

Research Article

Influence of the Date of Harvest on the Olive Oil Quality with Focus on Effect of Olive Ripening on Oxidative Stability

Miloudi Hilali^{1*}, Hanae El Monfalouti¹, Larbi El Hammari², Farid Amar³, Zakaria Mennane⁴, Malika Haddam⁵, Nadia Maata⁶ and Badr Eddine Kartah¹

¹Laboratory of Plant Chemistry and Organic and Bioorganic Synthesis, Faculty of Science, University Mohammed-V, Av. Ibn Battouta, BP 1014 Agdal-Rabat, Morocco; ²Laboratory of Applied Chemistry of Materials, Faculty of Science, University Mohammed-V, Av. Ibn Battouta, BP 1014 Agdal-Rabat, Morocco; ³Laboratory of sciences and society cases, Faculty of arts and humanities, University Mohammed-V, Rabat, Morocco; ⁴Department of bacteriology, National Institute of Hygiene, Av. Ibn Battouta, BP 769 Agdal-Rabat, Morocco; ⁵Lesieur Cristal, 1, street Caporal Corbi, Roches Noires, 20300 Casablanca, Morocco; ⁶Official Laboratory of Chemical Analysis and Research 25, street Nichakra Rahal, Casablanca, Morocco.

Abstract | The olive Harvest time of northern Morocco where veterinary technology and control are absent depends, on local agricultural culture and community experiences local, therefore harvesting periods are related to the degree of maturity of the olives from the point of view of the village women, thus determines maturity for them, is not a scientific criterion, but a specific period of time or a particular season. The aim of present study was to determine the optimal olive harvest time, this study was carried out in the circle of Massmouda region in the city of Ouezzane northern Morocco. It was made in order to evaluate the influence of ripening on the olive oil chemical composition. To carry out this work an olive tree of the Moroccan Picholine variety selected, the olives were harvested at different stages of maturity from the same tree in October, November and December 2020, the extraction was made by mechanical cold pressing. After extracting the olive oil, the physico-chemical analysis such as acidity, humidity, fatty acids and sterols was assayed. The results obtained the sample which extracted in the month of december contains a higher percentage of β -sitosterol (95.2%) and higher percentage of oleic acid C18:1 (75.4%). Time of the maturation is a factor that can significantly influence the quality of olive oil. Oleic acid, criterion of nutrition and quality of olive oil. Also, the amount of stearic acid increases with the ripening of the olive fruit. The sterol result shows that the percentage of stigmasterol and β -sitosterol (95.2%) increased with the ripening of the olives. The influence of ripening parameter is significant on the main quality criteria of olive oil such as acidity, fatty acid and sterols.

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***Correspondence** | Miloudi Hilali, Laboratory of Plant Chemistry and Organic and Bioorganic Synthesis, Faculty of Science, University Mohammed-V, Av. Ibn Battouta, BP 1014 Agdal-Rabat, Morocco; **Email:** hilali400@yahoo.com

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Introduction

Olive oil is obtained from the fruit of the olive tree. This tree was first grown on the shores of the Mediterranean Sea (Pérez-Jordà *et al.*, 2021).

Olive oil is obtained by extracting olive fruit, which is high in unsaturated fat (Guo *et al.*, 2018). Oil is produced by the complete crushing of olives and the separation of the oil by mechanical or chemical methods. This oil has various applications in cooking,

cosmetics, medicine and soap (Wiesman, 2009; Guo *et al.*, 2018). In ancient times, olive oil was also used as a fuel for oil lamps (Wiesman, 2009). Olive oil is used all over the world, and it is mostly consumed in countries around the Mediterranean (Wiesman, 2009; Guo *et al.*, 2018).

