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Physico-Chemical Characterization of Olive Oils produced in the rural commune of Tagzirt, province of Beni Mellal, Morocco

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ABSTRACT: The objective of the present work is the evaluation of the nutritional and organoleptic properties of olive oils from oil mills in the rural commune of Tagzirt, area of Beni Mellal (center of Morocco) by a physicochemical characterization of their compositions.

Fifteen samples of olive oils extracted from the Moroccan Picholine variety were collected from traditional oil mills. Physicochemical analyzes of free acidity, peroxide value, refractive index, density, K_{232}, K_{270} and ΔK, the chlorophyll content, the content of phenolic compounds, the α-tocopherol content and oleic acid proportion were conducted according to the standards of the International Olive Council (IOC).

The results were used to classify the oils studied according to their quality standards. The data obtained confirm that the conditions of harvesting, crushing and storage of olive oils affect the quality of produced oil. Therefore, we must educate farmers on the importance of improving practices and cultivation techniques and the owners of oil mills as regards the storage, processing and storage of oils.

KEYWORDS: Olive oil, quality optimization, stability of the oil, bioactive compounds.

1 INTRODUCTION

Virgin olive oil, especially extra virgin olive oil, constitutes one of the most appreciated and consumed vegetable oils worldwide because of its renowned organoleptic properties. It is the primary source of lipids in the Mediterranean diet and has been linked with positive health benefits [1], [2]. In the last few decades there has been a significant increase in the global consumption of olive oil, even in countries where it is not produced, such as the Canada and Japan [3]. Indeed, due to the significant content in bioactive compounds, such as phenols, phytosterols or tocopherols, its regular consumption improves antioxidant status and blood lipid profile, reducing the incidence of some degenerative diseases such as atherosclerosis or cancer [4], [5], [6], [7], [8].

The evolution of the consumption of virgin olive oil at the international level depends on its quality. In fact, several parameters are associated with the assessment of the quality and purity of the olive oil. According to the standard of the International Olive Council, the quality of olive oils is a set of physical, chemical and organoleptic characteristics which classify oils into different categories [9].This quality is influenced by several factors, such as cultivation practices, irrigation system, time of harvest, extraction process and conditions of storage [10]. All these parameters require careful study and mastery in order to achieve good quality oil.

In order to assess the quality of olive oils produced in the region of Beni Mellal, we were interested in the study of the chemical composition of these oils. To do this, samples were collected from traditional oil mills. Physicochemical analyzes of free acidity, peroxide value, refractive index, density, the coefficients of specific extinctions K_{232}, K_{270}, the variation of the specific extinction ΔK, the chlorophyll content, the α-tocopherol content, the content of phenolic compounds and oleic acid proportion were carried out.

2 MATERIALS AND METHODS

2.1 SAMPLING

The study was focused on fifteen samples of monovarietal olive oils (S_1 to S_{15}) of traditional oil mills equipped with two-phase crushing system, in the rural commune of Tagzirt, Beni Mellal region, during the 2015/2016 crop season. The samples were preserved in dry and clean dark glass bottles in the refrigerator at 4°C to avoid the phenomenon of auto-oxidation prior analysis.

2.2 DETERMINATION OF REFRACTIVE INDEX AND DENSITY

Measurements of densities and refractive indices of olive oil samples were performed with a combined Meter, density meter and refractometer at a time. Delta Ranges METTLER TOLEDO is a fully automatic system for the simultaneous determination of these two physical parameters with high accuracy (four decimal places). Before each using, the device is calibrated with distilled water and air.

2.3 DETERMINATION OF FREE FATTY ACIDS (FFAS)

The most common method for the determination of the FFA level uses acid/base titrations with phenolphathlein as an indicator. The oil is dissolved in an organic solvent and titrated with an alkaline solution such as sodium hydroxide. The results are given as % FFA as oleic acid, as described by IOC regulation. Briefly, 5 g of oil is weighed in an Erlenmeyer flask and 10 ml of 95% ethanol and 0.5 ml of phenolphthalein are added; titration is then carried out drop by drop with 0.5% NaOH solution until the color changes. Acidity is calculated as a percentage by mass as follows:

$$\textit{Acidity (\%oleic acid)} = V.c\ M/10\ m$$

where V = volume in ml of standard volumetric NaOH solution; c = concentration (in moles per liter) of the standard volumetric NaOH; M = molar mass (in g per mole of oleic acid); and m = mass (in g) of the test portion.

