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Chemical Characterization and oxidative stability of two monovarietal virgin olive oils (Moroccan Picholine and Arbequina) grown in Morocco

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Abstract
The aim of this work was to study the changes in the physico-chemical composition and oxidative stability of two different virgin olive oil cultivar "Arbequina and Moroccan Picholine" after 9 weeks of storage at 60 °C. Quality parameters periodically monitored over the study were peroxide value, acid value, UV absorption (E232 and E270) tocopherol and fatty acid composition. Results showed that after the storage period, the peroxide value, acidity and UV absorption increase, the important losses in α-tocopherol content of oils occur and significant decreases were observed in the oxidative stability, while the composition of the fatty acids was the more stable compounds. Comparing the both cultivars, the Moroccan Picholine seems to be more stable than the Arbequina.

Keywords: Olive oil varieties; Quality index; Fatty acid; Tocopherol; Oxidative stability; Rancimat

1. Introduction
Olive oil is extensively consumed due to its nutritional value and its organoleptic characteristics. Besides, mention should also be made of its use in medicine, recommended already in ancient times and today for the prevention of cardiovascular disease and for its anti-oxidative capacity and its fatty acid composition [1-2]. Moreover, it is widely known, that the quality of virgin olive oil (VOO) is influenced by genotype (olive cultivar) and various agronomic factors such as climatic conditions, degree of maturation and agronomic practices related to irrigation treatment [3-4]. In fact, olive (*Olea europaea L.*) is one of the most important fruit crops throughout the Mediterranean Basin [4]. In Morocco, Agriculture is one of the main stays economies, and cultivation of the olive tree constitutes one of the principal economical and agricultural sectors.
The extension of the olive grove is currently experiencing a renewed interest from both government and private sectors [5]. The demand on virgin olive oil and its growing popularity among consumers, on the one hand, and the specific strengths of Morocco make it a potential exporter on the other hand, are at the origin of this increased interest. To support the development of national olive oil sector, many efforts have been made in improving varietal olive gene pool to diversify the varieties grown, consisting of up to 90% by the variety population Moroccan Picholine [6].
Despite its adaptability and its dual purpose (production of olive oil and canned olives), the variety population has some drawbacks, notably its heterogeneity, its average productivity and alternating and low oil olive (18-22%) against 26-30% for oil cultivar [6]. Structure varietal olive grove in Morocco is characterized by the predominance of the Moroccan Picholine, which represents more than 90% of the national heritage and is dual purpose [6-7]. The rest consists of several varieties, especially Languedoc Picholine, Meslalla, Gordal, and Manzanille Ascolana Dura, located in irrigated areas (AL Haouz, Tadla and El Kelaâ) and some Spanish and Italian varieties (Picual, Frantoio, Hojiblanca ...) grown in the northern area (including Chefchaouen and Tetouan). In recent years, growers have planted large areas of Arbequina which is a variety of low vigor to high densities [7]. The production of this small fruit variety is strictly for oil extraction [7]. The choice of plant cultivar was based on some orchards tracking behavior, but the data are mainly agronomic traits such as age of entry into production, productivity, oil content, the regular production. As a result, the chemical quality and nutritional oils from such varieties, planted in a different context, are partially unknown in particular; their oxidative stability. To the best of our knowledge, shelf life of Moroccan Picholine and Arbequina oils has only

been shallowly comparatively evaluated [6,7] . The aim of this work was to investigate the behavior of three introduced olive cultivars: Arbequina, by comparing the chemical composition and oxidative stability of their oils to those obtained from Picholine planted in Morocco.

2. Materials and methods

2.1. Materials:

Olive fruits from Arbequina, and Picholine (grown in Morocco) cultivars. Monovarietal extra VOOs were from arbequina, and were acquired from local whole salers Zitoun Al Atlas (Casablanca, Morocco). Oil extraction was performed using a Continuous three-phase centrifuge.

