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Chemical profiles and Sensory analysis of four varieties of olive oil cultivated in Morocco

S. GHARBY[A,B], H. HARHAR[B*], S.M. JADOUALI[A], A. HAJIB[B], I. NOUNAH[B], M. FARSSI[C], A AIT TALEB[A]

[a]Laboratoire de Physicochimie des Milieux Naturels et Matériaux Bioactifs, Faculté Polydisciplinaire de Taroudant, Université Ibn Zohr, Maroc
[b]Laboratoire de Chimie des Plantes et de Synthèse Organique et Bioorganique, Faculté des Sciences, Université Mohammed V-Agdal, BP 1014- Rabat, Maroc.
[c] Laboratoire de controle qualité, CIBELII, Agadir, Maroc

** Corresponding author:*
s.gharby@yahoo.fr/hichamoo79@yahoo.fr
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Abstract

To ensure olive oil quality, a combination of physico-chemical and sensory methods is necessary. Utility and importance of these methods in the international norm for olive oil are presente**.** The aim of the present study is to evaluate the chemical characterization of main compounds and Sensory analysis of four extra virgin olive oil varieties grown in Morocco (Moroccan Picholine, Picual, Arebiquine and Koroneiki). Quality parameters monitored over the study were acidity, peroxide value, UV absorption (E232 and E270), tocopherol, fatty acid composition, total polyphenol and Rancimat test. Results show that the *Koroneiki* variety presents the highest concentrations of C18:1. This variety is also rich in phenolic and tocopherol compounds. In contrast, Arebquina oil shows the lowest contents of C18:1, phenolic and tocopherol compounds. This explains the low stability values for this cultivar. From the sensory results the *Picholine*, *Picual* and *Koroneiki* variety was characterized as fruity, but *Arbequina* variety did not meet the criteria of extra virgin oil.

Keywords: Chemical composition; Olive oil; Oxidative stability, Sensorial and Quality

1. Introduction

Olives from the olive tree (*Olea europaea L.*) are one of the most important fruits throughout the Mediterranean Basin **[1].** Pertaining to several principal constituents such as monounsaturated fatty acids (MUFAs) and phenolic compounds in the olive oil, their consumption has been steadily increasing all over the world and is reinforcing olive cultivation in new areas such as Latin America, California, South Africa, China, and Australia **[2].** The cultivation of olive trees in Morocco constitutes one of the principal economical and agricultural sectors. Olive groves in Morocco are characterized predominantly by the Moroccan variety *Picholine*, which represents more than 96% of the national heritage **[3]** and the rest constitutes varieties endogenous to Spain, Greece and Italy (*Picual*, *Arebiquine*, *Koroneiki*).
Olive oil is extensively consumed due to its nutritional value and organoleptic characteristics **[4].** According to some authors, the beneficial impact of olive oil on health is mainly attributed to its wealth of monounsaturated and low unsaturated fatty acids as well as polyphenols (which act as natural antioxidants) that may contribute together for the prevention of several human diseases such as coronary heart disease, cognitive impairment, colon cancer and many others **[5].** Some of these effects are associated with the high content of phenolic compounds as well as the high amounts of oleic acid, tocopherols and phytosterols **[6; 7-8].** The amount of these compounds in olive oil is influenced to a large extent by the cultivar, soil, climatic conditions, irrigation, degree of ripeness, processing methods and lipid oxidation **[9-10].** Virgin Olive oil, one of the few oils consumed without any chemical treatment has a high resistance to oxidative deterioration mainly due to high proportion of monounsaturated fatty acids and several miniscule compounds with powerful antioxidant activity among which polyphenols stand out **[11-12].** The objectives of current research is to evaluate and compare the chemical profiles and carry out Sensory analysis of four olive oil varieties (Moroccan Picholine, Picual, Arebiquine and Koroneiki) grown in Morocco.

2. Experimental.

2.1. Quality parameter

Acidity index, peroxide value (PV) and extinction coefficients (K_{232} and K_{270}) determination were carried out following the analytical methods described in the Regulations EEC/2568/91 of the European Union Commission (1991) **[13].** Acidity was expressed as the amount of oleic acid. PV was expressed as milli-equivalents of active oxygen per kilogram of oil (meq O_2/ kg oil) and extinction coefficients K_{232} and K_{270} were expressed as the specific extinctions of a 1% (w/v) solution of oil in 2,2,4-trimethylpentane measured in a 1 cm cuvette. For the determination of the fatty acid composition **[14],** the methyl esters were analyzed on a CP-Wax 52CB column (30m x 0.25 mm i.d.) using helium (flow rate 1mL/min) as carrier gas. Initial oven temperature was set at 170 °C, injector temperature at 200 °C and detector temperature at 230 °C respectively. Injected volume was 1µL for each analysis.

