Variation of the Chemical Properties and the Antiradical Activity of Olive Fruits During the Maturity

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Abstract

The work undertaken in this study focused on the variation of chemical properties on the oil content of olive fruit and antiradical activity. The results obtained showed that the accumulation of oil increases with the maturity of the fruits 31.41% in November, immature fruits have a low yield of oil, rich in tocopherols, sterols, and carotenoids. The proportion of saturated acids decreases with fruit maturity (26.6 to 20.03%), while that of unsaturated acids increases (75.09 to 79.66%). Immature fruit cakes are the richest in phenolic compounds (9,386 mg/g). The antioxidant activity of oils and Cakes from immature fruits are the most important (0.681 and 25.56 µM/g respectively). THE extracts from immature fruit cakes are 74 times more potent than lipid extracts. Analysis of the evolution of oil content, acidity, fatty acids composition and antiradical activity of oil show that November is the right date for the olive harvest for nutrition and the fruit setting period (July) is the most appropriate to pick the fruits to use the oil for therapeutic purposes.

Keywords: olive oil, maturity, chemical properties, phenolic compounds, antiradical activity.

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Introduction

The olive tree is a typical Mediterranean tree that lives for a very long time. It adapts to the extreme conditions of the environment, but requires high light intensity and an aerated floor [1]. The olive tree is classified in the Oleaceae family where ash and lilac are also found. The genus is called *Olea* and has 30 different species spread over the surface of the globe; the species that is cultivated in the Mediterranean basin is the *Olea europea* [2].

This tree and its oil have been used since ancient times in food, therapy and cosmetics. The olive tree has aroused particular interest in studying the composition of its oil which comes only from the fruit of the olive tree (the olive) and its leaf [3]. The olive is rich in water (40-45%), carbohydrates (10-20%) and lipids that make up about 30% of the ripe fruit [1]. Olive oil is the Mediterranean product par excellence. If it is an interesting product from a nutritional point of view it is first of all for its composition of fatty acids, especially oleic acid. In addition to this particular composition of fatty acids, olive oil is especially interesting for its minority compounds such as polyphenols. The nutritional interest of these phenolic compounds lies in their strong antioxidant capacity that could prevent or slow down the onset of certain degenerative diseases as well as cardiovascular diseases because they trap free radicals and therefore protect the human body [4]. Indeed, it is largely unsaturated and contains a small part of essential fatty acids; it is also found in many cosmetic care products and widely used as an excipient in cosmetic products. It is found in many formulations of soaps, creams, ointments, milks or oils where it plays a role of penetration inducer [3].

The quality of olive oil is influenced by several factors including: cultivar and stage of maturation, of which maturity stage is the main factor determining the quality and quantity of oil. During ripening, several metabolic processes take place in olives with subsequent variations in the profiles of certain compounds. These changes are reflected in the quality, sensory characteristics, oxidative stability and/or nutritional value of the product obtained. Polyphenols, tocopherols, chlorophyllic pigments, carotenoids and the composition of fatty acids and sterols are examples of compounds involved in this phenomenon [5]. The olive, which is a fruit exposed to air, is obliged to defend itself against oxygen and must therefore synthesize a greater amount of antioxidant substances (oleuropein, ligstroside, etc.), which are subsequently found in the oil from which it is extracted. The main antioxidants in olive oil are derivatives of oleuropein and ligstroside and are therefore part of the class of phenolic compounds (the enzyme β-glucosidase hydrolyzes oleuropein to produce aglycone. These compounds will allow a good preservation of olive oil over time since these molecules as well as the tocopherol will prevent its oxidation [1,2].

The maturation of the olive follows several stages according to the seasons: flowering takes place in April to June; fruit set takes place from June to August and corresponds to the initial phase of olive formation which becomes fleshy; Veraison takes place from September to November (the olive changes from green to purple to become black. It is during this stage that the fatty acids and sugars of the fruits are transformed into oil, it is lipogenesis). Picking takes place from September to February [3].

In view of what we have mentioned, the objective of this work is to study the variation of the characteristics of olive oil, namely: oil content, acidity, fatty acid composition, unsaponifiable content and the antiradical activity during the maturity of the fruits in order to determine the

appropriate date for olive harvesting and to guide the use of the olive oil in nutrition or health (therapeutic).