2.4 DETERMINATION OF THE PEROXIDE INDEX

One method used to determine lipid oxidation is to measure the peroxide value, which is indicative of the amount of hydroperoxide in the oil. The standard method for measuring the peroxide value is to titrate a mixture of the oil, chloroform, acetic acid and saturated potassium iodide solution with sodium thiosulfate, as described by the International Olive Oil Council according to ISO 3960 (IOOC, 1995) and expressed as the milliequivalent (meq) of active oxygen per kilogram.

5 g of oil is placed in an Erlenmeyer flask and 50 ml of a mixed solution of acetic acid and isooctane (60:40 v/v) is added to it; 0.5 ml saturated potassium iodide solution is then added and the solution is mixed. Titration is performed with sodium thiosulfate solution 0.01 mol/l. When the solution turns light yellow, about 0.5 ml of starch solution (5 g/l) is added. The end-point is determined when the color of the solution changes from brown to transparent. The peroxide value is calculated by the following equation:

$$\textit{Peroxide value (meq/kg oil)} = 1000\ (V - V_0) \times c/m$$

where V = volume of sodium thiosulfate solution used (in ml); V_0 = volume of sodium sulfate used for the blank determination (in ml); c = concentration of the sodium thiosulfate in moles per liter; and m = mass of the test portion (in g).

2.5 SPECTROPHOTOMETRIC ANALYSIS IN THE ULTRA-VIOLET

The UV absorbance of oil was assessed using the PrkinElmer lambda35 spectrophotometer, which measures the quantity of oxidized compounds that resonate at wavelengths of 232 and 270 nm in the ultraviolet spectrum (UV). For UV absorption

testing, 0.1 g of olive oil sample is dissolved in 10 ml cyclohexane. After homogenization, were measured extinctions at 232 nm and 270 nm wavelengths. For the variation of the specific extinction ΔK, the absorbance of the olive oil sample at wavelengths of 266 nm and 274 nm were measured (IOC, 2011). The values of specific extinctions at 232 nm and 270 nm are calculated using the following formula:

$$K = A_k / C \times S$$

where A_k: Absorbance at the wavelength k, C: Concentration of the solution in g / 100 ml, S: optical path (1 cm).

This analysis also provides for the determination of the variation of the specific extinction ΔK using the following equation:

$$\Delta K = K_m - (K_{m-4} + K_{m+4}) / 2$$

where K_m is the specific extinction in the length of wave m maximum absorbance at around 270 nm.

2.6 DETERMINATION OF CHLOROPHYLL CONTENT

The content of chlorophyll pigments (mg/kg) in the olive oil samples was determined using a PrkinElmer lambda 35 spectrophotometer through the absorbance (A) of VOO at 630, 670 and 710 nm. Samples were filled directly into a 1 cm path length glass cell (L). Pure carbon tetrachloride was used as a reference [11].The chlorophyll pigment was calculated by:

$$Chlorophyll\ (mg/kg) = [A_{670} - (A_{630} + A_{710}) / 2] / (0.1086 \times L)$$

2.7 DETERMINING THE CONTENT OF PHENOLIC COMPOUNDS

An adaptation of the method proposed by Gutfinger was used [12]. The phenolic compounds were measured in the polar fraction obtained from 10 g olive oil dissolved in hexane and extracted by washing three times with a methanol:water (60:40 v/v) solution. The procedure consisted of reacting a 0.5 ml aliquot of the extract with 0.5 ml Folin-Ciocalteau reagent plus 1.0 ml of a saturated Na2CO3 solution, leaving the mixture at rest for 1 hour in the dark. Subsequently the adsorption was read in the visible region (760 nm) of a UV/Visible spectrophotometer. The result was expressed in equivalents of gallic acid using a standard curve from 10 to 100 µg gallic acid/ml.

