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Study of the Chemical Composition of Olive Oil According to Its Mode of Extraction and Its Age from the Olive Tree

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Abstract

A comparative study of the chemical composition of olive oil was carried out in the Massmouda district of Ouezzane, a city in northern Morocco. It was made in order to study the influence of the extraction temperature and the age of the olive tree on the chemical composition of olive oil. To carry out this work, we selected four samples of olive oil of the same kind (Moroccan Picholine), from the same place and extracted at different temperatures, but these samples were collected from olive trees of different ages. For this we performed physico-chemical analyzes such as acidity, peroxide index, absorbance in ultraviolet, fatty acids, sterols and triglycerides. The result of this work shows that the temperature of olive oil extraction can increase the peroxide index, acidity, the percentage of oleic fatty acid (C18: 1), the percentage of stigmasterol, and the percentages of triglycerides OOO, POO and can decrease the percentage of fatty acid such as: C18: 0, C18: 2 (vitamin F) and the percentages of triglycerides LLL, LOL, OLO, PLO. The results of the chemical composition according to the age of the olive tree reveal that the percentages of oleic acid (C18: 1), the percentages of the triglycerides LLL, OOO are decreased with the increase in the age of the olive tree. On the other hand, our study demonstrates that the percentage of linoleic acid C18: 2 (vitamin F) is increased with the increase of the age of the tree. In the end our study proved the high quality of olive oil extracted by cold mechanical pressing.

Keywords: Olive, Fatty acids, Sterols, Triglycerides, Extraction temperature, Olive age

1 Introduction

The olive tree is a Mediterranean fruit tree that belongs to the Oleaceae family. This tree produces olives; a fruit consumed in various forms and from which olive oil is extracted. In Morocco, the main cultivated fruit species is the olive of the popular variety "Moroccan Picholine" [1] which occupies an area of 560,000 ha. Olive groves contribute to employment in rural areas with 11 million working days annually. Olive production reaches the approximate figure of 560,000 T, generates 50,000 T of olive oils and 90,000 T of industrial table olives [2]. The olive tree develops in four periods [3].

- Period of youth (1-7 years): it is the period of growth, size and flowering. The olive tree settles, expands but produces nothing.
- Period of entry into production (7-35 years): it is in a way the adolescence period of the tree which prepares for the establishment of regular and significant productions.
- Adult period (35-100 years): full production period (yield of 15 to 25 kg of olives per tree). The olive tree is in the prime of life.
- Period of senescence (beyond 150 years): end of the tree's productive life, little by little it produces less. Carpenter branches die, trunk bursts.

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Olive oil is practically the only vegetable oil that can be consumed in its raw form without prior treatment. Appreciated for its flavor and nutritional characteristics, it is known for its multiple virtues in the prevention of diabetes, high blood pressure [4], certain cancers and aging [5]. It is also used in the pharmaceutical and cosmetic fields [6]. Making the pillar of the Mediterranean diet, several studies have been carried out, on the fruit and leaves, to confirm the ancestral virtues attributed to it [7-9]. This study is part of the continuity of the research series performed by The Laboratory of Plant Chemistry, Organic and Bio-organic Synthesis on vegetable oils. To enhance olive oil and improve its nutritional effect we followed some steps: (a) the study of the chemical composition of olive oil according to the age of the olive tree; (b) the study of the chemical composition of olive oil according to its mode of extraction and (c) to accomplish this work, 4 olive samples belonging to the same region of Morocco (the town of Ouezzane Douar Ghnioua) and of the same variety "Moroccan Picholine" were selected.

2 Materials and methods

2.1 Presentation of the study area

The study region is the city of Ouezzane which belongs to the southern margins of the Jebala area whose major tribes bordering the city are: Masmouda, Rhouna, Ghzaoua and Beni Mesara. The Ouezzane region, in the North of Morocco, extends over an area of 1861.2 km², and has an altitude of 614 meters [10] (figure 1).

Figure 1: Presentation of the study area

Table 1: Origin and method of extraction of the 4 samples

N° Sample	The age of the tree	The region	Extraction mode
1	10 years	Ghanioua	Extracted by mechanical cold pressing 25 °C
2	20 years	Ghanioua	Extracted by mechanical cold pressing 25 °C
3	40 years	Ghanioua	Extracted by mechanical cold pressing 25 °C
4	40 years	Ghniuoua	Same sample 3 but extracted by mechanical hot press 50°C

2.2 Biological material

In the present work, 4 different samples were selected and collected through the mode of extraction from olive trees having different ages or coming from the same region of Ouezzane commune of Massmouda Douar Ghanioua (the city of Ouezzane north of Morocco) during the month of December 2019. Table 1 provides information on the origin, method of extraction and age of the olive tree from each sample of olive oil.