Experimental

Materials

All standards, chemicals, and solvents in analytical grade were purchased from Sigma and Merck companies (Darmstadt, Germany). Petroleum ether, Ethanol, sodium sulfate, 1,10-orthophenantroline, α-tocopherol (vitamin E), Chloroform, β-sitosterol, acetic Anhydride, acetic Acid, sulfuric Acid, β-Carotene, Hydroxide potassium, DPPH (2,2-Diphenyl-1-picrylhydrazyl).

The olive fruits used in this study were harvested from a single olive tree from an agricultural land located in the Laghouat region of southern Algeria during the months of July, August, September, October and November 2019. The fruits were picked between dates 24 to 27 at the end of each month. After each harvest the sample is kept in the freezer at a temperature of -6°C until use. The 5 samples were recovered from the freezer one month before their analysis in order to dry them in a well-ventilated environment, in the shade. After the samples have undergone sorting, stripping, they were stored in paper bags until the time of use.

Methods

Evolution of the weight of olives during maturation

In order to determine the evolution of the weight of the olive fruits during ripening, we prepared 3 samples of 10 olive fruits for each harvest, taken randomly, and then, they were weighed with precision. Average weighing was calculated for each sample.

Extraction

To extract and determine the fat content of studied samples, liquid solid extraction by maceration was adopted. The dried fruits were crushed using a mortar and then grinded using an electric grinder, subsequently sieved to obtain a homogeneous powder. A quantity (82 g) of each vegetable powder was macerated in 500 mL of petroleum ether at room temperature for 24 h. After filtration, the extracts were then dried by a sufficient amount of anhydrous sodium sulfate and filtered. The solvent was evaporated under reduced pressure using a rotary evaporator (Rotavapor) at 40°C. The oils obtained were then placed in an oven to remove traces of the solvent, and then stored in the refrigerator in a sterile bottle hermetically closed until the time of analysis. The extraction yield is defined as the ratio between the mass of vegetable oil extracted and the mass of plant material having undergone the extraction.

Extraction and quantitative analysis of lipids

Acid value

This is the number of milligrams of potassium hydroxide needed to neutralize the free fatty acids contained in one gram of oil.

The modus operandi was carried out according to Wolff's protocol, 1968 [6]. A test portion of 0.5 g of olive oil was dissolved in 10 mL of hexane for samples from August to November and 0.25g in 20 mL of hexane for July oil. The prepared oily solutions are then determined with an ethanolic solution of potassium hydroxide 0,01N until the turn of the color indicator used.

Fatty acid composition

In a flask, a quantity of oil (200 mg) was mixed with sodium méthanoate (15 mL 0.5%). The mixture was brought to a reflux boiling for 30 min. Then distilled water (20 mL) was added. The aqueous phase containing the fatty acids was extracted in two stages (2 times) using dichloromethane, the organic phase obtained was washed six times with distilled water until neutralization, then dried with anhydrous sodium sulfate and evaporated under reduced pressure. The methyl esters thus obtained were kept in the refrigerator until their analysis.

A volume of methyl esters (0.8 µL) was injected into a chromatograph (Chrompack CP 9002) equipped with a DP-23 capillary column (50% Cyanopropyl) and a flame ionization detector (FID). The temperature of the injector was set at 280°C and the detector 250°C. The temperature programming of the oven was 150 to 250°C at a rate of 3°C per minute. The carrier gas used was nitrogen at 1 mL/min. Fatty acid methyl esters were identified according to their retention times at the column level by comparison to standards and the level of each fatty acid is determined by calculating the corresponding peak areas.

Determination of total tocopherols

We used the Emmerie-Engel colorimetric assay [7]. The reducing properties of tocopherols were used which, in alcoholic solution, reduce ferric iron to ferrous iron. The latter are complexed by 1,10-orthophenantroline, giving a stable red-orange complex whose molar extinction coefficient at 510 nm is high.