Sterol composition was determined using the International Organisation for Standardization method **[15].** Sterol composition was determined by trimethylsilylation of the crude sterol fraction using a Varian 3800 instrument equipped with a VF-1 ms column (30 m & 0.25 mm i.d.) and helium (flow rate 1.6 mL/min) as carrier gas. Column temperature was isothermal at 270 °C; injector and detector temperatures were 300 °C respectively. Injected quantity was 1µL for each analysis. Data was processed using Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA). International Organization for Standardization method was used to determine **Tocopherol composition [16].** High performance liquid chromatography (HPLC) was used for the determination of tocopherols, using a solution of 250 mg of oil in 25 ml of n-heptane. Shimadzu CR8A HPLC instrument (Champ sur Marne, France) equipped with a C18-Varian column (25 cm×4 mm; Varian Inc., Middelburg, The Netherlands) was utilized for the analysis. Detection

was performed using a fluorescence detector (excitation wavelength 290 nm, detection wavelength 330 nm). Eluent used was 99:1 isooctane/isopropanol (V/V) mixture and flow rate was 1.2 ml/min.

The polyphenol content was determined using Folin–Ciocalteu spectrophotometrically according to the Singleton method **[17]** using caffeic acid as standard.

2.2. Rancimat test

Induction time was determined using the International Organization for Standardization method **[18].** The oxidative stability of each sample was determined as the induction period (IP, h) recorded by a 743 Rancimat (Metrohm, Herisau, Switzerland) apparatus using 3 g of oil sample. Samples placed into Rancimat standard tubes were subjected to the normal operation conditions of the test by heating at 383 K with an air flow of 20 L/h.

2.4. Statistical Analysis

Values reported in tables and figures are the means ± SE of two to three replications. The significance level was set at P= 0.05. Separation of means was performed by Tukey's test at the 0.05 significance level.

3. Results and discussion

3.1. Initial quality of olive oils

3.1.1. Quality indices

Olive oil quality can be classified into different categories by use of chemical, physical and sensory parameters according to the definitions and standards defined by the Commission Regulation (EEC) No. 2568/91 on the characteristics of olive oil and olive-residue oil in addition to the relevant methods of analysis **[13].** The European Commission has defined the quality of olive oil based on certain parameters and indicators, mainly the degree of acidity, peroxide value, values of specific extinction in the UV absorbance at 232 nm and 270 nm (E_{232} and E_{270}). The acidity of oil is evaluated from the amount of free fatty acids, expressed as grams of oleic acid per 100 g of oil. It is a simple and effective method to assess and classify the grade of olive oil **[13].** Generally, if the oil is extracted from fresh fruits by best practices crushing, oil has a very low acidity **[19].** However, during storage, the oil may deteriorate and its acidity increases due to the release of fatty acids by hydrolysis of triacylglycerols. Based on this index, all analyzed varieties can be classified into the category of "extra virgin olive oil" as their content of free fatty acid was below 0.8% **[13].** However, the high level of free fatty acids in *Picholine* olive oil (0.62%) could be the result of hydrolysis of triacylglycerols during oil extraction **[20].** The second criterion for the quality of olive oil is the peroxide value (PV). This index is used to evaluate the oxidation state of oil during storage and must not exceed 20 Meq O_2/kg for all categories of olive oil **[13].** The PV of the analyzed olive oils (*Table. 1*) is between 1.06 and 3.2 meq O_2/kg being lower than the maximum values indicated by the regulations **[13].** Measurements of absorbance at specific wavelengths (K_{232} and K_{270}) in the UV region are used to provide information on the oxidative state (K_{232}) and (K_{270}) of olive oil **[22].** The absorbance E_{232} showed low values for all oils ranging from 1.4 to 2.1 without exceeding the limit (2.5) defined by the European regulations **[13].** The absorption at 270 nm which provides information on the performance of a bleaching step showed for all virgin olive oil samples values below the limit of 0.22 given by the European regulations **[13].** These results show that the cultivar type had no significant influence on these analytical quality parameters. These results are in agreement with data reported in the literature **[21].**

Table 1. *Physicochemical parameters of extra virgin olive obtained from four varieties: Picholine, Picual, Koroneiki and Arebiquina oils.*