2.3 Preparation of different samples of olive oil

Only the mechanical pressing extraction method was used to extract olive oil. It was done in the Amal cooperative (olive oil extraction cooperative province of Ouezzane, Morocco) according to extraction methods already described [11], avoiding chemical and enzymatic reactions which could change its natural composition. The extraction method includes four main operations: cleaning the fruit (defoliation, washing the olives), preparing the dough (grinding, kneading), separation of the solid phase (pomace) and liquid phase (oil and water vegetation), separation of the liquid phase (oil and water from vegetation). Olive oil is prepared in two different ways: (a) samples 1,2,3: the olive oil is extracted by mechanical cold pressing at 25 °C (the exit temperature of the olive oil after extraction) and (b) sample 4: same olive fruit as sample 3 but the olive oil is extracted by mechanical hot pressing at 50 °C (the outlet temperature of the olive oil after extraction). These oils are then analyzed directly after extraction at the Official Laboratory of Analysis and Chemical Research of Casablanca (LOARC). The physico-chemical characteristics and the chemical composition of all the samples are determined (acidity index, peroxide index, cis and trans fatty acids, triglycerides and sterols). The oils are analyzed according to the methods of analysis already described in the international olive oil advisory literature [12].

2.4 Physico-chemical analyzes of oils

The acidity expressed as a percentage of oleic acid and the peroxide index were measured according to standardized methods, respectively the French standard [13], and the French standard [14]. The specific extinction coefficients in the ultraviolet at 232 nm and 270 nm (K232) and (K270). They are calculated respectively from the absorption at 232 and 270 nm according to the French method [15], using a VARIAN type spectrophotometer.

2.5 Analysis of cis fatty acids

The fatty acid composition was determined after transformation into methyl esters obtained by trans-esterification of the triglycerides with methanolic potassium hydroxide. The methyl esters of fatty acids in the samples of olive oils are obtained according to the French international standard method [16]. Then, these esters were analyzed by gas chromatography according to the conditions described in ISO 5508: 1990, using a VARIAN chromatograph with flame ionization detector (FID), equipped with a capillary column (CPWAX) 30 m long and 0.25 mm inside diameter. The oven temperature is set at 200 °C, and that of the injector at 220 °C. The carrier gas used is helium at 1.2 ml / min and the volume of the injection is 1 µl, leakage (split on) at ratio: 15%.

2.6 Triglyceride analysis

To 0.15 g of olive oil is added 0.5 ml of hexane and 15 ml of a mixture of hexane / diethyl ether (87/13). This solution is poured into a cartridge of supelco brand containing 0.5 g of silica gel previously activated with hexane. The fraction of triglycerides is thus separated from diglycerides and monoglycerides. It is collected in a 100 ml flask. It is subjected to analysis after evaporation of the solvent and dilution with 1.5 ml of acetone. The triglycerides are analyzed by HPLC on a column in the reverse phase of C18 (250 mm × 4.6 mm, Φ silica 5 µm), according to the IUPAC method N° 2.0 324. The HPLC device is equipped with an HP refractometric detector 10 47A. The elution is carried out with a mixture (acetonitrile / acetone) (v / v) with a flow rate of 0.5 ml / min during the analysis time (90 min).

2.7 Determination of the composition and nature of total sterols [18]

Weigh 2.5g of olive oil in a 20ml flask. 25 ml of a potassium hydroxide solution (1N ethanol) is added to it. The flask is heated to boiling under reflux for 30 min until the solution becomes clear. Then add 25 ml of distilled water to stop the reaction. The extraction of the unsaponifiable is carried out using 75 ml of hexane or petroleum ether. The organic phase undergoes a series of washing with 15 ml of mixture (water / ethanol 95°) (90/10) in a separatory funnel. The hexane phase is transferred from the top of the vial to a 100ml flask. After evaporation of the solvent using a rotary evaporator, the unsaponifiable material is recovered. The unsaponifiable matter, diluted with 300 µl of hexane or petroleum ether, is filtered. The unsaponifiable is obtained according to standard NFT 50-205. It is fractionated by high performance liquid chromatography (HPLC) on a silica column (25cm × 4 mm). The HP device is equipped with a 205 nm-254 nm UV detector. The eluent is an isooctane / isopropanol mixture (99/1), the flow rate of which is 1.2 ml / min. The duration of the analysis is 15 min, and the sterol fraction recovered according to standard NF 12228 May 1999, is evaporated to dryness. Sterols are transformed into silylated derivatives (TMS) using a mixture of pyridine, hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS), (9/1/1), (v / v / v). The pyridine evaporates to dryness and the silylated derivative is diluted with 60 µl of heptane or hexane. TMS sterols are analyzed by gas chromatography (CPG) on an apolar column (Chroma pack) (30m × 0.32mm, ID: 0.25 µm, phase: CPSIL8CB). The HP Hewlett packard 6890 series GC system chromatograph is equipped with a FID detector (T °: 300 °C). The carrier gas is nitrogen and its flow rate is 1ml / min (P.E: 8.6 bar). The analysis is carried out in temperature programming (200 °C up to 270 °C with a speed of 10 °C / min and an isotherm at 270 °C for 35 min).