A calibration line was drawn from commercial α-tocopherol to relate the optical density and the concentration of tocopherol expressed in grams per liter (g/L). From a commercial solution of vitamin E, we prepared in ethanol solutions with well-determined concentrations between 0.01 and 0.05 g/L. A volume of 1 mL of each prepared solution was mixed with 1 mL of 1,10-orthophenantroline reagent (0.4%) and 0.5 mL of iron chloride (ethanolic solution 0.12%). The mixture was incubated in the dark for 5 min. The absorbance reading is carried out at 510 nm by UV/Vis spectrophotometer (Shimadzu 1800), against a blank, the lipid extracts of each sample

were treated according to the steps of the same protocol followed during the preparation of the α-tocopherol calibration curve, and the results are expressed in mg tocopherol equivalent per gram of oil (mg EVE/g oil). All measurements were carried out in triple.

Total sterol determination

This is a spectrophotometric assay following the Liebermann-Burchard test [8,9]. Sterols form a stable complex with acetic anhydride in an acidic medium that absorbs into the visible at a wavelength of 550 nm.

From chloroform solutions of β-sitosterol at different concentrations in a range of 0.424 to 2.12 g/L, we plotted a calibration curve of this sterol. A volume of 1mL of each dilute solution was mixed with 2 mL of the Liebermann reagent (Liebermann reagent prepared 60 mL acetic anhydride + 30 mL of acetic acid + 10 mL sulfuric acid). The mixture was incubated in the dark for 25 min at room temperature. The absorbance of each solution was determined at 550 nm against a blank on a UV/Vis spectrophotometer (Shimadzu 1800). The oil samples were treated in the same way and the total sterol content of each extract was determined from the β-sitosterol calibration curve. The measurements were repeated 3 times for each sample and the mean readings were recorded, and the results are expressed in mg equivalent of β-sitosterol per gram of oil (mg EβS/g oil).

Determination of total carotenoids

β-Carotene, is generally the most abundant and common compound in fatty substances of plant origin. The total carotenoids content was determined using the Talcott and Howard method (modified) [10].

For the realization of the calibration curve, different concentrations of β-carotene in chloroform from 0.012 to 0.063 g/L were prepared. 2 mL of each dilute solution was taken and absorbances were measured at 464 nm against a blank containing only the solvent. For the samples, the same steps were followed except that instead of β-carotene the extracts of the samples were introduced, the carotenoids content was determined based on the calibration curve carried out with β-carotene. The measurements were repeated 3 times for each sample and the average readings were recorded. The results are expressed in mg equivalent of β-carotene per gram of oil (mg EβC/g of oil).

Extraction and quantitative analysis of phenolic compounds

The extraction was carried out by ultrasound at a temperature of 44°C by putting 2 g of defatted fruit cake in 50ml of a methanol / water solvent system (8: 2 v/v) for 45 min. The extracts were filtered on a filter paper. After removal of methanol under reduced pressure in a rotary evaporator (steam Rota) at 45°C, two successive extractions of light petroleum removed residual pigments and lipids (exhaustion). Then the aqueous solution was extracted twice with ethyl

acetate. To remove traces of water, the extracts were dehydrated by adding anhydrous sodium sulphate, after filtration, the ethyl acetate is evaporated under vacuum using a steam Rota at 40°C, the dry residue obtained is weighed and solubilized in 5 ml of methanol. The extracts thus obtained are transferred to hermetically sealed vials covered with aluminum foil and then stored in the refrigerator until the time of analysis. The contents of the crude extracts are calculated by mass difference.

Determination of total phenolic compounds

The determination of total phenols was carried out according to the protocol described by Vermerris and Nicholson (2006) [11]. With some modifications.

100 µl of each diluted extract (prepared in methanol) were introduced using a micropipette into test tubes, followed by the addition of 500 µl of the Folin-Ciocalteu reagent (10 times diluted in distilled water). After incubation for 2 minutes, 2 ml of sodium carbonates $Na_2 CO_3$ to 2% were added, then the solutions were shaken immediately and kept in the dark for 30 minutes at room temperature. The absorbance of each solution was determined at 760 nm against a blank on a UV/Vis spectrophotometer (Shimadzu 1800). The phenolic content of each extract was calculated from a gallic acid calibration curve (different concentrations of gallic acid ranging from 0.03 to 0.3 g/l were prepared in distilled water) and expressed in milligrams gallic acid equivalent per gram of cake (mg EAG/g oilcake). All measurements were carried out in triple.