2.2. Quality parameter

Oils were analysed at the time of purchase and after 9 weeks of storage at 60° in oven. Acidity index, peroxide value (PV), and extinction coefficients (K270) determination were carried out following the analytical methods described in the Regulations EEC/2568/91 of the European Union Commission (1991). Acidity was expressed as the amount of oleic acid as %. PV was expressed as milliequivalents of active oxygen per kilogram of oil (mEq. O2/kg oil), and extinction coefficient K270 was expressed as the specific extinctions of a 1% (w/v) solution of oil in 2, 2, 4-trimethylpentane in 1 cm cellpath length. For the fatty acid composition determination, the methyl esters were analyzed on a CP-Wax 52CB column (30m x 0.25 mm i.d.) using helium (flow rate 1mL/mn) as a carrior gas. Initial oven temperature was set at 170°C; injector temperature 200°C; detector temperature 230°C. Injected quantity was 1uL for each analysis. Sterol composition was determined after 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 using helium (flow rate 1.6 mL/mn) as carrier gas. Column temperature was isothermal at 270 °C, injector and detector temperature was 300 °C. Injected quantity was 1uL for each analysis. Data were processed using Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA). Tocopherols were analyzed by HPLC using Shimadzu CR8A instruments (Champ sur Marne, France) equipped with a C18-Varian column (25 cm×4 mm; Varian Inc., Middelburg, The Netherlands). Detection was performed using a fluorescence detector (excitation wavelength 290 nm, detection wavelength 330 nm). Eluent used was a 99:1 isooctane/isopropanol (V/V) mixture, flow rate of 1.2 ml/min.

2.2. Rancimat test

The oxidative stability of each sample was determined as the induction period (IP, h) recorded by a 743 Rancimat (Metrohm, Switzerland) apparatus using 3 g of oil sample. Samples placed into Rancimat standard tubes were subjected to the normal operation of the test by heating at 110°C with an air flow of 20 L/h.

2.3. 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 the Picholine and Arbequina olive oil

3.1.1. Quality indices

Oil quality is defined as the set of chemical, physical and sensory, to classify virgin olive oil into different categories according to the definitions and standards adopted by the commercial International Olive Oil Council (IOC) [9]. From a regulatory perspective, the IOC 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 (E232 and E270). The acidity of oil is evaluated by the amount of free fatty acids, expressed as grams of oleic acid per 100 g of oil. It appeared as a simple and effective means for quantitative assessment and classification by commercial grade olive oil [10]. Generally if the oil is extracted from fresh healthy fruits and according to best practices crushing, oil has a very low acidity [10-11]. However, during storage, the oil may deteriorate and its acidity increases due to the release of fatty acids by hydrolysis of triglycerides. Based on this index, the both varieties are analyzed and seem to be classified in the category of "extra virgin oil" as their free fatty acid content is below 0.8% [9].

Thus for both cultivar studied, the lowest initial acidity is detected for the Picholine is 0.62% and for Arbiquina the acidity was 0.8% (Table-1). The variation of free acidity between samples analyzed can be attributed to technological practices in the process of crushing and also to the residence time before crushing olives [11]. The

second criterion of the quality measuring is the peroxide value. This index is used to evaluate the oxidation state of oil during storage and must not exceed 20 Meq (O2)/kg for all categories of olive oil [9]. The initial values of the peroxide value of olive oils were found well below the limit agreed by the standards [9]. The initial values of the peroxide value of the oils is 3.2 Meq (O_2)/Kg for the Arbiquina cultivar and 2.1 Meq (O_2)/kg for the Picholine cultivar (Table-1). Measuring the peroxide value could be verified by determining the UV absorbance at 232nm, correlated with the presence of conjugated dienes forms that appear on fatty acids having at least two double bonds [12-13]. Based on our findings, we detected the same variations than the peroxide value. The absorbance E232 showed low values for both oils ranging from 1.71 to 2.1 without exceeding the limit (2.5) required by the standard [9]. For extinction E270 which provides information on the degree of formation of secondary products of oxidation, showed low values for both oils, (0.13 and 0.16; respectively Arebiquina and Picholine).

