (Alessandri, 1997). These compounds determine the stability of the oil, its resistance to oxidation and consequently its keeping properties (Çavusoglu *et al.*, 1994).

The influence of soil on the quality of olive oil is quite a complex phenomenon where several factors such as soil type, pH and chemical composition are at play (El Murr, 2005).

Generally speaking, rich soils produce less aromatic oils than do poor soils with lower yielding trees (Çavusoglu *et al.*, 1994). Moreover, oils produced from olives grown on calcareous oils have a lower acidity than those obtained from olives cultivated on clay soils.

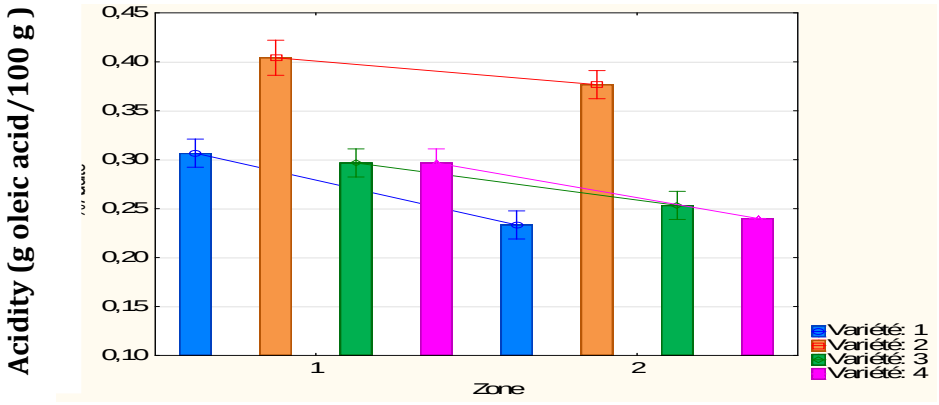


Figure 6: Variation in the free acidity levels of the olive oil produced from four varieties cultivated in two different areas (2010/11 crop year).

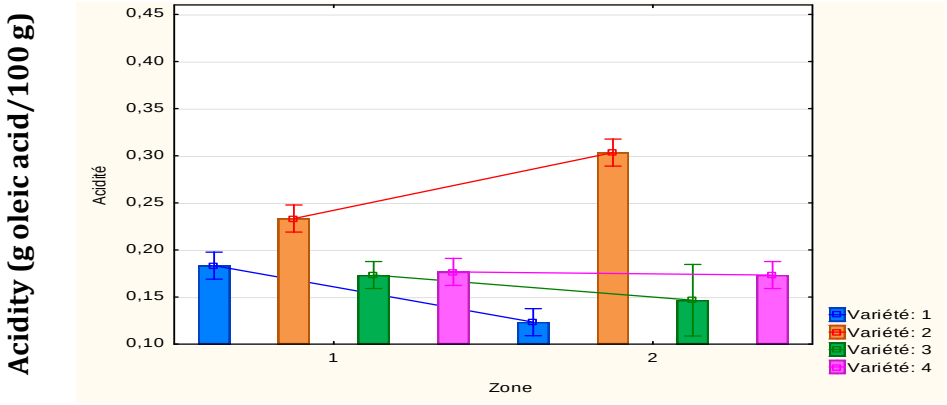


Figure 7: Variation in the free acidity levels of the olive oil produced from four varieties cultivated in two different areas (2011/12 crop year).

3.5. Peroxide value

The peroxide value, which is the number of hydroperoxides that form in a fat during storage, provides information about the oxidative status of the oil.

The analytical results plotted in **Figures 8 and 9** show that the peroxide content of the oils obtained from the four varieties in both areas ranged between 4.29 and 6.06 meq O_2/kg oil. When compared with the limits fixed in the IOC trade standard, it can be seen that all the samples analysed comply with the standard and can therefore be graded as extra virgin (PV≤20). These values indicate that the oil was extracted quickly after the olives had been harvested.

Geographically speaking, the results for the 2010/11 crop year show that the oils obtained from the 'Arbequina' and 'Haouzia' varieties in Area 1 had a higher peroxide value than the oils produced from the same varieties in Area 2, whereas the reverse was the case for the 'Picholine marocaine' and 'Menara' varieties. For the 2011/12 crop year, the peroxide value of the oils from the four varieties studied in Area 1 was slightly higher than that of the same varieties in Area 2.

Overall, the results reveal that in the majority of cases the oils obtained from the four varieties in 2010/11 had a higher peroxide value than those produced a season later in 2011/12. Peroxide compounds exist because olive oil oxidation begins after the olives are picked from the tree and continues through fruit storage and processing (Bouhadjra, 2011). Tanouti *et al.* (2011) attribute this improvement to the effectiveness of the technical extension services provided for olive growers and good olive oil practices.

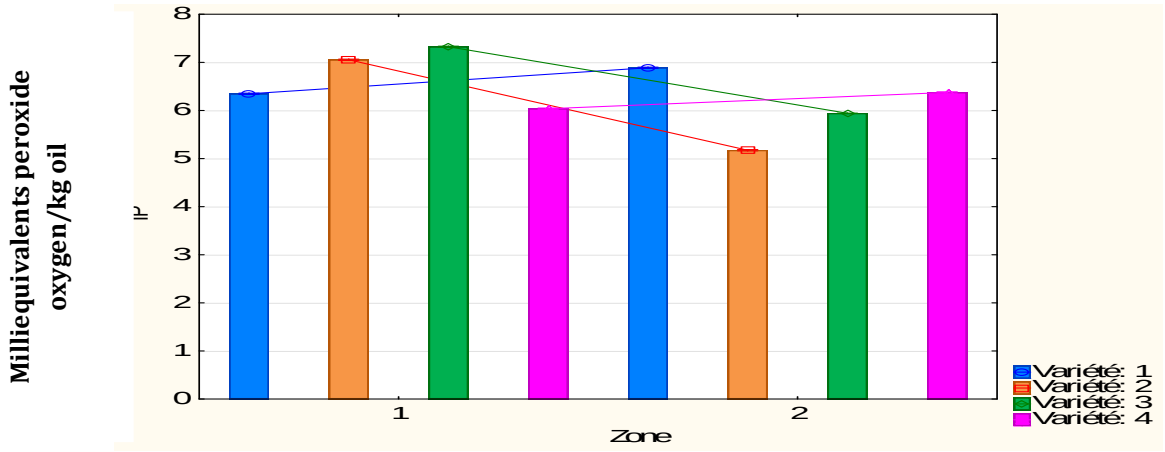


Figure 8: Peroxide value of the olive oil obtained from four varieties cultivated in two different areas (2010/11 crop year).

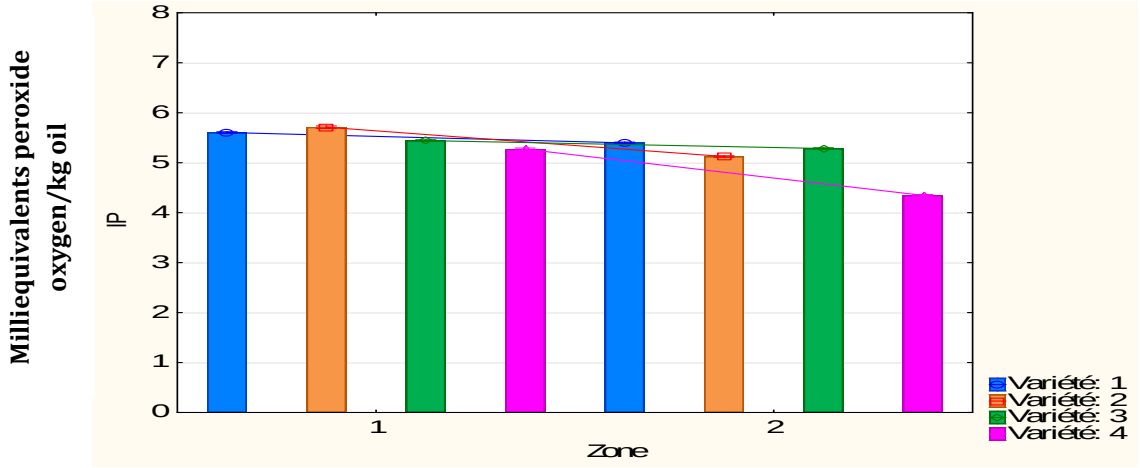


Figure 9: Peroxide value of the olive oil obtained from four varieties cultivated in two different areas (2011/12 crop year).

3.6. Absorbance in the ultraviolet region

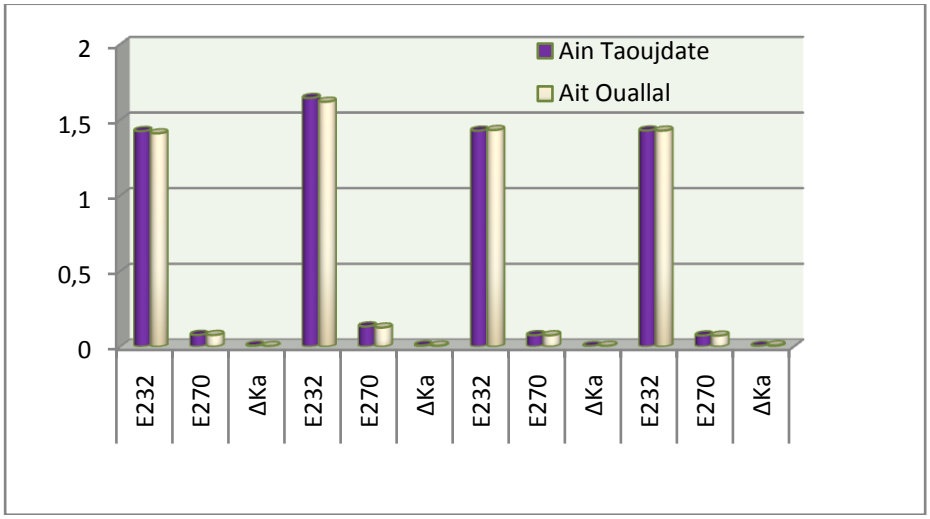
PV values ≤ 20meq O_2/kg of oil do not always mean that oxidation has not occurred. Determining the extinction coefficients in the ultraviolet region (K_{232}, K_{270}) provides information on the presence or absence of secondary oxidation products in the oil. The hydroperoxides that form during the initial stages of oxidation absorb at 232 nm whereas secondary oxidation products such as unsaturated ketones-diketones do so at around 270 nm (Ollé, 2002; Jeantet *et al*., 2006).

The measurement of the absorbance in the ultraviolet is a way of evaluating the keeping status of the oil. It is also an indicator of the sensitivity of the extraction method as well as of oxidation caused by overexposure of the oil to air during crushing. The lower the extraction temperature (<28°), the less contact there is between the oil and the air during extraction and the lower the K_{232} and K_{270} values.

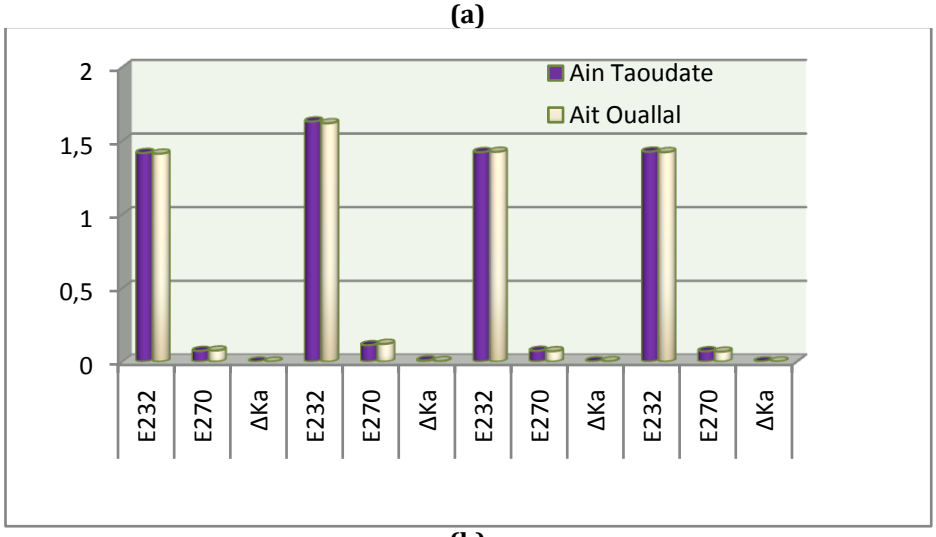
The results obtained for the absorbance in ultraviolet (**Figure 10**) reveal that the samples of olive oils from the 2010/11 and 2011/12 crop years comply with the limits recommended in the IOC standard (COI/T.15/NC No 3/Rev.5/2010) : K_{232}≤ 2.5; K_{270}≤ 0.25, Δ K≤ 0.01. The K_{232} and K_{270} values of the oils comply with the requirements stipulated in the IOC standard for classification as extra virgin olive oil.

Comparison of the mean values for the absorbance in ultraviolet in the two crop years (**Figure 10**) in both areas reveals no between-area differences. Ranalli *et al*. (1996) and Kiritsakis (1998) reported that geographical origin has no significant influence on these analytical parameters which are basically affected by factors that cause fruit damage such as attacks from olive fruit fly or damage from harvest equipment or during fruit transportation and storage.

When analysed by variety, the K_{232} and K_{270} values of 'Arbequina' were higher than those of the other varieties.



(a)



(b)

Figure 10 (a, b): Mean K_{232}, K_{270} and ΔK values of the olive oils produced from four varieties in two crop years (a): 2011/2012 crop year; (b): 2010/2011 crop year.

3.7. Fatty acid composition

The triacylglycerol structure of olive oil varies according to the percentage of fatty acids in each cultivar. Oleic acid is the predominant fatty acid in olive oil, followed by linoleic acid and palmitic acid. These fatty acids are an important parameter in determining the quality and authenticity of olive oil.

As can be seen from **Tables 1 and 2**, the fatty acid composition of the oils that were tested was clearly influenced by variety.

Several factors such as degree of fruit ripeness, climate and variety affect the fatty acid composition profile of olive oil (Bruni *et al.*, 1994; García *et al.*, 1996; Ollé, 2002; Judde, 2004;). Some authors have used this profile as a parameter for classifying olive oils by origin (Ranalli *et al.*, 1997) while others have noted minimal variations in the content of the chief fatty acid (C18 :1) in the same variety of olive when cultivated in different places (USAID/MAROC, 2006).

The analytical results for the oils produced in the two areas in the 2010/11 and 2011/21 crop years show that the content of oleic acid, the main fatty acid in olive oil, was lowest in 'Arbequina' (64.37% and 63.58%; 65.48% and 62.42%) whereas it was quite high for 'Picholine marocaine' (76.80% and 76.71%; 76.80% and 76.62%) and intermediate for the 'Haouzia' and 'Menara' varieties.

The highest percentages of linoleic and palmitic acid were recorded for the 'Arbequina' oils in both areas and both crop years.

Saturated fatty acid content (SFA) varied according to the crop year, ranging from 12.27% ('Haouzia') to 19.21% ('Arbequina') for the 2010/2011 crop year and from 12.27% ('Menara') to 18.85% ('Arbequina') for the 2011/2012 crop year. Similarly, the percentage of unsaturated fatty acids (UFA) varied slightly according to variety, ranging from 80.4% to 82.8% in 'Arbequina' in the

2010/11 crop year and from 80.68% to 81.34% in 2011/12, thus revealing a slightly higher level in the oils from Area 2. The highest UFA content was recorded for the 'Picholine marocaine', 'Haouzia' and 'Menara' varieties and varied from 85.53% to 88.76% in both areas and both crop years.

Table 1. Fatty acid composition of the oils produced from four olive varieties (% total fatty acids, TFA) IOC standard (1998) - (2010/11 crop year)

Varieties studied	Name																					
	C16-0		C16-1		C17-0		C17-1		C18-0		C18-1		C18-2		C18-3		C20-0		C20-1		C22-0	
	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2
'P.M.'	10.7	11.56	0.69	1.27	0.1	0.02	0.1	0.1	2.2	2.2	76.8	76.71	9.4	9.4	0.88	0.88	0.3	0.3	0.4	0.4	0	0
'Arbeq.'	16.2	17.21	1.69	2.1	0.09	0.1	0.2	0.2	1.6	1.7	64.37	63.58	13.12	15.9	0.72	0.72	0.2	0.2	0.3	0.3	0	0
'Haou.'	9.82	10.69	0.87	0.66	0.06	0.03	0.04	0.08	2.1	2.31	75.5	75.25	10.6	9.41	0.82	0.8	0.29	0.21	0.35	0.35	0	0
'Mena.'	9.85	11.26	0.86	0.75	0.05	0.04	0.04	0.09	2.1	2.32	74.66	75.2	10.62	9.42	0.81	0.79	0.29	0.21	0.34	0.36	0	0
	7.5 - 20		0.3 - 3.5		≤ 0.3		< 0.3		0.5 - 5		55 - 83		3.5 - 21		< 1		≤ 0.6		≤ 0.3		≤ 0.2	
	Limits for total fatty acids																					

Table 2. Fatty acid composition of the oils produced from four olive varieties (% total fatty acids, TFA) IOC standard (1998) - (2011/12 crop year)

Var.	Name																					
	C16-0		C16-1		C17-0		C17-1		C18-0		C18-1		C18-2		C18-3		C20-0		C20-1		C22-0	
	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2
'P.M.'	10.88	9.37	0.71	0.7	0.1	0.02	0.1	0.1	2.18	2.24	76.8	76.62	9.38	9.4	0.91	0.88	0.32	0.3	0.29	0.4	0	0
'Arb.'	16.22	16.79	1.54	2.1	0.1	0.1	0.22	0.2	1.63	1.76	65.48	62.42	12.38	15.6	0.75	0.72	0.2	0.2	0.31	0.3	0	0
'Haou.'	10.25	10.15	0.89	0.66	0.06	0.03	0.05	0.09	2.12	2.31	75.77	75.2	10.75	9.41	0.86	0.8	0.32	0.21	0.36	0.36	0	0
'Mena.'	9.85	9.7	0.86	0.65	0.05	0.04	0.04	0.1	2.16	2.32	74.83	74.2	10.62	9.42	0.81	0.79	0.29	0.21	0.34	0.37	0	0
	7.5 - 20		0.3 - 3.5		≤ 0.3		< 0.3		0.5 - 5		55 - 83		3.5 - 21		< 1		≤ 0.6		≤ 0.3		≤ 0.2	
	Limits for total fatty acids																					

3.8. Sterol composition

The analytical results show that the sterol composition of the oils produced from the four varieties in the two crop years lie inside the limits fixed in the IOC standard (2011).

As can be seen from **Tables 3 and 4**, β-sitosterol was predominant in the oils produced from all four varieties, ranging from 78.82% to 85.56% in 2010/11 and from 79.12% to 87.6% in 2011/12. The lowest value was observed for 'Arbequina'. Olive oil is the only oil to contain a particularly large amount of β-sitosterol, which obstructs intestinal absorption of cholesterol (ONH, 2009).

The oil produced from the 'Arbequina' variety had a lower cholesterol content (0.02-0.03) than the other varieties studied. 'Haouzia' had a higher content while 'Picholine marocaine' and 'Menara' had intermediate levels.

In the case of campesterol, the highest content was recorded for the 'Arbequina' oil in both areas and both crop years. Campesterol content is always higher than stigmasterol content. The composition and content of all the sterols were in line with the ranges fixed by the IOC (2011) and the EU (2002).

All the oils obtained from the varieties studied contained stigmasterol at levels lying within the limits specified in the latest version of the IOC standard (COI/T.15/Doc. No 3). The stigmasterol content of the 'Arbequina' oils was higher in both crop years.

Despite interregional variations, the values for Δ-5-avenasterol, Δ-7-stigmasterol and Δ-7-avenasterol fell within IOC limits (2009).

Table 3. Sterol composition of the oils obtained from four varieties of olive (2010/11crop year)

Varieties studied	Name													
	Cholesterol		Campesterol		Stigmasterol		β-sitosterol		Δ-5-avenasterol		Δ-7-stigmasterol		Δ-7-avenasterol	
	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2
'Picholine marocaine'	0.1	0.09	2.8	2.7	0.6	0.6	85.01	84.86	6.97	6.95	0.3	0.29	0.3	0.27
'Arbequina'	0.02	0.02	3.7	3.6	1	0.97	79.12	78.82	6.56	6.54	0.36	0.37	0.4	0.36
'Haouzia'	0.2	0.08	2.6	2.45	0.76	0.58	85.5	85.46	5.88	5.87	0.2	0.18	0.36	0.34
'Menara'	0.08	0.09	3.13	3.22	0.62	0.6	85.56	85.41	5.74	5.8	0.2	0.17	0.36	0.33
	≤ 0.5		≤ 4		≤ 4		80-90				≤ 0.5			
	Limits													

Table 4: Sterol composition of the oils obtained from four varieties of olive (2011/12crop year)

Varieties studied	Name													
	Cholesterol		Campesterol		Stigmasterol		β-sitosterol		Δ-5-avenasterol		Δ-7-stigmasterol		Δ-7-avenasterol	
	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2	Area 1	Area 2
'Picholine marocaine'	0.1	0.08	3	3	0.6	0.6	85.01	86.58	6.97	7.76	0.3	0.3	0.3	0.3
'Arbequina'	0.02	0.03	3.9	3.72	0.9	0.9	79.12	81.45	6.56	6.32	0.36	0.39	0.4	0.4
'Haouzia'	0.08	0.09	3.2	3.3	0.62	0.59	85.5	86.85	5.88	5.98	0.2	0.3	0.36	0.3
'Menara'	0.08	0.09	3.03	3.4	0.65	0.74	85.5	87.6	5.88	5.62	0.2	0.36	0.36	0.34
	≤ 0.5		≤ 4		≤ 4		80-90				≤ 0.5			
	Limits													

4. CONCLUSIONS

The results obtained in the experimental conditions described here show that fresh olive weight increases as ripening advances until maximum weight is reached at full maturity. The 'Picholine marocaine' variety recorded the highest value for fruit weight on a fresh matter basis.

The four varieties studied recorded maximum oil yields as of 1 December in **Area 1** (**Ain Taoujdate-El Hajeb**) and 7 December in **Area 2** (**Ait Ouallal-Meknes**), when the maturity index of all the varieties was around 4.

Comparison of the free acidity values of the oils in 2010/11 reveals that those in Area 2 were slightly lower than those in Area 1. The same tendency was recorded in 2011/12, except for 'Arbequina'.

The results for the peroxide value show that the majority of the oils produced from the four varieties had higher levels in 2010/2011 compared with 2011/2012 and ranged from 4.29 to 6.06 meq O_2/ kg. These values comply with the IOC standard (PV≤20).