3 Results and Discussion

3.1 The yield of the extraction

From these results, since the temperature of the extraction (50 °C) increases it is quite possible that the yield of the extraction of the olive oil is higher. Consequently we found a difference from 5 to 6 liters of olive oil in each 100 kg of olive fruit if the extraction has been hot.

Table 2: The yield of olive oil as a function of the temperature of the extraction

N° Sample	1	2	3	4
Oil yield in 100kg / liter	15	14	14	20

3.2 Analysis of the physico-chemical characteristics

All the acidity values observed are less than 1%. This result shows that virgin olive oil is characterized by low acidity (acidity of olive oil must be less than or equal to 2%) [19]. The acidity results show that the acidity of four samples is higher (0.93%) than the acidity of sample 3 (0.7%) (3 and 4 same sample but extracted at two different temperatures). These results suggest that the extraction temperature may influence the acidity values of olive oil. The temperature therefore appears as a parameter influencing the acidity value of olive oil. Indeed, the acidity value of the oil samples prepared from mechanical cold pressing is uniformly lower than those of oils prepared from mechanical hot pressing.

The results of the peroxide index of the four samples of olive oil show that the samples have a peroxide index of less than 10 meq O2 / kg (peroxide index of olive oil ≤ 20 meq O2 / kg) (COI / T.20 / DOC. No 42-2 / Rev.2 - 2017). It is quite likely that the value of the peroxide index observed in our four samples is lower. This is related to the freshness of the oil. In fact, it is extracted directly after the recovery of the olive fruit and analyzed after its extraction (the analyzes were made after 15 days). The result of the peroxide index shows that the peroxide index of sample 4 is higher. Indeed, this sample is extracted by mechanical pressing at a higher temperature (50 °C). This result clearly indicates that some components of olive oil are extremely sensitive to oxidation [20]. The high peroxide content is observed for sample 4. This is linked to the extraction temperature. Our result is consistent with the literature which indicates that extraction methods, geographic origin and climatic factors influence the chemical characteristics of oils. In a study carried out in Italy and taking into account two different methods of storing olive oil, it was established that the peroxide index which represents one of the quality parameters of olive oil increased rapidly and above tolerated threshold according to Torres and Maestri [21]. On the other hand, Kiritsakis [22], considers that the place of culture has no significant influence on these analytical parameters (acidity, UV absorbances and peroxide index). This author points out that the deterioration in the quality of olive oil is rather fundamentally affected by factors damaging the fruit such as attack by parasites (flies) or the use of improper systems of harvesting as well as transport and storage of olives. Table 3 shows the results of the acidity value and the peroxide index of 4 samples.

Table 3: Results of acidity and peroxide index

N° sample	Acidity	Peroxide index meq O_2 / kg
1	0.84%	8
2	0.90%	10
3	0.7%	4
4	0.93%	10

3.3 Determination of absorbance in ultraviolet

The specific extinction of olive oil was determined at 247 nm, 270 nm and 266 nm. In general, the values found vary between 0.1410 to and 0.1650 to 270 nm. The specific extinction of all the samples is lower (lower than 0.1757). It is a virgin olive oil prepared cleanly from olive fruit. We found that the specific extinction values of sample 4 prepared from olive fruit by mechanical hot pressing have values higher than sample 3 (same batch). From this result, it was concluded that extraction by mechanical hot pressing can increase the specific extinction values. This result clearly shows that there is a formation of carbon-carbon bonds or carbon-oxygen bonds in the form of secondary auto-oxidation products during hot extraction. All of these compounds cause an increase in absorption in the region between 225 nm and 325

nm. The result of the absorbance in the ultraviolet is collated in table 4.