Determination of flavonoids

The flavonoid content of the extracts obtained is determined by the method of La maison and Carnat (1990) [12]. Flavonoids form yellowish complexes by chelation of metals (iron and aluminum).

For the preparation of the standard range, a dilution series ranging from 0,003 to 0,03 g/l quercetin solution (prepared in methanol). Subsequently, in a test tube is put a volume of 1ml of quercetin and 1ml of aluminum chloride solution (2%). Incubate for 20 minutes at room temperature. The reading is carried out at 409 nm by UV/Vis spectrophotometer (Shimadzu 1800), and the absorbance values found for each solution are used to plot the calibration curve. For the samples, the same steps were followed except that instead of quercetin the sample extracts were introduced, and the results are expressed in mg quercetin equivalent per g of cake (mgEQ/g of oilcake). All measurements were carried out in triple.

Evaluation of the anti-radical activity of lipid and phenolic extracts by the DPPH test

In our study we used the chemical test: the free radical 2.2'-diphenyl-1-pycrilhydrazyl ($DPPH^{\cdot}$). The reduction of the DPPH radical by an antiradical can be monitored by visible UV spectrophotometry, by measuring the decrease in absorbance at 517 nm caused by the presence of the extracts. This stable free radical has a dark purple coloration, when reduced, the coloration

becomes yellow. The DPPH test then makes it possible to obtain information on the antiradical power which is proportional to the disappearance of DPPH· radicals [13].

The DPPH· was solubilized in absolute ethanol to reach a solution of 100 µM. The lipid extracts were dissolved in chloroform to prepare various sample solutions with different concentrations, and then 1 mL of each prepared solution was added to 1 mL of a DPPH· solution. The reaction mixture was shaken immediately at the vortex and then kept in the dark for 30 min at room temperature for the reaction to take place. The absorbance of the reaction medium was measured at 517 nm against a blank by UV/Vis spectrophotometer (Shimadzu 1800). Vitamin E was used as a standard for the calibration curve. All measurements were carried out in triplicate. The results were expressed in µM in Vitamin E equivalent per g of oil (VEEAC).

It should be noted that for phenolic extracts, we proceeded with the same protocol except that DPPH was solubilized in methanol and the antiradical activity was expressed in µM equivalent of ascorbic acid per g of oilcake.

Results and Discussion

Evolution of the weight of olives during maturation

The weight of the fruit is the most important component of olive yield. It is a highly sought-after agronomic character, from this value depends the importance of production. The results of monitoring fruit weights according to maturity are presented in Table 1. On notice that the average value of fruit weight increases during ripening, the weight of fruit (samples of 10 fruits) varied by 5.30 g in July at initial phase of olive formation (fruit set) at 8.76 g in November maturation phase and picking.

Acid value

The acidity value of oil (Free fatty acid levels) is the first measure of its quality. The values of the acid value are given in table 1. These values indicated a high acidity of the oils during the months of July to October (5.08 to 3.23).

The acidity value decreased with the degree of maturity; from 5.08 to 2.15 in July and November respectively. In the months of July, August, September and October the fruits were immature, the biosynthesis of triacylglycerols remains low. At this stage, the biosynthesis of glycerides, free fatty acids and phospholipids predominates over the synthesis of triacylglycerols. The acidity measured in this stage could correspond to both the presence of free fatty acids and phosphatidic acid. These components are the substrates for triacylglycerol biosynthesis [14]. As the acid value reaches its minimum value of 2 in November, the value is within the range of the standards established by AFNOR 1984. Hence, November was the best date to use olive oil for nutrition.

Oil extraction yield

The oily extracts appeared in two strong colors: dark green at the first month of harvest and yellow at the end of the harvest. This change in color is related to the variation in the composition of pigments (chlorophylls, carotenoids, etc.).