Table 1. Physicochemical parameters of virgin Picholine and Arebiquina oils

	Norm IOC [8]	Picholine oil	Arebiquina oil
Acidity (%)	Extra virgin < 0.8	0.62 ± 0,01	0.8 ± 0.02
PV (MeqO2/Kg)	<20	3.2 ± 0.5	2.1 ± 0.5
E232	<2.5	2.10 ± 0.01	1.71 ± 0.01
E270	<0.22	0.13 ± 0.01	0.16 ± 0.01
Palmitic acid C16:0	7.5 – 20	9.2 ± 0.1	14.3 ± 0.1
Stearic acid C18:0	0.5 – 5	2.9 ± 0.1	2 ± 0.1
Oleic acid C18:1	55 – 83	74.6 ± 0.1	67.1 ± 0.1
Linoleic acid C18:2	3.5 – 21	10.7 ± 0.1	13.2 ± 0.1
Linolenic acid C18:3	<1	0.9 ± 0.1	0.8 ± 0.1
SFA[a](mg/100 mg)	-	12.4± 0.1	16.6± 0.1
UFA[a](mg/100 mg)	-	86.8± 0.1	81.4± 0.1
Campesterol	<4	2.7 ± 0.2	3.1 ± 0.3
Stigmasterol	<Campesterol	1.7 ± 0.1	1.9 ± 0.2
Beta–sterol (Other sterols)	>93	93.8 ± 0.5	94.8 ± 0.5
7 Stigmastenol	<0.5	0.2 ± 0.1	0.3 ± 0.1
7 Avenasterol	-	0.1± 0.1	-
Tocopherol (mg/kg)	-	202 ± 21	182 ± 30
α-Tocopherol	-	166.3 ± 5	167 ± 5
β-Tocopherol	-	11.7 ± 3	10.5 ± 2.5
γ-Tocopherol	-	1.9 ± 0.3	2.3 ± 0.3
δ-Tocopherol	-	21.9 ± 6	20.1 ± 6

[a] SFA: saturated fatty acids, UFA: unsaturated fatty acids.

3.1.2. Fatty acid composition

Fatty acid composition of evaluated in the two oils, was also found to be satisfactory in terms of International Olive Council-imposed rules (Table-1). As expected, a larger range of values was observed between Picholine and Arebiquina oils. Particularly for palmitic acid content went from 9.2% (Picholine oil) to 14.3% (Arbequina oil), oleic acid: from 67.1% (Arbequina oil) to 74.6% (picholine oil), and linoleic acid: from 10.7 (Picholine oil) to 13.2% (Arbequina oil). Linolenic acid is a minority, according to the standard [9], its concentration must be less than 1%. Its content increased from 0.8% to 0.9% for both varieties. The minimum content of linolenic acid can be used to detect adulteration of olive oil with other oils rich in linolenic acid such as rapeseed oil and soybean oil[14]. Accordingly the unsaturated fatty acids/saturated fatty acids ratio went from 4.9 for arbequina oil to 7 for picholine oil.

3.1.3. Sterol composition

Sterols are important constituents in vegetable oils and they are widely used to verify the authenticity of vegetable oils [15]. This study showed that the composition of sterols in olive oils, contrary to the literature [16], which states that the cultivar of olive oil influences the proportion of sterols, we have no record of substantial differences in the proportions of sterols between the cultivars of our samples. According to our results, the olive oils studied are characterized by a high content of β-Sitosterol more than 93% in both varieties

(Table-1). This is in agreement with other results already reported in the literature [16-17].

3.1.4. Tocopherols composition

Tocopherols are important molecules to analyze because of their vitamin, nutrition and their role in intercepting free radicals [18-19]. The α-tocopherol is the major element in the olive oil. Analysis of tocopherols showed that the cultivar of olive oil has no significant variations were observed in the both oils tocopherol content (Table-1). Indeed, our results show that α-tocopherol is not influenced by the two cultivars (Arebiquina and Picholine 167mg/kg). For its part the δ-tocopherol, the γ-tocopherol and the β-tocopherol are not also influenced by the cultivars.

3.1.5. Rancimat

To get a complete picture of both olive oils oxidative stability, we decided to determine the induction period by Rancimat test, an instrument for automatic determination of the oxidation stability of oils and fats. During the oxidation process, volatile acids were formed in the distilled water and were measured conductimetrically [18]. The induction period was defined as the necessary time to reach the inflection point of the conductivity curve. The results show that the olive oil cultivar has clearly influenced the stability of the oil. Picholine cultivar exceeds 43.6 h at 100°C. At de same temperature, we found the Rancimat induction time of Arbiquina oil to be only 25.1 hours. To confirm this result, we also determined the induction time of our samples at 110, 120, 130, and 140°C. Independently of the temperature, a much shorter induction time was consistently observed for Arebiquina oil, compared with the Picholine oil (Table-2).These results are in agreement with the results of some varieties of olive oil [20-21].