	European Regulations (**1991**) for olive oil extra virgin	*Picholine*	*Picual*	*Koroneiki*	*Arebiquina*
Acidity (%)	< 0.8	0.6 ± 0.02^{c}	**0.2** ± 0.05^{a}	0.4± 0.1^{b}	0.8 ± 0.1^{d}
PV (MeqO2/Kg)	<20	3.2 ± 0.5^{c}	1.4 ± 0.5^{ab}	**1.06**± 0.50^{a}	2.1 ± 0.5^{bc}
E232	<2.5	2.1 ± 0.01^{c}	1.7 ± 0.01^{b}	1.4± 0.01^{a}	1.7 ± 0.01^{b}
E270	<0.22	0.1 ± 0.01^{a}	0.1 ± 0.01^{a}	0.1± 0.01^{a}	0.1 ± 0.01^{a}
Palmitic acid C16:0	7.5 – 20	9.2 ± 0.1^{a}	12.7 ± 1.5^{b}	12.5± 1.5^{b}	14.3 ± 0.1^{b}
Stearic acid C18:0	0.5 – 5	2.9 ± 0.1^{b}	2.9 ± 0.5^{b}	2.5± 0.1^{a}	2 ± 0.1^{a}
Oleic acid C18:1	55 – 83	74.6 ± 0.1^{b}	76.3 ± 2.5^{b}	**76.5**± 1.5^{b}	67.1 ± 0.1^{a}
Linoleic acid C18:2	3.5 – 21	**10.7** ± 0.1^{b}	5.4 ± 1.5^{a}	6.4± 0.1^{a}	13.2 ± 0.1^{c}
Linolenic acid C18:3	<1	0.9 ± 0.1^{a}	0.7 ± 0.1^{a}	0.7 ± 0.1^{a}	0.8 ± 0.1^{a}
SFA^{a}(mg/100 mg)	-	12.4± 0.1^{a}	15.6 ± 0.1^{c}	15± 0.1^{b}	16.6± 0.1^{d}
UFA^{a}(mg/100 mg)	-	86.8 ± 0.1^{d}	82.4 ± 0.1^{b}	83.6± 0.1^{c}	81.4± 0.1^{a}
UFA^{a}/ SFA^{a}	-	**7.00^{a}**	5.28^{a}	5.57^{a}	4.90^{a}
oleic acid/linoleic acid		6.97	**14.13**	11.95	5.08
Campesterol	<4	2.7 ± 0.2^{a}	3.1 ± 0.5^{ab}	3.2± 0.2^{b}	3.1 ± 0.3^{ab}
Stigmasterol	<Campesterol	1.7 ± 0.1^{a}	2.1 ± 0.1^{a}	1.8± 0.2^{a}	1.9 ± 0.2^{a}
Beta–sterol (other sterols)	>93	93.8 ± 0.5^{a}	94.2 ± 7^{ab}	94.7± 1.1^{ab}	94.8 ± 0.5^{b}
7 Stigmastenol	<0.5	0.2 ± 0.1^{a}	0.4± 0.1^{c}	0.3± 0.1^{b}	0.3 ± 0.1^{b}
7 Avenasterol	-	0.1± 0.1^{a}	0.3± 0.1^{a}	0.5± 0.1^{a}	-
Tocopherol (mg/kg)	-	202 ± 21^{a}	205 ± 33^{a}	**360.5 ±** 25^{b}	182 ± 30^{a}
α-Tocopherol (mg/kg)	-	166.3 ± 5^{a}	164 ± 15^{a}	324± 25^{b}	167 ± 5^{a}
Polyphénol (mg/kg)	-	275 ± 20^{b}	295± 25^{b}	**320**± 30^{c}	136± 25^{a}

*Values are means of three replicates ± standard deviation. Values in the same row with different superscripts are significantly different ($p \leq 0.05$).

SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid.

3.1.2. Fatty acid composition

Table 1 shows the results of the principle fatty acids of the four olive oil cultivars. Major fatty acid components present in all virgin olive oil samples were oleic acid (C18:1), linoleic acid (C18:2) and palmitic acid (C16:0). Palmitoleic acid (C16:1), stearic acid (C18:0), and linolenic acids (18:3) constitute minor composition. The fatty acid composition of the four oils was found to be in agreement with the European Regulations. On the other side significant differences were observed between the different cultivars. Palmitic acid is the major saturated fatty acid in olive oil **[22]** and its content vary between 9.2% (*Picholine*) and 14.3% (*Arebiquina)* according to cultivars with a mean value of 12.6%. The highest percentage of Oleic acid which constitutes main mono-unsaturated fatty acid of olive oil **[22]** was found in *Picual* and *Koroneiki* varieties (76.3% and 76.5%) respectively while varieties of *Arebiquina* (60.43%)