The olive tree is the main fruit specie cultivated in Morocco. With an area of around 918,385 ha, with around 1,500,000 tonnes of oil per year (Loukili *et al.*, 2021), and it occupies the first arboreal area in Morocco. For these considerations, national olive growing ensures intense agricultural activity generating more than 15 million days of work per year (Loukili *et al.*, 2021). “Virgin olive oil” is the oil obtained from the fruit of the olive tree only by mechanical or other physical processes under conditions, particularly thermal, which do not lead to deterioration of the oil, and not having undergone any treatment other than washing, decantation, centrifugation and filtration (Gutiérrez *et al.*, 1999; Jimenez-Lopez *et al.*, 2020). At room temperature, virgin olive oil is a shiny liquid and its color varies from amber yellow to greenish yellow (Khah *et al.*, 2021). Its flavor is sweet or fruity, this fruitiness can be light or accentuated. The density of olive oil is 0.914 to 0.920 at 20 ° C (Carbone *et al.*, 2001). Olive oil and especially virgin olive oil is widely used in ethno-medicine with attention to high level of Tocopheryl and other bioactive components (Oguegbulu and Nwadiibia, 2020).

The composition of olive oil varies widely not only depending on the variety of olives, but also on the latitude of cultivation and the time of harvest (Gutiérrez *et al.*, 1999; Dag *et al.*, 2011). According to the international standard such as ISO 665 (2000) applicable to olive oils and olive-pomace oils, the chemical constituents of virgin olive oil can be subdivided into two categories namely the saponifiable fraction (triglycerides, phospholipids, *etc.*) and the unsaponifiable fraction (sterols, tri-terpene alcohols, *etc.*). The glyceridic part represents about 98% of virgin olive oil and a little less in the case of pomace oil (Jabeur *et al.*, 2017). There is a clear predominance of monounsaturated oleic acid, a low percentage of saturated fatty acids and an acceptable percentage of polyunsaturated fatty acids (Al-Bachir and Sahloul, 2017; Jabeur *et al.*, 2017). Olive oil contains a large number of minor components of a glyceridic and non-glyceridic nature (Al-Bachir and Sahloul, 2017; Jabeur *et al.*, 2017) The optimal time of harvest should be determined accord-

ing to the content of the oil in the olive fruits, the quality of the oil and the cost of harvest (Camposeo *et al.*, 2013). In order to maintain the quality characteristics that olives possess when they are harvested on the tree, it is necessary to transport them immediately to the mills. The batches of olives, once weighed, are stored in an individualized manner, according to the origin, the degree of maturity and the sanitary state of the fruits, *etc.* The storage time of olives before processing should be as short as possible, and in any case less than 2 days, because prolonged storage is a main cause of deterioration in the quality of the oil. This study is part of the continuity of the research series carried out by our Plant Chemistry and Organic and Bio-organic Synthesis laboratory on vegetable oils. To enhance olive oil and improve their nourishing effect, it consisted of: Influence of the olive harvest date on the chemical composition of olive oil. To carry out this work, an olive tree belonging to the region of Massmouda (the town of Ouezzane Douar Ghnioua) and of the “Moroccan Picholine” variety was selected (Khadari and Moukhli, 2016).

Materials and Methods

Presentation of the study region

The study region is the city of Ouezzane which belongs to the southern margins of the Jebala country whose large tribes bordering the city are: Masmouda, Rhouna, Ghzaoua and Beni Mesara. The Ouezzane region extends to the north of Morocco over an area of 1861.2km², and has an altitude of 614 meters (Chohin-Kuper *et al.*, 2010).

Biological material

In the present work, a olive tree of the Moroccan Picholine variety, or from the region of Massmouda Douar Ghanioua (the city of Ouezzane in northern Morocco) were selected, during the months of October, November and December 2020. Table 1 presents information on origin, extraction method, and olive tree age.

Preparation of different olive oil samples

the cold mechanical pressing extraction method (25°C, the temperature of the olive oil outlet after extraction) was used to extract the olive oil, the extraction was done in the Dar Dmana cooperative (olive oil extraction cooperative, province of ouezzane, Morocco) according to the extraction methods already described (Fanali *et al.*, 2018), avoiding all chemical

Table 1: Origin and method of extraction of the 3 samples.

Samples	Tree age	The region	Date of harvest	Method of extraction
1	20 Years	Ghanioua	October 2020	Extracted by mechanical cold pressing 25°C in October
2	20 Years	Ghanioua	November 2020	Extracted by mechanical cold pressing 25°C in November
3	20 Years	Ghanioua	December 2020	Extracted by mechanical cold pressing 25°C in December

and enzymatic reactions that could change the natural composition of the olive oil.