Comparison of the mean absorbance in ultraviolet of the oils obtained from the four varieties studied in the two areas and two crop years reveals no difference between the areas.

In the two crop years, the fatty acid composition of the oils varied in both areas and was clearly influenced by variety. The highest percentages of linoleic and palmitic acid were observed in the 'Arbequina' oils, which in contrast recorded the lowest level of oleic acid.

Olive oil is the only oil to contain a particularly large amount of β-sitosterol, which obstructs intestinal absorption of cholesterol (ONH, 2009). The level of this sterol is strikingly low in the case of the 'Arbequina' oils, whereas the opposite is observed for its campesterol content. These results were recorded in both areas and both crop years.

Despite interregional variations, the values for Δ-5-avenasterol, Δ-7-stigmasterol and Δ-7-avenasterol fell within IOC limits (2009).

Overall, the qualitative and quantitative parameters varied from season to season according to the geographical area of each variety studied.

Generally, the oils analysed could be classified as extra virgin on the basis of their physico-chemical characteristics, irrespective of the growing area.

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Physico-chemical characterisation and oxidative stability of olive oils produced from the 'Picholine marocaine', 'Haouzia', 'Koroneiki' and 'Arbequina' varieties in the central olive growing region of Morocco (Chaouia-Ouardigha)

M. Haddam[1,2], H. Chimi[3], A. El-Antari[4], M. Zahouily[5], R. Mouhibi[5], A. Zaz[2], M. Ibrahimi[2] and A. Amine[1]

[1] Laboratoire des Analyses Chimiques et Biocapteurs, Faculté des Sciences et Techniques de Mohammedia, Morocco.
[2] Lesieur Cristal, 1, Rue Caporal Corbi 20300 Roches Noires – Casablanca, Morocco. E-mail: haddam@lesieur-cristal.co.ma or haddam3@hotmail.fr
[3] Institut Agronomique et Vétérinaire Hassan II. Département des Sciences Alimentaires et Nutritionnelles.B.P 6202 – Instituts, 10101, Rabat, Morocco. E-mail: chimihammadi@yahoo.fr
[4] Institut National des Recherches Agronomiques (INRA, MARRAKECH), Morocco.
[5] Laboratoire de Catalyse, Chimiométrie et Environnement, Faculté des Sciences et Techniques de Mohammedia, Morocco.

ABSTRACT

The aim of this research was to characterise the olive oil produced in the central Chaouia-Ouardigha region of Morocco by studying the olive oils obtained from four varieties grown between the Settat and Berchid districts of this region through two successive crop years. The olives were harvested between 11 and 18 November of each crop year at the same maturity index and in the same region.
The results of all the analyses performed were compared with the values fixed in the standard referenced COI/OH/Doc. No 1 of November 2011 and showed that the oils produced from all four varieties were extra virgin grade and devoid of organoleptic defects.
This research showed that the total phenol, ortho-diphenol and tocopherol content of the olive oils studied, and hence their oxidative stability determined according to the Rancimat method, was basically variety-dependent given that the region and maturity index were the same in all cases.
Total polyphenol content varied between 106 and 478 mg/kg: the highest content in both successive crop years was noted in the olive oil produced from the 'Picholine marocaine' variety (313-478 mg/kg), followed by the 'Haouzia' variety (268-288) while the lowest level was found in the oil obtained from the 'Arbequina' variety (106-125). Ortho-diphenol content varied similarly to the polyphenols, ranging from 15 to 22 mg/kg. Oxidative stability, measured in terms of the Rancimat induction rate, varied between 27 hr and 40.9 hr. The highest rate was recorded for the 'Koroneiki' oil (40.9 hr) produced in 2009–2010, followed by the oil obtained from the 'Picholine marocaine' variety in 2009–2010. The lowest rate was observed in the 'Arbequina' oil (27 hr) from the 2008–2009 season. An attempt was made to establish a correlation between the oxidative stability of the oils, measured according to the Rancimat method, and their chemical composition.

Key words: Ouardirha region; olive oil; quality; characterisation; PCA

1. INTRODUCTION

Olive oil production has always been concentrated in the Mediterranean countries: Spain, Italy, Greece, Turkey, Tunisia and Morocco. Taken together, they account on their own for more than 90% of the olive oil produced in the world. The commercial quality of virgin olive oil is internationally defined in the standards of the International Olive Council and the Codex Alimentarius. These standards make a distinction between four grades of virgin olive oil - extra virgin, virgin, ordinary virgin and lampante virgin - which are defined in terms of their physico-chemical (free acidity, peroxide value, UV absorbency) and organoleptic criteria. In terms of stability, olive oil is well known for its resistance to oxidation mechanisms. This is closely linked to its low content of polyunsaturated fatty acids (Velasco *et al*., 2002) and its composition of natural antioxidants, notably ortho-diphenols, polyphenols (Idrissi *et al*.,) and tocopherols which trap the free radicals in oxygen and preserve the quality (Boskou, 1996) and stability of olive oil during storage.

Olive is the chief fruit crop cultivated in Morocco. Thanks to its ability to grow and bear crops in a variety of growing conditions and its adaptability to critical soil and climatic conditions, it has developed virtually nationwide. Nevertheless, the olive oil produced by Morocco accounts for no more than 4% of world production and domestic olive oil consumption in Morocco is very low, standing at 2 kg/capita/year, far below average consumption elsewhere in the Mediterranean region (6 kg in Tunisia, 12 kg in Spain, 14 kg in Italy and 24 kg in Greece). However, through Morocco's development plan for the olive sector known as the *Plan Maroc Vert*, there has been a considerable improvement in the quality of Moroccan olive oil and how it is perceived on international markets. In fact, between 2006/07 and 2010/11, Morocco's production climbed from 75,000 t to 135,000 t.

The 'Picholine marocaine' population variety is the chief variety grown in Morocco where it represents more than 96% of olive resources. The remaining 4% is made up of several varieties, in particular 'Picholine du Languedoc', 'Dahbia' and 'Meslala', which are concentrated in irrigated orchards (Haouz, Tadla, El Kelâa) and a few Spanish, Italian and Greek varieties, such as 'Picual', 'Manzanilla', 'Gordal', 'Frantoio', 'Arbequina' and 'Koroneiki'. Some years ago several Moroccan researchers undertook surveying of the predominant 'Picholine marocaine' variety as part of a genetic improvement programme designed to select the best clones. This research led to the selection of two interesting clones called 'Haouzia' and 'Menara'. (Zaher *et al*., 2011; Indicateur Macroéconomique et agricole-Maroc, 2005).

The target area of this study was the Chaouia-Ouardigha region, which extends over an area of almost 16 510 km² in the centre of Morocco. It is characterised by predominantly calcimagnesic soil with an area of high agricultural potential (with black *tirs* soils and red *hamri* soils). The rainfall is moderate, rarely exceeding 500 mm (the mean rainfall over five successive years was 333 mm). This region forms a corridor which is famous for the production of cereals, fodder crops and legumes but olive growing (5% of domestic production) is not very widespread despite the very conducive soil and climatic conditions. However, several farmers have made an enormous effort to set up and develop the olive sector in this region, which has also recently been taken into account by Morocco's Ministry of Agriculture and Sea Fisheries and has benefited from the advantages offered for olive growing under the *Maroc Vert* plan (Monographie agricole région Chaouia – Ouardigha, 2009). Nevertheless, at present no data are available on the olive oils produced in the Ouardigha region, with the exception of a few publications (A. Mahhou *et al*., 2011).

The aim of this research was therefore to undertake a comparative evaluation of the physico-chemical characteristics and oxidative stability of the olive oils produced from the varieties cultivated in a new olive growing region of Morocco.

2. MATERIALS AND METHODS

2.1. Varieties and sampling

Four varieties were studied, two of which were Moroccan ('Picholine marocaine' and 'Haouzia') and two were foreign ('Arbequina' and 'Koroneiki'). The orchards belonged to private farmers in the Settat and Berchid olive growing districts of the Ouardigha region.

The sampling method referred to in the Guide for the determination of the characteristics of oil-olives was used (COI/OH/Doc. No 1, November 2011).

Four plots comprising ten homogeneous trees were chosen at random for the four varieties and marked with paint. Two batches of olives of each variety were collected from the selected plots, which belonged to three orchards, and underwent testing.

2.2. Methods of analysis.

Determination of optimal harvest time

This was determined according to the method described in the literature (Uceda and Frias, 1975; Arnon et al., 2011).

Physico-chemical characterisation

The olives of each variety were crushed in a modern, continuous two-phase mill. Four 5-litre batches of oil were obtained from each variety and were kept out of light until the physico-chemical and stability determinations were performed.

The ***free acidity*** of the oils obtained from the four varieties, expressed as a percentage of oleic acid, and the peroxide value were measured according to ISO standards 660 and 3960, respectively

The ***specific extinction coefficients***[1] in ultraviolet at 232 nm and 270 nm (K_{232} and (K_{270}) were calculated respectively from the absorption at 232 and 270 nm according to method NF EN ISO 3656, with the aid of a Varian-type spectrophotometer.

The ***moisture and volatile matter content and insoluble impurities content*** were determined according to ISO standards 662 and 663, respectively.

Polyphenol and ortho-diphenol[2] ***composition***: Total phenols were extracted according to the method described by Gutfinger (1981). Ten grams of olive oil were dissolved in 50 ml of hexane in a separating funnel and the solution was extracted successively with three 20-ml portions of aqueous methanol (60/40, v/v). The methanol phase was recovered in a 100-ml volumetric flask and made up with distilled water. The concentration of total polyphenols was determined by spectrophotometry using Folin-Ciocalteau reagent and the extinction was measured at 725 nm according to the Folin-Cicalteau method (Vazquez *et al*., 1973).

The ortho-diphenols were extracted according to the method described by Tsimidou *et al*. (1992) and validated by A. Amine *et al*. (2012). Twenty-five grams of olive oil were dissolved in 25 ml of hexane in a separating funnel and the solution was extracted successively with three 15-ml portions of aqueous methanol 60/40. The methanol phase was recovered in a 50-ml volumetric flask and made up with distilled water. The concentration of ortho-diphenols was determined by spectrophotometry using sodium or ammonium molybdate as reagent and measuring the extinction of the phenol solutions at 370 nm (Denis *et al*., 2004).

The concentrations of ortho-diphenols and polyphenols are expressed (ppm) in mg caffeic acid/kg of olive oil.

Tocopherol composition: The tocopherols were analysed by HPLC according to ISO 9936: 2006, using a Lichrospher 100 DIOL C18 silica column, length 250 mm and diameter 4.6 mm , equipped with 5-µm diameter particles. The HPLC apparatus was equipped with a fluorimetric detector with an excitation length of 295 nm, an emission length of 330 nm and a mobile phase of 3.85% tetrahydrofurane in n-heptane.

Total fatty acid composition[3] was determined after conversion into methyl esters obtained by transesterification of the triacylglycerols with methanol potassium. The fatty acid methyl esters of the olive oil samples were obtained according to the international standard ISO 5509. These esters were then analysed by gas chromatography according to the conditions described in ISO 5508:1990 with the aid of a Varian chromatograph with a flame ionisation detector (FID), equipped with a capillary column (CPWAX), length 30 m and internal diameter 0.25 mm. The working conditions were as follows: oven temperature: 200 °C; injector temperature: 220 °C; carrier gas: helium at a rate of 1.2 ml/min; and quantity injected: 1µl.

The ***sterol fraction***[4] was determined according to method COI/T.20/Doc No 10/Rev. 1. After saponification with an ethanolic potassium hydroxide solution, the unsaponifiable matter was extracted with ethyl ether and the sterol fraction was separated from the unsaponfiable extract by thin-layer chromatography using basic silica gel plates. The sterols were recovered from the silica gel, converted into trimethylsilyl ethers and underwent gas chromatography using a Varian 3800 chromatograph equipped with a non-polar capillary column (VF-5HT), length 30m, internal diameter 0.25 mm and film thickness of 1µm. The working conditions were as follows: oven temperature: 270 °C; injector temperature: 300 °C; detector temperature: 300 °C; carrier gas: helium at a rate of 0.5 ml/min; and quantity injected: 1µl.

[1] The IOC reference method is method COI/T.20/Doc. No 19 – Spectrophotometric investigation in the ultraviolet (*all footnotes to this article have been inserted by the IOC Executive Secretariat*)

[2] The IOC reference method is method COI/T.20/Doc. No 29 – Determination of biophenols in olive oils by HPLC

[3] The IOC reference method is method COI/T.20/Doc. No 24, 2001 – Preparation of fatty acid methyl esters from olive oil and olive pomace oil

[4] This method has been replaced by method COI/T.20/Doc. No 30, 2013 – Determination of the composition and content of sterols and triterpene dialcohols by capillary column gas chromatography.

2.3. Rancimat oxidative stability[5]

The Rancimat test is an official method that is recognised internationally (ISO 6886) as well as by numerous countries such as the United States, Japan and Switzerland. This test was therefore performed to evaluate the oxidative stability of the four samples of olive oil and gave the induction time (IT), expressed in hours, which is the length of time the fat resisted oxidative stress. Three grams of the test olive oil were placed in a test tube where it was subjected to thermal degradation at 110 °C by bubbling a stream of air at a rate of 10 l/hr. The IT value is reported on a PC directly connected to the Rancimat apparatus.

2.4. Organoleptic analysis[6]

The organoleptic profile was determined in accordance with the IOC trade standard (COI/T.20/Doc. No 15/Rev..4 .November 2011) by a skilled panel from the *Institut National de Recherche Agronomique* (INRA) in Marrakech.

2.5. Statistical processing

Principal Component Analysis (PCA) of the olive oils

The data on the polyphenol, ortho-diphenol, sterol, fatty acid and tocopherol composition of the four samples studied from each crop year were assembled and underwent Principal Component Analysis using Excel Stat software. Principal Component Analysis is considered the basic method for the analysis of multidimensional data when all the observed variables are numerical and the aim is to ascertain whether there are links between variables and samples. Its objective is to describe the data contained in a table with *n* rows (individuals) and *p* columns (variables) (Bouroche and Saporta, 1994; Benabid, 2009).

Mean and standard deviation

The results are the means of tests performed in duplicate and triplicate and are presented as the mean ± standard deviation. The standard deviations of the results were calculated using Excel 2007.

3. Results and Discussion

3.1. Determination of optimal harvest time

The maturity index of the four varieties through the two crop years varied between 2.58 and 3.66 (**Table 1**). These values correspond to the optimal harvest time and concur with those described in the literature, ranging from 2.8 to 3.5 (Bendriss, 2010). They also concur with the interval (2.59-3.93) reported by Mahhou *et al.*, 2011. This is the interval where the highest polyphenol content coincides with the maximum oil content of the olive fruits.

Table 1. Maturity index of the olive varieties studied

Olive variety	Maturity index	
	2008-2009 crop year	2009-2010 crop year
'Haouzia'	3.03 ± 0.1	3.0 ± 0.1
'Arbequina'	3.32 ± 0.2	3.52 ± 0.2
'Koroneiki'	3.34 ± 0.1	2.58 ± 0.2
'Picholine marocaine'	3.16 ± 0.1	3.66 ± 0.1

3.2. Physico-chemical characterisation of the oils

The test results show that the quality criteria - acidity, E270, moisture, volatile matter and peroxide value - were compatible with the criteria for the extra virgin olive oil grade stipulated in the IOC trade standard applying to olive oils and olive pomace oils (COI/OH/Doc. No 1, November 2011) (**Table 2**).

[5] This is not an official IOC method.

[6] This method was revised in 2013, COI/T.20/Doc. No 15/2013 – Method for the organoleptic assessment of virgin olive oil

Table 2. Results of the physico-chemical analyses

Variety	Acidity (%)		E(270)		E(232)		Moisture (%)		Impurities (%)		Peroxide value (meq/kg)	
	*C08-09	*C09-10	C08-09	C09-10	C08-09	C09-10	C08-09	C09-10	C08-09	C09-10	C08-09	C09-10
'Picholine marocaine'	0.25 ±0.02	0.28 ±0.01	0.11 ±0.01	0.10 ±0.02	1.60 ±0.02	1.82 ±0.01	0.11 ±0.02	0.14 ±0.02	0.03 ±0.01	0.04 ±0.01	1.27 ±0.1	1.19 ±0.1
'Koroneiki'	0.29 ±0.01	0.42 ±0.01	0.12 ±0.02	0.13 ±0.01	1.56 ±0.01	1.72 ±0.03	0.12 ±0.03	0.12 ±0.03	0.03 ±0.01	0.04 ±0.01	0.00 ±0.01	0.15 ±0.01
'Arbequina'	0.22 ±0.01	0.22±0.02	0.10 ±0.01	0.10 ±0.03	1.55 ±0.02	1.95 ±0.01	0.10 ±0.02	0.10 ±0.03	0.04 ±0.01	0.04 ±0.01	0.82 ±0.04	0.85 ±0.03
'Haouzia'	0.37 ±0.01	0.46 ±0.02	0.09 ±0.01	0.10 ±0.02	1.43 ±0.02	1.75 ±0.02	0.13 ±0.03	0.15 ±0.02	0.05 ±0.01	0.07 ±0.01	3.12 ±0.2	3.20 ±0.1

*C08-09: 2008-2009 crop year C09-10: 2009-2010 crop year

3.3. Physico-chemical characterisation of the oils in terms of fatty acids, sterols, tocopherols, total phenols and ortho-diphenols

***Fatty acid composition*:**

The total fatty acid (FA) composition is key to the nutritional quality of olive oil. The originality and health-promoting benefits of olive oil lie in its content of monounsaturated fatty acids in which oleic acid can account for up to 83%. Several factors such as olive ripeness, climate and variety affect the fatty acid composition profile of olive oil (García *et al*., 1996; Ollé ,2002; Judde,2004).

The results obtained in this research on analysing the olive oil oils produced from the varieties studied in the Ouardigha region show that their fatty acid composition complies with the specifications laid down in the IOC trade standard although it does vary and is clearly influenced by variety. For instance, the FA profile of the 'Arbequina' oil differs distinctly from that of the oils produced from the other varieties in that it has a palmitic acid content of 16-17%, a palmitoleic acid content of 1.7-1.2%, the highest content of saturated fatty acids (SFA) (18.5-19.9%), the lowest content of monounsaturated fatty acids (MUFA) (63.7-68.6%) and hence the lowest MUFA/PUFA ratio (4-5.3%) for the two crop years. The other three varieties -'Picholine marocaine', 'Haouzia' and 'Koroneiki' - had quite a similar fatty acid profile: palmitic acid (8.7-12.4%, palmitoleic acid (0.4-0.8%), total SFA (11.6-15.2%) and MUFA content (75-77.8%). In addition, a very high MUFA/PUFA ratio was observed for the Koroneiki variety (**Table 3**). Review of the FA composition of the olive oils produced from the varieties studied reveals that linolenic acid is a minority FA ranging in content from 0.5% to 0.9% (**Table 3**), in line with the IOC standard (max. 1%). This limit is also an indicator of the adulteration of olive oil with high-linolenic seed oils such as rapeseed and soyabean oil (Ollivier, 2003a).

Table 3. Fatty acid composition of the olive oils obtained from the varieties studied

Fatty acid	'Picholine marocaine'		'Koroneiki'		'Arbequina'		'Haouzia'	
	C08_09	C09_10	C08_09	C09_10	C08_09	C09_10	C08_09	C09_10
Palmitic acid	10.9±0.1	9.6±0.1	12.4±0.1	12.3±0.2	16.1±0.1	17.9±0.1	8.7±0.1	8.9±0.1
Palmitoleic acid	0.6±0.1	0.5±0.1	0.8±0.1	0.7±0.1	1.7±0.1	2.1±0.1	0.4±0.1	0.4±0.1
Heptadecenoic acid	0.1±0.1	0.1±0.1	0.1±0.1	0.0±0.0	0.3±0.1	0.2±0.1	0.0±0.0	0.00±0.0
Stearic acid	2.1±0.1	2.2±0.1	2.3±0.1	2.4±0.1	1.8±0.1	1.7±0.1	2.5±0.1	2.6±0.1
Oleic acid	76.1±0.1	75.9±0.1	76.6±0.1	75.9±0.2	66.3±0.1	61.2±0.1	74.2±0.1	75.2±0.2
Linoleic acid	8.7±0.1	10±0.1	6.4±0.1	7±0.1	12.3±0.1	15.6±0.1	12.3±0.1	11.2±0.1
Linolenic acid	0.7±0.1	0.9±0.1	0.6±0.1	0.6±0.1	0.5±0.1	0.5±0.1	0.8±0.1	0.8±0.1
Arachidic acid	0.3±0.1	0.2±0.1	0.4±0.1	0.4±0.1	0.4±0.1	0.2±0.1	0.3±0.1	0.3±0.1
Gadoleic acid	0.3±0.1	0.4±0.1	0.3±0.1	0.3±0.1	0.3±0.1	0.2±0.1	0.4±0.1	0.3±0.1
Behenic acid	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.0±0.0	0.1±0.1	0.1±0.1
Total *SFA	13.4±0.5	12.1±0.4	15.2±0.6	15.2±0.5	18.5±0.5	19.9±0.5	11.6±0.5	11.9±0.6
Total *MUFA	77.1±1	76.9±1	77.8±1	76.9±1	68.6±1	63.7±1	75.0±1	75.7±1
Total*PUFAs	9.4±0.6	10.9±0.5	7.0±0.3	7.6±0.2	12.8±0.5	16.1±0.5	13.1±0.5	12.0±0.5
*MUFA/PUFA	8.2 ±0.4	7.1±0.5	11.1±0.5	10.1±0.4	5.3±0.4	4.0±0.3	5.7±0.3	6.3±0.4

*FAs: Saturated fatty acids - SFAs: Monounsaturated fatty acids -MUFAs: Polyunsaturated fatty acids - PUFAs

Total polyphenol and ortho-diphenol composition

Olive oil contains a considerable quantity of phenolic compounds which pass from the fruit to the oil during extraction. These fine compounds are natural antioxidants. Ortho-diphenols such as hydroxytyrosol, caffeic acid and oleuropein are considered to be the most powerful antioxidants. They protect olive oil from oxidation, make it more stable during storage (Boskou, 1996) and lend it a bitter taste and pungent sensation (Gutiérrez *et al*., 2001; Ben Temime *et al*., 2006). Their content in the oil likewise depends on several factors, namely variety and olive fruit ripeness (Ucella *et al*., 1994), conditions of extraction (Ranalli *et al*., 2003) and storage conditions (Fielding *et al*., 2003).