2.8 DETERMINATION OF α-TOCOPHEROL CONTENT

α-Tocopherol was evaluated according to IUPAC 2432 method (IUPAC, 1992), [12]. Oil samples of 2 g was dissolved in 25 mL hexane, filtered (0.45 lm) and injected (20 µL) into the HPLC system (Agilent 1100, Agilent Technologies) with a Zobrax C18 column (150 mm × 6 mm × 3.5 µm). The mobile phase was n-hexane/2-propanol (99.5:0.05, v/v) and the flow rate 0.8 mL/min. Individual tocopherol were identified at 295 nm and quantified as mg/100 g of oil using the corresponding external standard, α-tocopherol (Sigma–Aldrich). The concentration of α-tocopherol in the samples is determined in relation to the peak area of the standard, and the results are adjusted for the average specific gravity of olive oil.

2.9 DETERMINATION OF THE OLEIC ACID PROPORTION

The fatty acid profile is generally determined by assessing fatty acid methyl esters by gas chromatography, as suggested by IOC regulation. In brief, 0.1 g oil is weighed and diluted with 2 ml heptane and 0.2 ml of 2N methanolic KOH. The combined solution is shaken vigorously for 30 s and left to stratify until the upper solution becomes clear. The upper solution is collected, and evaporated to dryness under a nitrogen gas flow. The methyl ester is re-suspended in 1 ml of heptane and injected into the GC for determination. Analysis was carried out using a gas chromatograph HP 6890 equipped with a flame ionization detector (T = 250 °C). The column used is a Carbowax capillary column of type size (30 m x 0.32 mm x 0.25 microns). The carrier gas was nitrogen at a flow rate of 2.5 ml / min. The oven temperature program was 140 °C to 200 °C, from 210 to 245 ° C, the temperature gradient is 10 °C / min for 10 min. Identification of the peaks was carried out in the presence of witnesses and calculating the oleic acid percentage was done by mean of an automatic integrator. All analyzes were performed triple and the results are expressed as mean.

3 RESULTS AND DISCUSSION

The following Table reports the results of free acidity, peroxide value, refractive index and density of olive oil samples studied.

Table 1. Free acidity, peroxide value, refractive index and density of olive oil samples studied

Sample ID	refractive index	density (g/cm3)	free acidity	peroxide value
S_1	1.4693	0.9138	1.14±0.01	7.31±0.02
S_2	1.4697	0.9141	1.02±0.01	14.53±0.02
S_3	1.4695	0.9139	1.98±0.01	09.11±0.02
S_4	1.4694	0.9138	1.17±0.01	11.85±0.02
S_5	1.4696	0.9138	1.00±0.01	09.13±0.02
S_6	1.4693	0.9138	0.80±0.01	13.45±0.02
S_7	1.4693	0.9135	2.24±0.01	5.95±0.02
S_8	1.4694	0.9138	1.35±0.01	15.34±0.02
S_9	1.4692	0.9133	1.56±0.01	12.97±0.02
S_{10}	1.4692	0.9135	0.7±0.01	11.70±0.02
S_{11}	1.4693	0.9136	1.72±0.01	10.43±0.02
S_{12}	1.4694	0.9137	1.64±0.01	15.26±0.02
S_{13}	1.4694	0.9136	2.12±0.01	08.09±0.02
S_{14}	1.4690	0.9129	1.33±0.01	09.48±0.02
S_{15}	1.4693	0.9137	0.96±0.01	13.22±0.02

3.1 REFRACTIVE INDEX AND DENSITY

The results of density and refractive index are shown in the Table 1 above. All density values appear to be identical and are within the standard codex for virgin and refined olive oils (0.910 to 0.916) g/cm^3. The density of the oils is generally related to the degree of unsaturation and oxidation state. The refractive indices of the analyzed samples are very similar and comply with the food codex standard for oils (1.4677 to 1.4705). The density and the refractive index are physical parameters to identify and judge the purity of olive oil.