From the results of the table 1, it is clear that the oil yield increases with fruit ripening. Recorded values vary by 3.57% in July to 31.41% in November pick-up date. This increase is attributed to the continuation of the triglyceride-forming biosynthesis pathway until the fruit reaches full ripening. This yield was lower than that obtained by other authors [15].

The study of oil accumulation during olive ripening is important to decide the best time for their harvests. Indeed, in our study, the largest amount of oil was observed in November, which corresponds to the best time to harvest olive fruits and guarantees a significant oil production. If the harvest is advanced the fruits are not ripe (month of July) which induces a reduction in the quantity of oil as well in its organoleptic quality (taste and smell) and chemical composition. A late picking in our study (month of November) the olives become much ripped and the fruit will be very rich in oil.

A good correlation was recorded between the oil yield and the mass of fruit from each crop. The correlation coefficient was 0.99, indicating that oil accumulation peaks when the fruits were fleshy.

Fatty acid composition

The results of the chromatographic analysis of methyl esters of fatty acids are given in table 1 where the relative proportions of the different fatty acids contained in olive oils are given. We noted the presence of fatty acids usually found in vegetable oils namely palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids.

The saturated fatty acids in the oils were palmitic and stearic whose proportions varied from 18.78 to 24.18% and from 1.55 to 2.19% respectively. Palmitic acid was the most dominant among saturated fatty acids. Its proportion reaches a maximum value of 24.18% in olive oil for immature fruits, and a minimum value of 18.78% for mature fruits. Stearic acid was found in all oil samples but with lower proportions, not exceeding the value 2.19% in the oil of fruits collected in the month of August considered immature stage.

Table 1: Fatty acid composition, acid value and Yield content of olive oil.

Fatty acids		July	August	September	October	November
Palmitic	**C16:0**	24.18	23.39	20.1	21.06	18.78
Palmitoleic	**C16:1**	2.65	2.81	2.3	2.66	2.35

Stearic	**C18:0**	1.72	2.19	1.72	1.73	1.55
Oleic	**C18:1**	61.32	58.21	65.4	64.28	65.05
Linoleic	**C18:2**	9.09	11.2	9.2	9.21	11.28
Linolenicacid	**C18:3**	2.03	2.17	1.18	1.03	0.98
Saturated fatty acids (SFAs)		25.9	25.58	21.82	22.79	20.33
Monounsaturated fatty acids		62.97	61.02	67.7	66.94	67.4
Polyunsaturated fatty acids		11.12	13.37	10.38	10.24	12.26
Unsaturated fatty acids (UFAs)		74.09	74.39	78.08	77.18	79.66
UFAs/SFAs		2.86	2.90	3.58	3.38	3.92
Yield w/w (%)		3.57	8.88	20.88	25.87	31.41
Acid value		5.08	6.90	5.92	3.23	2.15

For unsaturated fatty acids, we noticed the existence of oleic, linoleic and linolenic acids. They represent 74.09 to 74.39% in the oils of immature fruits (July and August) and 78.08 to 79.66% in mature fruits (September to November).

Oleic acid was the major component among unsaturated fatty acids, it represented proportions of 58.21 to 61.32% in immature fruit oils (July and August) and 64.28 to 65.4% in mature fruit oils. The second position goes to linoleic acid where their proportions recorded ranged between 9.09 and 11.2% for immature fruits and 11.28% for mature fruits. Finally, linolenic acid took the third position with proportions 2.03 to 2.17% for immature fruits and 0.98% for mature fruits.

The analysis of the results obtained allowed us to notice that the content of saturated fatty acids (palmitic and stearic) decreases during the ripening of the fruits, while for unsaturated fatty acids with the exception of linolenic acid increases. It should be noted that olive oil contained a significant amount of linolenic acid in immature fruits. Why it is important to pick the fruits at this stage to use the oil for therapeutic purposes, as linolenic acid is known for important biological activities. The ratio of unsaturated fatty acids to saturated acids was slightly high 2.86 for oils of immature fruits and 3.92 for mature fruits, which gives them remarkable stability.