The polyphenol and ortho-diphenol content of the varieties of oils studied (**Table 4**) oscillate respectively between 106 and 478 ppm and 15 and 22 ppm. This concurs closely with the values cited by Maestro *et al*. (1994), who reported that the total polyphenol concentration of olive oil may vary from 100 to 800 mg/kg, and by Owen et al. (2000) who recorded a value of 232 ± 15 mg/kg in extra virgin olive oils. Consequently, the results obtained in this research reveal that the content of these natural antioxidants is influenced by the varietal criterion. The 'Picholine marocaine' variety has the highest content of polyphenols and ortho-diphenols (313-478 and 22-20.4, respectively), followed by 'Haouzia' and then 'Koroneiki'. The lowest contents were recorded for the oil produced from the 'Arbequina' variety (106-125 and 15.3-15.2, respectively). In addition, the polyphenol and ortho-diphenol contents of the oils produced from the four varieties were higher in the 2009–2010 season (**Table 4**). This was probably due to the effect of climatic conditions, which concurs with the literature (Ollivier *et al*., 2004).

Table 4. Total polyphenol and ortho-diphenol composition

Variety	Crop year	Total polyphenols (ppm)	Ortho-diphenols (ppm)
'Picholine marocaine'	*C08-09	313.0±5	20.4±3
	C09-10	478.3±3	22.0±4
'Koroneiki'	C08-09	130.0±7	16.4±4
	C09-10	178.0±5	18 ±2
'Arbequina'	C08-09	106.0±3	15±3
	C09-10	125.5±6	15.2±3
'Haouzia'	C08-09	268.0±2	19±2
	C09-10	287.7±4	20±2

*C08-09: 2008-2009 crop year C09-10: 2009-2010 crop year

Tocopherol composition

Tocopherols are important analytical parameters because of their vitamin and nutritional properties and their role in protecting olive oil from free radicals (Reboul *et al*., 2007). Tocopherol analysis during the two successive crop years has shown that olive variety has an effect on tocopherol content. Alpha-tocopherol, which has vitamin properties, predominates over the other tocopherols in all the varieties of olive oil studied and is influenced by the varietal profile; this concurs with other findings reported in the literature (Gharby *et al*., 2011). The highest percentage was recorded in the 'Arbequina' oils (90-91%), followed by 'Koroneiki' (89%) and 'Haouzia' and 'Picholine marocaine' (70-83%). Conversely, the 'Picholine marocaine' and 'Haouzia' varieties had quite high gamma-tocopherol values (10-19%) compared with 'Koroneiki' and 'Arbequina' (5-7.5%). This moderately high content of gamma-tocopherol in the 'Picholine marocaine' and 'Haouzia' oils could have a positive influence on their stability (**Table 5**) since the antioxidant activity of gamma-tocopherol is greater than that of alpha-tocopherol (Evrard *et al*., 2007; Combe and Castera, 2010).

Table 5. Tocopherol content (%) of oils

Tocopherol (%)	'Picholine marocaine'		' Koroneiki'		'Arbequina'		'Haouzia'	
	C08-09	C09-10	C08-09	C09-10	C08-09	C09-10	C08-09	C09-10
Alpha-tocopherol	83.9 ±0.8	70.4±0.9	89.7±0.6	89.5±0.7	91.7±0.5	90.7±0.5	82.7±0.6	80.2±0.6
Beta-tocopherol	2.0±0.2	2.4±0.3	1.6±0.4	1.6±0.3	0.8±0.3	0.9±0.3	1.9±0.3	1.5±0.3
Gamma-tocopherol	10.8±0.5	19.8±0.4	7.5±0.6	6.9±0.3	5.1±0.5	6.1±0.6	12.5±0.4	16.5±0.6
Delta-tocopherol	3.3±0.4	7.5±0.6	1.2±0.3	2.0±0.4	2.3±0.5	2.3±0.5	3.0±0.5	1.8±0.4

Sterol content

The sterol composition of vegetable oils is an important criterion for the identification of their botanical origin (Karlenskind, 2002). In contrast with the findings reported in the literature (Aparicio *et al*., 2002) to the effect that olive variety influences sterol content, this research did not observe substantial differences in the sterol content of the samples of any of the varieties over the two crop years (**Table 6**).

Table 6: Sterol composition

Sterols (%)	'Picholine marocaine'		'Koroneiki'		'Arbequina'		'Haouzia'	
	C08-09	C09-10	C08-09	C09-10	C08-09	C09-10	C08-09	C09-10
Cholesterol	0.3±0.1	0.3±0.1	0.4±0.1	0.2±0.1	0.3±0.1	0.2±0.1	0.4±0.1	0.3±0.1
Campesterol	3.3±0.2	3.3±0.3	3.2±0.2	3.1±0.2	3.3±0.4	3.4±0.4	3.4±0.1	3.3±0.2
Stigmasterol	1.2±0.1	1.4±0.2	1.3±0.2	1.4±0.2	1.2 ±0.3	1.4±0.3	1.2±0.1	1.2±0.1
Beta-sitosterol	86. ±0.5	86.8±1	86.1±1.5	86.5±0.7	86.4±0.7	86.5±0.6	86.8±0.7	86.2±0.5
Delta-5-avenasterol	8.0±0.3	7.3±0.4	8.1±0.5	7.9±0.4	7.9±0.3	7.6±0.4	7.3±0.3	8.0±0.3
Delta-7-stigmasterol	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1
Delta-7-avenasterol	0.4±0.1	0.4±0.1	0.4±0.1	0.4±0.1	0.4±0.1	0.4±0.1	0.4±0.1	0.4±0.1

3.4. Rancimat oxidative stability

The Rancimat test measures resistance to accelerated oxidation (Matthaus, 1996; Rahmani 2007). The results obtained (**Table 7**) show that variety clearly influences oil stability in that the highest values were recorded for the 'Koroneiki' variety (2009-2010 crop year: 40.9 hr), followed by 'Picholine marocaine' (39.8 hr) and Haouzia (36.3 hr). The lowest value was recorded for the 'Arbequina' variety (27 hr), These results agree with the results reported by other authors for various varieties of olive oil (Abaza *et al*., 2005; Ben Temime *et al*., 2008a) as well as for the low induction time of the 'Arbequina' variety compared with other varieties (Gutiérrez *et al*., 2002a; Ceballos *et al* , 2003; Mateos et al., 2006).

Table 7: Oxidative stability of the oils measured according to the Rancimat method at 110 °C

Variety	Crop year	Stability (IT in hours)
'Picholine marocaine'	*C08-09	38 .4±0.5
	C09-10	39 .8±0.5
'Koroneiki'	C08-09	37.2±0.4
	C09-10	40.9±1
'Arbequina'	C08-09	27.0±2
	C09-10	28. 3±2
'Haouzia'	C08-09	30.8±0.5
	C09-10	36.3±1

Hence, 'Haouzia', 'Picholine marocaine' and 'Koroneiki' are more stable than 'Arbequina' (**Table 7**). In the case of the first two varieties, this can be explained by the fact that the oils have a higher content of total polyphenols, ortho-diphenols and gamma-tocopherol, all of which are acknowledged to be antioxidants in oils. As for the 'Koroneiki' variety, besides having quite a high content of these antioxidant compounds, it has a low content of polyunsaturated fatty acids and a high MUFA/PUFA ratio. In contrast, the poor stability of the 'Arbequina' variety versus the other oils is basically because of its low content of total polyphenols, ortho-diphenols and gamma-tocopherol and its lower MUFA/PUFA ratio. This concurs with the literature (Gharby *et al*., 2011) (33). Hence, a positive correlation was recorded between the polyphenol, ortho-diphenol and gamma-tocopherol content of the 'Arbequina', 'Haouzia' and 'Picholine marocaine' varieties for the two crop years and their oxidative stability (Figures 1, 2,3). This also concurs with the findings reported in the literature (Chimi *et al*., 1990).

Figure 4 also shows a positive correlation between oxidative stability and the MUFA/PUFA ratio in the oils produced from the four varieties studied in this study.

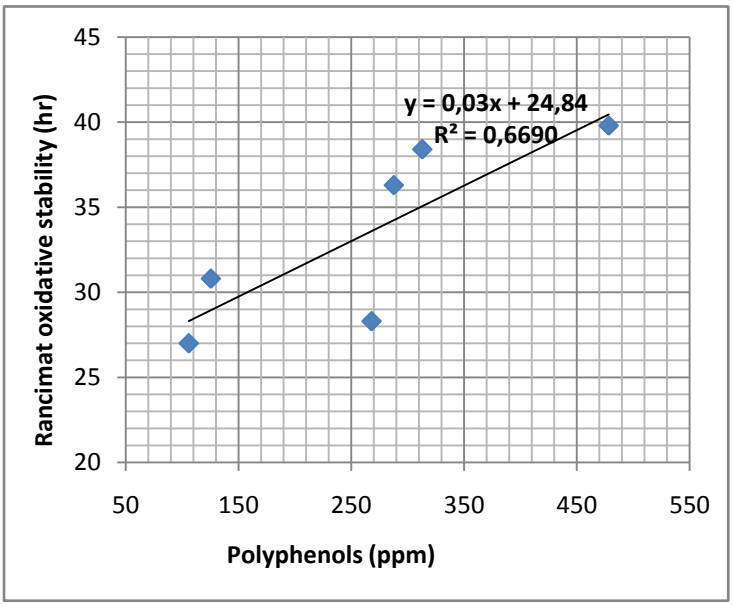


Figure 1. Correlation between the oxidative stability and total polyphenol content of the oils produced from the 'Picholine marocaine', 'Haouzia' and 'Arbequina' varieties.

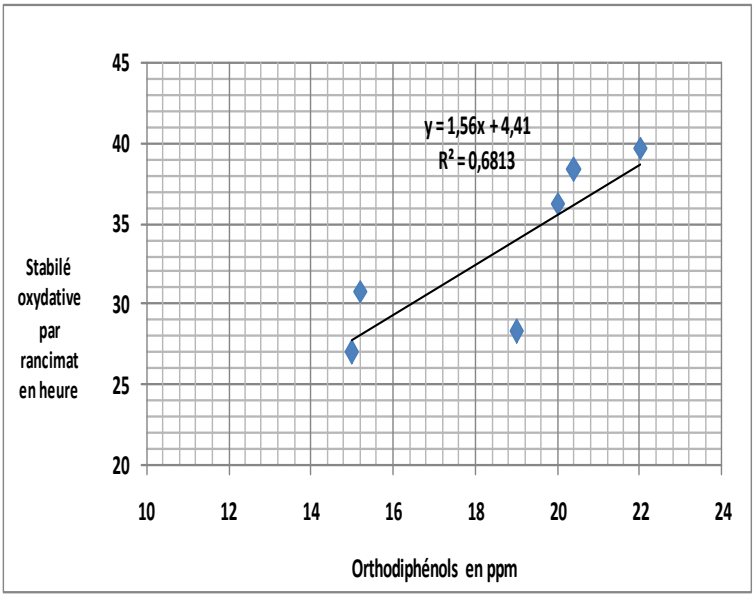


Figure 2. Correlation between the oxidative stability and ortho-diphenol content of the oils produced from the 'Picholine marocaine', 'Haouzia' and 'Arbequina' varieties.

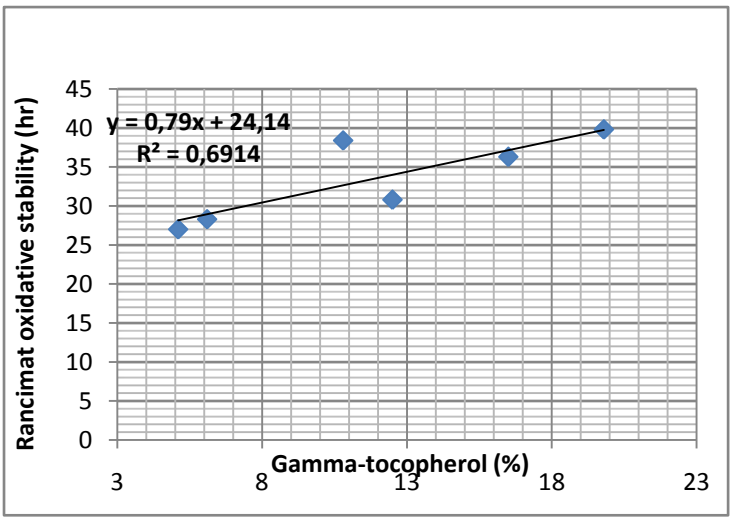


Figure 3. Correlation between the oxidative stability and percentage gamma-tocopherol content of the oils produced from the 'Picholine marocaine', 'Haouzia' and 'Arbequina' varieties.

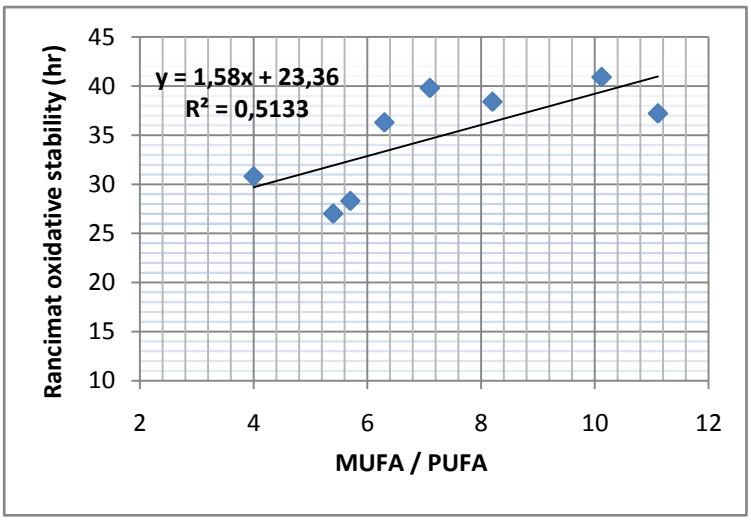


Figure 4. Correlation between the oxidative stability and MUFA/PUFA ratio of the oils produced from the four varieties studied.

3.5. Principal Component Analysis (PCA) of the olive oils

PCA provides a schematic representation and very simplified summary of all the interpretations already reported for ortho-diphenol, total polyphenol, tocopherol and fatty acid composition.

PCA phenol profile of the oils

The results for the polyphenol and ortho-diphenol composition of the olive oils studied for the 2008–09 and 2009–2010 crop years are described in Figures 5 and 6 respectively.

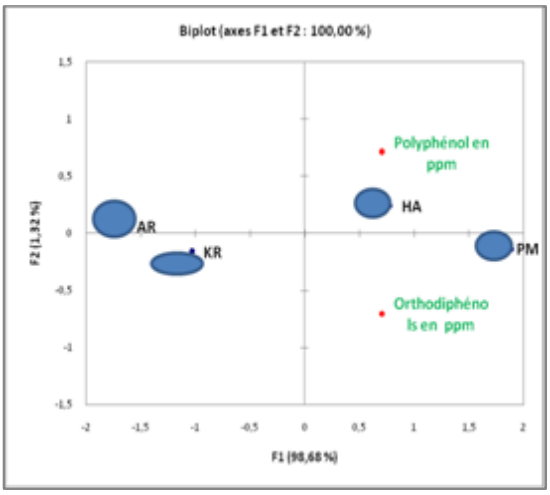


Figure 5. Principal Component Analysis (PCA) of ortho-diphenol and total polyphenol composition, 2008-2009 crop year.

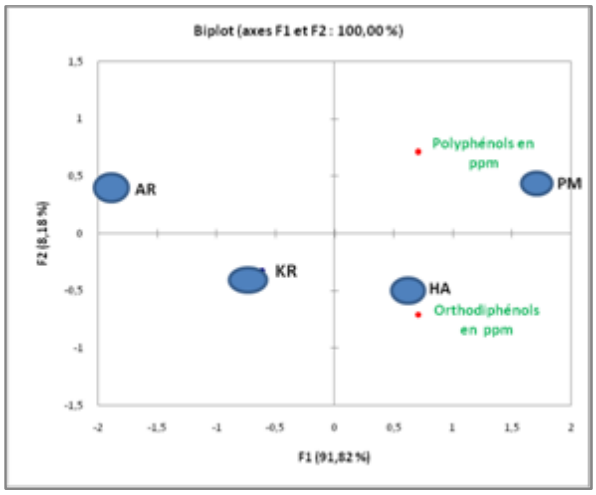


Figure 6. Principal Component Analysis (PCA) of ortho-diphenol and total polyphenol composition, 2008-2009 crop year.

The 'Picholine marocaine' and 'Haouzia' oils had a very similar phenol profile with high values for the two successive crop years, followed by the 'Koroneiki' variety; the 'Arbequina' variety lies in last place.

PCA of the tocopherol composition of the oils.

Figures 7 and 8 present the Principal Component Analysis of the tocopherol composition of the oils.

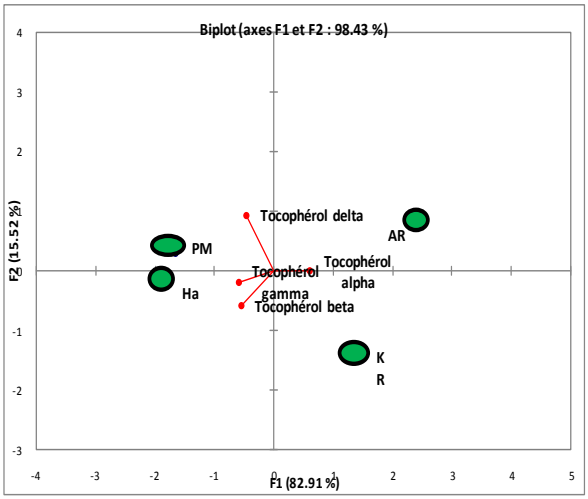


Figure 7. Principal Component Analysis (PCA) of tocopherol composition, 2008-2009 crop year.

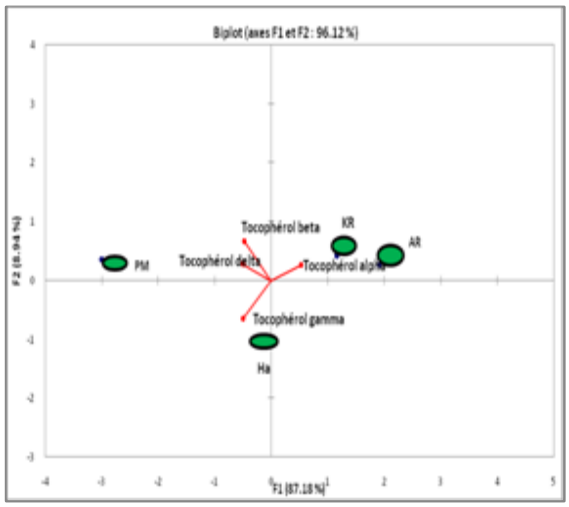


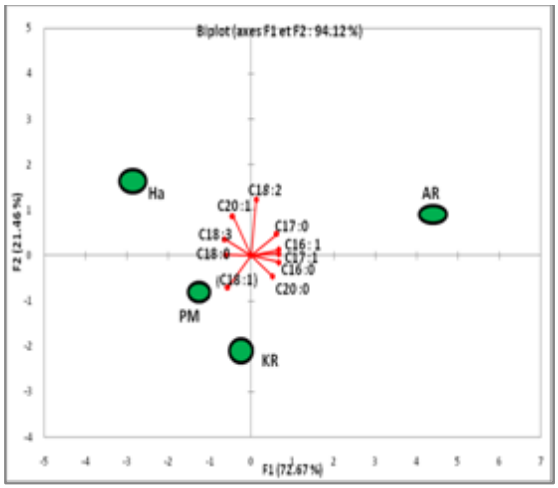
Figure 8. Principal Component Analysis (PCA) of tocopherol composition, 2009-2010 crop year.

Ar: Arbequina-**KR**: Koroneiki-**Ha**: Haouzia-**PM**: Picholine marocain

Alpha-tocopherol is the most neutral component (lying on the axis) and has a high content in all the varieties studied. The 'Picholine' and 'Haouzia' varieties have almost the same percentage contents of the other tocopherols, notably gamma-tocopherol, which are quite high compared with 'Arbequina'.

PCA of fatty acid composition

PCA reveals a perfectly different fatty acid profile for the 'Arbequina' variety compared with the other varieties in the two consecutive crop years. This is apparent above all in its high content of palmitic (C16:0) and palmitoleic (C16:1) acid. Its oleic acid content was comparable to that of the other three varieties studied (**Figures 9 and 10**).



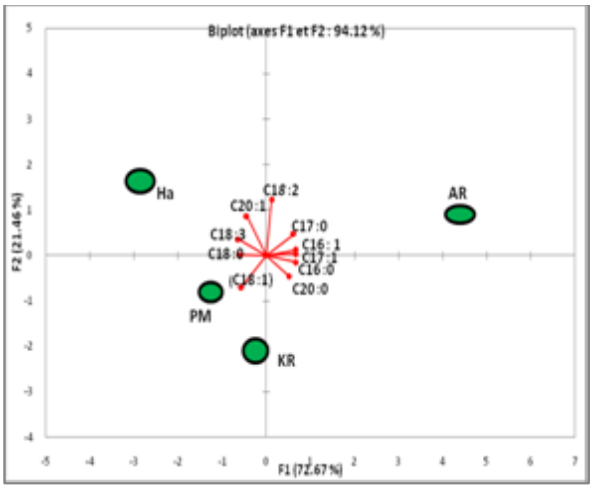


Figure 9. PCA of the fatty acid composition of the varieties, 2008-2009 crop year.

Figure 10. PCA of the fatty acid composition of the varieties, 2009/10 crop year.

C16 :0 Palmitic acid **C16 :1** Palmitoleic acid, **C18 :0** Stearic acid, **C17 :0** Heptadecanoic acid **C17 :1** Heptadecenoic acid **C20 :0** Arachidic acid **C18 :2** Linoleic acid **C20 :1** Gadoleic acid , **C18 :3** Linolenic acid C18 :1 Oleic acid

3.6. Organoleptic profile

A simple chemical analysis does not suffice to determine the quality of an olive oil. The volatile compounds that develop during oil extraction and storage can modify the smell and taste of an oil. Hence, it was decided to perform sensory analysis in order to provide a further insight into the quality of the samples, which therefore underwent organoleptic assessment according to the IOC standard.

The results of the sensory analysis of the oils produced from the four varieties in the two consecutive seasons confirmed that they were extra virgin olive oils with scores of 3-4.55 for fruitiness, 1.2-3 for bitterness and 1.5-4 for pungency. The specific aromas noted were grass, tomato, apple, artichoke and almond. No negative attribute was reported. The sensory analysis results are given in **Figures 11 and 12**.