Table 2. Rancimat induction periods [h] of virgin Picholine and Arebiquina oil at various temperatures.

Temperature [°C]	Picholine oil	Arebiquina oil
100	43.6 ± 1	25.1 ± 1
110	21.3 ± 1	11.9 ± 1.5
120	9.7 ± 1.5	5.9 ± 1
130	4.8 ± 1	3.1 ± 1
140	1.9 ± 1	1.4 ± 0.5

3.2. Changes in physico-chemical properties of stored oils

We evaluated the oxidative stability during 9 weeks of storage at 60 °C. The physico-chemical parameters were regularly monitored during this period. Acidity, peroxide value, E232 and E270 were followed on a weekly basis while the Rancimat "induction time" were monitored every three weeks whereas the fatty acids and the composition of tocopherols were analyzed at the initial and after 9 weeks.

3.2.1. Changes in free fatty acids

Acidity index evolution is shown in Figure-1. The acidity of the Picholine which was originally slightly lower (0.62%) compared to that of the Arbequina, it is relatively stable, it reach 0.94 after 9 weeks (51% increase). The latter lost the extra virgin olive oil labeling after only 7 weeks of storage (Figure-1). Arbequina oil with acidity recorded initial upper limit of 0.8% allowed for labeling extra virgin. After 9 weeks, it reached the highest acidity 1.92%, that is to mean an increase of 140%, through two regimes, a fast one for two weeks to reach the value of 1.6 with an average change of 0.4% unit per week and a second one 7 weeks from 1.6 to 1.9% with an average 10 times less than that of the first plan. These results are also similar to those reported by [21-22]. The hydrolysis of triglycerides is favored at 60 °C, which results in the increase of free fatty acids in both oils studied. However, this increase appears more in the Arbiquina cultivar.

3.2.2. Changes in peroxide value

Oxidation, and the formation of peroxides, occurs during oil extraction and processing and can continue after bottling and during storage. Peroxides are intermediate oxidation products of oil which lead to the formation of a complex mixture of volatile compounds such as aldehydes, ketones, Hydrocarbons, alcohols and esters responsible for the deterioration of organoleptic proprieties [23]. Therefore, their formation dramatically impacts oil shelf life and consumer acceptance. High temperature and light are two well-known factors generally promoting peroxide formation [24-25]. The first observation of the Figure-2 is that both oils have undergone an evolution of the peroxide (the hydro peroxides) during storage at 60 °C. This trend is stronger for

the Arbequina oil which recorded values ranging from 3.2 to 62.1 Meq (O_2)/kg after 9 weeks of storage. The latter has lost its quality of extra virgin olive oil after only 3 weeks of storage. In fact, the largest increase of the peroxide value in Arbiquina cultivar explains the low stability of this oil; this confirms our results obtained by the Rancimat test and acidity, which is consistent with previous results [26].