The method of extraction comprises four main operations: cleaning of the fruits (defoliation, washing of the olives), preparation of the paste (churning, malaxation), separation of the solid phase and liquid phase (oil and vegetation water), separation of the liquid phase (oil and vegetation water). These oils are then analysed directly after extraction at the Quality Control Laboratory of Lesieur Cristal, Ain Harouda, Casablanca of Morocco. The physicochemical quality and characteristics of all samples are determined including acidity index, fatty acid, sterols and moisture. The oils are analyzed according to analytical methods described in the international olive oil advisory literature (Afnor, 1985).

Determination of acidity

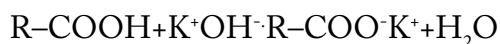
The acidity expressed as a percentage of oleic acid was measured according to the standardised method, the French standard (NF EN ISO 660 :2020).

Acidity (Ia) is the quantity of free fatty acids expressed as a % in a fat. It is measured in relation to oleic, palmitic or lauric acid. In our case, this parameter is measured in % oleic acid.

Procedure

The acidity is measured by simple acid-base dosage. Weighed 5g of olive oil in an Erlen and then add 25 ml of the mixture (ethanol/diethyl oxide) (v/v). The olive oil is then neutralized with a solution of potassium hydroxide (ethanolic KOH) of known (0.1N). Phenolphthalein is used as a colour indicator.

The KOH neutralisation reaction is as follows:



Fatty acid base soap water

Calculation of acidity: The acidity is measured by the acid-base dosage:

$$n1 \times V1 = n2 \times V2$$

(V×T of KOH) mi equivalent P (g)
 Quantity of free fatty acids 100 g
 $[Q] = (V \times T \text{ of KOH}) \times 100 \text{ g} / P$ (e.g. / 100g sample)
 $[Q] = (V \times T \text{ KOH}) \times 100 \text{ g} \times 10^{-3} / P$ (eq. / 100g sample)
 $[Q] = (V \times T \text{ of KOH}) \times 10^{-1} / P$ (eq. / 100g sample)
 Q acidity in % oleic acid] = $(V \times T \text{ KOH}) \times 10^{-1} \times 282 / P$ (eq. / 100g sample)

The result of the acidity is expressed in % oleic acid by the following formula:

$$\text{Acidity \%} = (V \times T \times 282) / 10 \times P$$

- V: Volume of the burette fall (in cm3).
- T: The title of the KOH solution.
- P: The test sample in g.
- 282: Molecular weight of oleic acid.

Determination of moisture content: (ISO 665, 2004). The moisture content were determined in accordance with the method for the determination of moisture and volatile matter in oilseeds ISO 665 (2004), 5 g of sample were weighed fresh and placed in an oven set at 50°C for overnight. The dry matter is removed from the oven after cooling in a desicator, and the dried sample is weighed.

The moisture content is calculated according to the following formula:

$$H\% = ((Pf - Ps) / Pf) \times 100$$

Pf: Weight of the “fresh plant” sample; Ps: Weight of the “dry plant” sample; H %: Moisture content expressed as a percentage.

Analysis of cis fatty acids: (Kane et al., 2012), The fatty acid composition was determined after transformation into methyl esters obtained by transesterification of triglycerides with methanoic potash. The fatty acid methyl esters of olive oil samples are obtained

according to the French international standardised method (NF EN ISO 5509 June 1995). These esters were then analysed by gas chromatography according to the conditions described in ISO 5508: 1990, using a VARIAN chromatograph with flame ionisation detector (FID), equipped with a capillary column (CPWAX) 30 m long and 0.25 mm internal diameter. The oven temperature is set at 200 °C and the injector temperature at 220 °C. The carrier gas used is helium at 1.2 ml/min and the injection volume is 1 µl, leakage (split on) at a ratio: 15 %.

Determination of the composition and nature of total sterols was according to method of Bardaa et al., (2016).

Results and Discussion

Extraction yield (Table 2)

Table 2: The yield of olive oil according to maturation.

Samples	1	2	3
Oil yield in 100kg/litre	15	18	16

Based on these results, since the date of ripening can influence the yield of olive oil extraction. Also, a difference of 2 to 3 litres of olive oil in each 100 kg of olive fruit depending on the ripening date was found.