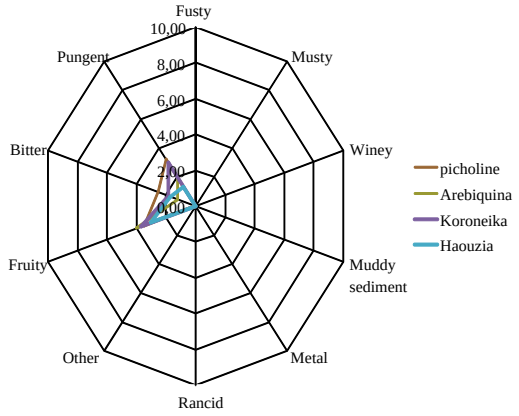


Figure 11. Sensory profile of the oils 2009-2010 crop year.

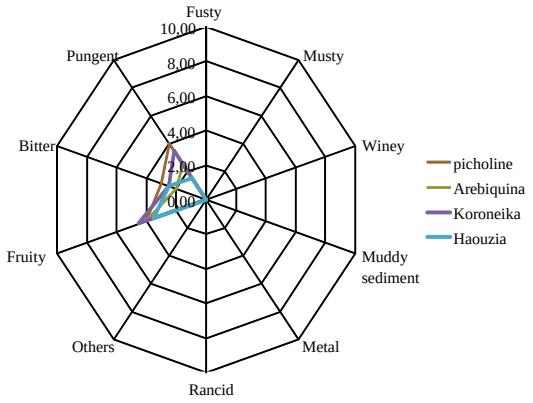


Figure 12. Sensory profile of the oils – 2009-2010 crop year.

4. CONCLUSIONS

The oils studied in this research were produced in the Chaouia-Ouardigha region and were all extra virgin grade according to IOC standards; they were obtained from olive fruits with a maturity index between 2.58 and 3.6.

The quality of these extra virgin olive oils is heavily correlated with their minor components, namely polyphenols, ortho-diphenols and tocopherols as well as with the type of monounsaturated and polyunsaturated fatty acids, in particular the MUFA/PUFA ratio, which is a determinant of oxidative stability. Furthermore, the physico-chemical and organoleptic characteristics of the four varieties of oil produced show that the Chaouia-Ouardigha region appears to be suited to the production of superior quality olive oil although oil quality in terms of antioxidant content and oxidative stability is variety-dependent. It emerges that the Spanish 'Arbequina' variety, which has lately come into great favour with olive farmers because of its early start of bearing and its high oil yield, is an unstable variety due to its lower oxidative resistance and its low content of natural antioxidants compared with the two Moroccan varieties 'Picholine marocaine' and 'Haouzia' and the Greek variety 'Koroneikei'. Hence, in order to enhance the quality of olive oil produced in the Chaouia-Ouardigha region, olive farmers should opt for olive varieties that produce superior quality oil such as the Moroccan and Greek varieties just mentioned. Likewise, they should opt to blend oils obtained from high-yielding varieties ('Arbequina') with more stable varieties (Moroccan varieties, 'Koroneiki', etc.) in order to aim simultaneously at quality and productivity.

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Creation of a database of the fatty acid and triacylglycerol composition of virgin olive oils produced from 34 French varieties, eight French designations of origin and two foreign varieties grown in France (Part I)

D. Ollivier[1], C. Pinatel[2], V. Ollivier[1] and J. Artaud[3]

[1] Laboratoire de Marseille, Service Commun des Laboratoires, Ministère des Finances, 146 traverse Charles-Susini, 13388 Marseille Cedex 13, France.
[2] Association Française Interprofessionnelle de l'Olive, (AFIDOL), Maison des Agriculteurs, 22 avenue Henri-Pontier, 13626 Aix-en-Provence Cedex, France.
[3] Aix-Marseille Université, Faculté des Sciences de St Jérôme, avenue Escadrille Normandie-Niemen, 13397 Marseille cedex 20, France.

ABSTRACT

France has approximately 200 olive varieties in addition to eight designations of origin (DO) which are either registered (RDO) or protected (PDO) and spread across 13 departments in the south of the country. Some 15 varieties are of general relevance because they are grown over large areas or they are constituent varieties in the composition of DO oils. With the growing demand for agricultural foodstuffs to be traceable, non-subjective tools are required to determine the origin of both the raw materials and the final product. Effective, swift means of authentication are needed to ensure that retail product is true to its stated varietal or geographical origin, that the very precise provisions of DO regulations (PDOs, CDOs, PGIs) are fulfilled and that trading practices are fair. The mean fatty acid composition and triacylglycerol composition of 34 French varieties, two foreign varieties grown in France and eight designations of origin (n= 2035) were determined. The French oils differed strikingly in composition. Indices created on the basis of the fatty acids and the composition of the chief triacylglycerols helped to classify the oils. The results were confirmed by Principal Component Analysis of the 34 variables determined. These data are part of an original database comprising more than 3000 samples, which was the starting point for statistical processing aimed at oil authentication. A simple, swift, reliable and visual method is proposed for this purpose in Part II.

Key words: French virgin olive oils, fatty acids, triacylglycerols, traceability, database.

1. INTRODUCTION

The Mediterranean region has an extensive pool of olive resources comprising more than 2000 cultivars so far [1], approximately 200 of which are to be found in France [2, 3]. French cultivars are of general or local importance depending on the size of crop area. Eleven varieties are of general importance according to the Official French Catalogue of Species and Varieties [4] whereas Moutier *et al*. speak of 13 [1]. Certain general or local varieties are the foundation stone of the oils produced under the eight registered or protected designation of origin schemes currently in place in France. Until recently, there were no comprehensive data on the chemical composition of French olive oils. This dearth of data was detrimental to olive growing in France which, though modest compared with elsewhere in the Mediterranean, produces oils displaying original and highly varied characteristics, particularly from the organoleptic standpoint [5, 6]. Furthermore, one of the main problems facing the agri-food industry is that it needs objective tools to determine the origin of raw materials and final products and so ensure they can be traced from producer to consumer. The authentication of the origin of virgin olive oils is one facet of this challenge. The fact that virgin olive oil is more expensive than other edible oils and that it differs greatly in price depending on its origin and quality has led to numerous studies proposing ways of determining its varietal origin or quality certification. Effective, swift means of authentication are therefore needed to ensure that retail product is true to its stated varietal or geographical origin, that the very precise provisions of DO regulations (PDOs, CDOs, PGIs, ...) are fulfilled and that trading practices are fair. Four different yet complementary approaches have been developed for oils: sensory analysis [6], molecular biology [7], infrared spectroscopy [8, 9] and nuclear magnetic resonance (NMR) [10, 11], and analysis of chemical composition. Varietal characterisation based on chemical composition has investigated several families of compounds: fatty acids [5, 12], sterols [13], volatile compounds [14], hydrocarbons [16], ... The spectroscopic and chemical approaches are associated with chemometric data processing methods such as Principal Component Analysis (PCA), Soft Independent Modelling of Class Analogy (SIMCA) and Partial Least Squares Discriminant Analysis (PLS-DA).

For several years, the authors have been conducting a widespread survey of French monovarietal or DO virgin olive oils (VOOs) on the basis of their fatty acid and triacylglycerol composition [5, 16, 17]. Fatty acids and triacylglycerols were chosen as testing parameters because they are relatively easy to analyse and they remain stable over time compared with the compounds in the other chemical families found in VOOs. The chromatographic data underwent chemometric analyses enabling discrimination of the oils produced from general varieties [13] and DO oils [6, 17]. The above study was extended to cover numerous French monovarietal or DO oils, oils produced from foreign varieties grown in France and foreign oils in order to create a database (known by its French acronym, AGTG 33) [18] encompassing approximately 3000 olive oils of different origins.

This article presents the mean fatty acid and triacylglycerol composition of 34 French monovarietal oils, two monovarietal oils produced from foreign varieties cultivated in France and eight DO oils and helps to give a better insight into their composition. The results were entered in the AGTG 33 database [18] and are applied an original statistical processing approach in Part II permitting swift, visual interpretation.

2. MATERIALS AND METHODS

2.1. Materials

The samples of monovarietal (n=1009) and DO (n=1026) virgin olive oils were supplied by the *Association Française Interprofessionnelle de l'Olive* (AFIDOL), Aix-en-Provence, France and the *Service Commun des Laboratoires* (SCL), Marseille, France. The samples were collected over 10 successive harvest seasons (2001–2010).

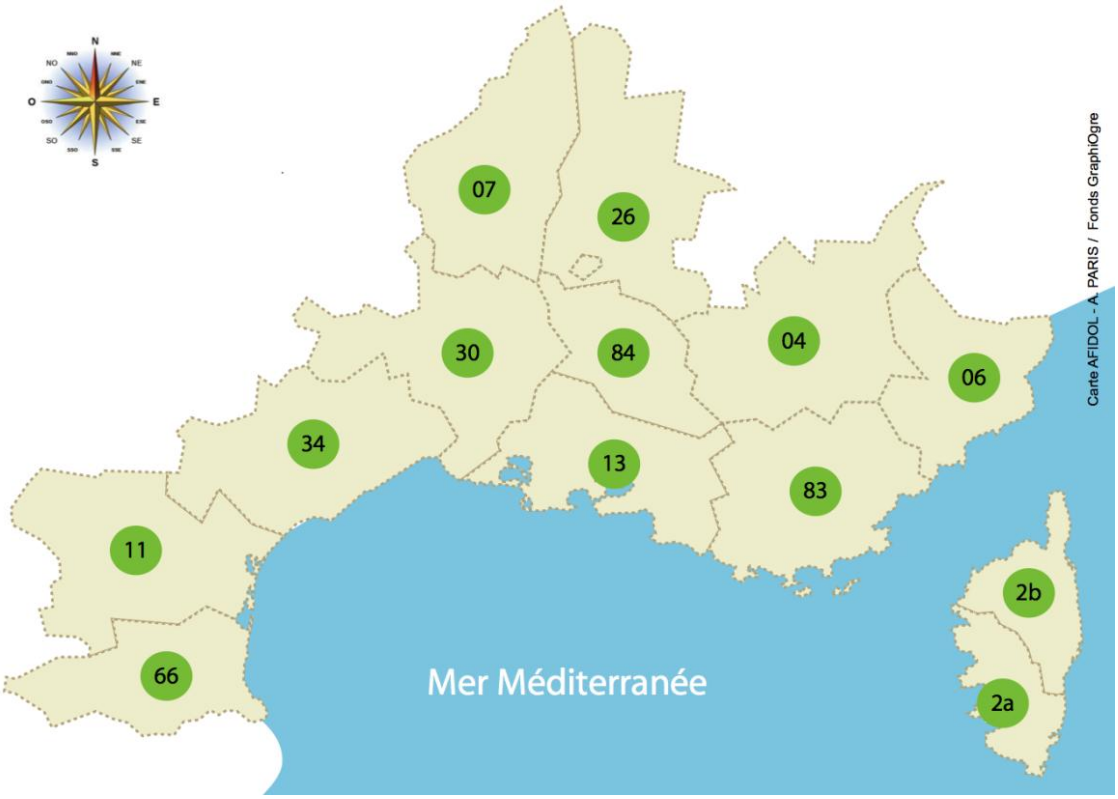

Figure 1. Chief locations of French varieties.

Départements : **04** : Alpes de Haute-Provence ; **06** : Alpes Maritimes ; **07** : Ardèche ;:**11** : Aude ; **13** : Bouches-du-Rhône ; **2a** et **2b** : Corse ; **26** : Drome ; **30** : Gard ; **34** : Hérault ; **66** : Pyrénées Orientales ; **83** : Var ; **84** : Vaucluse.
Variétés : Aglandau (**04, 13, 84**), Aubenc (**07**), Baguet (**07**), Bé-dé-Cézé (**07**), Blanche de Paysac (**07**), Brun (**83**), Cailletier (**06**), Clermontaise (**34**), Coucourelle (**83**), Cayanne (**13**), Cayet roux (**83**), Cayon (**83**), Dent de Verrat (**06**), Grassois (**83**), Grossane (**13**), Lucques (**34**), Négrette (**30**), Olivière (**11, 34, 66**), Petit ribier (**83**), Petite noire (**06**), Petite violette (**07**), Pointue de l'Ardèche (**07**), Picholine du Languedoc (**13, 30, 2a** et **2b**), Rougette de l'Ardèche (**07**), Rougette de l'Hérault (**34**), Roussette du Var (**83**), Sabine (**2a** et **2a**), Salonenque (**13**), Tanche (**26, 83**), Tripue (**06**), Verdale 13 ou Verdale des Bouches-du-Rhône (**13**), Verdale 34 ou Verdale de l'Hérault (**34**), Verdale 66 ou Verdale de Millas (**66**).

2.2. Preparation and analysis of the fatty acid methyl esters

One hundred and twenty milligrams of virgin olive oil were placed in 2 ml of iso-octane and transesterified with a 2 ML cold methanolic solution of potassium (1 mL). The reaction mixture was shaken in a vortex mixer for 2 min and then centrifuged and 2 mL of iso-octane were added to the upper phase containing the fatty acid methyl esters. An aliquot was drawn off for analysis. The analyses were performed using a Perkin-Elmer Autosystem 9000XL chromatograph equipped with a split/splitless injector (T=250 °C), flame ionisation detector (FID) (T=250 °C) and autosampler. The characteristics of the DB WAX (JW) capillary column were as follows: L=60 m, Φ_{int}= 0.25 mm, e_f= 0.25 µm. Hydrogen was the carrier gas used (154 kPa, split vent flow 70). The oven temperature was programmed as follows: 13 min at 200 °C, from 200 °C to 230 °C at 6 °C/ min, 17 min at 230 °C. All the analyses were performed in duplicate. The fatty acid methyl esters were identified in a previous paper [5, 17]. The fatty acid contents were determined by area normalisation without taking into account response factors. Only fatty acids with a content of more than 0.01% were taken into account. A reference sample from the laboratory proficiency recognition scheme run by the International Olive Council (IOC) was systematically tested before each batch of analyses in order to validate the results. The coefficients of variation calculated on the basis of 60 analyses of the same sample were below 5% for the chief fatty acids and below 10% for certain minor fatty acids [17].

2.3. Squalene analysis

Squalene was determined during analysis of the fatty acid methyl esters by area normalisation without taking into account the difference in response coefficients between the fatty acid methyl esters and the squalene.

2.4. Triacylglycerol analysis

The triacylglycerols were analysed with the aid of a Merck chromatograph Model LaChrom equipped with a Merck RP-18 Superpher 100 column (L=250 mm, d_i= 4 mm, temperature 28 °C) coupled to a Merck L-7490 refractometric detector. Ten µL of a 5% (p/v) triacylglycerol solution in propionitrile (CHEM-LAB NV, Belgium) (19) were injected using an autosampler (Merck L-7200) and a 100 µL injection loop. Propionitrile was used as the elution solvent at a linear gradient flow rate of 0.5 to 1 mL/min for 47 min. All the analyses were performed in duplicate.

The triacylglycerols were separated according to their equivalent carbon number (ECN), defined as CN-2n, where CN is the total number of carbon atoms in the acyl chains and n is the total number of double bonds in the acyl chains. Triacylglycerol identification was performed by consulting data reported in the literature [20] and after collection of the HPLC peaks and methyl ester analysis [17].

As no reference sample values were available, a reference sample was created by constructing a control chart and taking as a reference the mean values obtained for the triacylglycerols [17]. The coefficients of variation, calculated on the basis of 33 analyses, were below 5% for the triacylglycerols with a content of more than 2%. In the case of those with a content between 1 and 2%, the coefficients of variation were less than 10% [17].

2.5. Mean indices

Four mean indices were calculated to characterise the VOOs, namely:

- The monounsaturated index (**MI**), which is the ratio of total monounsaturated fatty acids to total saturated fatty acids;
- The polyunsaturated index (**PI**), which is the ratio of polyunsaturated fatty acids to total saturated fatty acids;
- The total unsaturated index (**TUI**), which is the ratio of monounsaturated and polyunsaturated fatty acids to total saturated fatty acids;
- The odd-chain index (**OCI**), which is the ratio of total odd-numbered C17 fatty acids to total even-numbered acids, multiplied by 100.

2.6. Nomenclature

Fatty acids : palmitic acid (16:0), (hexadecanoic acid); hypogeic acid (16:1ω9), (7-hexadecenoic acid); palmitoleic acid (16:1ω7) (9-hexadecenoic acid); margaric acid (17:0), (heptadecanoic acid); margaroleic acid (17:1ω8), (9-heptadecenoic acid); oleic acid (18:1ω9), (9-octadecenoic acid); *cis*-vaccenic acid (18:1ω7), (11-octadecenoic acid); linoleic acid (18:2ω6), (9,12-octadecadienoic acid); linolenic acid (18:3ω3), (9,12,15-octadecatrienoic acid); arachidic acid (20:0), (eicosanoic acid); gondoic acid (20:1ω9), (11-eicosenoic acid) [13]; behenic acid (22:0), (docosanoic acid); lignoceric acid (24:0), (tetracosanoic acid).

Triacylglycerols: The triacylglycerols are designated by letters corresponding to the abbreviated names of the fatty acids fixed to the glycerol: P, palmitoyl; Po, palmitoleyl S, stearoyl; O, oleoyl; L, linoleoyl; Ln, linoleolenyl; A, arachidoyl.

3. Results and Discussion

All the samples analysed contain the same 14 fatty acids (**Tables 1a, 1b and 2**), the percentage contents of which show within- and between-variety variations. The within-variety variations can be attributed to various environmental and cultivational parameters – *terroir*, climatic conditions, cultural practices and fruit ripeness – while the between-variety variations stem from genetic differences. **Tables 1a and 1b** report the mean values of each of the fatty acids with a content of more than 0.01% as well as the four mean indices MI, PI, TUI and OCI.

Table 1a. Mean fatty acid[1] (%) and squalene composition of virgin olive oils produced from 14 varieties of general relevance

Fatty acids	Aglandau	Bouteillan	Cailletier	Cayanne	Cayet roux	Cayon	Grossane	Lucques	Olivière	PicholineL	Salonenque	Tanche	Verdale 13	Verdale 34
	n=128	n=63	n=163	n=12	n=4	n=14	n=25	n=21	n=32	n=107	n=52	n=151	n=5	n=11
16 :0	12,78	12,02	10,88	11,14	12,80	10,29	14,19	11,98	11,96	10,75	14,58	8,43	13,13	12,70
16 :1ω9	0,14	0,13	0,10	0,17	0,10	0,20	0,11	0,14	0,14	0,13	0,12	0,15	0,10	0,14
16 :1ω7	1,03	0,63	0,63	0,71	0,89	0,94	1,75	0,86	1,42	0,58	1,07	0,39	1,00	0,88
17 :0	0,17	0,13	0,05	0,05	0,12	0,05	0,05	0,15	0,11	0,06	0,07	0,05	0,05	0,14
17 :1ω8	0,34	0,21	0,10	0,12	0,34	0,09	0,12	0,28	0,28	0,10	0,12	0,08	0,09	0,26
18 :0	2,48	2,53	2,13	1,76	1,43	2,12	1,94	2,34	1,75	2,24	2,60	2,66	2,04	2,02
18 :1ω9	71,97	68,24	75,55	75,09	66,70	78,83	69,26	72,91	74,43	73,73	64,13	79,51	65,12	68,44
18 :1ω7	2,49	1,96	2,13	20,51	3,26	2,25	3,33	2,21	3,49	1,84	2,46	1,47	2,81	2,43
18 :2ω6	7,19	12,28	6,99	6,86	12,92	3,82	7,70	7,53	4,04	8,92	13,38	5,81	14,21	11,51
18 :3ω3	0,60	0,96	0,60	0,60	0,64	0,63	0,71	0,73	0,69	0,84	0,59	0,61	0,67	0,79
20 :0	0,40	0,43	0,37	0,38	0,30	0,36	0,38	0,40	0,31	0,36	0,44	0,38	0,36	0,33
20 :1ω9	0,25	0,30	0,31	0,40	0,33	0,26	0,29	0,32	0,27	0,32	0,25	0,31	0,25	0,21
22 :0	0,12	0,13	0,12	0,15	0,12	0,22	0,12	0,11	0,08	0,09	0,13	0,10	0,10	0,10
24 :0	0,05	0,06	0,05	0,06	0,06	0,05	0,05	0,06	0,04	0,05	0,07	0,04	0,05	0,04
Squal.	0,81	0,93	0,43	0,50	0,87	0,57	0,91	0,46	0,65	0,70	0,64	0,92	1,01	0,75
MI	*4,77*	*4,71*	*5,78*	*5,83*	*4,86*	*6,45*	*4,46*	*5,12*	*5,66*	*5,65*	*3,79*	*6,99*	*4,40*	*4,72*
PI	*0,49*	*0,87*	*0,56*	*0,55*	*0,92*	*0,35*	*0,50*	*0,55*	*0,33*	*0,72*	*0,78*	*0,55*	*0,94*	*0,80*
TUI	*5,25*	*5,58*	*6,34*	*6,38*	*5,78*	*6,80*	*4,96*	*5,67*	*5,99*	*6,37*	*4,56*	*7,54*	*5,34*	*5,52*
OCI	*3,21*	*2,19*	*1,06*	*1,28*	*3,11*	*1,11*	*1,02*	*2,81*	*2,73*	*1,14*	*1,05*	*1,06*	*0,93*	*2,58*

[1]determined as methyl esters, % of areas of total fatty acids

Table 1b. Mean fatty acid[1] (%) and squalene composition of virgin olive oils produced from 22 local varieties