and *Picholine* (64.33%) showed significant lower amounts. Concerning linoleic acid, which is much more susceptible to oxidation than monounsaturated fatty acids **[22],** the highest percentage was observed in variety of *Arebiquina* (13.2%) whereas the lowest amount was found in olive oil from *Picual variety* (5.4%). Other samples showed percentages at 6.4% and 10.7% in varieties of *Koroneiki* and *Picholine*, respectively (Table 1). Linolenic acid belongs to the family of minor fatty acids of olive oil and according to the European Regulations **[13]** and the concentration must be less than 1%. The investigated oils were in agreement with European Regulations with values of linolenic acid between 0.8% and 0.9% amongst all the investigated varieties. Also the amount of the other minor fatty acids including palmitoleic acid and stearic acid varied in different oils. For almost all oils the oleic acid to linoleic acid ratio was superior to the minimum value of **7 [23],** only variety *Arebiquina* showed a ratio of 5.08 (*Table-1*). This ratio can be useful for the characterization of olive cultivars and for the interpretation of stability effects **[24].** Additionally the ratio between unsaturated and saturated fatty acids was found between 4.90 for olive oil from *Arebequina* variety and 7.00 for olive oil from *Picholine*.

3.1.3. Sterol composition

Sterols are important minor constituents in vegetable oils and they are widely used to verify the authenticity **[25].** Besides, their determination is of major interest due to their health benefits, as discussed before **[26].** Table 1 shows the sterol composition of different olive oils. The sterol composition shown in this study is in contrast to the literature **[27],** which stated that the cultivar of the olive tree influences the proportion of sterols. In the present work no significant difference in the composition of sterols between the different cultivars was found. According to the obtained results, the olive oils studied were characterized by a high content of β-sitosterol, comprising more than 93% of the total sterols in the four varieties.

3.1.4. Tocopherols composition

Tocopherols are important molecules due to their role as vitamins in nutrition or their ability to intercept free radicals **[27-28].** α-tocopherol is the major vitamin-E-active compound in the olive oil **[13].** As shown in Table-1, significant differences between the cultivars were found for the total tocopherols content and the content of α-tocopherol. The highest amount of total tocopherols was observed in the variety *Koroneiki* (360 mg/kg); whereas, the lowest amount was recorded in *Arebiquina* cultivar (182 mg/kg), with amounts for varieties *Picholine* and *Picual* of 166.3 mg/kg and 164 mg/kg, respectively. The amount α-tocopherol in virgin olive oil depends on several factors such as variety, fruit ripeness, and agro-climatic conditions. Among these factors, variety is the most important reason for variation **[29].**

3.1.4. Total phenol content

Phenolic compounds contribute to the nutritional importance and benefit to human health of virgin olive oil and they are responsible for bitter taste and the antioxidant activity of the oil **[30- 31].** Ttherefore the content of phenolic compounds is an important parameter which determines the characteristics and quality of olive oil **[32-33].** The total amounts of phenolic compounds in olive oil depend on various factors such as cultivar, climate and irrigation, altitude and technological conditions during extraction **[30-32].** The amounts of total phenols in the analyzed oils show significant differences between different varieties. The highest content of these components was detected in oil from variety *Koroneiki* (320.5 mg/kg), whereas the lowest amount was recorded for oil from variety *Arbequina* (136 mg/kg).

3.2. Oxidative stability

The Oxidative stability of the olive oils from the studied varieties analyzed with Rancimat equipment show significant differences according to cultivar. It ranged from 11.9 (*Arbequina*) to 59 h (*Koroneiki*). *Picholine* and *Picual* oils present intermediate values (18.4 and 26.1 h respectively). The induction times for the oils from the four varieties can be ranked as *Koroneiki* > *Picual* > *Picholine* > *Arebquina*. Nevertheless, *Koroneiki* behaved more satisfactorily under Rancimat conditions and its notorious high tocopherol and polyphenol levels is generally advanced to explain its oxidative stability. In addition to that Regression analysis was done in order to establish the compounds that better explained the oxidative stability. Results showed that phenolic and tocopherol compounds were the major contributors to the oxidative stability, a high correlation values were obtained (R^2 = 0.9, and 0.9,). This is in agreement with other results already reported in the literature. Indeed several authors have established a correlation between phenol content and oxidative stability of virgin olive oil **[34].**