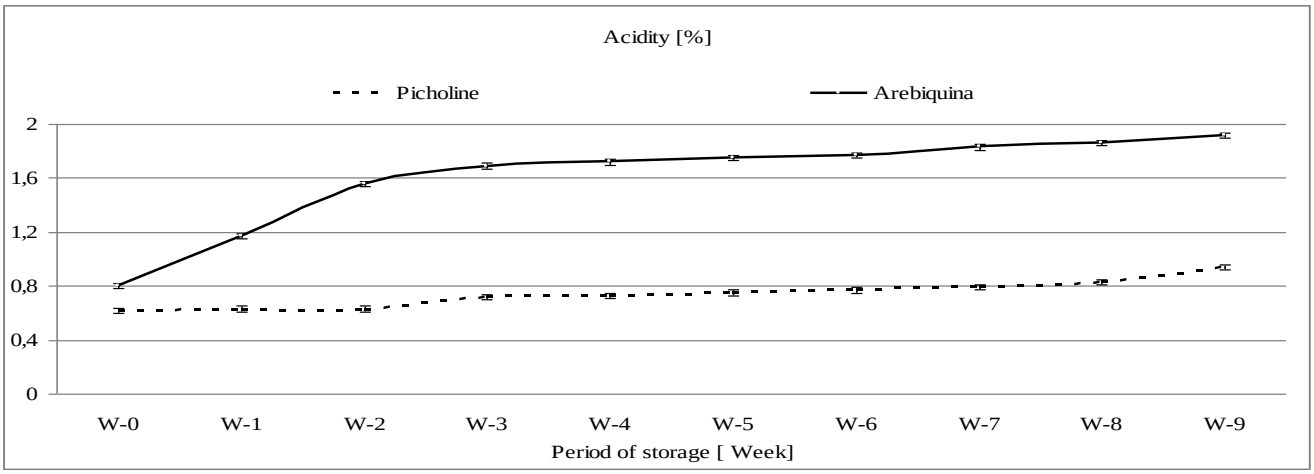

Figure 1. Changes in the acidity of the oils stored at 60 °C.

For the other cultivar (Picholine) it suffered a less intense compared to that of the Arbiquina, from 2.1 to 33.1 Meq (O_2)/kg, thus losing labeling of extra virgin olive oil after 5 to 6 weeks of storage. This period is twice as large as the one we observed for oil Arbiquina cultivar, which is also confirmed by the Rancimat test. In fact, the formation of hydro peroxides after 9 weeks of storage is more important in Arbiquina oil because it is twice higher than in Picholine oil.

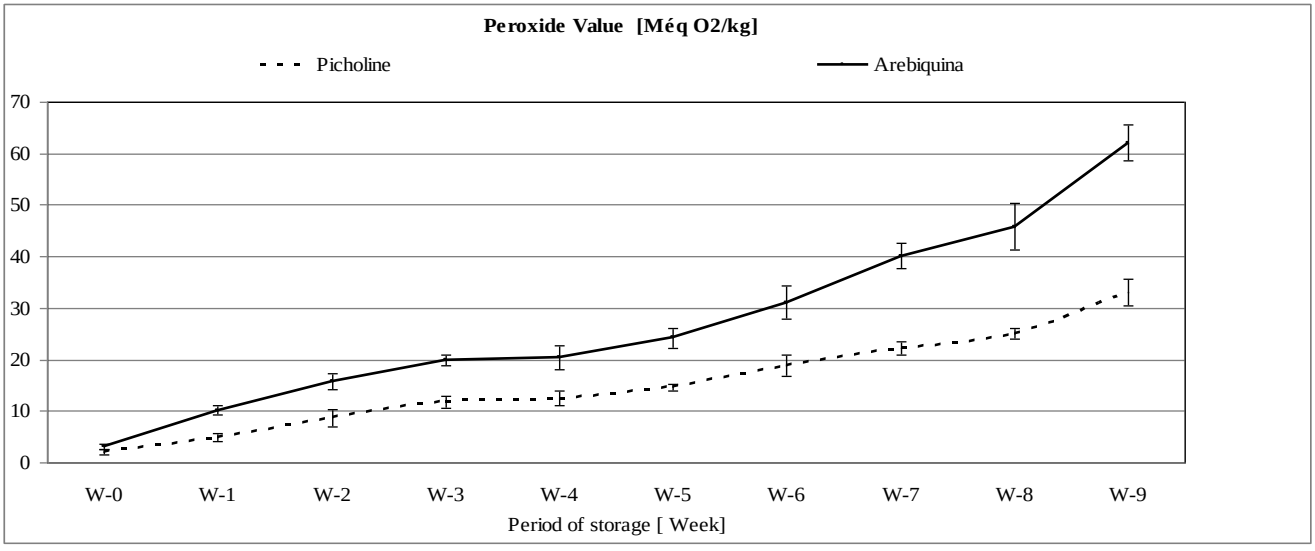

Figure 2. Evolution of the peroxide value of oils stored at 60 °C.