Fatty Acids	Aubenc	Arbéquine F[2]	Arboussane F[2]	Baguet	Bé-dé-Cézé	Blanche de Paysac	Brun	Clermontaise	Coucourelle	Dent de Verrat	Grassois	Négrette	Petit ribier	Petite noire	Petite violette	Pointue 07[3]	Rougette 07[3]	Rougette 34[4]	Roussette 83[6]	Sabine	Tripue	Verdale 66[5]
	n=5	n=38	n=10	n=3	n=6	n=6	n=27	n=4	n=5	n=5	n=3	n=27	n=19	n=5	n=3	n=3	n=27	n=13	n=5	n=9	n=5	n=77
16 :0	10,46	14.22	12.88	13,76	13,66	11,18	12,53	11,67	14,06	12,71	11,86	9,88	11,24	12,52	8,13	12,89	11,76	10,24	10,78	11,22	13,70	11,00
16 :1ω9	0,10	0.14	0.08	0,12	0,14	0,11	0,14	0,13	0,16	0,10	0,13	0,14	0,06	0,12	0,13	0,14	0,15	0,14	0,09	0,18	0,08	0,10
16 :1ω7	0,51	1.51	1.12	1,51	1,12	0,54	1,39	0,75	1,66	1,20	0,72	0,61	0,60	00,61	0,51	0,75	1,05	0,63	0,60	0,057	1,43	0,52
17 :0	0,04	0.11	0.13	0,04	0,04	0,05	1,12	0,18	0,09	0,05	0,04	0,13	0,05	0,04	0,16	0,14	0,05	0,12	0,15	0,05	0,05	0,06
17 :1ω8	0,07	0.23	0.28	0,09	0,07	0,09	0,24	0,27	0,22	0,10	0,08	0,22	0,09	0,09	0,25	0,25	0,09	0,30	0,24	0,06	0,09	0,09
18 :0	2,86	1.75	2.11	1,30	2,20	2,36	2,03	2,81	1,53	2,21	1,81	3,68	1,97	1,78	2,75	2,43	2,70	1,56	2,78	2,54	1,74	3,13
18 :1ω9	78,08	69.39	74.49	71,43	68,12	72,66	70,68	71,08	68,54	74,31	75,13	73,57	76,05	72,45	82,30	71,71	72,46	72,97	77,63	71,55	68,49	74,88
18 :1ω7	1,71	3.48	2.86	3,91	2,63	2,04	2,85	2,11	3,75	2,56	2,81	1,91	2,11	2,68	1,32	2,14	2,16	2,14	1,61	1,64	3,06	1,73
18 :2ω6	4,92	7.78	4.54	6,54	10,16	9,57	8,76	9,37	8,53	5,48	6,01	8,12	6,39	8,23	2,86	8,20	8,10	10,49	4,62	10,45	9,94	6,99
18 :3ω3	0,38	0.53	0.57	0,76	1,08	0,64	0,55	0,67	0,57	0,55	0,70	0,87	0,63	0,67	0,71	0,54	0,62	0,69	0,61	0,91	0,68	0,58
20 :0	0,44	0.38	0.41	0,23	0,38	0,37	0,34	0,47	0,35	0,35	0,30	0,44	0,34	0,32	0,43	0,40	0,44	0,28	0,42	1,40	0,33	0,49
20 :1ω9	0,24	0.30	0.30	0,22	0,22	0,26	0,23	0,28	0,36	0,22	0,28	0,28	0,30	0,32	0,31	0,23	0,25	0,29	0,28	0,27	0,26	0,25
22 :0	0,14	0.12	0.16	0,07	0,13	0,10	0,10	0,14	0,12	0,12	0,09	0,11	0,11	0,11	0,11	0,12	0,13	0,09	0,14	0,09	0,12	0,13
24 :0	0,06	0.06	0.06	0,04	0,05	0,04	0,04	0,06	0,06	0,04	0,04	0,05	0,05	0,05	0,05	0,06	0,05	0,04	0,05	0,06	0,04	0,05
Squa.[7]	0,92	0,43	0,43	0,68	0,73	0,75	0,33	0,64	0,94	0,28	0,35	0,73	0,86	0,67	1,00	0,95	0,72	0,63	0,53	0,39	0,97	0,73
MI	*5,78*	*4.52*	*5.01*	*5,01*	*4,37*	*5,38*	*4,94*	*4,86*	*4,58*	*5,08*	*5,60*	*5,40*	*5,75*	*5,14*	*7,30*	*4,69*	*5,02*	*6,18*	*5,51*	*5,19*	*4,62*	*5,26*
PI	*0,38*	*0.50*	*0.32*	*0,47*	*0,68*	*0,72*	*0,61*	*0,66*	*0,56*	*0,39*	*0,47*	*0,63*	*0,51*	*0,60*	*0,31*	*0,54*	*0,57*	*0,91*	*0,36*	*0,80*	*0,67*	*0,541*
TUI	*6,16*	*5.02*	*5.33*	*5,48*	*5,05*	*6,10*	*5,55*	*5,51*	*5,14*	*5,47*	*6,07*	*6,03*	*6,26*	*5,74*	*7,61*	*5,23*	*5,59*	*7,09*	*5,87*	*5,99*	*5,28*	*5,77*
OCI	*0,76*	*2.02*	*2.60*	*0,84*	*0,68*	*0,97*	*2,40*	*2,96*	*1,87*	*0,97*	*0,83*	*2,45*	*0,96*	*0,90*	*3,53*	*2,41*	*0,92*	*3,38*	*2,67*	*0,74*	*0,86*	*1,01*

[1]determined as methyl esters, % of areas of total fatty acids
Foreign varieties grown in France: [3]**07Ardèche**; [4]34 : **Hérault** ; [5]66 : **Pyrénées Orientales**; [6]83 : **Var** ; [7]**Squalene**

Table 2. Mean fatty acid[1] (%) and squalene composition of virgin olive oils produced from eight French designations of origin

Fatty Acids	Aix-en-Provence	Corse	Haute-Provence	Nice	Nîmes	Nyons	Provence	Vallée des Baux de Provence
	n=181	n=35	n=141	n=163	n=70	n=151	n=87	n=198
16 :0	13.78	12.76	11.76	10.73	10.82	8.43	12.24	14.12
16 :1ω9	0.12	0.11	0.14	0.10	0.13	0.15	0.13	0.12
16 :1ω7	1.06	0.94	0.88	0.61	0.60	0.39	0.91	1.13
17 :0	0.12	0.04	0.18	0.05	0.06	0.05	0.12	0.08
17 :1ω8	0.21	0.08	0.35	0.10	0.10	0.08	0.22	0.14
18 :0	2.57	2.09	2.41	2.10	2.35	2.68	2.52	2.54
18 :1ω9	68.05	72.38	73.89	75.88	73.95	79.48	71.56	65.71
18 :1ω7	2.45	2.63	2.29	2.10	1.87	1.47	2.27	2.55
18 :2ω6	10.17	7.46	6.68	6.87	8.46	5.83	8.55	12.12
18 :3ω3	0.61	0.69	0.59	0.60	0.83	0.61	0.65	0.64
20 :0	0.43	0.37	0.39	0.37	0.37	0.38	0.40	0.43
20 :1ω9	0.25	0.30	0.26	0.32	0.31	0.31	0.26	0.25
22 :0	0.12	0.11	0.12	0.12	0.09	0.10	0.13	0.12
24 :0	0.06	0.05	0.05	0.05	0.05	0.04	0.05	0.06
Squa[2]	0.72	0.55	0.82	0.44	0.69	0.91	0.75	0.71
MI	*4.23*	*4.96*	*5.22*	*5.89*	*5.57*	*6.99*	*4.89*	*4.03*
PI	*0.63*	*0.53*	*0.49*	*0.56*	*0.67*	*0.55*	*0.60*	*0.73*
TUI	*4.86*	*5.49*	*5.71*	*6.44*	*6.25*	*7.54*	*5.49*	*4.76*
OCI	*1.92*	*0.83*	*3.53*	*1.09*	*1.19*	*1.04*	*2.19*	*1.29*

[1]determined as methyl esters, % of areas of total fatty acids
[2]Squalene

Oleic (18 :1ω9), palmitic (16 :0), linoleic (18 :2ω6) and stearic acid (18 :0) are the chief fatty acids commonly found in VOOs. The monounsaturated isomers of the C16 (hypogeic, 16 :1ω9 ; palmitoleic, 16 :1ω7) and C18 fatty acids (oleic, 18 :1ω9; *cis*-vaccenic, 18 :1ω7) were considered separately, contrary to what is done in the IOC trade standard [21] and the European Union regulation where they are taken together [22]. This more in-depth approach is useful for distinguishing between varieties and DOs for some of which several minor fatty acids are markers.

Figure 2 plots the mean indices for each variety and DO as well as the mean indices for the 36 varieties and eight DOs determined on the basis of 2035 samples.

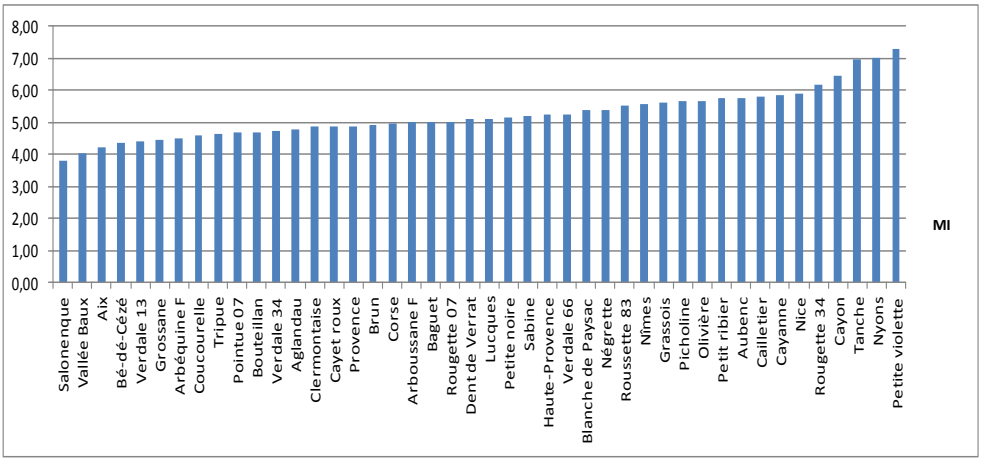

Figure 2. Classification of varieties and PDOs according to four indices.

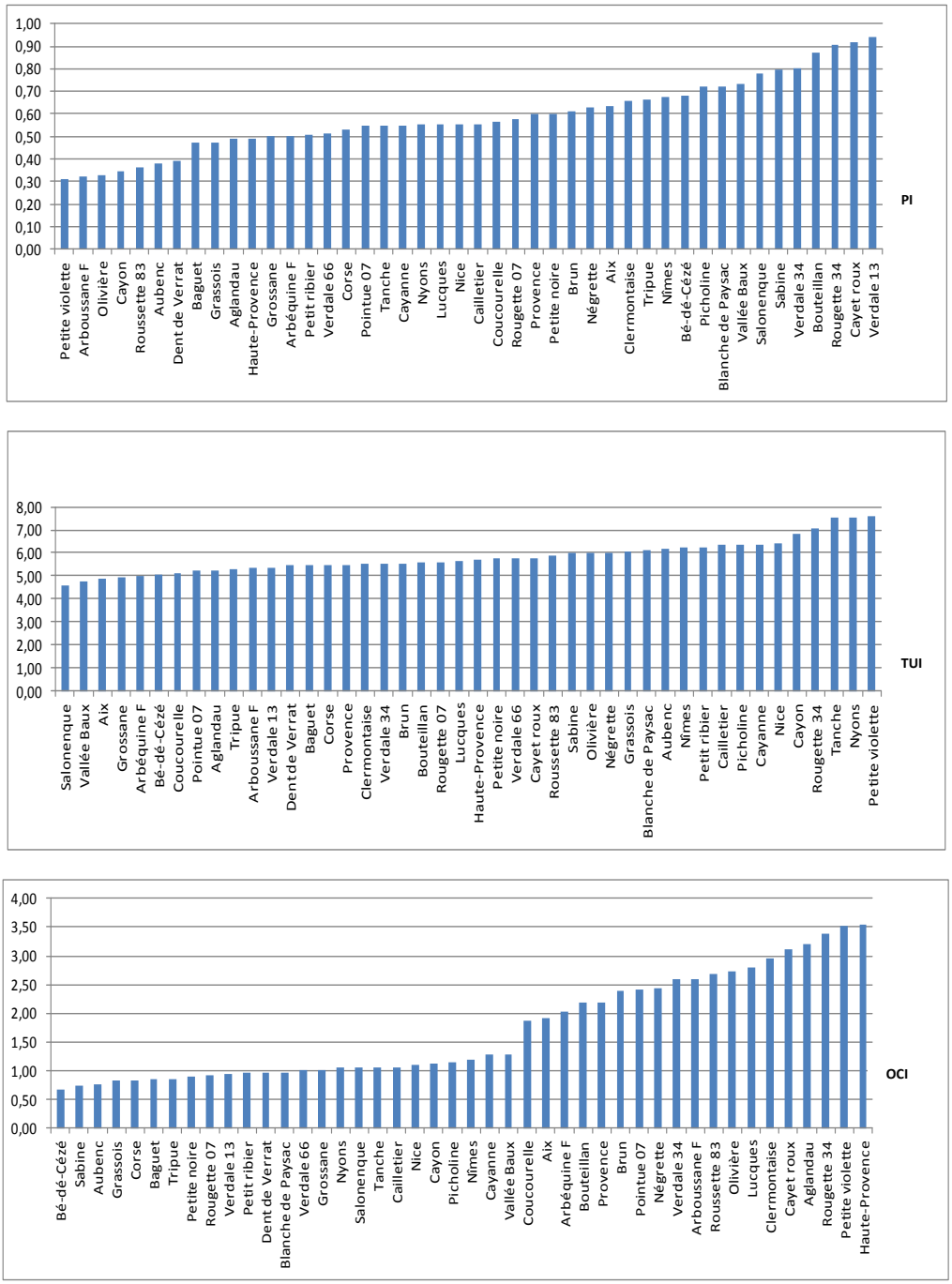

Figure 2 (contd). Classification of varieties and PDOs according to four indices.

The MI and TUI indices, in which oleic acid (18 :1ω9) and linoleic acid (18 :2ω6) are predominant respectively, were lowest (3.79 and 4.56) for 'Salonenque' and highest (7.30 and 7.61) for 'Petite Violette'. The PI index, which is largely correlated with the percentage of linoleic acid (18 :2ω6), recorded a minimum value (0.31) for 'Petite Violette' and a maximum (0.94) for 'Verdale 13'. The OCI index is directly related to the % content of margaric (17 :0) and margaroleic acid (17 :1ω8).'Bé-dé-Cézé' recorded the lowest value (0.68), contrasting with 'Petite Violette' which recorded the highest (3.53). Index-based classification of DO oils must be close to that of their constituent varieties, particularly when one is a majority or ultra-predominant variety.

For instance, the indices for the Nyons and Nice PDO oils, in which 'Tanche' and 'Cailletier' account respectively for 95% of their varietal composition, are identical to these source varieties. Likewise, the index ranking of Nîmes PDO oils, in which the 'Picholine' variety is predominant, is similar to that

of their majority source variety. 'Aglandau', the main French variety, has one of the highest OCI indices of all the French varieties studied. It is the predominant variety in oils produced under the Haute-Provence (~80%) and Aix-en-Provence (~50%) PDOs while it represents a smaller percentage of the varietal composition of oils from the Vallée des Baux PDO (~15%). The OCIs of these three PDOs are, in descending order, 3.53 (Haute-Provence PDO), 1.92 (Aix-en-Provence PDO) and 1.29 (Vallée des Baux de Provence PDO). While made up of different varieties the Corse and Provence DOs have similar MI, PI and TUI indices. However, their OCI values differ, being higher for the Provence DO than for the Corse DO because the former contains the 'Aglandau' variety. The four indices permit swift classification of the different oils but do not always suffice for their official identification. The lowest squalene content of all the varieties and DOs was recorded for 'Brun' oils (0.33%) and the highest for oils produced from the 'Verdale 13' variety (1.01%).

The triacylglycerol composition of the 36 varieties and eight DOs are given in **Tables 3a, 3b and 4**.

Table 3a. Mean triacylglycerol[1] composition (%) of virgin olive oils produced from 14 varieties of general relevance

Triacylglycerols	Aglandau	Bouteillan	Cailletier	Cayanne	Cayet roux	Cayon	Grossane	Lucques	Olivière	PicholineL	Salonenque	Tanche	Verdale 13	Verdale 34
	n=128	n=63	n=163	n=12	n=4	n=14	n=25	n=21	n=32	n=107	n=52	n=151	n=5	n=11
LLL	0,09	0,41	0,06	0,09	0,22	0,06	0,09	0,10	0,07	0,07	0,31	0,06	0,41	0,20
OLnL	0,19	0,56	0,20	0,21	0,38	0,14	0,27	0,25	0,13	0,19	0,32	0,17	0,48	0,49
PLnL	0,05	0,15	0,05	0,05	0,13	0,03	0,07	0,06	0,03	0,05	0,11	0,03	0,10	0,12
LOL	1,42	3,99	1,36	1,51	4,24	0,68	1,51	1,66	0,79	1,19	4,05	1,25	4,80	3,31
OLnO	1,44	1,91	1,49	1,57	1,64	1,73	1,86	1,77	1,57	1,47	1,30	1,59	1,78	2,03
PLL	0,51	1,21	0,40	0,46	1,07	0,23	0,67	0,49	0,33	0,37	1,66	0,21	1,68	1,14
PLnO	0,75	0,97	0,63	0,65	0,78	0,62	0,92	0,85	0,73	0,63	0,72	0,47	0,81	1,20
LOO	11,60	17,10	12,84	12,23	19,61	7,90	11,92	13,25	6,99	11,32	16,94	12,11	18,87	17,12
PoOO	2,05	1,28	1,35	1,59	1,19	2,20	3,15	1,66	3,08	1,23	1,53	0,98	1,79	1,57
PLO	5,62	7,8/2	4,85	5,22	8,80	2,97	6,49	5,48	3,07	4,67	10,15	3,26	10,06	8,67
PoOP	1,34	0,69	0,53	0,75	1,00	0,79	1,51	1,09	1,63	0,53	0,79	0,28	0,77	0,98
PLP	0,57	0,95	0,36	0,53	0,89	0,21	0,69	0,44	0,25	0,43	1,22	0,18	1,19	0,97
OOO	41,16	34,87	47,71	45,15	34,59	52,36	37,92	42,90	48,94	48,28	29,94	54,29	30,03	33,74
SLO	0,78	1,18	0,62	0,58	0,62	0,32	0,54	0,58	0,28	0,53	1,16	0,79	1,11	1,03
POO	22,27	18,61	20,29	21,56	18,99	21,01	23,56	21,04	23,25	21,30	21,13	17,17	19,09	19,71
POP	3,87	3,18	2,81	3,25	3,33	2,80	4,03	3,60	3,70	3,17	4,00	1,98	3,19	3,34
SOO	3,81	3,27	3,39	2,88	1,82	3,68	2,74	3,50	3,05	3,29	3,25	4,39	2,53	2,78
SOP	0,97	0,79	0,70	0,61	0,43	0,67	0,76	0,86	0,68	0,76	1,01	0,64	0,71	0,66
POA	0,48	0,47	0,48	0,45	0,26	0,46	0,42	0,47	0,42	0,47	0,42	0,50	0,31	0,36

[1] % of areas of total triacylglycerols

Picholine L: Picholine du Langedoc; **Verdale 13**: Verdale des Bouches du Rhône; **Verdale 34**: Verdale de l'Hérault.

Table 3b. Mean triacylglycerol[1] composition (%) of virgin olive oils produced from 22 local varieties

Triacylglycerols	Aubenc	Arbéquine F[2]	Arboussane F[2]	Baguet	Bé-dé-Cézé	Blanche de Paysac	Brun	Clermontaise	Coucourelle	Dent de Verrat	Grassois	Négrette	Petit ribier	Petite noire	Petite violette	Pointue 07[3]	Rougette 07[3]	Rougette 34[4]	Roussette 83[6]	Sabine	Tripue	Verdale 66[5]
	n=5	n=38	n=10	n=3	n=6	n=6	n=27	n=4	n=5	n=5	n=3	n=27	n=19	n=5	n=3	n=3	n=27	n=13	n=5	n=9	n=5	n=77
LLL	0,02	0,07	0,05	0,06	0,16	0,12	0,14	0,16	0,08	0,07	0,13	0,13	0,07	0,12	0,04	0,11	0,13	0,24	0,10	0,20	0,17	0,10
OLnL	0,11	0,18	0,14	0,31	0,51	0,29	0,28	0,32	0,22	0,18	0,27	0,34	0,19	0,23	0,10	0,30	0,25	0,48	0,15	0,45	0,29	0,22
PLnL	0,02	0,04	0,03	0,07	0,14	0,06	0,06	0,09	0,07	0,04	0,07	0,08	0,05	0,05	0,02	0,07	0,06	0,09	0,03	0,10	0,07	0,06
LOL	0,88	1,59	0,75	1,39	2,50	2,56	2,24	2,34	1,89	1,08	1,30	1,87	1,19	2,00	0,32	1,97	1,84	3,38	0,98	3,27	2,87	1,58
OLnO	1,22	1,60	1,41	2,31	2,42	1,56	1,88	1,62	1,81	1,64	1,87	2,04	1,47	1,64	1,69	1,70	1,67	1,98	1,58	1,98	1,82	1,37
PLL	0,26	0,63	0,23	0,50	0,91	0,68	0,80	0,69	0,74	0,43	0,42	0,51	0,37	0,56	0,09	0,64	0,63	0,84	0,27	0,85	1,16	0,47
PLnO	0,43	0,78	0,68	1,13	1,21	0,62	0,86	0,76	0,91	0,68	0,88	0,76	0,63	0,69	0,54	0,86	0,67	0,84	0,60	0,83	0,97	0,56
LOO	10,43	12,41	8,76	11,94	14,65	16,35	13,87	14,72	13,66	10,30	11,29	13,45	11,32	14,37	5,92	14,56	13,36	17,90	8,59	17,06	14,18	12,15
PoOO	1,37	2,69	2,45	3,23	1,96	1,23	2,70	1,60	2,82	2,81	1,64	1,31	1,23	1,33	1,28	1,70	2,22	1,40	1,45	1,18	2,39	1,30
PLO	3,82	6,65	4,03	6,01	7,73	6,23	6,72	6,70	7,34	4,60	4,88	5,22	4,67	6,53	1,91	7,01	5,89	6,56	3,11	6,78	8,24	5,08
PoOP	0,48	1,53	1,46	1,52	0,89	0,44	1,39	0,93	1,52	1,22	0,63	0,77	0,53	0,52	0,86	1,10	0,75	0,96	0,78	0,43	1,24	0,62
PLP	0,40	0,74	0,42	0,56	0,98	0,67	0,62	0,67	0,59	0,41	0,40	0,44	0,43	0,68	0,14	0,69	0,42	0,60	0,22	0,58	1,13	0,52
OOO	49,69	38,59	44,92	39,82	35,27	42,39	39,48	39,37	36,52	44,85	18,43	44,08	48,28	41,70	60,17	38,54	43,34	40,95	51,31	38,87	34,62	44,30
SLO	0,70	0,51	0,48	0,32	0,89	1,15	0,69	1,09	0,42	0,52	0,42	1,33	0,53	0,63	0,42	0,99	0,86	0,74	0,55	1,04	0,51	1,14
POO	20,99	23,82	24,18	24,13	21,45	18,47	21,26	19,98	24,76	22,91	20,77	17,71	21,30	21,75	17,44	21,12	20,72	17,05	21,00	18,97	21,78	20,76
POP	2,96	4,27	4,03	3,74	3,70	2,60	3,03	3,26	4,11	3,61	2,74	2,64	3,17	3,44	2,37	3,80	2,73	2,64	2,89	2,76	3,43	2,86
SOO	4,88	2,56	3,64	2,16	3,23	3,39	3,07	4,29	2,23	3,44	2,78	5,76	3,29	2,63	5,22	3,43	4,13	2,30	4,41	3,49	2,36	5,00
SOP	1,06	0,76	0,95	0,53	0,90	0,77	0,72	1,02	0,54	0,86	0,67	1,03	0,76	0,59	0,79	0,94	0,86	0,54	0,75	0,60	0,65	1,04
POA	0,59	0,43	0,60	0,27	0,42	0,41	0,43	0,60	0,40	0,51	0,40	0,54	0,47	0,37	0,69	0,46	0,55	0,33	0,55	0,36	0,38	0,50