3.4 Analysis of cis fatty acids

The fatty acid composition of the different oils was determined after methylation of the oil and analysis of the methyl esters by gas chromatography on a capillary column [23, 24]. Table 4 groups together the results obtained for the four samples. he fatty acid composition of olive oil corroborates with data from the standards (COI / T.20 / DOC. No 42-2 / Rev.2 - 2017). Olive oil contains 86% unsaturated fatty acids. It is of the oleic – linoleic type and contains 13% of essential fatty acids: linoleic acid (11 to 13%) (Vitamin F). This acid is said to be essential because it cannot be synthesized by the body and must be provided by food. Unsaturated fatty acids play an essential role in the prevention of cardiovascular diseases, and the omega 6 family (such as linoleic acid) is vital for the growth of children [25]. Olive oil is rich in oleic acid C18:1 Its oleic acid content makes olive oil particularly interesting in regulating cholesterol. Studies in progress seem to show that ingesting 2 tablespoons a day of olive oil for a month could significantly lower blood cholesterol levels. The other fatty acids present are: palmitic C16: 0 (9 to 10%) and stearic C 18: 0 (2.5 to 3%). The percentage of linolenic acid (C18: 3) in olive oil does not exceed 1%. The presence in olive oil of long chain fatty acids such as C20: 0 (0.3%), C20: 1 (0.3%) is noted. Comparing samples 3 and 4 (same sample but the extraction temperature is different) we found that the percentage of stearic acid (C18: 0) and linoleic acid (C18: 2) is reduced because of hot extraction. This is probably due to a deterioration of the fatty acid during the hot extraction. These variations can be considered as useful markers to ensure the extraction method. From this study we noticed that the percentage of linoleic acid (C18: 2) is increased with the increase in the age of the olive tree (samples 1, 2 and 3). The variation in fatty acid resulting from our samples is consistent with studies which indicate that the percentage of oleic acid may influence the climate [26].

3.5 Analysis of trans fatty acids

The trans fatty acid composition of the different oil samples was determined after methylation of the oil and analysis of the methyl esters by gas chromatography. Table 6 groups together the results obtained for the four samples. It appears from this result that the percentage of trans oleic, linoleic and linoleinic acid (C18: 1, C18: 2 and C18: 3), (elaidic acid) in virgin olive oil is very low and does not give any information on extraction methods or age. The presence of trans fatty acids in "virgin" olive oils, suitable for consumption, is an indication of the fraudulent presence of refined oil. For this reason, the content of trans fatty acids has been limited by the standard to 0.05% [27] both for elaidic acid and for the sum of the trans isomers of linoleic acids and linolenic.

3.6 Triglyceride analysis

The triglycerides of the different olive oil samples analyzed by high performance liquid chromatography are grouped in Table 7

Analysis of the triglyceride fraction of olive oil by HPLC allowed the separation of the individual triglycerides. The predominance of OOO triglycerides is noted (41.2% to 45.7%). POO (19.3 to 20.6%), OLO (18.9 to 20.3%), LOL (4%), PLO (5.5 to 7.1%). It should also be noted that the majority of the oleic and linoleic acids occupy the Sn-2 position. Our results are in agreement with data from the literature [26, 28] which indicate that the triglycerides OOO, POO, OLO, LOL, PLO are predominant in olive oil. Triglyceride results show that extraction temperature can influence the compositions of the triglycerides. We have also found that the extraction by mechanical hot pressing decreases the percentages of triglycerides such as LLL, LOL, OLO and PLO. Analysis of the triglyceride fraction shows that the age parameter of the olive tree decreases the percentage of triglycerides LLL and OOO. Our results are in agreement with data from the literature [29].

3.7 Analysis of sterols

The sterol composition of the different olive oil samples was determined by gas chromatography after silylation of the sterol fraction. The latter is obtained by fractionating the unsaponifiable of olive oil by HP on a normal phase. The various sterols encountered were identified by gas chromatography. Table 8 summarizes the results obtained for the 4 selected samples.