3.2 FREE ACIDITY

Analysis of free acidity of the studied samples gave the following data (Table 1 above). They are expressed in percentage of oleic acid. The free acidity of olive oil samples studied is between 0.7 and 2.24%. Based on these results and according to the trade standard of the IOC, there is no sample analyzed is kind of lampante virgin olive oil (Acidity greater than 3.3%). Similarly, studied oils can be classified into three distinct categories (IOC, 2011): the class of extra virgin olive oil whose free acidity is less than or equal to 0.8%. This class contains the samples S_6 and S_{10}. The class of virgin olive oil with a free acidity is between 0.8 and 2%, this class contains the samples S_1, S_2, S_3, S_4, S_5, S_8, S_9, S_{11}, S_{12}, S_{14} and S_{15}, and the class of common virgin olive oil with a free acidity is between 2 and 3.3%, this class contains the samples S_7 and S_{13}.

The first two categories present a percentage of 86.7% of all samples analyzed (n = 15), 2 extra virgin olive oils and 11 virgin olive oils, this result indicates good overall quality, which may be due to both the freshness of the crushed olives and olive oil of samples studied and also to a good post-harvest fruit conservation.

The results show that there is high variation in the acidity of the olive oil of different samples; this can be attributed to the various practices in the process of crushing and also to the residence time of olives before crushing. Free fatty acids result from the action of lipase on triglycerides, or other hydrolytic activity of these triglycerides; this can occur before, during or after pressing olives.

The high acidity, which we have obtained for the third category (13.3%), can be explained by the state advanced of fruit maturity, lack of precautions taken during harvesting or storage of olives which cause the deterioration of fruits and consequently increase the content of free fatty acids, under the action of lipases [14].

However, the values observed in this study are lower than those reported by Boulfane et al. who have obtained values between 1.77 and 5.83% for different olive oils olive-growing region of Chaouia [15], and they are also lower than those reported by Benabid et al. who have obtained values between 0.77 and 9.26% for different olive oils olive-growing region of Algeria [16]. By cons, our results are higher compared to those reported by Tanouti et al. who noted that the free acidity remains below 0.8% for olive oil produced in eastern Morocco [17].

3.3 PEROXIDE VALUE

The peroxide value (PV) of an olive oil is a crude measurement of its primary oxidation due to oxygen exposure. This oxidation leads to secondary oxidation products which give undesirable flavors and odors. In general, high PVs imply oxidized oil and, therefore, a lower quality product [18]. The results for the peroxide content of samples analyzed are presented in Table 2 above. They are expressed in milliequivalents of active oxygen per kilogram of oil (meq active O_2/kg olive oil).

We notice that the PVs range from 5.95 for the sample S_7 to 15.34 (meq active O_2/kg) of olive oil for the sample S_8. These values remain below the limit established by the trade standard of the IOC for extra virgin olive oils (≤ 20 meq active O_2 /kg olive oil) (IOC, 2011). This means that the olive oil studied will have a good shelf-life. High levels at the bottling stage are not a good indication of a long shelf-life.

The highest PV obtained was 15.34 (meq active O_2/kg) (S_8). This can be explained by oxidation of olive oils following the conditions of harvest and post-harvest of olives [19].

Tanouti et al. have reported similar values for olive oils produced in eastern Morocco (7 to 15.4 meq active O2/kg olive oil) [17].While Abu-Reidah et al. have reported much lower values (8.20 to 11.37 meq O2/kg of olive oil) for olive oils from Palestine [20]. By against Boulfane et al. have reported higher values (10.96 to 18.7 meq active O2/kg of olive oil) for olive oils from region of Chaouia [15].

