

## Lipid Components Of Olive Oil From Tadla Azilal Area Of Morocco: Characterization and authenticity

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### Abstract

This work was carried out on the characterization of virgin olive oils from the Moroccan picholine, the main variety cultivated in this country. The picholine samples were obtained from five different locations in Tadla Azilal area (Moroccan centre): Ksiba (KS), Bradia (BR), Beni Mellal (BM), Souk Sebt (SS) and Fkih Bensalah (FBS). They were analyzed for their composition in fatty-acids, triglycerides and sterols during the crop years 2010/2011.

The sterols profile of Tadla azilal virgin olive oils produced by Moroccan picholine cultivar was established by gas chromatography using a flame ionization detector. More than ten compounds were identified and characterized. As expected for virgin olive oil, the main sterols found in all olive oil zones were  $\beta$ -sitosterol,  $\Delta 5$ -avenasterol, campesterol and stigmasterol. Cholesterol, campestanol,  $\Delta 7$ -stigmastenol and  $\Delta 7$ -avenasterol were also found in all samples, but in lower amounts. Most of these compounds are significantly affected by the geographical origin.

For the fatty acids composition, many of them were detected in the virgin olive oils of the studied zones. Indeed, the five olive oils analyzed have shown a fatty acid composition close to the EEC established limits (EEC, 2003). However, the Variations in linoleic acid contents were observed in olive oil samples of other varieties.

**Keywords:** Tadla azilal area, Virgin Olive Oil, Moroccan Picholine, Sterols, fatty acids.

### 1. Introduction

Olive oil production is of great importance in the Mediterranean area, where Spain, Italy, Greece, Tunisia and Morocco are the main producing countries. It is of dietetic importance in the so-called Mediterranean diet (Hrncirik & Fritsche, 2005; Tawfik & Huyghebaert, 1999). This is mainly due to its fatty acid composition, characterized by a high monounsaturated-to-polyunsaturated fatty acid ratio, and to the presence of minor compound. Some of these compounds have a powerful anti-oxidant activity as well as biological and nutritional functions, and endow added value to extra virgin olive oil in terms of vitamin E content and anticancer properties (Psomiadou & Tsimidou, 2002; Velasco & Dobarganes, 2002).

In the last years the demand of olive oil increased outside the Mediterranean region (Bandelj, Jakše, & Javornik, 2002). This is a need to improve olive cultivation, both to produce more and to enhance its quality. Also, in sight of importance of the olive growing, like lever of national and regional socio-economic development and in front of the deficiency of data on the olive oils produced in Tadla Azilal area; we had been brought and for a few years to undertake research tasks on the olive oils produced in this region. The present work aimed to complete the characterization of olive oil in Tadla Azilal area, which was started by studying the quality of the olive oil of the "Moroccan Picholine" while basing ourselves on physico-chemical analyzes relating to free acidity, the peroxide index, the measurement of the values of UV absorption standards and the content of total polyphenols. To complete this chemical description, fatty acid, triacylglycerol and sterolic compositions have been performed and may constitute a genuine fingerprint of olive oil in Tadla Azilal area.

## 2. Materials and methods

### 2.1. Sampling

The samples of the olive oil were collected directly from several units of trituration located in various zones in Tadla Azilal area during crop seasons 2010/2011. All samples belonged to the variety known under the name of "Moroccan Picholine". The method of collection, conditions of storage and the conservation were the same for all samples. Research was concentrated on the olive oil samples selected in the zones called Ksiba (KS), Bradia (BR), Beni Mellal (BM), Souk Sebt (SS) and Fkih Bensalah (FBS). Units producing these oils are equipped with a system of trituration constituted of two phases. The olive oil samples were put in clean and dry bottles of dark glass of a minimal size of 250 mL and refrigerated according to the standard methods of EC (ECC, 2008).