[1] % of areas of total triacylglycerols
Foreign varieties grown in France: [3]**07Ardèche**; [4]34 : **Hérault** ; [5]66 : **Pyrénées Orientales**; [6]83 : **Var** ;

Table 4. Mean triacylglycerol[1] composition (%) of virgin olive oils produced from eight French PDOs

Triacylglycerols	Aix-en-Provence	Corse	Haute-Provence	Nice	Nîmes	Nyons	Provence	Vallée des Baux de Provence
	n=181	n=35	n=141	n=163	n=70	n=151	n=87	n=198
LLL	0.20	0.09	0.08	0.06	0.15	0.06	0.13	0.29
OLnL	0.30	0.23	0.18	0.20	0.36	0.17	0.24	0.36
PLnL	0.08	0.07	0.04	0.05	0.08	0.03	0.07	0.11
LOL	2.78	1.46	1.34	1.36	2.26	1.25	2.05	3.63
OLnO	1.61	1.52	1.48	1.51	1.81	1.58	1.47	1.72
PLL	1.08	0.53	0.43	0.39	0.58	0.21	0.67	1.52
PLnO	0.84	0.74	0.74	0.61	0.73	0.46	0.70	0.89
LOO	14.65	11.92	11.83	13.85	14.48	12.11	13.44	15.91
PoOO	1.98	1.86	1.90	1.31	1.32	0.98	1.79	2.06
PLO	7.92	5.82	5.10	4.78	5.49	3.30	6.45	9.37
PoOP	1.06	0.87	1.23	0.52	0.57	0.28	1.00	1.07
PLP	0.88	0.62	0.43	0.36	0.49	0.19	0.74	1.16
OOO	35.21	43.01	44.39	18.32	43.98	54.20	41.48	31.85
SLO	1.06	0.69	0.75	0.63	0.91	0.79	0.98	1.19
POO	21.56	21.62	21.69	20.13	18.90	17.11	19.42	20.27
POP	3.73	3.67	3.38	2.74	2.85	1.99	3.35	3.67
SOO	3.30	3.29	3.76	3.33	3.67	4.44	3.95	3.24
SOP	0.92	0.83	0.85	0.67	0.78	0.66	0.97	0.95
POA	0.43	0.50	0.48	0.49	0.44	0.50	0.55	0.43

[1] determined as methyl esters, % of areas of total triacylglycerols

Nineteen triacylglycerols were identified [5, 17] but some were co-eluted with minor triacylglycerols due to the difficulties of HPLC separation. All the oils had four main triacylglycerols: triolein (OOO), dioleylpalmitin (POO), diolelylinolein (OLO) and linoleyloleylpalmitin (PLO). In addition, they contained small percentages of dioleylstearin (SOO), oleyldipalmitin (POP) and dilinoleylolenin (LLO) as well as of minor triacylglycerols. The percentage content of triacylglycerols varied within and between varieties, as was the case for the fatty acids. **Figure 3** ranks the varieties and DOs according to their percentage content of the four major triacylglycerols.

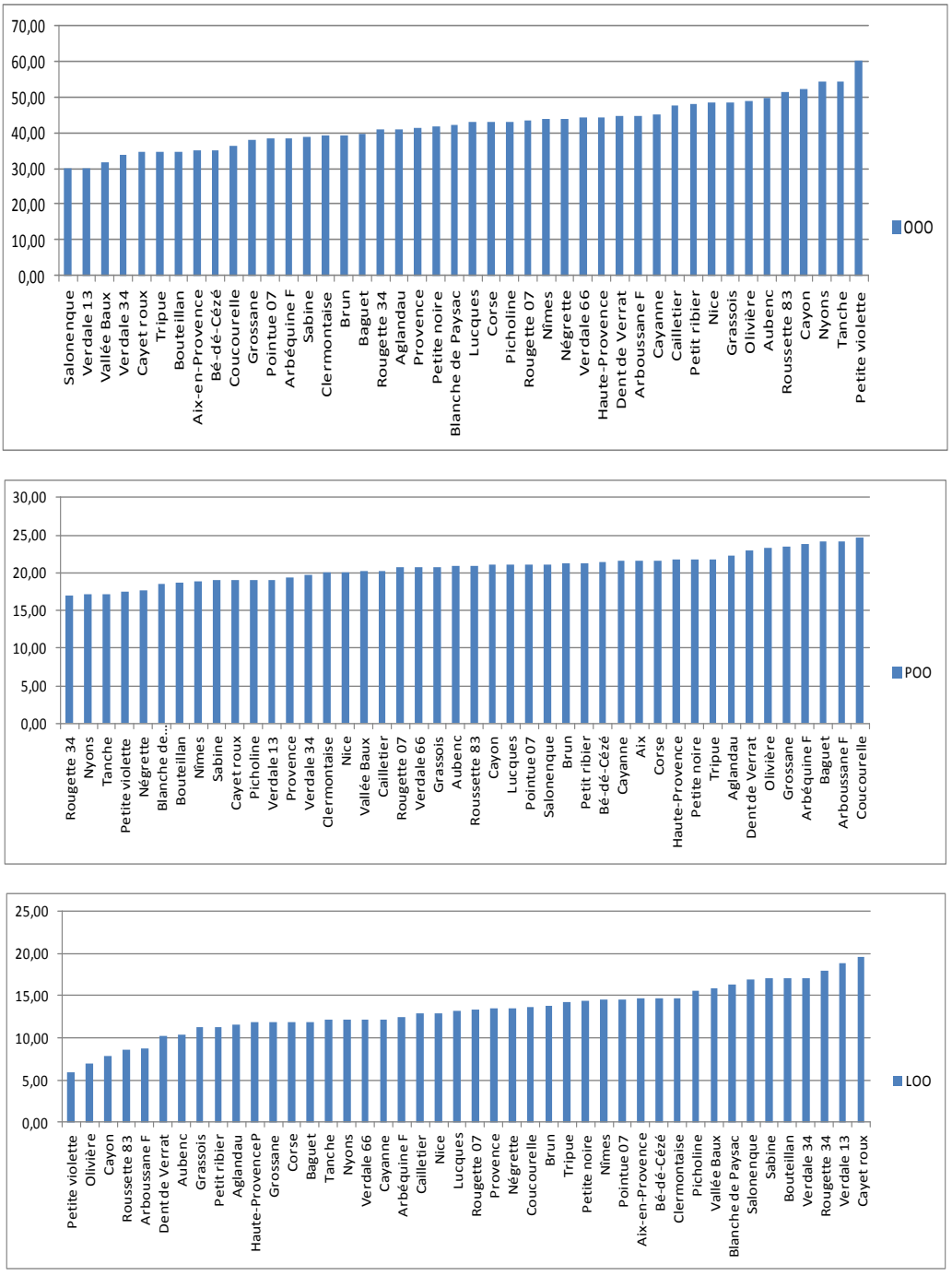

Figure 3. Classification of varieties and PDOs according to the four major triacylglycerols.

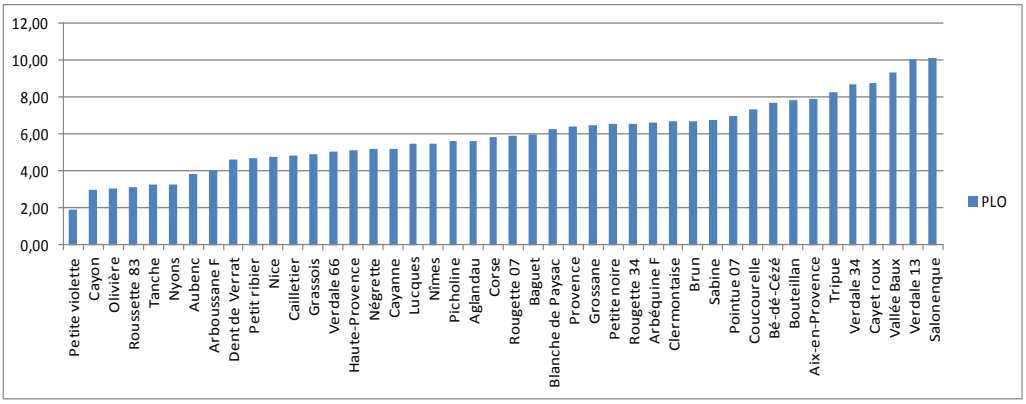

Figure 3 (contd). Classification of varieties and PDOs according to the four major triacylglycerols.

Triolein (OOO) was the majority triacylglycerol in all the varieties and DOs. Mean percentage OOO content ranged from 29.94% in 'Salonenque' to 60.17% in 'Petite Violette'. Oil ranking on the basis of OOO was very similar to that based on MI and hence on oleic acid content (18 :1ω9) (**Figure 2**). Dioleylpalmitin (POO) was the second main triacylglycerol. 'Rougette 34' had the lowest percentage (17.05%) of all the varieties and DOs whereas 'Coucourelle' had the highest (24.76%). Linoleyloleylpalmitin (PLO) ranged from 1.91 in 'Petite Violette' to 10.15 in 'Salonenque'. The relationship between the percentage contents of POO, LOO and PLO and the contents of palmitic and linoleic acid is not simple. Hence, the ranking of these triacylglycerols and these two acids, by ascending order, is not the same.

Triacylglycerol analysis enabled confirmation of the fatty acid analysis. These two determinations are complementary and give a better insight into the lipid composition of virgin olive oils than fatty acid analysis or triacylglycerol analysis on their own.

Principal Component Analysis (PCA) (**Figure 4)** of the 34 variables measured shows the diversity of the oils obtained from the French varieties and DOs on the plane defined by the first two principal components (Expl.: 58% of the explained variance; PC1, 37% and PC2, 21%).

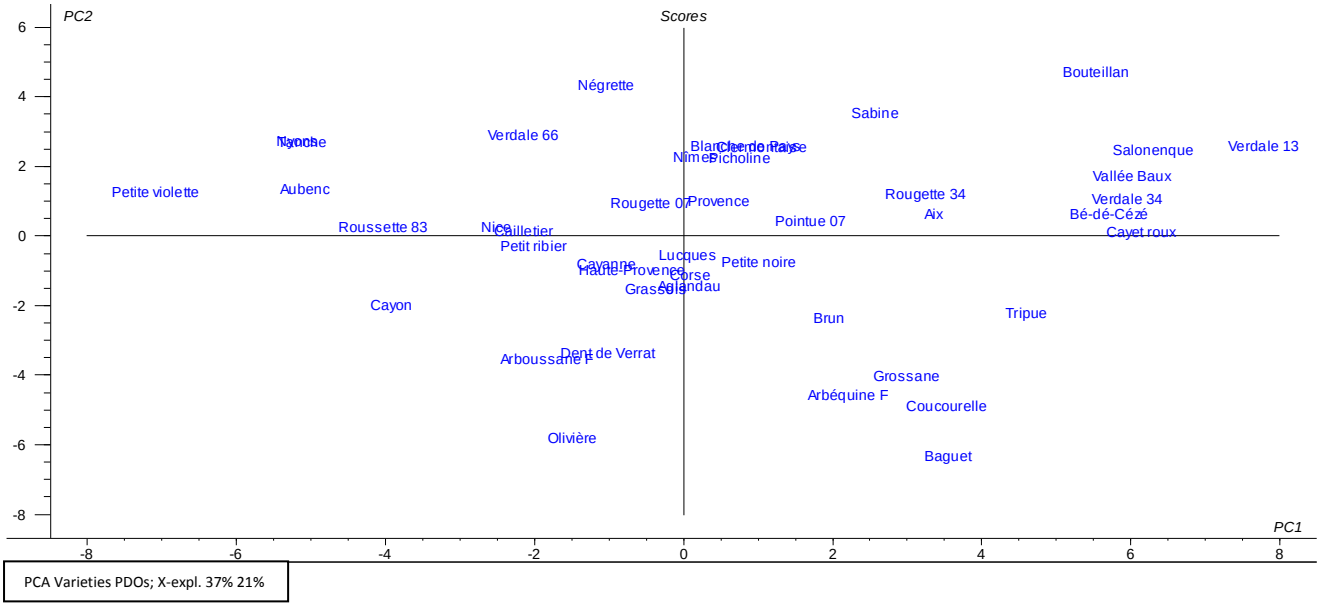

Figure 4. Principal Component Analysis (PC1 and PC2) of the 36 French varieties and eight French designations of origin.

For PC1, the varieties and DOs with the highest positive coordinates (**Figure 4**) are correlated with the variables with the biggest positive coordinates (**Figure 5**), and similarly for the negative coordinates. The same occurs with PC2. The most discriminant variables on principal components 1 and 2 are those closest to ±1 (**Figure 5**). PCA enables confirmation of the strong analogies reported earlier between 'Aglandau' and the Haute-Provence DO, 'Cailletier' and the Nice PDO, 'Picholine' and the Nîmes PDO, 'Salonenque' and the Vallée des Baux de Provence PDO and 'Tanche' and the Nyons PDO.

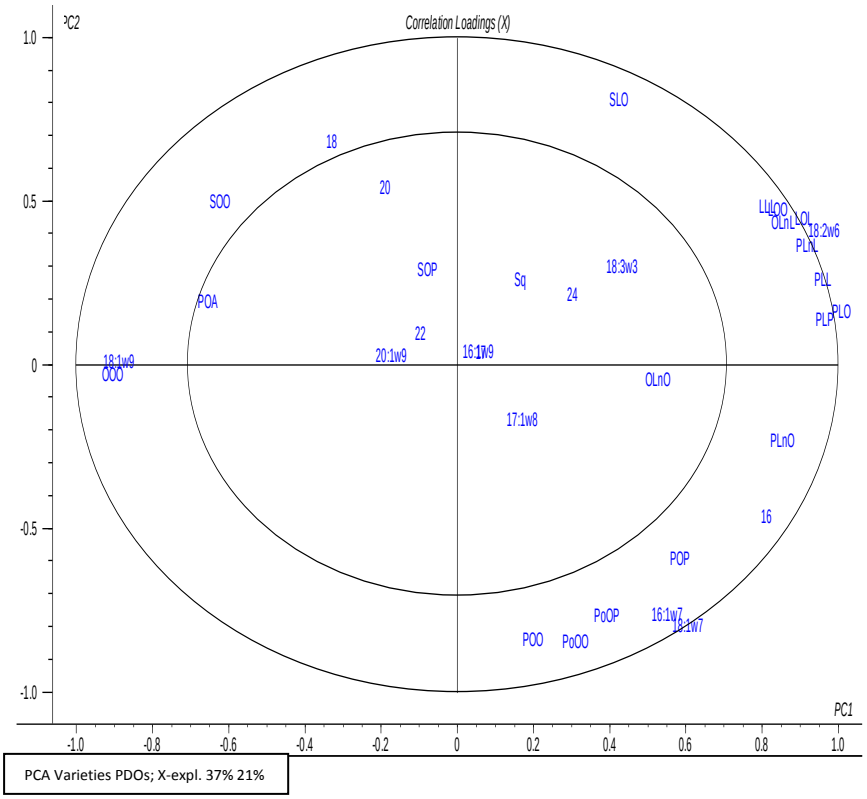

Figure 5. Circle of correlation of the variables on the PC1 and PC2 plane.

Creating a database is an essential prerequisite for checking the varietal or geographical origin of olive oils (18). At this stage, in numerous cases chemometric data processing based on fatty acid and triacylglycerol composition enables determination of the source varieties of French or DO olive oils (CDO, PDO) [5, 16]; however, chemometric methods are not always easy to use and require specific software and specialists to interpret the results correctly. An attempt was therefore made to develop a simple, swift, easy and visual method to remedy this problem. The proposed method and its applications are the subject matter of Part II of this study (23).

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New approach to the determination of the origin of olive oils: morphograms and morphotypes (Part II)

C. Pinatel[1], D. Ollivier[2], V. Ollivier[2] and J. Artaud[3]

[1] Centre Technique de l'Olivier (CTO), Maison des Agriculteurs, 22 avenue Henri-Pontier, 13626 Aix-en-Provence Cedex, France.
[2] Laboratoire de Marseille, Service Commun des Laboratoires, Ministère des Finances, 146 traverse Charles-Susini, 13388 Marseille Cedex 13, France.
[3] Université d'Aix-Marseille, Faculté des Sciences de Saint Jérôme, Laboratoire LISA, 13397 Marseille cedex 20, France.

1. Introduction

The first registered designation of origin for olive oil in France (AOC in French) was set up in 1994 in the region of Nyons. In 2006, EU Regulation No 510 defined the terms Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI). At present, there are around 100 registered PDOs and PGIs in the European Union and a further 10 or so are in the pipeline [1]. Further afield, the Maghreb olive growing countries are currently setting up designations of origin for their olive oils too.

As yet, consumers are still not very aware of these quality seals. Designations of origin must be reliable and meaningful if they are to win and keep the confidence of consumers; they must allow consumers to distinguish DOs from other products without any risk of error. Besides the tools stipulated in DO specifications to permit product traceability, other tools are needed to allow direct checks of the origin of finished product, whether they be internal or external checks or checks performed by the public authorities at point-of-sale.

Several papers have reported the possibility of using fatty acid and triacylglycerol composition to identify the origin of olive oils [2-6]. In addition, in the case of the 'Tanche' variety the authors have demonstrated [7] that fatty acid composition does not vary through the harvest period; however, this does not apply to all varieties.

Chemometric processing of chromatographic data shows that it is possible to identify groups of samples belonging to different designations. These methods enable identification of groups but do not permit easy, case-by-case verification of whether a sample belongs to a specific group. Various statistical packages (Pirouette®, Unscrambler®) do permit speedy performance of this type of work and once models have been developed, they can be circulated to users for sample tests, but these models are laborious to manage and the software is expensive. Furthermore, it is complicated for testing organisations to exchange models because they must have the same software and suitable training.

An attempt has therefore been made to simplify the recognition of oil origin on the basis of fatty acid and triacylglycerol composition. The aim of this article is to put forward a tool which enables swift sample analysis using solely Excel® as the software and which is based on optimised global representation of fatty acid and triacylglycerol composition. The distribution of the fatty acid and triacylglycerol data contained in a database were examined for this purpose in order to choose the most effective parameters while maintaining an adequate field of application. Two practical examples help to illustrate the advantages of the proposed approach.

2. Materials and Methods

2.1 Database

A database was created containing data on the fatty acid and triacylglycerol composition of some

3900 samples of oils collected between the 1998 and 2011 production seasons. Only samples (3,500) for which at least one of the following three criteria was clearly known were taken into account: variety, designation of origin and producing country. These samples had:
- been collected from producers (56% of samples, category P);
- obtained from olives crushed in the laboratory using a mini-mill similar to the Abencor system but modified in order to obtain a larger quantity of oil from each batch (22% of samples, category L);
- supplied by a research institute, testing laboratory, producer association or other body connected with olive oil production (15% of samples, category R); or
- purchased in shops (7% of samples, category S).

These samples were tested between 1999 and 2012. Testing was performed according to the procedures described in earlier articles [2, 3, 5, 6]. All the results were examined before they were entered in the database. Samples were discarded if they gave anomalous results or there was any uncertainty about their authenticity. After this screening process, the database held data on 2,588 samples from 23 countries, 35 designations of origin and over 200 varieties. However, all these subsets are not represented by the same number of samples. Hence, there are up to 200 samples for some French DOs whereas there are only 1–5 samples for DOs from other countries. For instance, 'Aglandau', the top oil variety in France, is represented by 140 samples whereas 80 other varieties are only represented by one.

2.2 Statistical measures

Excess kurtosis is a measure of the dispersion of the results of a random variable. A high value indicates a 'peaked' dispersion.
The coefficient of skewness is a measure of the distribution asymmetry of a real-valued random variable. It can be positive or negative depending on whether the asymmetry occurs to the right or left of the mean.
The truncated or trimmed mean (0.25) is the mean calculated from 25% of the values positioned positively and negatively around the median.

2.3 Nomenclature

Fatty acids: C16:0, palmitic acid (hexadecanoic acid); C16:1ω9, hypogeic acid (7-hexadecenoic acid); C16:1ω7, palmitoleic acid (9-hexadecenoic acid); C17:0, margaric acid (heptadecanoic acid); C17:1ω8, margaroleic acid (9-heptadecenoic acid); C18:1ω9, oleic acid (9-octadecenoic acid); C18: 1 ω7, *cis*-vaccenic acid (11-octadecenoic acid); C18: 2 ω6, linoleic acid (9,12-octadecadienoic acid); C18:3 ω3, linolenic acid (9,12,15-octadecatrienoic acid); C20:0, arachidic acid (eicosanoic acid); C20:1ω9, gondoic acid (11-eicosenoic acid); C22:0, behenic acid (docosanoic acid); C24:0, lignoceric acid (tetracosanoic acid).
The triacylglycerols are designated by letters corresponding to the abbreviated names of the fatty acid carbon chains attached to the glycerol: P : palmitoyl; Po : palmitoleyl; S : stearoyl; O : oleoyl; L : linoleoyl; Ln : linolenoyl; A ; arachidoyl.

3. Systems of Graphical Representation and Data Selection

3.1 Choice of system

The results for each sample are presented as two series of percentages, one of which characterises the fatty acid composition and the other the triacylglycerol composition. These series contain 14 and 19 values, respectively.
Since each of these series gives an exhaustive picture of sample composition, it can be represented graphically as a pie chart. This highlights the distinctive features of olive oil in general but does not indicate the differences between oils produced from different varieties.
Figure 1 shows the pie charts of the fatty acid composition of very typical samples of oils from the French Haute-Provence and Nyons PDOs. Although the composition of these samples is very different, it is only possible to see the major differences between them such as their content of oleic acid or palmitic acid. The composition of these two samples is reported in **Table 1**.