3.2.3. Changes in K270 and K232 parameters

- K232 analysis

All oils contain unsaturated fatty acids greater or lesser extent. The oxidation of fatty acids leads to the formation of hydro peroxides (the primary oxidation products) that absorb light in the vicinity of 232nm (E232), while changes in the specific absorption at 232nm (E232) are useful for evaluate the formation of primary oxidation products [27]. Reading the Figure-3, which shows the evolution of the absorption of our samples by the time of storage at 60 °C showed that both oils have undergone a change in content of hydro peroxides, but in different ways. Generally for both oils studied, they were increased at E232 from 76 to 98%. After 9 weeks of storage at 60 °C, the absorption at 232 increased from 2.1 to 3.71 for Arbiquina and from 1.71 to 3.39 for Picholine.

- K270 analysis

After the formation of hydro peroxides in the early stages of the oxidation products are unstable, they are rapidly converted to secondary oxidation products especially unsaturated ketones and diketones that absorb light in the vicinity of 270 [28-29]. Specific absorption 270 nm (K270) is a marker for the formation of secondary oxidation products [29]. Thus, we can say that the specific absorption (E232 and E270) is an image of the oxidation state of oil. More the absorption E232, the higher the oil is peroxidized and more the absorption E270 the higher the oil is rich in secondary oxidation products [30]. From the results shown in Figure-4, the increased absorption E270 is between 244% to Picholine. The largest increase was recorded in Arbequina oil (400%). Indeed, the rate of secondary oxidation products increased from (0.13 to 0.65) for Arbiquina oil and (0.16 to 0.55) for Picholine oil. According to the results of the absorption E270, 2 to 3 weeks at 60 ° C was sufficient for the two varieties to cross the limit of 0.22 for the extra virgin olive oil allowed by the standard [31]. From these results it can be concluded that the formation of primary and secondary products of oxidation appears to be much lower in Arbiquina than in Picholine [31].

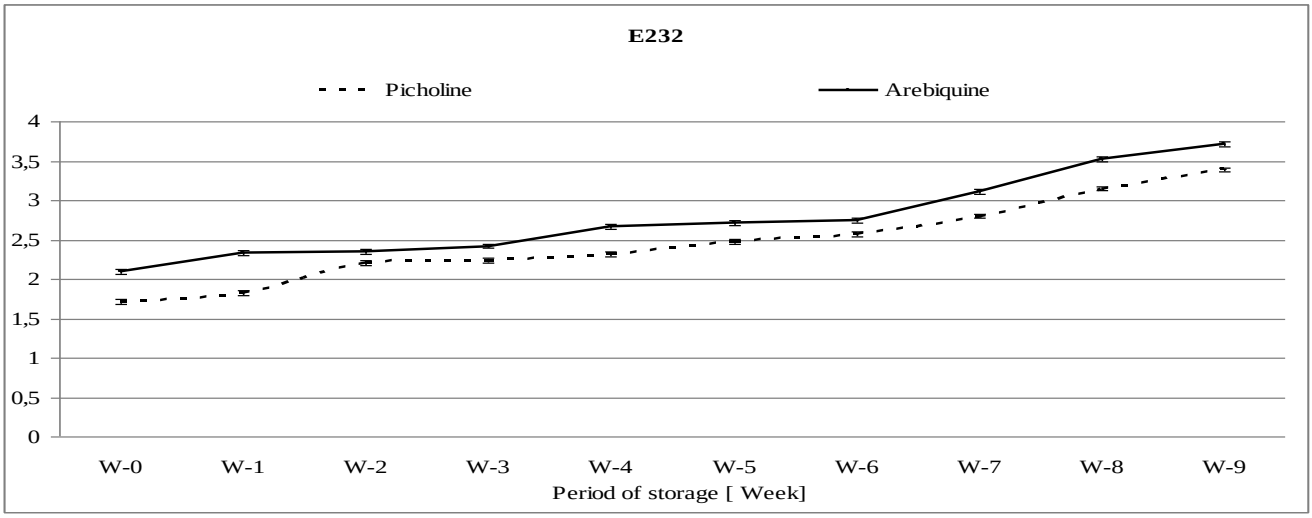

Figure 3. Evolution of the absorption E232 of the oils stored at 60 ° C.