3.4 ABSORBANCE IN THE ULTRAVIOLET

The values of specific extinctions obtained for the samples studied by ultraviolet at 232 nm and 270 nm and ΔK are shown in the fig. 1 below:

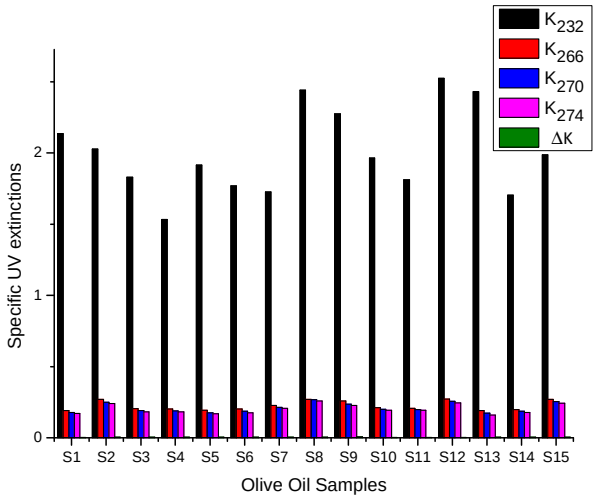

Fig. 1. UV specific extinctions and ΔK of different studied samples

The values of specific extinctions ultraviolet K_{232} and K_{270} obtained for all samples, indicate that they do not exceed the limits set by the International Olive Council for virgin olive oils (IOC, 2011), which are respectively less than or equal to 2.60 and 0.25, with the sole exception of the sample S_8 (k_{270} = 0.2663). This exception may be explained by several factors, such as late harvest of olives, excessive exposure of olives and oil extracted to oxygen from air and to light and also to a possible warming of the dough during crushing [17]. It is noted that this oil showed the highest peroxide index value (15.34 meq active O_2/kg oil). The specific extinction at 232 nm and 270 nm of oil reflects its oxidation state. More the extinction at 232 nm is greater, more it is peroxidized. Similarly, more the extinction at 270 nm is higher, more the oil is rich in secondary

oxidation products and reflects its low storability [11]. As for the variation of the specific extinction, it varies from sample to sample but its value is comparable to that established by the International Council ($\Delta K < 0.01$), (IOC, 2011).

3.5 CHLOROPHYLL PIGMENT CONTENT

The level of chlorophyll is considered as one of the most important factors in olive oil. Chlorophyll plays a vital role in determining the olive oil color, and the color plays a key role in acceptability among consumers. In fact, many consumers preferred a deep green color in olive oil, as in virgin oils [21]. The chlorophyll level also affects the oxidative stability of the olive oil, because it is implicated in auto-oxidation in the dark and the photo-oxidation mechanism in the light. Color is an important attribute to consumers, who associate the green hues from the chlorophyll in the oil with freshness of product [22].

The contents obtained for chlorophyll of the samples studied, expressed in ppm, are shown in fig. 2 below:

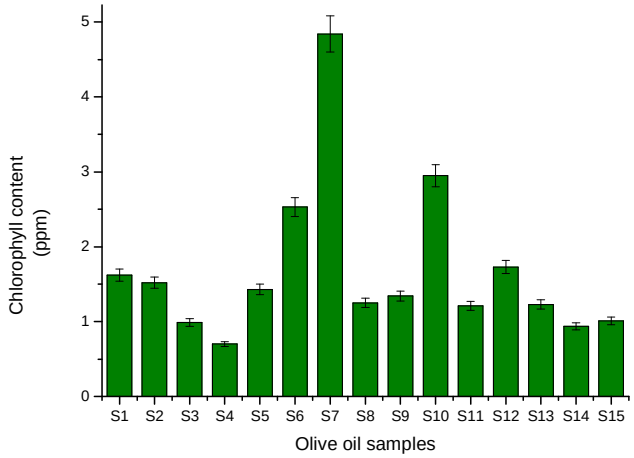

Fig. 2. Chlorophyll content of the samples studied

The contents of chlorophyll for most of the samples tested are strictly less than 2 ppm. These low levels are desired to avoid pro-oxidant action of chlorophyll pigments and thus ensure appropriate conservations of oils [23]; hence the interest to produce olive oils from ripe olives and to remove leaves during the extraction of the oil. Indeed, some researchers have found that the concentration of chlorophyll is high – at up to 80 mg/kg of oil – early in the ripening period, and very low – about 2 mg/kg oil – when fruit is very ripe [24]. This decrease is due to the degradation of chlorophyll producing pheophytin which give the oil its yellow color [25]. Samples S_6, S_7 and S_{10} have slightly higher levels, respectively: 2.53, 4.84, and 2.95 ppm. This can be explained by an early harvest of olives pressed.