Table 4: Determination of absorbance in ultraviolet

N° sample	absorbance in 247 nm	absorbance in 270 nm	absorbance in 266 nm	Extinction
1	0.1529	0.1650	0.1757	0.0007
2	0.1392	0.1513	0.1619	0.0008
3	0.1290	0.1410	0.1544	0.0007
4	0.1435	0.1554	0.1669	0.0002

Table 5: The results of cis guys-acids depending on the temperature of the extraction and the age of the tree

N° sample/ fatty acid	Retention time	1	2	3	4
C16:0	11.284	8.92	10.08	9.32	9.16
C16:1	11.932	0.58	0.80	0 .60	0.58
C17:0	12.709	0.04	0.04	0.05	0.03
C17:1	13.332	0.04	0.05	0.04	0.05
C18:0	14.192	2.65	2.78	3.02	2.56
C18:1	14.886	73.94	71.40	72.17	74.83
C18:2	15.797	12.23	13.23	13.04	11.20
C18:3	17.116	0.84	0.88	0.97	0.86
C20:0	17.324	0.32	0.33	0.33	0.32
C21:1	18.092	0.39	0.36	0.36	0.37

Table 6: the result of the trans fatty acid composition according to the temperature of the extraction and the age of the tree

N° sample	1	2	3	4
%C18:1trans (TR13.03min)	0.03%	0.02%	0.03%	0.03%
%C 1%C18:2trans+%C18:3trans (TR13.96min)	0.02%	0.00%	0.02%	0.02%

Table 7: The triglyceride composition according to the temperature of the extraction and the age of the tree

Sample/TG	TR min	Sample 1	Sample 2	Sample 3	Sample 4
LLL	32.57	0.71	0.52	0.53	0.27
OLL	36.34	0.11	0.52	0.57	0.1
LOL	44.71	4.27	4.54	4.41	3.53
OLO	47.55	-	1.38	1.62	1.69
PLO	49.59	0.45	-	-	0.60
OLO	59.41	19.83	20.34	19.64	18.89
PLO	66.44	6.13	7.13	6.61	5.53
PLP	74.22	0.56	0.82	1.08	0.37
OOO	81.67	44.26	41.18	42.10	45.69
POO	92.60	19.33	20.57	19.88	20.39
POP	106.49	1.63	1.67	0.94	2.22

(P: palmitic acid, L:linoleic acid, O: oleic acid)

Table 8: Composition in sterols of the 4 samples.

N° Samples/ Sterols	TR min	Sample 1	Sample 2	Sample 3	Sample 4
Cholesterol	30.23	0.12	0.12	0.15	0.18
Campesterol	33.32	2.89	2.73	2.85	2.89
Stigmasterol	34.33	2.34	2.45	1.64	2.22
Delta-7-stigmasterol	35.79	1.01	1.05	1.11	1.03
β-sitosterol	36.57	81.80	83.10	82.56	82.75
Delta-5-avenasterol	37.04	10.92	9.73	10.70	10.10
Delta-5-stigmasterol-stadineol- Tosterol-	38.64	0.64	0.61	0.62	0.60

The sterolic composition is in accordance with the data in the literature [30]. These are essentially β-sitosterol. The main products are β-sitosterol and Delta-5-avenasterol. Their proportion varies respectively between 81.80% and 83.10%, and from 9.73 and 10.92%. The content of campesterol found in olive oil varies between 2.7% and 2.9%. The result shows that the percentage of cholesterol varies from 0.12% to 0.18%. This value is consistent with the standards which indicates that the percentage of cholesterol in virgin olive oil must be less than 0.5%. The result of the sterols shows that the percentage of stigmasterol is higher in sample 4 which is extracted by mechanical hot pressing. In spite of these results, we have not found a significant variation on the sterol composition of the different samples according to the temperature of the extraction and age of olive trees.

4 Conclusion

As part of the promotion of olive oil, we conducted a comparative study of the different physico-chemical parameters of olive oil as a function of the extraction temperature and its olive age. Our study shows that the extraction method appears to be a parameter influencing the acidity value and the index of peroxide in olive oil. Indeed, the acidity values and the peroxide index are higher in sample 4 which was prepared from mechanical hot pressing. This result suggests that the temperature of the extraction may influence the acidity values and the peroxide index. The analysis of fatty acids and sterol composition are in accordance with data from the literature. The main products are β-sitosterol (83%) and Δ-5-avenasterol (10%). The results related to the chemical compositions show that the temperature of the extraction by mechanical hot pressing can influence the percentage of sterol (cholesterol), the percentage of fatty acid and the percentages of triglycerides such as: OOO, POO, LLL, LOL, OLO, PLO. These results agree with those reported in the literature. This shows that the extraction method influences the dietary qualities of olive oil. On the other hand, the results of the chemical composition according to Olivier's age do not give any significant variation between the results obtained.

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Ethical issue

Authors are aware of, and comply with, best practice in publication ethics specifically with regard to authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests and compliance with policies on research ethics. Authors adhere to publication requirements that submitted work is original and has not been published elsewhere in any language.

Competing interests

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Authors' contribution

All authors of this study have a complete contribution for data collection, data analyses and manuscript writing

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