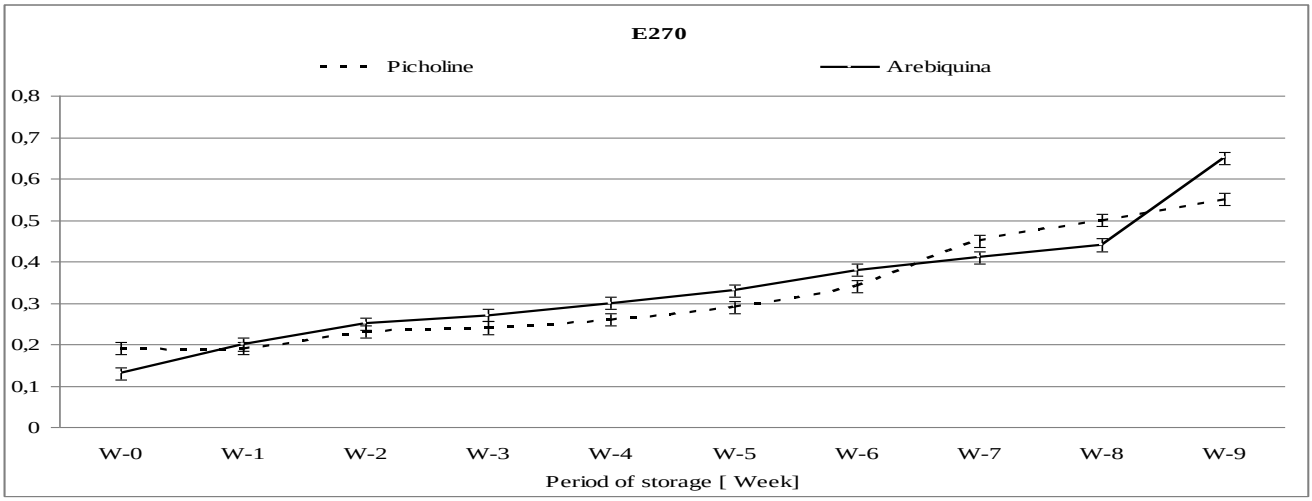

Figure-4. Evolution of the absorption E270 of the oils stored at 60 ° C.

3.2.4. Rancimat study

Resistance to the oil oxidation accelerated by Rancimat, shows that all the oils studied have experienced a decrease in time resistance Rancimat test (Figure-5). This can be explained by the degradation of antioxidants at 60 °C, but this degradation varies from oil to another. Indeed, the decrease is more intense for the Arbequina which was already very low compared to Picholine at the beginning of our study, after 9 weeks of storage, the oil resisting was only 1.4 h this represents a decrease of 88%. This sharp decrease confirms our results on the evolution of the peroxide value, acidity and extinctions E232 and E270 [31]. For the Picholine the Rancimat induction period remained close to 5 hours after 9 weeks.

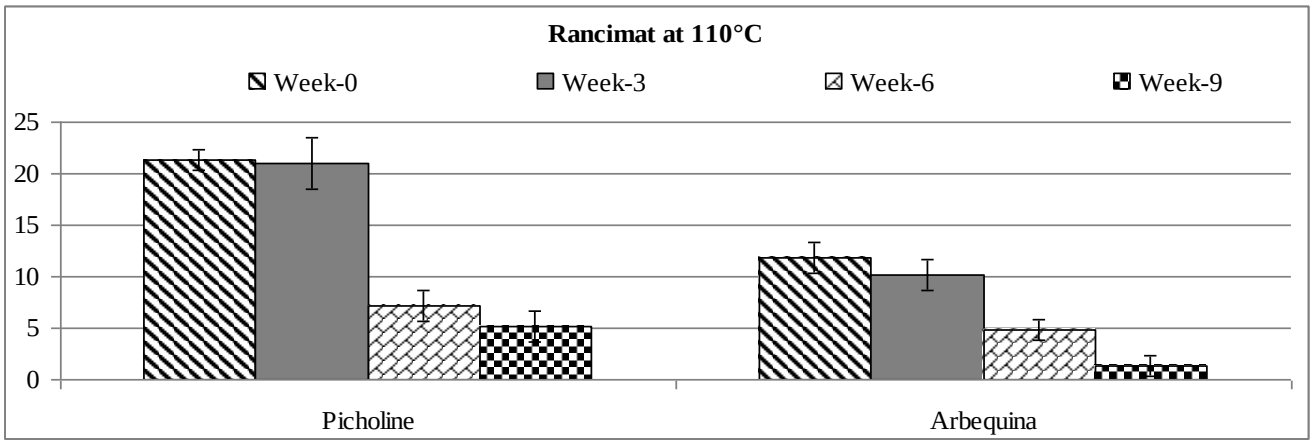

Figure 5. Evolution of the induction time (Rancimat) during storage at 60 °C**.**