3.6 PHENOLIC COMPOUNDS CONTENT

Phenolic compounds are perhaps the most important of the minor components in olive oil, owing to their powerful antioxidant effect on the oil and the resulting contribution to shelf-life stability. The contents of phenolic compounds obtained for samples studied, expressed in ppm, are shown in fig. 3 below:

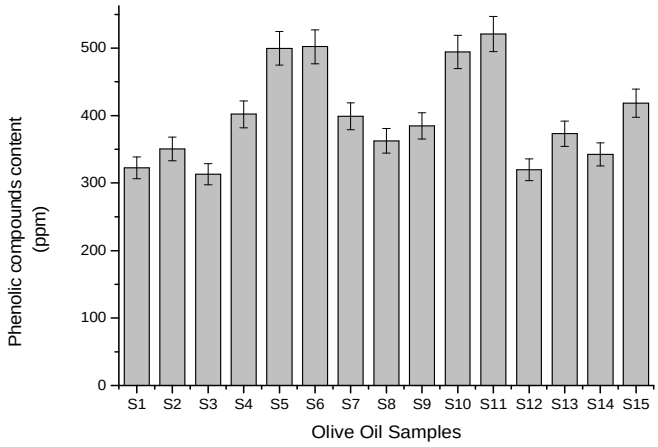

Fig. 3. Phenolic compounds content of the samples studied

The results obtained show that the studied olive oils contain a very substantial amount of phenolic compounds. This amount varies between 313.23 (S_3) and 520.85 ppm (S_{11}). The results we obtained are consistent with those reported by Abu Reidah et al. who found values ranging from 318.99 to 469.96 ppm for Palestinian oils [20]. However, they are much higher than those obtained for olive oil in the region of Tadla (179.9 to 281.35 mg/kg) [26]. Phenolic compounds pass into the oil when extracted. They are considered natural antioxidants that protect the oil against oxidation and ensure better stability during storage and a bitter taste [17]. The changes observed of concentrations may be due to the difference in maturity of the olives before crushing (early harvest olives) but also depends on the cultivar and the geographical area [27]. Indeed, the olive oil located in altitude, are richer in phenols that olive groves of the plains [28]. The presence of leaves in olive milling may also increase the concentration of phenolic compounds in olive oils [29]. Moreover, it is generally known that phenols are bitter in taste, contributing pungency and bitterness to the oil; however, even though high polyphenol contents were found in the Tagzirt olive oils, organoleptically they did not demonstrate any specific bitterness or pungency that adversely affected quality.

3.7 α-TOCOPHEROL CONTENT

Tocopherols are collectively known as vitamin E, and represent an important class of antioxidants that occur naturally in vegetable oils and function to maintain oil quality by terminating free radicals [30], [31].

In olive oil, more than 90% of total tocopherols are represented by α-tocopherol, which shows high variation according to soil and climatic conditions and agronomic factors, such as area of origin, cultivar, and fruit ripening stage [32]. Figure 4 below shows the values of α-tocopherol contents in samples analyzed.