Physicochemical characteristics

The acidity and moisture values of three olive oil samples is presented in Table 3.

Table 3: Results of acidity and humidity.

Samples	Acidity	Humidity
1	0.54%	0.31%
2	0.95%	0.20%
3	1.96%	0.13%

The acidity results show that the acidity of sample 3 is higher (1.96%) than the acidity of samples 2 and sample 3 (0.54% and 0.95% respectively). These results suggest that the harvest date of the olive fruit may influence the acidity values of the olive oil.

The harvest date therefore considers as a parameter influencing the acidity of olive oil. In fact, the acidity of samples of oils prepared in October is uniformly lower than those of oils prepared from mechanical

pressing in November and December (in morocco climate).

Present result was in consistent with the studies which indicates that extraction methods, geographical origin and climatic factors influence the chemical characteristics of the oils (Romero et al., 2016; Conte et al., 2019; Hilali et al., 2020).

Also, present study showed that the alteration of olive oil quality is rather fundamentally affected by factors damaging the fruit, such as attack by pests or the use of unsuitable systems for harvesting, transporting and storing the olives.

Analysis of cis fatty acids

The fatty acid composition of the olive oils was determined by gas chromatography method. The results obtained for the three oil samples is presented in Table 4.

Table 4: Results of cis-acids by harvest date.

Samples / fatty acid	Sample 1	Sample 2	Sample 3
C16:0	10.51	9.65	9.81
C16:1	0.76	0.56	0.70
C18:0	2.37	2.78	3.03
C18:1	73.36	70.90	75.42
C18:2	10.13	13.47	10.33
C18:3	1.11	1.24	1.19
C20:0	0.22	0.28	0.20
C20:1	0.30	0.30	0.17

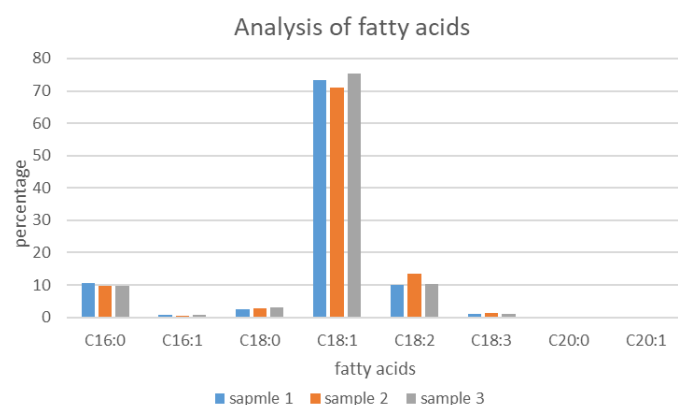


Figure 1: Fatty acid profile of olive oil samples.

The fatty acid composition of olive oil was documented and acceptable in accordance with the international standards (COI/T.20/document No 42-2/ Rev.2, 2017). Olive oil contains 86% unsaturated fatty acids, and it's mainly oleic linoleic type and contains essen-

tial fatty acids up to 13.5%; linoleic acid (10 to 13%). This fatty acid is essential fatty acids cannot be synthesized by the human body and must be provided by the diet (Kostik *et al.*,2013).

Olive oil is rich in C18:1 oleic acid. Its oleic acid content makes olive oil particularly interesting in the regulation of cholesterol level in dietary regimens (Gavahian *et al.*, 2019; Rahiman *et al.*, 2019). Palmitic C16:0 (in the range of 9 to 10%) and stearic C 18:0 (in the range of 2.3 to 3%) was determined in samples. Amounts of linolenic acid (C18:3) in olive oil was not more than 1%.

In present study, long-chain fatty acids such as C20:0 (0.28%) and C20:1 (0.3%) were not presented in olive oil samples.

By comparing the three samples (the oils were extracted from the same olive tree but with a different date) it was found that the percentage of stearic acid (C18:0) increases with the maturation time.

The variation in the fatty acid results of our samples is consistent with studies indicating that the percentage of oleic acid can be influenced by climate (Bouchenak *et al.*, 2018).