### 2.2. Fatty acids

In order to determine fatty acid composition (%), the fatty acids methyl esters (FAME) were analyzed on capillary tube by gas chromatography (CPG). The FAME of the olive oil samples were prepared according to the standard method recommended by the COI (COI 2001). Olive oil in n-heptane (0.12 g/2mL) was transmethylated with a cold solution of KOH (2M) (1mL). After agitation during 30 seconds, one lets rest until the higher phase of the solution becomes clear, the higher phase heptanic methyl esters of fatty-acids thus obtained, is analyzed in CPG with a Hewlett-Packard (HP 6890) chromatograph equipped with a FID Detector. The results were expressed as peak area (relative) percent.

### 2.3. Triacylglycerols determination

The Triacylglycerols (TAG) compositions were determined by HPLC, applying the modified method of Abaza and al (Abaza *et al.* 2005). The TAG purified by thin layer chromatography of silica gel are split by a chromatograph HPLC of mark Shimadzu CBM 20A (equipped with a Detector with index of refraction RID 10A) using a non-polar Column in phase transfers ODS C18 (250 x 5 mms interior diameter, diameter of the particles 5 $\mu$ m). The mobile phase is a polar mixture of two solvents acetone and acetonitrile (63.6 /36.4 V/V) with a flow of 1 ml/mn, in isocratic mode. Triacylglycerols in olive oils were separated according to equivalent carbon number (ECN), defined as  $CN-2n$ , where CN is the total acyl carbon number and  $n$  is the number of double bonds of fatty acids.

### 2.4. Sterolic composition

2.5 grams of oil sample were subjected to cold saponification. The unsaponifiable fraction was dried under nitrogen flow (Sander *et al.* 1989), added to 30 ml of ethanolic potassium hydroxide solution (for free fatty acid methylation) and dried under nitrogen flow. The treated unsaponifiable fraction was then dissolved in n-hexane-isopropanol (4:1, v/v) and the solution was loaded on 15 cm of a TLC plate. A spot containing sterol standards (b-sitosterol and campesterol) was loaded on each TLC plate in order to correctly identify the sterol band. The mobile phase was a mixture of n-hexane/diethyl ether (65:35, v/v). The sterol TLC band was visualized under UV light (254 nm) after being sprayed with a 0.2% ethanolic solution of 2',7'-dichlorofluorescein sodium salt. The sterol bands of the TLC plates were then scraped off, and extracted with 10 ml of chloroform. To eliminate silica residues, the solution was filtered and then transferred to another vial. Separation and quantification of the silanised sterol fractions was carried out by capillary gas chromatography. The relative percentage of each sterol was calculated as the percent ratio of the chromatographic area of the each sterol peak to the total area of sterols peaks.

## 3. Results and discussion

The composition in sterols, fatty acids and Triacylglycerols of olive oil is an essential aspect of its qualitative assessment. These parameters are very useful for detecting adulterations or to check authenticity since they can be considered as a fingerprint (Cert *et al.* 1997; Vichi *et al.* 2001).

### 3.1. Fatty acid

As shown in table I, many fatty acids were detected in the virgin olive oils of the studied zones. Oleic, linoleic and palmitic acids were considered as major components while stearic, palmitoleic, linolenic and arachidic acids were present in small amounts. The five samples of olive oils analyzed showed a fatty acid composition close to the EEC established limits (EEC, 2003). The monounsaturated fatty acids have great importance because of their nutritional implication and effect on oxidative stability of oils (Aguilera *et al.* 2000; Aparicio *et al.* 1999; Beltrán, 2000; Gutiérrez *et al.* 1999; Martínez de Victoria & Mañas, 2001). Oleic acid, the major

monounsaturated fatty acid, is present in higher concentrations (67.42-71.96%). The level of palmitic acid (C16:0), the major saturated fatty acid in olive oil, showed a minimum value of 8.81 % observed for the FBS sample and a maximum value of 10.84 observed for the BM olive oil.

The observed variations in linoleic acid contents (16.31% for BR, 15.4% for FBS and 13.87% for KS) are probably related to the cultivar-environmental interactions during the development and the maturity of the fruit (lavee & Wodner, 1995). The delay in harvesting tends to increase the content of unsaturated fatty acids, especially linoleic, at the expense of palmitic acid (Osman *et al.* 1994; Schiratti, 1999). For the other fatty acids (palmitoleic (C16:1); stearic (C18:0); linolenic (C18:3) and arachidic (C20:0)), although their small amounts they varied between oil samples.