We studied the variation of fatty acid percentages according to the date of collection (July corresponds to zero days). A good negative regression was recorded between the saturated fatty acid content and the harvest date (R^2 = 0.83) Figure 1, which shows that the saturated fatty acid content decreases with the month of fruit harvest. In general, our results are consistent with those reported by Beltran et al 2004 [16], who studied the influence of the date of collection and production of olive oil on oil yield and chemical composition.

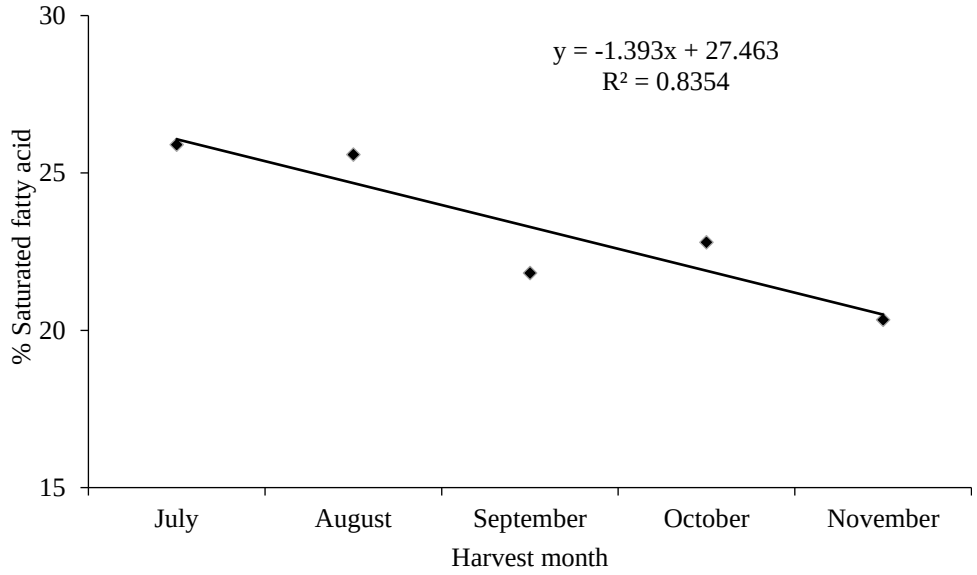

Figure 1: Variation of saturated fatty acids as a function of harvest month

Determination of total tocopherols

The results of the quantification of total tocopherols are grouped in table 2. The values of the quantities of tocopherols range from 0.12 to 3.82 mg/g of oil. The tocopherols content decreased throughout maturation stage until the last sampling date. The lowest value was recorded for fruit in the ripening stage (November), while the highest value was recorded for fruit set fruit. The same finding has also been reported by several authors who have shown that the content of total tocopherols decreased during fruit ripening [15].

Table 2: Contents of tocopherols, sterols and carotenoids values during maturation

Harvest date	July	August	September	October	November
Tocopherols (mg/g oil)	3.82±0.13	0.56±0.01	0.24±0.01	0.16±0.01	0.12±0.01
Sterol (mg/g oil)	45.61±0.06	18.85±0.24	8.38±0.13	6.31±0.04	5.02±0.01
Carotenoid (mg/g oil)	2.31±0.06	0.34±0.01	0.12±0.01	0.08±0.01	0.04±0.01

The results obtained show that July is the optimal month to pick olives and use their oils for therapeutic purposes. If we compare our results with those published, we can say that the oils studied are rich in tocopherols [1].

Total sterol determination

Sterols are the main constituents of the unsaponifiable fraction and their content corresponds to about 20% of the unsaponifiable olive oil material. Studies have shown that each oily fruit has a characteristic sterol profile that makes determination an important tool for verifying the authenticity of an oil. They are important components for oil stability because at high temperatures they act as inhibitors of polymerization reactions [5].

Changes in sterol amounts in oil samples throughout maturation are given in table 2. The sterol quantity values varied between 5.02 and 45.61 mg/g of oil. The sterol content decreases with fruit ripening. This decrease can be explained by the fact that at a certain specific time during the ripening of olives, the plant leads to the conversion of their existing sterols into steroid hormones and vitamins that regulate the growth and development of immature tissues [17]. These results were consistent with those obtained by Matos et al [5], where they studied the variation of the chemical composition of three different varieties of olive oils according to different harvest periods.