Analysis of trans fatty acids

The trans-fatty acids of the various olive oil samples was determined by gas chromatography (Table 5).

Table 5: The result of the trans fatty acid composition according to the ripening of the olive fruit.

Samples	1	2	3
%C18:1trans (TR13.03 min)	0.03%	0.02%	0.03%
%C 1%C18:2trans+%C18:3trans (TR13.96min)	0.02%	0.00%	0.02%

According to the Table 5, the percentage of oleic-, linoleic- and trans- linoleic- acid (C18:1, C18:2 and C18:3), (elaidic acid) in virgin olive oil is very low and does not give any information on the ripening date of the olive fruit.

The presence and levels of trans-fatty acids in “virgin olive oils”, is in acceptable level for dietary consumption. For this propose, the content of trans fatty acids has been limited in virgin olive oil by the standard to 0.05% (COI/OT/NC n° 1 December 2004) both for

elaidic acid and also for the sum of the trans isomers of linoleic and linolenic acids.

Sterols content

The sterol content of the olive oil samples was determined by method of Bohacenko and Kopicova (2001) with gas chromatography after sterol fraction-silylataion (Table 6).

Table 6: Sterol composition of the three samples.

Samples / Sterol	1	2	3
Cholesterol	0.06	0.04	0.01
Campesterol	3.73	3.73	2.49
Stigmasterol	0.89	1.17	1.64
Delta-7-stigmasterol	0.01	0.13	0.15
β-sitosterol	92.1	93.1	95.2
Delta-7-avenasterol	0.28	0.37	0.37

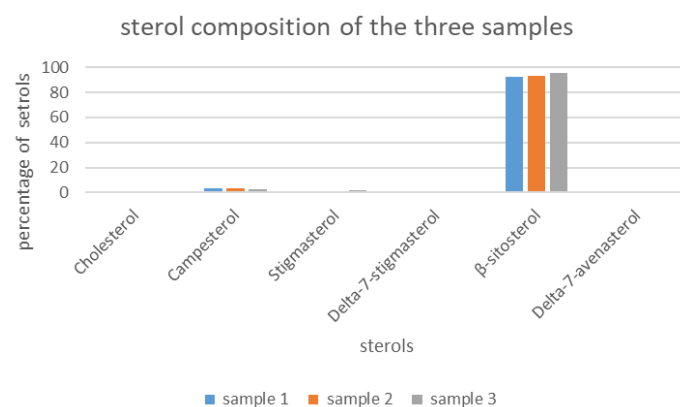


Figure 2: Sterol composition of olive oil.

The sterol composition is in accordance with the data in the literature (IOC/OT/NC No. 1 December 2004). These are essentially β-sitosterol. Their proportion varies between 92.1% and 95.2%. the campesterol content in olive oil varies was between 2.49% and 3.73%.

Our result shows that the percentage of cholesterol varies between 0.01% and 0.06%. This value is in accordance with the norms which indicate that the percentage of cholesterol in virgin olive oil must be inferior to 0.5%.

The sterol result shows that the percentages of stigmasterol and β-sitosterol are higher in sample 3, this sample is extracted by mechanical pressing in the month of December 2020, from these results it can be concluded that the percentages of stigmasterol and β-sitosterol in olive oil increases according to the date of harvest of the olive fruit.

Conclusion and Recommendations

It's concluded that the date of harvest of the olive fruit is a key factor in physico-chemical parameters of extracted olive oil. Present study shown that maturation factor is a parameter influencing the acidity value of olive oil. Indeed, the acidity values of olive oils harvested in October and December (Mediterranean climate) are higher than those of harvested in other months (November). As well as, ripening can influence the percentage of sterols (stigmaterol and β -sistosterol), the percentage of fatty acids such as stearic acid (C18:0). So, the date of harvest of the olive fruit influences the dietetic qualities of the olive oil.

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Novelty Statement

To the our knowledge this is first study conducted on date of harvest on the olive oil quality with focus on effect of olive ripening on oxidative stability in morocco climate.

Author's Contribution

All of authors had similar roles and attempt in conducting, analyzing and writing of present study.

Conflict of Interest

The author declare that there is no conflict of interests regarding the publication of this paper

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