Table 1: Fatty acid composition of oils from two French PDOs

Table 1	C16:0	C16:1ω9	C16:1ω7	C17:0	C17:1ω8	C18:0	C18:1ω9	C18:1ω7	C18:2ω6	C18:3ω3	C20:0	C20:1ω9	C22:0	C24:0
Haute-Provence	12,17	0,13	0,94	0,15	0,30	2,39	70,00	2,35	6,37	0,56	0,38	0,24	0,11	0,05
Nyons	8,43	0,15	0,39	0,05	0,08	2,68	79,48	1,47	5,83	0,61	0,38	0,31	0,10	0,04

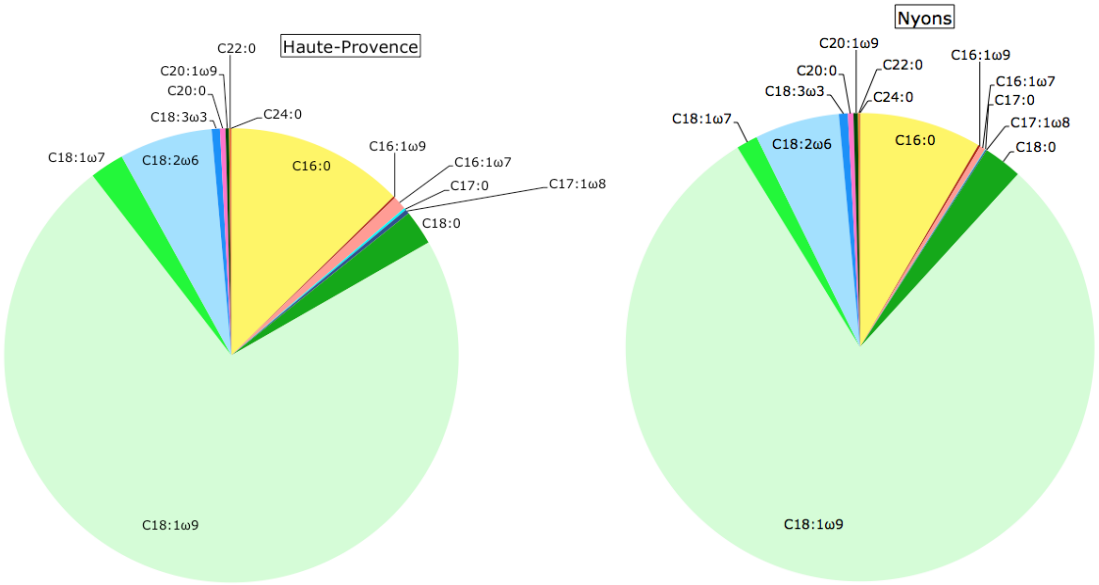

Figure 1: Pie charts of the mean fatty acid composition of oils from two French PDOs.

A radar chart, also known as a spider chart or star chart, can give a very interesting graphical representation using a logarithmic scale. Although it highlights the most important components and its shape is easy to memorise, the differences between the two samples are not indicated any better than with the pie charts (**Figure 2**).

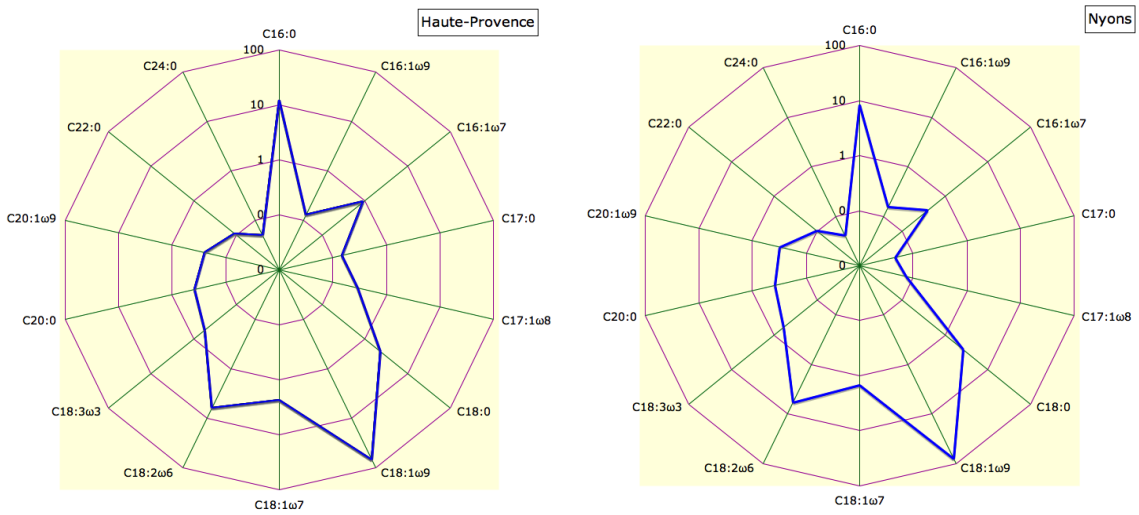

Figure 2. Radar chart of the mean fatty acid composition of oils from two French PDOs.

3.2. Adaptation of the range of variation

The difficulty in finding an effective form of graphical representation is that although there are major compositional differences between the two varieties, the hierarchy of compounds is always the same. Each compound varies over a specific range of variation which can be defined by a mean, a minimum value and a maximum value. The value of a compound in a specific sample can be positioned inside this range according to its deviation from the mean compared to the maximum known deviation of the compound (**Figure 3**).

Hence, each axis represents a range of variation extending from -100% to +100% where origin is the mean. To give an example, the hypogeic acid content (16:1ω9) of the Nyons sample, equal to 0.15%, is represented by the value (0.15-0.13)/(0.29-0.13) where 0.13 is the mean of this compound in the entire database and 0.29 is the maximum value observed, i.e. 0.02/0.16=12.5%. Specific shapes are obtained which help to identify the two samples visually. However, while all the oils have a very different fatty acid composition, the resultant shapes are still very similar to a circle (**Figure 3**). The chart space is heavily underused.

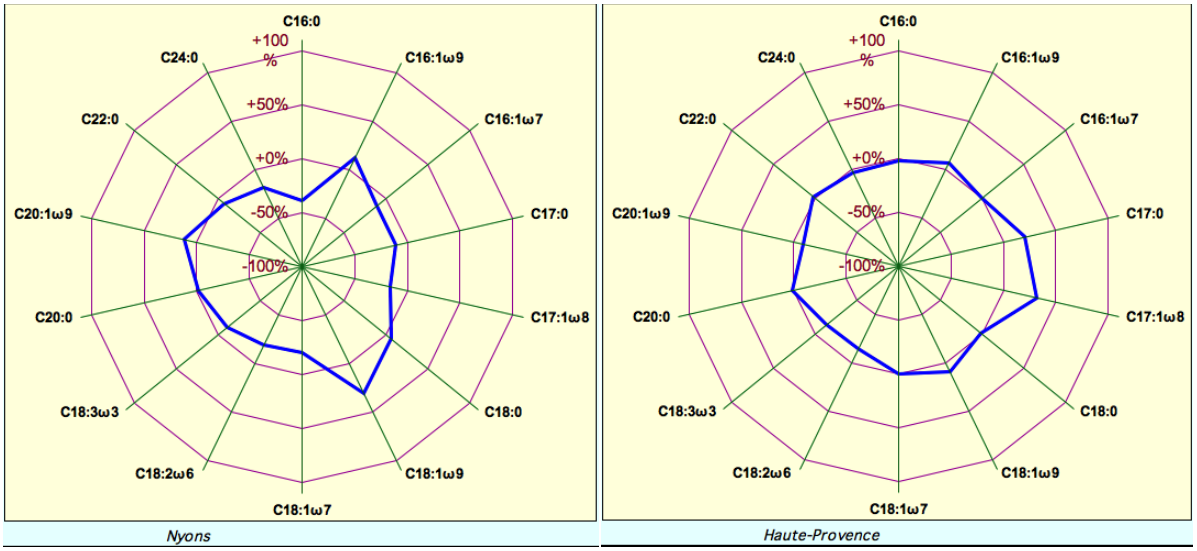

Figure 3. Radar chart showing the fatty acids in two samples of Nyons and Haute-Provence PDO oils on an adapted range of variation for each component.

3.3. Reduction of the range of variation

Another approach aimed at improving the possibilities of visual identification of commonplace samples is to use values defined according to the standard deviation, for instance (mean +/- standard deviation), as the bounds of the range of variation. However, this makes it necessary to begin by examining the distribution of the values for each compound (fatty acids and triacylglycerols).

Distribution of the values in the database

The distribution of most of the compounds is positively skewed (**Table 2**). Only two compounds - oleic acid (C18:1ω9) and triolein (OOO) - are negatively skewed. Moreover, these distributions are both heavily clustered around the mid values and are very long-tailed on the positive side. **Table 2** reports the distribution characteristics of the 14 fatty acids. For each one, with the exception of palmitic acid, over 68% of the values lie within an interval of one standard deviation on either side of the mean. Excess kurtosis is generally high, thus revealing very 'peaked' distributions: the distribution is heavily grouped around the median. Excess kurtosis is only close to zero (corresponding to a normal distribution) for palmitic acid, the distribution of which is more pyramid-like. The coefficient of skewness is generally positive, with a long tail extending over the high values. Only the distribution of oleic acid is negatively skewed to the left.

Table 2: Fatty acid distribution characteristics of the samples entered in the database

	Mean	Median	Standard deviation	Min	Max	Excess kurtosis	Coefficient of skewness
C16:0	12.02	11.98	1.97	7.02	20.89	0.22	0.19
C16:1ω9	0.13	0.13	0.03	0.04	0.29	1.57	0.27
C16:1ω7	0.90	0.87	0.40	0.18	5.55	11.31	1.99
C17:0	0.10	0.08	0.06	0.02	0.50	4.52	1.49
C17:1ω8	0.18	0.14	0.11	0.04	0.74	1.04	1.02
C18:0	2.44	2.43	0.49	1.05	6.11	3.06	0.85
C18:1ω9	71.93	72.63	4.88	45.49	83.96	1.24	-0.71
C18:1ω7	2.32	2.31	0.57	0.72	6.30	2.98	1.00
C18:2ω6	8.48	7.87	2.91	2.04	23.63	1.19	0.96
C18:3ω3	0.66	0.64	0.13	0.34	1.41	2.85	1.39
C20:0	0.39	0.39	0.05	0.21	0.62	1.02	0.01
C20:1ω9	0.28	0.27	0.04	0.16	0.53	1.35	0.72
C22:0	0.11	0.12	0.02	0.05	0.28	3.98	0.50
C24:0	0.05	0.05	0.01	0.00	0.12	1.18	0.14

Distribution of some specific compounds

Generally speaking, the distribution of the triacylglycerols is further away from a normal distribution than that of the fatty acids and the excess kurtosis value and coefficient of skewness are higher. Some compounds have a distinctive distribution, now outlined.

Margaric acid

Margaric acid (C17:0) has one of the most skewed distributions of the fatty acids, with a coefficient of skewness of 1.49. The mean and the median are respectively 0.10 and 0.08% while the minimum value is 0.02% and the maximum is 0.50%. This extremely asymmetric distribution is apparent when the values are plotted in a histogram with class intervals of 0.04% (**Figure 4**).

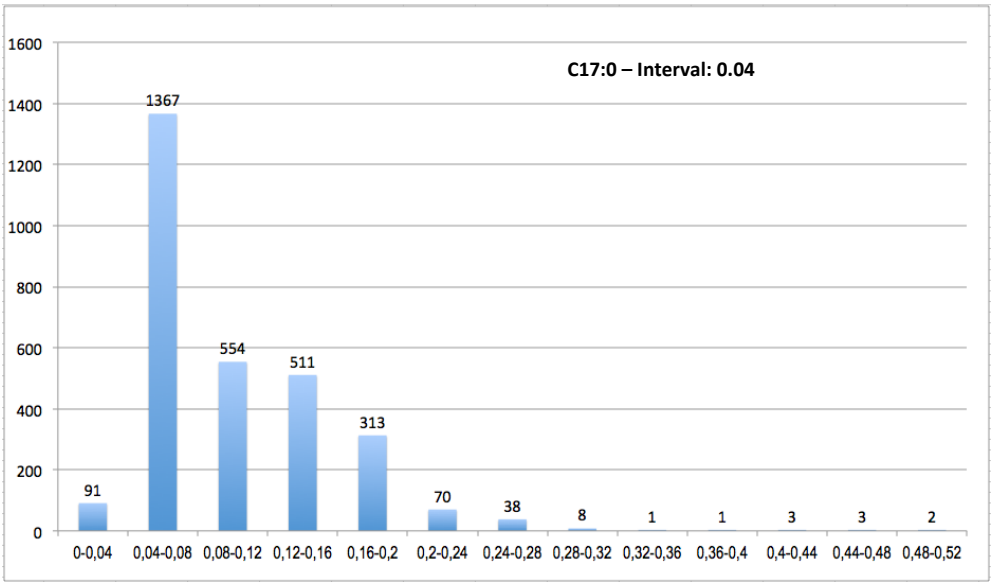

Figure 4: Distribution of the values for margaric acid (C17 :0) in class intervals of 0.04%.

The mean (0.10), median (0.08) and mode (0.04-0.08%) are far from the centre of the range of variation (0.26%). Consequently, in the large majority of the oils, the position of this compound in the radar chart would be very close to 0%. Only 17 of the samples lie in the upper half of the right-hand side of the distribution, i. e. beyond 0.28%. This subset comprises 12 L-category samples produced from the 'Aglandau' variety and collected in a study designed to observe the characteristics of the oils produced in specific drought conditions in the 2007 season. Four other L-category samples are from the minor local variety 'Ventoulane'. Only one sample, a monovarietal 'Aglandau' oil, belongs to the P-category.

Palmitoleic acid

With a coefficient of skewness of 1.99, palmitoleic acid (C16:1ω7) has the most asymmetric distribution of all the fatty acids. It also has the most 'peaked' distribution, with an excess kurtosis value of 11.3. There are only four samples in the upper, right-hand side of the distribution (>3.28). These are a P-category monovarietal 'Olivière' oil supplied by a producer from a single, drought-sensitive plot with a very stony soil, two L-category samples of 'Olivière' oil from the same plot and a single R-category sample produced in Argentina from the 'Arbequina' variety (**Figure 5**).

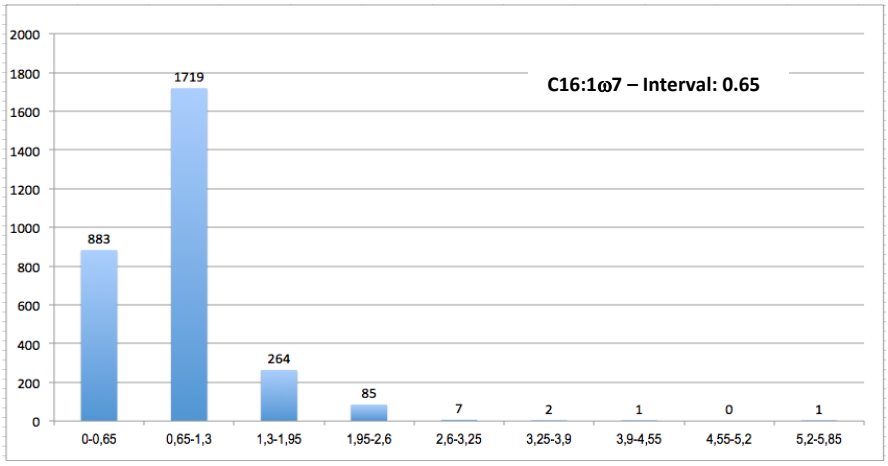

Figure 5. Distribution of the values for palmitoleic acid (C16:1ω7) in class intervals of 0.65%.

Oleic acid

Oleic acid is the only fatty acid to have a negatively skewed distribution due to several very low values which therefore lie far away from the mode (72–75%). Twenty-three samples have values below 54%. Sixteen of these are Tunisian oils produced from the 'Chemlali' variety. The remaining seven are oils produced from the 'Arbequina' variety (five from Argentina, one from Morocco and one from Tunisia). One sample is from the 'Cerisier' variety, a French variety of very marginal importance with a very low oil content (**Figure 6**).

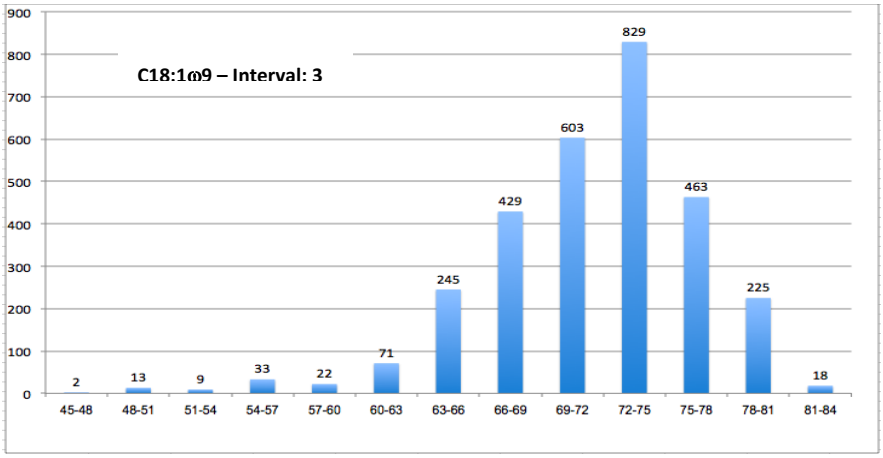

Figure 6: Distribution of the values for oleic acid (C18:1ω9) in class intervals of 3%.

Triacylglycerol distribution

Table 3 shows the distribution of the triacylglycerols. In this case likewise, excess kurtosis is generally positive and very high (LLL, PLnL, PoOP). The coefficient of skewness of these compounds is very high and their distribution is very striking, being heavily grouped around the median but with a very long tail over the high values.

Table 3: Characteristics of the triacylglycerol distribution of the samples entered in the database

	Mean	Median	Standard Deviation	Min	Max	Excess kurtosis	Coefficient of skewness
LLL	0.16	0.11	0.14	0.00	1.84	21.42	3.42
OLnL	0.28	0.25	0.14	0.04	1.49	7.98	1.99
PLnL	0.07	0.06	0.05	0.00	0.48	13.24	2.57
LOL	2.15	1.78	1.23	0.24	9.42	2.24	1.35
OLnO	1.62	1.61	0.32	0.61	3.17	1.32	0.17
PLL	0.73	0.56	0.56	0.03	4.79	6.58	2.07
PLnO	0.76	0.74	0.20	0.25	2.37	4.57	1.10
LOO	13.31	13.08	2.85	4.37	24.45	0.30	0.13
PoOO	1.75	1.76	0.66	0.14	9.23	9.26	1.26
PLO	6.15	5.73	2.22	1.74	16.42	0.92	0.87
PoOP	0.93	0.89	0.44	0.11	6.17	10.89	1.54
PLP	0.64	0.54	0.40	0.03	4.09	8.07	2.01
OOO	41.74	42.45	7.51	14.33	63.15	-0.15	-0.23
SLO	0.85	0.82	0.36	0.01	3.27	1.73	0.57
POO	20.67	20.64	2.56	12.67	34.84	1.76	0.56
POP	3.29	3.28	0.76	1.43	7.07	0.86	0.41
SOO	3.65	3.52	0.86	1.14	8.22	1.68	0.90
SOP	0.85	0.83	0.22	0.33	2.09	1.36	0.66
POA	0.47	0.46	0.11	0.06	1.00	0.69	0.49

The distribution of the triacylglycerols shows the same features, i.e. most of the distributions are long tailed on the right, except for triolein, which has the most skewed distribution.

Atypical samples are again found in the areas farthest away from the distribution zones. In the case of trilinolein, the samples concerned are of oils produced from the 'Arbequina' variety grown in Argentina. These 'Arbequina' samples are positioned in the far, right-hand part of the PLP distribution area along with numerous Tunisian samples produced from the 'Chemlali' variety.

Discussion

Analysis of the distribution of the different compounds, particularly of the extreme values, reveals the existence of two types of samples which are atypical for diametrically opposed reasons. The first type comprises samples that are inevitably atypical because they are the result of special atypical conditions. Two examples are samples of oils obtained from the 'Aglandau' or 'Olivière' varieties in very specific conditions. The samples of these two varieties are included in the database because they were collected during the course of particularly exhaustive sampling for a specific survey. Others are produced from rare varieties such as 'Ventoulane'. The second type of samples are only atypical because the database is angled, i.e. composed of samples obtained under our collection system as opposed to samples collected under a planned scheme. They are samples that are from countries or have been produced from varieties that are underrepresented in the database. They are atypical because the database contains very few samples from such sources.

If an effective tool is to be developed for swift identification to check oil provenance, the top priority is to search for the best possible visual graphic discrimination of commercial oils. The range of variation can be narrowed to the detriment of samples with extreme values in order to improve graphic processing of the most usual samples. A new, more balanced database was therefore needed to replace the raw initial database. This would be made up solely of identified groups: countries, producing regions and varieties. Groups were only incorporated when the data for a minimum number of samples was processed. The limits were fixed in such a way as to favour non-French groups in order to balance the database. **Table 4** shows the minimum number of samples per category and the resultant number of groups obtained per category.

Table 4. Database groups

Category	Limit	Nr of groups
Foreign varieties grown in France	10	2
Foreign varieties grown in their country of origin	4	12
French varieties cultivated in France	10	16
Producing countries	4	15
Foreign designation of origin	2	12
French designation of origin	10	7

The much narrower ranges of values made it possible to build more explicit graphical representations (**Tables 5a and 5b**). Furthermore, the medians and means were shifted because of the more balanced composition of this database (FATG-DB-06). For instance, in the case of margaric acid (C17:0), the mean is much lower because the distribution is less centred on the French data.

Table 5a. Fatty acid distribution characteristics based on the six categories of sample cited in Table 4

FAT-DB-06	Mean	Median	Trimmed mean 0.25	Standard deviation	Min	Max
C16:0	12.07	11.68	11.87	1.91	8.43	19.68
C16:1ω9	0.12	0.13	0.12	0.03	0.06	0.20
C16:1ω7	0.92	0.81	0.84	0.44	0.26	2.80
C17:0	0.08	0.07	0.08	0.04	0.04	0.21
C17:1ω8	0.15	0.12	0.14	0.08	0.05	0.35
C18:0	2.54	2.53	2.51	0.53	1.56	4.13
C18:1ω9	71.55	72.90	72.24	5.30	48.94	79.48
C18:1ω7	2.33	2.20	2.27	0.60	1.20	4.87
C18:2ω6	8.68	7.88	8.29	3.37	2.93	19.93
C18:3ω3	0.68	0.64	0.66	0.11	0.49	1.01
C20:0	0.41	0.41	0.41	0.06	0.28	0.58
C20:1ω9	0.29	0.28	0.29	0.04	0.20	0.40
C22:0	0.12	0.12	0.12	0.02	0.08	0.18
C24:0	0.06	0.06	0.06	0.01	0.04	0.08

Table 5b. Triacylglycerol distribution characteristics based on the six categories of sample cited in Table 4

FAT-DB-O6	Mean	Median	Trimmed mean 0.25	Standard deviation	Min	Max
LLL	0.17	0.12	0.14	0.15	0.04	0.97
OLnL	0.28	0.24	0.26	0.14	0.08	0.90
PLnL	0.07	0.06	0.06	0.04	0.02	0.29
LOL	2.21	1.86	2.01	1.27	0.37	6.17
OLnO	1.64	1.58	1.61	0.25	1.17	2.65
PLL	0.77	0.58	0.65	0.65	0.12	4.10
PLnO	0.77	0.73	0.73	0.21	0.46	1.92
LOO	13.18	13.21	13.19	3.10	5.50	20.26
PoOO	1.80	1.72	1.75	0.54	0.98	3.17
PLO	6.26	5.75	6.00	2.42	2.33	15.65
PoOP	0.94	0.81	0.89	0.40	0.28	2.28
PLP	0.72	0.59	0.63	0.52	0.19	3.65
OOO	41.08	42.94	41.62	7.37	16.58	54.20
SLO	0.93	0.87	0.89	0.35	0.28	1.97
POO	20.41	20.47	20.45	2.12	14.68	26.38
POP	3.35	3.27	3.29	0.63	1.99	5.59
SOO	3.88	3.75	3.83	0.93	1.72	6.00
SOP	0.90	0.87	0.89	0.19	0.54	1.53
POA	0.51	0.50	0.50	0.11	0.27	0.80

On using the values listed in **Table 5b** to represent the composition of the two samples taken as examples (Nyons and Haute-Provence PDOs), the resultant charts provide much more information.