Determination of total carotenoids

The total amount of pigments in olive oils is an important parameter because it is correlated with color, which is one of the factors that influence the selection made by consumers. In addition, pigments are also involved in auto-oxidation and photo-oxidation mechanisms [18].

From table 2 it can be seen that carotenoid concentrations decreased markedly with fruit ripening. Their concentrations range from 2.31 to 0.04 mg/g of oil. These results were consistent with the conclusions reached by other authors [18]. The change in the color of olive oils during the ripening process was explained not only by the reduction in the concentration of pigments but also by the formation of other colored compounds, such as anthocyanins.

During the ripening process, anthocyanins accumulate in the fruits (purple color) at the same time as the oil content increases further. As maturation progresses, photosynthesis activity decreases and chlorophyll and carotenoid concentrations gradually decrease [19].

Quantification of phenolic compounds in oilcake

Contents of crude extracts

Table 3 summarizes the results for the mg/g content of crude extracts of ethyl acetate in olive meal, total phenol content, flavonoid content and anti-radical activity during maturation.

Table 3: Contents of crude extracts, total phenols and flavonoids during maturation

Harvest date	July	August	September	October	November
Crude extract content (mg/g)	25.59	20.58	9.49	3.38	8.86
Total phenol content (mgEAG/g)	9.39±0.33	4.74±0.20	2.42±0.07	1. 87±0.07	2.14±0.10
Flavonoid content (mgEQ/g)	0.53±0.008	0.54±0.03	0.21±0.004	0. 24±0.01	0.41±0.002

From Table 3, it is clear that the contents of crude extracts decrease during maturation, these contents vary by 25.59 to 8.86 mg/g of oilcake. If we compare the contents of oils (lipid extract) in olive fruits with those of these extracts (extract from oilcake), we find that the content of non-polar extracts varies in the opposite direction with that of polar extracts. This result is justified by the fact that the olive is an oleaginous fruit (source of oil).

Determination of total phenolic compounds

The results are given in Table3. From these results we notice that the contents of crude extracts are higher than those of total phenols, which proves that crude extracts contain compounds other than phenols that are extractable by methanol. The total phenol content reaches its maximum value at the first harvest (9.39 mg/g), then gradually decreases until it reaches a minimum value in October with an amount equal to 1.87 mg/g, after which it increases to 2.14 in November.

The total level of total phenolic compounds in the present study showed a clear negative correlation with increased ripening levels in fruits.

Determination of flavonoids

According to the results of Table 3, it is noted that the quantities of flavonoids do not vary in the same direction with those of total phenols and the date of collection, the samples of the two months of August and July have the highest quantities of flavonoids (0.54 and 0.53 mg /g), followed by the month of November with an amount of 0.42 mg/g, and lastly the samples of September and October which contain similar amounts of flavonoids which are of the order of 0.2 1 to 0.24 mg/g. However, it is noted that flavonoid levels are always lower than those of total phenolic compounds.

Anti-radical activity

We assessed the anti-radical activity of the different extracts. Anti-radical activity is expressed as VEEAC (µM/g oil) for lipids and VCEAC (µM/g oilcake), which is defined as the concentration of µM of a vitamin E/vitamin C solution with the same anti-radical activity as a 1µM solution of the test substance.

The higher the values of VCEAC or VEEAC, the more potent the extract is. In the extracts and according to the results mentioned in Table 4.

Table 4: The values of VEEAC and VCEAC in µM/g of lipid and phenolic extracts.

Harvest date	July	August	September	October	November
VEEAC (µM/g)	0.68±0.03	0.10±0.01	0.08±0.01	0.08±0.01	0.08±0.01
VCEAC (µM/g)	25.56±0.87	15.35±0.19	7.62±0.26	6.58±0.22	7.42±0.20

We note for lipid extracts the anti-radical activities range between 0.08 and 0.68 µM/g. If we compare the antiradical activities of the oils, we should find that the oil of July is the most powerful (0.68 µM/g), followed by that of August (0.10 µM/g). The samples of the months of September, October and November have practically the same antiradical activity, as they recorded VEEAC values almost close to the order of 0.08 µM/g. The results showed that the more immature the fruits, the greater their anti-radical abilities, which has already been found for unsaponifiable (tocopherols, sterols and carotenoids).