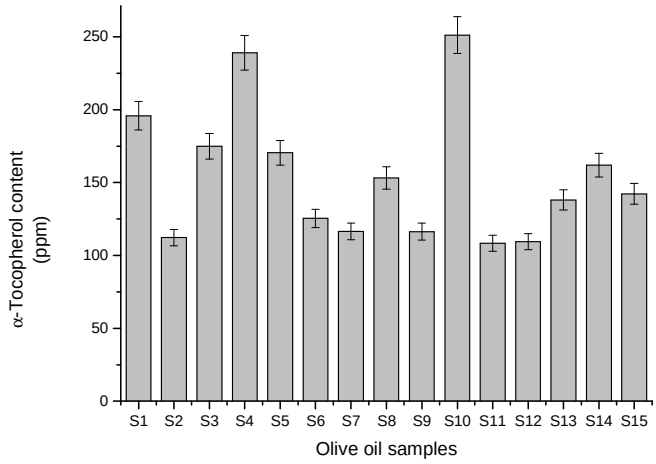

Fig. 4. α-Tocopherol content of the samples studied

The α-Tocopherol content values of the samples studied are between 108.45 (S_{11}) and 251.17 ppm (S_{10}). These data show that the Tagzirt-grown olives contain level of α-tocopherols in the same range compared to results of other monovarietal olive oils from different traditional olive-growing areas, ranging from 109–250 ppm of olive oil [33], [34], [35].

3.8 OLEIC ACID PERCENTAGE

The fatty acid profile (FAP) of the oil is a measure of the proportions of individual fatty acids in the oil, and is therefore an important factor in oil quality. The ratio of the different fatty acids in the oil influences the stability of the oil, as well as determining its nutritional value. Some fatty acids are considered to be better than others; in the case of olive oil, oleic acid is more desirable than the others from the nutritional point of view. Oils that have high levels of monounsaturated oleic acid are considered to be of the highest nutritive value (in fact, oleic acid is named after the olive, "olea"). So, we determined the percentage of oleic acid for each sample by gas chromatography. The data from study are presented in the table 2 below:

Table 2. Percentages of oleic acid of the samples studied

	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}	S_{15}	**IOC standards**
Oleic C18:1	68.11	70.28	69.27	69.65	69.53	73.19	74.10	72.28	78.21	75.65	80.53	71.19	80.91	79.33	74.98	55.0–83.0

The results for the fifteen samples analyzed show that the proportions of oleic acid in olive oils studied are in compliance with established limits by (IOC, 2003). Indeed, the percentage of oleic acid (C18:1) vary between 68.11% for sample S_1 and 80.91% for the sample S_{13}. Studies have shown that the fatty acid profile of olive oil is related to the cultivar and have also shown that fruit maturity affects the FAP [36], [37]. The level of oleic acid increases during ripening due to active triacylglycerol biosynthesis [38].

The percentages of oleic acid in the studied olive oils are very higher than values reported by Issaoui et al. for Tunisian olive oils (54.6 to 66.8%) [38]. However, they are somewhat higher also than the values found by Abu-Reidah et al. for Palestinian oils, which are ranging from 67.24 to 72.27% [20].

4 CONCLUSION

To sum up, in the present article, fifteen samples of monovarietal olive oils from the Moroccan Picholine variety, produced in the rural commune of Tagzirt, province of Beni Mellal, Morocco, have been physically and chemically analyzed. Eleven physicochemical analysis have been obtained for each sample; (free acidity, peroxide value, refractive index,

density, specific extinction coefficients K_{232}, K_{270} and ΔK, the chlorophyll content, the content of phenolic compounds, the α-tocopherol content and oleic acid proportion); to classify these olive oils according to the standards of the International Olive Council (IOC) and thus to assess their nutritional and organoleptic properties.

73.33% of the samples studied were classed in the category "virgin olive oil", 13.3 % in the class "extra-virgin olive oil", and 13.3% in the category "common virgin olive oil". Based on these results, we can deduce that the studied olive oil reveals great potential as regards the nutritional value. Indeed, all quality parameters of the majority of the samples studied (Free fatty acids, UV absorbency, peroxide value) prove that these olive oils meet the standards of IOC for virgin olive oils and even extra-virgin olive oils for some. The purity parameters evaluated (chlorophyll content, level of phenolic compounds, α-tocopherol content, and oleic acid percentage) indicate also good global quality of these olive oils.

The results of this study confirm also that the quality of olive oil is influenced by many factors, which can be grouped into those that act during oil formation in the fruit, during fruit collection, and during the processing and storage of oil. Genetic (varietal), climatic and environmental factors might affect all of the above.

Therefore, to enhance further these regional oils and give them an added value, it is necessary to educate farmers to improve practices and techniques cultivation and the owners of traditional oil mills in regard to the storage, processing and storage of oils.

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