3.2.5. Changes in Fatty acids composition

Table-3 summarizes the results of the degradation of fatty acids throughout the storage period. It is clear from these results that the storage for 9 weeks at 60 °C does not have a great influence on the fatty acid composition of the two varieties. Slight changes in the composition of fatty acids in the Arbequina was detected, these changes are as follows: a decrease was observed in the unsaturated fatty acid type linolenique C18: 3 during the storage period from 0.8% to 0.3% in the same finding was observed for the same oil linoleic acid C18: 2 it decreased from 12.7% to 10.2%, but these decreases are acceptable and still in the standards [31], at the end of our study. It can be said that storage at 60 °C during 9 weeks did not influence clearly the composition of fatty acids in the both cultivar.

Table 3. Fatty acid composition (initial and final) in Picholine and Arebiquina oil samples stored for 9 weeks at 60°C.

	Fatty Acid Composition			
	Picholine		Arebiquina	
	Initial	After storage	Initial	After storage
Palmitic acid C16:0	9.2 ± 0.1	9.8 ± 0.5	14.3 ± 0.1	15.9 ± 1
Stearic acid C18:0	2.9 ± 0.1	2.9±0.5	2 ± 0.1	2.1 ± 0.5
Oleic acid C18:1	74.6 ± 0.1	74.9 ± 1.5	67.1 ± 0.1	68.7 ± 1.5
Linoleic acid C18:2	10.7 ± 0.1	10.2 ± 1	12.7 ± 0.1	10.2 ± 1
Linolenic acid C18:3	0.9 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.3 ± 0.1

3.2.6. Changes in tocopherols composition

The most intense degradation was recorded in Arbequina cultivar. Indeed, the concentration of tocopherols decreased by 58,5% after 9 weeks of storage at 60 °C from 182 to 75.5 mg/kg (Table-4). Qualitatively, the four types of tocopherols have been affected by the oxidation process, but their percentage distribution remained almost unchanged in both oils studied. These results are in agreement with other results already reported in the literature [31].For the Picholine oil, it decreased 53,3% from total tocopherols (202 to 94.3 mg/kg).In conclusion, the Arbequina oil has lost a large amount of tocopherols, which leads to rapidly lose its nutritional properties and pharmacological.

Table 4. Tocopherol composition (initial and final) in Picholine and Arebiquina oil samples stored for 9 weeks at 60°C.

	Tocopherols Composition			
	Picholine		Arebiquina	
	Initial	After storage	Initial	After storage
α-Tocopherol	166.3 ± 15	82.2 ± 11	167 ± 15	60 ± 10
β-Tocopherol	11.7 ± 3	8.6 ± 1	10.5 ± 2.5	4.1 ± 1.5
γ-Tocopherol	1.9± 0.3	2 ± 0.2	2.3 ± 0.3	3 ± 0.5
δ-Tocopherol	21.9 ± 6	0.5 ± 0.1	20.1 ± 6	9.8 ± 1
Total	202 ± 21	94.3 ± 15	182 ± 30	75.5 ± 12

Conclusion

The study of the oxidative stability of olive oil at 60 ° C shows the influence of the cultivar of olive fruit on the chemical composition and oxidative stability of the oil. Indeed, the Arbiquina cultivar gave an oil more prone to oxidation and loses its designation as extra virgin olive oil after a few weeks of storage. Picholine cultivar shows a stability average. This study confirms the results obtained by other authors [6, 8]. However, it should be clearly stated that the purpose of our study was not to demonstrate that the stability of a type of oil is higher than in another because on the one hand, the number of samples oils studied is limited and secondly the phenomenon of oxidation is very complex to master. These two factors prevent us from advancing claims in relation to stability

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