For phenolic extracts the values of VCEAC vary by 6.58 and 25.56 µM/g. The July extract recorded the highest anti-radical activity with a VCEAC value equal to 25.56 µM/g, the least anti-radical is the October extract with a value of 6.58 µM/g. The results obtained show that the antiradical activity decreases steadily with the maturity of the fruit from July to October.

It is well to note that the anti-radical activity of phenolic extracts of immature fruit cakes (July and August) are gifted by the highest activities because they are twice as powerful as that of mature fruits (November). In contrast to the results obtained for lipid extracts, phenolic extracts of olive fruit cakes are more potent than vitamin C and even vitamin E (EC50 values are 2.35 and 4.65 mg/l for vitamins C and E respectively). The two phenolic extracts of the two months July and August are theoretically 20 times more active than vitamin C.

Correlations between the contents: tocopherols, sterols, carotenoids and VEEAC; total phenols, flavonoids and VCEAC were investigated. Positive correlations were found between the contents of tocopherols, sterols, carotenoids and VEEAC. The values of the correlation coefficients determined (Table 5) were of the order of almost 1, which proves that the quantities of the constituents of the unsaponifiable matter of the oils studied varied regularly and in the same direction according to the maturity of the fruits. Also, it can be said that the anti-radical activity of lipid extracts depends on the contents of the constituents of the unsaponifiable fraction, which varied in the same direction with these constituents.

For phenolic extracts the antioxidant activity calculated during fruit ripening was strongly correlated with the total phenol content (R = 0.99), which shows the strong contribution of

phenolic compounds in the antioxidant activity of olive fruit meal extracts. Smaller correlations were found between total phenol and flavonoid content (R=0.72) and between antioxidant activity and flavonoid content (0.77).

Table 5: Correlation between tocopherols, sterols, carotenoids and VEEAC; total phenols, flavonoids and VCEAC

	Tocopherols	Sterols	Carotenoids	VEEAC
Tocopherols	1	0.976	0.999	0.996
Sterols		1	0.978	0.955
Carotenoids			1	0.995
VEEAC				1

	phenols	flavonoids	VCEAC
phenols	1	0.721	0.997
flavonoids		1	0.796
VCEAC			1

Conclusion

The present work allowed us to study the variation of the chemical characteristics and antiradical activity of olive oil during the maturity of fruits.

As the ripening process continues, a number of physical and chemical changes occurred in the fruit. They include variations in the fatty acid profile, where the content of saturated fatty acids (palmitic and stearic) decreased during fruit ripening, while unsaturated fatty acids with the exception of linolenic acid increased. Oleic acid, the main monounsaturated fatty acid was present in high concentrations, 64.28 to 65.05% in mature fruit oils.

The contents of tocopherols, sterols, carotenoids and antiradical activity decreased during maturation inversely to the oil yield which reached its maximum (31.41%) in November. The chemical parameter studied: the acidity value has decreased and reached its minimum value at the last harvest date.

The results showed that the more immature the fruits, the greater their antiradical abilities. For oil, the extract of the month of July was the most powerful antiradical.

Despite the low oil content, the immature fruits (July) of the olive tree could be a major source of beneficial compounds such as linolenic acid, compounds of the unsaponifiable fraction (tocopherols, sterols and carotenoids).

The phenolic extracts of immature fruit cakes are endowed by the highest anti-radical activities (July and August), the phenolic extracts of olive fruit cakes are more potent than vitamin C and even vitamin E. For phenolic extracts, the antioxidant activity calculated during fruit ripening was strongly correlated with the total phenolic compound content (R=0.99), which shows the strong contribution of phenolic compounds to the antioxidant activity of olive fruit meal extracts.

Finally, we can say that our work has brought very interesting and original preliminary results in support of the fact that olive fruits before full maturity could be a healthy diet and a valuable source of natural bioactive molecules find in the unsaponifiable fraction

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