Concerning the quantitative characterization of the fatty acid compounds, results of literature showed that it differ from one olive oil to another. Indeed, Tanouti *et al.* 2011 reported that olive oil produced in oriental region of Morocco contains about 76.5 % of oleic acid, 13.5% of palmitic acid and 9.36% of linoleic acid. Also, Salvador *et al.* reported that olive oil from Cornicabra cultivar (Spain) contains about 80% of oleic acid, 10% of palmitic acid and 4% of linoleic acid. However, Buccioni *et al.* mentioned that the percentages of these fatty acids in Italian olive oil samples were respectively, 38.74, 21.35 and 19.6%. Thus, G. Bianchia *et al.* (2001) and R. Bucci *et al.* (2002) explained this difference in the fatty acids quantitative characterization by the fact that their content is affected by geographical growing area and differences in olive varieties.

The percentages of saturated, monounsaturated and polyunsaturated fatty acids and oleic acid/linoleic acid ratio for the olive oil samples studied here were also evaluated. It was observed that BR olive oil is rich in total SFAs (13.6), essentially due to its higher contents of palmitic acid, while KS olive oil has the lowest SFAs value (11.87). The other regions present average values which are ranging between these two measures. For the MUFAs ratio, KS olive oil presents the highest percentage (73.12) due to its high content in oleic acid. BR olive oil is rich in total PUFAs (17.5) because of its high contents of linoleic acid. The oleic acid/linoleic acid ratio varies between 4.13 and 5.18 according to the zones. This ratio can be useful to characterize olive cultivars which can constitutes a marked relationship with stability.

### 3.2. Triacylglycerols (TAG) determination

The majority of TAG identified in the olive oil samples studied here is given on table II. The TAG type OOO is expressed at a higher level which represents a minimum value of 33.22% observed for BR olive oil and a maximum value of 39.06 for the KS olive oil. Moreover, LOO TAG (21.37%-20.19%) and POO (18.72%-16.67) were present in a relatively high percentage. Beside these compounds, several minor TAG molecular species were identified for the olive oils of Tadla Azilal area as LOP (8.34%-6.37%), SOO (4.41%-3.38%), POP (2.46%-1.66%), LLO (2.05%-1.79%), LnOO (1.23%-0.82%) and SOP (0.83%-0.71%). In the olive oil samples studied here, the percentages of the main TAG species were present in this order OOO > LOO > POO > LOP. After this quantitative characterization of TAG molecular species, we deduce that the fatty acids contents of olive oil predict the TAG composition of the studied olive oils. In fact, the percentage of the main fatty acids determined in olive oil of Tadla Azilal area were in order of Oleic > Linoleic > Palmitic acids. The different TAG species and their percentages in the studied cultivars were in agreement with those reported in the literature. Indeed, Ollivier *et al.* reported that OOO (45.36%), POO (21.69%) and LOO (12.05%) were the main TAG species detected in French olive oils collected from the area of Haute-Provence. However, results from the quantitative characterization of TAG from olive samples studied by Jakab *et al.* have inverse ratios. In deed, they reported that the OOO (46.43%) was the highest component followed by POO (18.14%) and LOO (12.34%).

### 3.3. Sterolic composition

As shown on table III, many sterols were detected in the virgin olive oils of the studied zones. This composition presents small variations between olive oil samples. The highest phytosterol levels were found for  $\beta$ -Sitosterol, followed respectively by  $\Delta$ 5-Avenasterol and Campesterol which characterize the virgin olive oil and is located in the pulp (Cornforth, 2002). Small amounts of cholesterol, Stigmasterol,  $\Delta$ 7-Stigmasterol and  $\Delta$ 7-avenasterol were also detected in all samples. These results are in agreement with data published elsewhere on olive oils (Alves *et al.* 2005; Rivera del Alamo *et al.* 2004; Sánchez Casas *et al.* 2004).