The distinctive features of the oils can be seen straight away, for instance the very high margaroleic acid content of the Haute-Provence sample (C17:1ω8) (**Figure 7**).

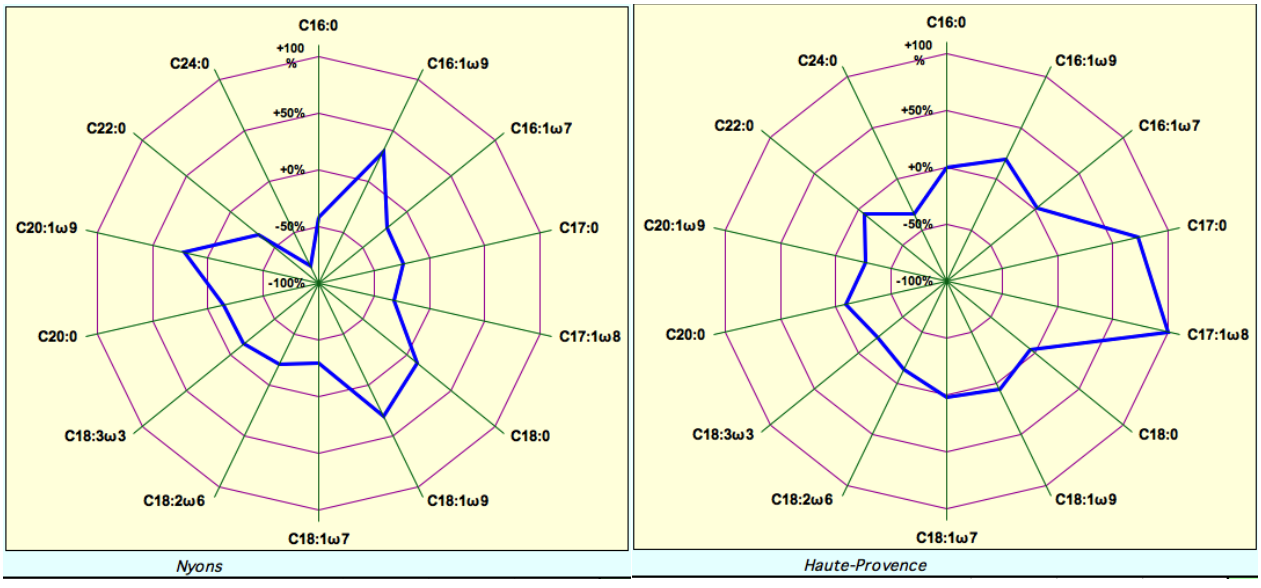

Figure 7. Radar chart of two samples of Nyons and Haute-Provence PDO oils according to the new database.

To ensure the tool is solid, the bounds must be defined from the statistics. If the bounds of the range of variation are merely determined by the minimum and maximum values, the inclusion of new elements may occasionally lead to major changes in the charts. Furthermore, graphic representation can be improved by shifting the

centre of the range of variation according to the distribution of the values.

After several simulations, the best results for calculating the centre of the range of variation were obtained by using the truncated or trimmed mean to which the absolute difference between the mean and the median was added. The lower and upper bounds were calculated by respectively subtracting or adding twice the standard deviation at the centre of the range (**Tables 6a and 6b**).

Table 6a. New characteristics of fatty acid distribution to optimise graphical representation

FAT-DB-06	Centre of range of variation	Lower bound	Upper bound
C16:0	12.22	8.40	16.05
C16:1ω9	0.12	0.07	0.17
C16:1ω7	0.94	0.06	1.82
C17:0	0.10	0.01	0.18
C17:1ω8	0.18	0.02	0.33
C18:0	2.51	1.46	3.56
C18:1ω9	70.88	60.28	81.48
C18:1ω7	2.40	1.19	3.61
C18:2ω6	9.10	2.35	15.85
C18:3ω3	0.70	0.47	0.93
C20:0	0.41	0.30	0.52
C20:1ω9	0.29	0.21	0.37
C22:0	0.12	0.08	0.17
C24:0	0.06	0.04	0.07

Table 6b. New characteristics of triacylglycerol distribution to optimise graphical representation

FAT-DB-O6	Centre of range of variation	Lower bound	Upper bound
LLL	0.18	-0.11	0.48
OLnL	0.29	0.01	0.57
PLnL	0.07	-0.01	0.15
LOL	2.36	-0.18	4.91
OLnO	1.67	1.18	2.16
PLL	0.84	-0.45	2.13
PLnO	0.77	0.34	1.19
LOO	13.15	6.95	19.36
PoOO	1.83	0.76	2.90
PLO	6.52	1.68	11.37
PoOP	1.02	0.22	1.81
PLP	0.76	-0.27	1.80
OOO	39.76	25.03	54.49
SLO	0.95	0.24	1.66
POO	20.39	16.16	24.62
POP	3.37	2.11	4.63
SOO	3.97	2.11	5.83
SOP	0.92	0.54	1.29
POA	0.52	0.30	0.73

In the case of the samples from the two origins taken as examples, the graphical representations were optimised by using this new set of values.

The resultant shapes fully express the specific features of the oils and are easy to identify visually (**Figure 8**).

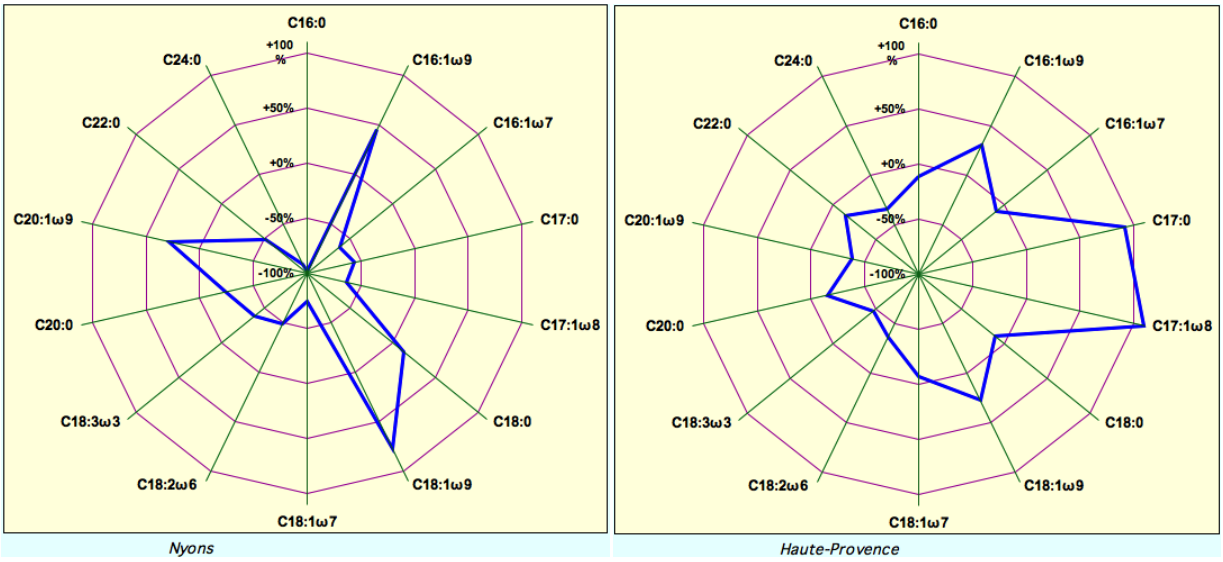

Figure 8. Optimised radar chart of the fatty acids of two samples of Nyons and Haute-Provence PDO oils.

Application to the verification of oil origin

The samples of French PDO oils were identified and found to be homogeneous on the basis of their fatty acid and triacylglycerol compositions. The next two subsections detail the procedure for checking sample compliance with these two PDOs (Nyons and Haute-Provence).

Concepts of morphograms and morphotypes

The term 'morphogram' means the graphical representation of the fatty acid and triacylglycerol composition of a sample according to the method explained above. It is a real 'fingerprint' of the origin of the sample.

When representing groups (variety, PDO, country), the chart is completed by adding the first and third quartiles (dotted line) in order to better reflect internal variations. This type of representation is termed a 'morphotype'. To give an example, the Nyons PDO morphotype is constructed from the fatty acid composition of 151 samples of olive oil from Nyons, collected over 15 crop years. The Haute-Provence morphotype is constructed from the fatty acid composition of 141 samples of olive oil from Nyons, collected over 12 crop years.

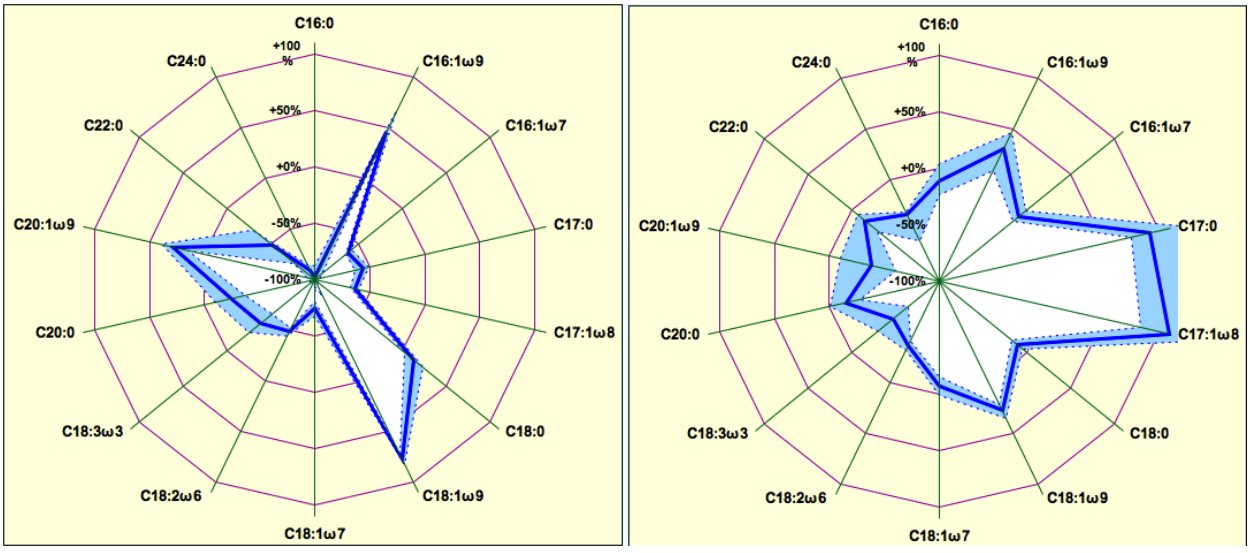

Figure 9. Morphotypes of the fatty acid composition of the Nyons and Haute-Provence PDOs.

Collation of a morphogram and morphotype very quickly makes it possible to diagnose the real provenance of the sample. For example, it is apparent straight away that the sample represented in **Figure 10** does not correspond to either of the two PDOs plotted in **Figure 9**. It is in fact a sample of the 'Picual' variety.

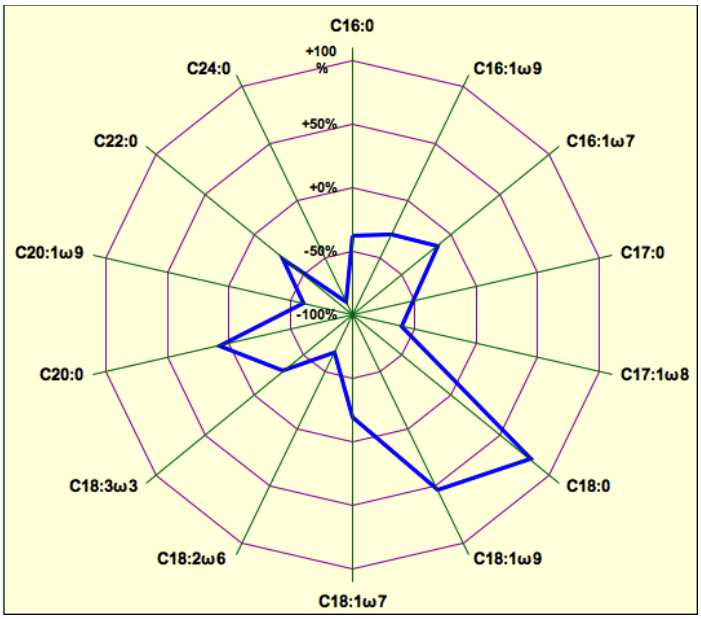

Figure 10: Fatty acid morphogram of a sample produced from the 'Picual' variety.

Test with blended samples

Two tests were performed on fictitious groups obtained from the compositions of real samples chosen at random from the database.

The first test was performed on a fictitious sample constructed by combining 20% of the 'Arbequina' sample with 80% of the Nyons PDO sample, the fatty acid composition of which was calculated.

The morphogram of this sample (shown in red) was compared with the morphotype of the Nyons PDO (shown in blue) (**Figure 11a**). There is a rough correspondence between the graphical representations of the two oils, but numerous points of the red line move away from the blue line, notably in the case of palmitic, palmitoleic, margaric, margaroleic, oleic, *cis*-vaccenic and linoleic acid. This finding raises doubts that this sample is from the Nyons PDO and makes more indepth analysis necessary. **Figure 11b** shows the full range of variation of the fatty acids of all the samples from the Nyons PDO (n=151) entered in the database without being limited to the 1st and 3rd quartiles. The test sample is positioned on the borderline of the ranges of numerous compounds (palmitic acid, margaric acid, margaroleic acid, oleic acid, vaccenic acid); above all, it lies clearly outside the range of variation for palmitoleic acid (**Figure 11b**, red arrow). To confirm this result, a similar approach was taken to the triacylglycerol composition of the fictitious blend and the Nyons PDO. **Figure 11c** shows several borderline triacylglycerol limits for the fictitious sample compared with the Nyons PDO samples and one triacylglycerol (PoOP, red arrow) outside the maximum range of variation. It is therefore possible to reject this sample on the grounds of lack of compliance with the composition of Nyons oil.

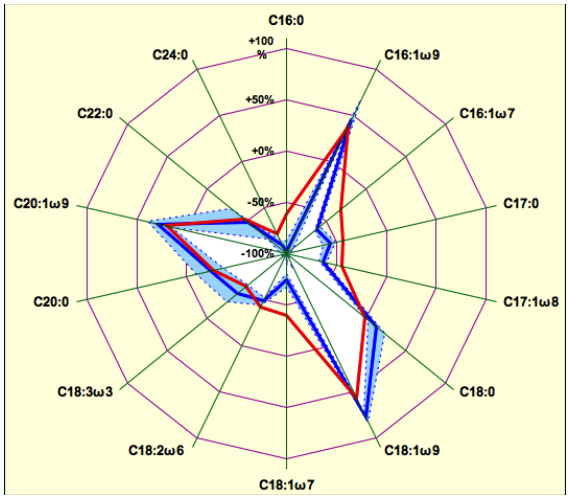

Figure 11a: Superimposition of the fatty acid morphogram of the fictitious sample on the fatty acid morphotype of the Nyons PDO limited to the 1st and 3rd quartiles

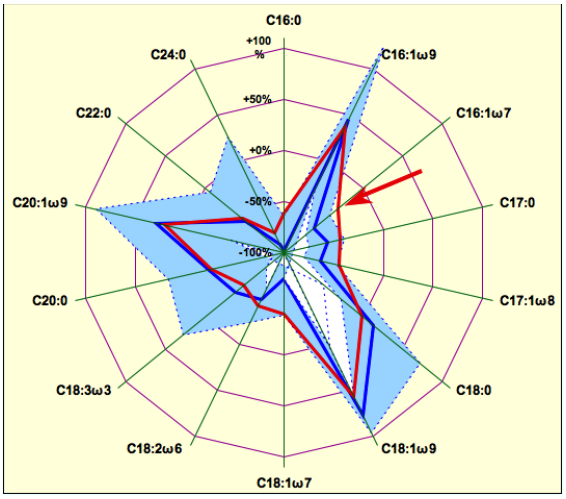

Figure 11b: Superimposition of the fatty acid morphogram of the fictitious sample on the fatty acid morphotype of the Nyons PDO with the maximum range of variation

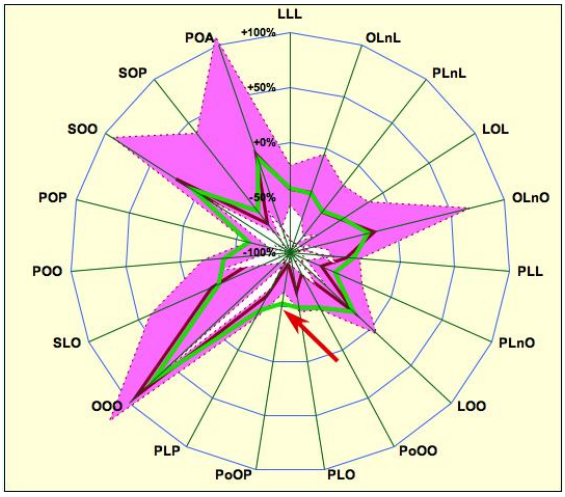

Figure 11c: Superimposition of the triacylglycerol morphogram of the fictitious sample on the triacylglycerolmorphotype of the Nyons PDO with the maximum range of variation

The second test was performed on a combined sample of Tunisian oil chosen at random (20%) and a sample from Haute-Provence (80%). The same result can be obtained by applying the same working approach. Five suspect points were identified in **Figure 12a**: C16:0, C16:1ω7, C17:0, C17:1ω8, C18:1ω9, C18:2ω6. In **Figure 12b**, one point lies outside the blue area and the rest are on the borderline. As a result, this sample is ruled out as a possible sample from the Haute-Provence PDO. **Figure 12c** permits confirmation of this conclusion on the basis of the triacylglycerol composition.

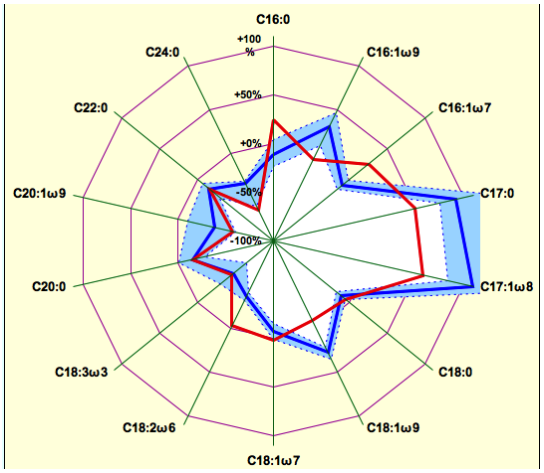

Figure 12a: Superimposition of the fatty acid morphogram of the fictitious sample on the fatty acid morphotype of the Haute-Provence PDO limited to the 1st and 3rd quartiles

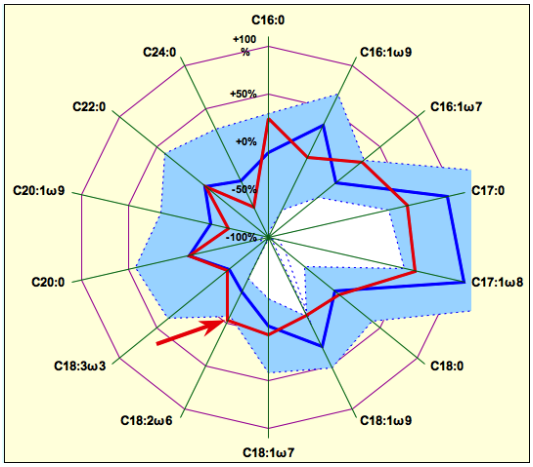

Figure 12b: Superimposition of the fatty acid morphogram of the fictitious sample on the fatty acid morphotype of the Haute-Provence PDO with the maximum range of variation

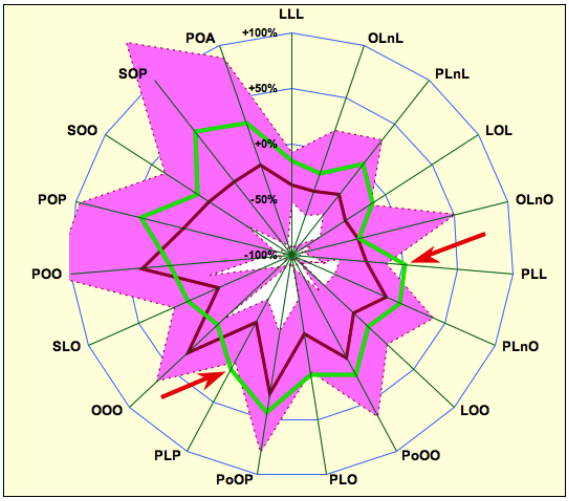

Figure 12c: Superimposition of the triacylglycerol morphogram of the fictitious sample on the triacylglycerolmorphotype of the Haute-Provence PDO with the maximum range of variation

4. Conclusions

The graphical representation of the fatty acid and triacylglycerol composition of samples by morphograms and of groups (varieties, PDOs, countries) by morphotypes enables swift verification of sample compliance with origin. The databases that help to construct the morphotypes can be amplified each production season in order to incorporate the distinctive features of each season.

The samples incorporated need to be checked one by one but this method makes this task easy and fast.

This method can be implemented by any laboratory capable of performing the necessary analyses. The reference morphotypes for the available PDOs and the tools for processing them in order to verify compliance can be downloaded from the AFIDOL website.

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