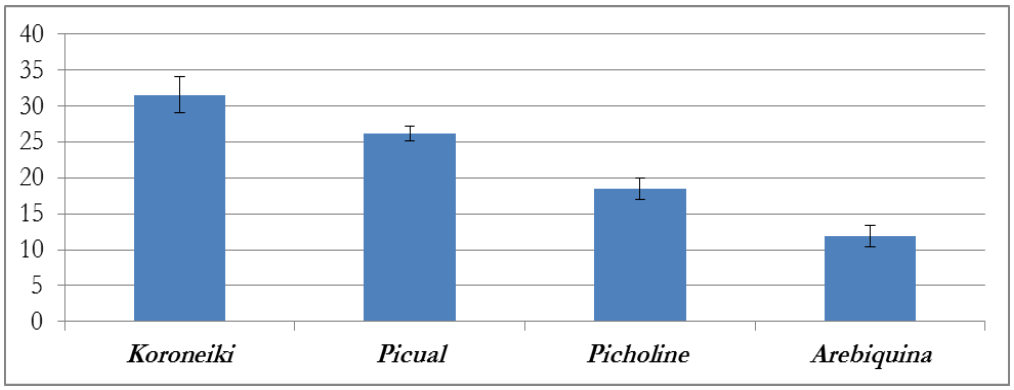
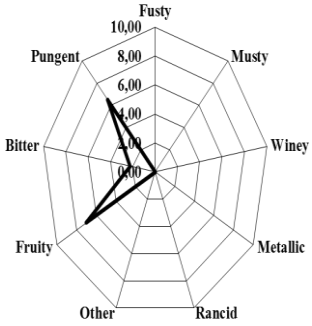
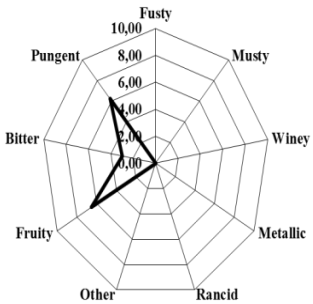


Fig.1: Rancimat induction period in hour of virgin olive oils for the Moroccan olive cultivars at 110°C.

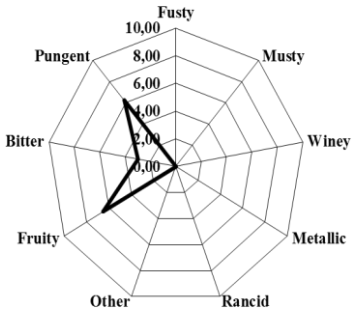
3.3. Sensory analysis

If olive oils are interesting from a nutritional point of view, they are especially appreciated for their taste and particular aromas. A simple chemical analysis is not sufficient to determine the quality of oil. Indeed, the volatile compounds which develop during oil production process and during storage are capable of modifying the odor and flavor of an oil. For this, we decided to use sensory analysis to further evaluate the quality of our samples. Thus, the organoleptic evaluation of oils was performed according to standard for olive oils **[35].** According to Figures 3,4 and 5, the oils of *Picholine* varieties *picual* and *Koroneiki* gave a median of defects equal to 0 and a median of fruitiness greater than 5, which classifies them in extra virgin oil **[35].** *Arbequina* oil did not meet the criteria of extra virgin oil (Figure-2) as it recorded the median of the defect Me = 0.35. The result of the sensory test and the initial high acidity indicate that at the time of purchase, arbiquina variety of oil does not have the necessary criteria to be labeled extra virgin.

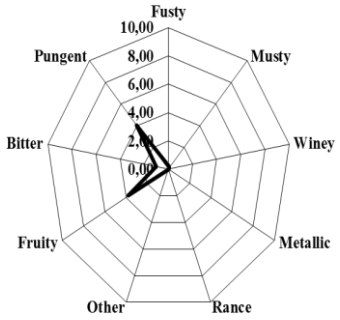




Koroneiki



Picual



Picholine

Arebquina

Fig-2. Sensorial wheels of virgin olive oils for the Moroccan olive cultivars

CONCLUSION

The cultivar appears to play a significant role for the qualitative characteristics and the sensory attributes of the olive oils analysed. The results obtained showed that the Picholine, Picual and Koroneiki variety was characterized as fruity, but Arbequina variety did not meet the criteria of extra virgin oil. The Koroneiki variety demonstrated excellent nutritional characteristics in terms of antioxidant compounds. In fact, the olive oils showed a higher content of total tocopherols. In contrast, Arebquina oils show the low contents of C18:1 phenolic and tocopherol compounds. This explains the low stability values in this cultivar. The four olive oil varieties studied can be efficiently authenticated using multivariate statistics both on the basis of chemical characteristics and of certain attributes included in their sensory profiles.

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