$\beta$ -sitosterol, the most abundant phytosterol in olive oil, represents more than 87% of total sterols. The highest mean per cent value of  $\beta$  -sitosterol is observed in BM oil (87.83%), whereas the SS oil has the lowest one (84.01%) (Table III). These values are similar to those reported for other olive oil varieties (Pardo *et al.* 2007; Rivera del Alamo *et al.*, 2004). The health aspects of  $\beta$ -sitosterol like have been recently reported in several

studies ( $\beta$ -sitosterol inhibits HT-29 human cancer colon cell growth and alters membrane lipids) (Awad *et al.* 2000; Awad *et al.* 1996).

Concerning the  $\Delta 5$ -Avenasterol content, the FBS virgin olive oil showed the highest value (7.85%), whereas the BM sample recorded the lowest one (5.75%). The content of  $\beta$ -sitosterol generally decreases during ripening, while  $\Delta 5$ -avenasterol increases. Some authors reported also that  $\beta$ -sitosterol is minimal and  $\Delta 5$ -avenasterol is maximal when olives are harvested at their ripening optimum (Koutsaftakis *et al.* 1999).

Regarding campesterol content, all the olive oil samples analyzed here showed low concentrations, ranging from 2.89% (BM) to 3.6% (SS). The campesterol content was below the threshold established by EU Regulations (4%) in all olive oils studied indicating a peculiarity of this olive oil variety.

As regards the other authenticity indices established by the current legislation (EEC, 2003), cholesterol percentages were below the established limits of 0.5% and those of stigmasterol were lower than those of campesterol in about 50% of the samples analyzed (Table III).

In general, the Sterols composition differed, at least for the main sterols, depending of the growing location. The olive oils from SS, KS and BM had higher levels of the main sterols.

#### 4. Conclusion

In summary, taken together the results from the analysis of triacylglycerols, fatty acids composition and the sterol fractions of olive oil growing in Tadla Azilal area, we can conclude that: (1) This olive oil have physico-chemical characteristics of virgin to extra virgin; (2) The fatty acids composition is closed to the EEC established limits; and it was differed, at least for the main fatty acids, depending of the growing location. (3) The main sterols found in all olive oils of different zones were  $\beta$ -sitosterol,  $\Delta 5$ -avenasterol, campesterol and stigmasterol. Cholesterol, campestanol,  $\Delta 7$ -stigmastenol, and  $\Delta 7$ -avenasterol were also found in all samples; (4) the most compounds of the sterols and fatty acids are significantly affected by the geographical origin. For the fatty acids composition, there was an increase for the monounsaturated fatty acids from KS olive oils. These results suggest that, besides the genetic factor, environmental conditions influence the sterolic and fatty acid fractions.

This type of experimental approach provides a more realistic picture of the effect of the geographical origin on the compounds of olive oil. In those conditions, we could suggest in a future time the NIR spectroscopy analysis instead of chromatography analysis. Therefore, a simple, quick and reliable overall characterization of quality of virgin olive oils may be obtained at a lowcost.

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Table I: Mean fatty acid content of the olive oils and standard deviation of the three of virgin olive oil from Moroccan Picholine grown in five geographical areas (KS: Ksiba, BR: Bradia, BM: Beni Mellal, SS: Souk Sebt, FBS: Fkih Bensalah).

Fatty acid	BR	BM	SS	FBS	KS	EVOO (EEC, 2003)
<b>C16:0 (%)</b>	<b>10.67</b>	<b>10.84</b>	<b>9.04</b>	<b>9.17</b>	<b>8.81</b>	<b>7.5-20.0</b>
<b>C16:1 (%)</b>	<b>0.95</b>	<b>0.83</b>	<b>0.68</b>	<b>0.77</b>	<b>0.76</b>	<b>0.3-3.5</b>
<b>C18:0 (%)</b>	<b>2.63</b>	<b>2.22</b>	<b>2.81</b>	<b>2.77</b>	<b>2.74</b>	<b>0.5-5.0</b>
<b>C18:1 (%)</b>	<b>67.42</b>	<b>69.17</b>	<b>71.18</b>	<b>69.97</b>	<b>71.96</b>	<b>55.0-83.0</b>
<b>C18:2 (%)</b>	<b>16.31</b>	<b>14.9</b>	<b>14.34</b>	<b>15.4</b>	<b>13.87</b>	<b>3.5-21.0</b>
<b>C18:3 (%)</b>	<b>1.19</b>	<b>1.21</b>	<b>1.02</b>	<b>1.06</b>	<b>0.98</b>	<b>≤1.0</b>
<b>C20:0 (%)</b>	<b>0.29</b>	<b>0.29</b>	<b>0.37</b>	<b>0.32</b>	<b>0.32</b>	<b>≤0.6</b>
<b>∑ SFAs (%)</b>	<b>13.6</b>	<b>13.35</b>	<b>12.22</b>	<b>12.26</b>	<b>11.87</b>	
<b>∑ PUFAs (%)</b>	<b>17.5</b>	<b>16.11</b>	<b>15.36</b>	<b>16.46</b>	<b>14.85</b>	
<b>∑ MUFAs (%)</b>	<b>68.71</b>	<b>70.34</b>	<b>72.24</b>	<b>71.1</b>	<b>73.12</b>	
<b>oleic/linoleic</b>	<b>4.13</b>	<b>4.64</b>	<b>4.96</b>	<b>4.54</b>	<b>5.18</b>	
<b>MUFAs/PUFAs</b>	<b>3.92</b>	<b>4.36</b>	<b>4.7</b>	<b>4.31</b>	<b>4.92</b>	

C16:0 palmitic, C16:1 palmitoleic, C18:0 stearic, C18:1 oleic, C18:2 linoleic, C18:3 linolenic, C20:0 arachidic, SFAs saturated fatty acids, PUFAs polyunsaturated fatty acids, MUFAs monounsaturated fatty acids, EVOO extra virgin olive oil. Values are the mean of three experiments. The standard deviation is less than 2%.

Table II: Triacylglycerols (TAG) composition of virgin olive oil samples analyzed by HPLC (L = linoleic; Ln = Linolenic; O = oleic; P = palmitic; A = arachidic and S = stearic acids). (KS: Ksiba, BR: Bradia, BM: Beni Mellal, SS: Souk Sebt, FBS: Fkih Bensalah).

TAG	BR	BM	SS	FBS	KS
LLO (%)	1.91	2.05	1.84	1.88	1.79
LnOO (%)	1.23	1.12	0.87	1	0.82
LOO (%)	20.79	20.19	20.83	21.37	20.56
LOP (%)	8.34	8.1	6.43	7.06	6.37
OOO (%)	33.22	34.24	38.24	36.26	39.06
POO (%)	18.47	18.72	17.47	17.29	16.67
POP (%)	2.46	2.53	1.89	1.95	1.66
SOO (%)	3.8	3.38	4.23	4.32	4.41
SOP (%)	0.81	0.71	0.79	0.83	0.76

Values are the mean of three experiments. The standard deviation is less than 1%.

Table III: The sterolic composition of virgin olive oil samples (KS: Ksiba, BR: Bradia, BM: Beni Mellal, SS: Souk Sebt, FBS: Fkih Bensalah).

Sterols	BR	BM	SS	FBS	KS
Cholesterol (%)	0.13	0.11	0.13	0.16	0.1
Campesterol (%)	3.23	2.89	3.6	3.12	3.39
Stigmasterol (%)	0.9	0.77	1.37	1.11	1.29
$\beta$ -Sitosterol (%)	86.21	87.83	84.01	85.4	85.69
$\Delta^5$ -Avenasterol (%)	6.88	5.75	7.72	7.85	7.24
$\Delta^7$ -Stigmasterol (%)	0.14	0.1	0.36	0.12	0.42
$\Delta^7$ -Avenasterol (%)	0.37	0.25	0.29	0.17	0.27

Values are the mean of three experiments. The standard deviation is less than 2%.

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