Influence of the heating process on the total phenols and fatty acids composition of virgin olive oil originated in Tunisia, Italy, and Spain

Amor Slama[1,2] ✉
Abdelmajid Zouhir[3]
Ammar Cherif[1,4]

[1] University of Tunis El Manar, Faculty of Sciences of Tunis, Laboratory of Neurophysiology, Cellular Physiopathology and Biomolecules Valorisation, Tunis, Tunisia

[2] University of Carthage, Faculty of Sciences of Bizerte, Jarzouna-Bizerte, Tunisia

[3] Research Unit at Institute of Applied Biological Sciences of Tunis (ISSBAT), Tunis, Tunisia

[4] Department of Biology, College of Science, Qassim University, Qassim 5142, Saudi Arabia

✉ CORRESPONDING AUTHOR:
Amor Slama
Email: slamaamor@yahoo.fr
University of Carthage
Faculty of Sciences of Bizerte
Jarzouna-Bizerte 7021, Tunisia
Phone: +21697622401

Received: February 6, 2023
Accepted: December 5, 2023

ABSTRACT

Olive oil is considered a strategic species for human nutrition across the world particularly in the Mediterranean countries, the major producers of this oil. The objective of this research was to determine the influence of the temperature variation and the origin of the oil on the total phenolic contents and fatty acids composition of virgin olive oil originated in Tunisia, Italy, and Spain. The total polyphenols were determined by spectrophotometer and the fatty acids composition was analysed by means of a chromatographic GC-FID. Results showed that the content of polyphenols reduced slightly during the temperature treatment from 60 to 150°C. However, the amount of polyphenols and polyunsaturated fatty acids decreased mainly at high temperatures (180°C), while, saturated and monounsaturated fatty acids increased. The results also show a positive correlation between the polyphenol content and the total polyunsaturated fatty acids during heat treatment. Based on the fatty acids composition and the polyphenols content, which are an essential indicator of the nutritional value of the oil, a decrease in the nutritional quality of the three types of olive oil was recorded. So, it moved on to the ovoid heating process of olive oil, if necessary the temperature should be inferior to 150°C to preserve the quality and the nutritional value of virgin olive oil.

Keywords: polyphenols, fatty acids, virgin olive oil, heating process

1. INTRODUCTION

Polyphenols are important organic molecules of the unsaponifiable fraction found in many plants and many components of the Mediterranean diet, including olive oil, fruit, and vegetables [1]. The polyphenols are subdivided into simple phenols and polymerised forms. These compounds are secondary plant metabolism products [2]. Their structure contains one or more benzene rings carrying one or more hydroxyl functional groups and other constituents [3]. Polyphenols have powerful anti-inflammatory and antioxidant roles; they also have a beneficial role in the prevention of hypertension. They represent an important contribution to the oxidative stability of olive oil. Their presence in the diet would make it possible to fight against cardiovascular diseases, cancers, and osteoporosis [4,5].The results reported by Manna et al. (1997) suggest that olive oil polyphenols could lower the risk of reactive oxygen metabolite–mediated diseases such as some gastrointestinal diseases and atherosclerosis. Other studies mentioned that the protection of olive oil against cardiovascular diseases and cancer was due to its fatty acid profile and the presence of minor amounts of phenolic constituents [7]. These studies have also emphasised the importance of the phenolic compounds of olive oil as modulators of key mechanisms implicated in the development of atherosclerosis. The Antioxidants have an important role in

protecting humans against infections and degenerative diseases [8].
The types of phenols and their concentrations differ greatly among olive oils, depending on fruit varieties and their maturation degree as well as other agronomic and technological factors, such as the extraction procedures [9]. Referring to the total phenol concentrations, olive oils can, therefore, be divided into three groups, containing low (0.05-0.2 g/kg), medium (0.2-0.5 g/kg) and high (0.5-1.0 g/kg) of total phenol concentrations [10]. It is widely known that the quality of virgin olive oil and polyphenol levels in olives are influenced by various agronomic factors such as olive cultivar, climatic conditions, agricultural practices, and ripeness at harvest [11].
Olive oil is characterised by a high content of monounsaturated fatty acids, in particular, oleic acid; moreover, olive oil is a valid source of essential fatty acids: αlinolenic acid and linoleic acid. This composition has contributed to the reduction of LDL-cholesterol levels and the increase of HDL-cholesterol content in plasma.
The method used in oil's processing has a direct impact on its antioxidant capability [12]. During heat treatment, oil is subjected to hydrolysis, oxidation, and polymerisation. The mechanism of the oxidation processes is the same for different fats and oils, the reaction rates vary for different types of fats and oils [13].The changes in lipids after heat treatment, influence their nutritive value which are in agreement with the results of Gharby et al. (2016b).
The objective of this study was to describe and compare the influence of heating on the content of saturated and unsaturated fatty acids and the total polyphenols of Tunisian, Italian, and Spanish virgin olive oil and then identify the possible correlations between the total polyphenols and fatty acids composition.

2. MATERIALS AND METHODS

2.1 SAMPLES

The virgin olive oil of Tunisia was purchased from the local market, the Italian and Spanish virgin olive oils were purchased from the Quebec market (Canada).

2.2 HEATING CONDITIONS

To study the effect of the increased temperature and duration on the olive oil composition, a heat treatment was carried out as below: 150 ml of each oil sample of the three edible oils was heated to 60, 90, 120, 150 and 180°C for 12 hours each one.

2.3 EXTRACTION AND DETERMINATION OF PHENOLIC COMPOUNDS

The Total phenolic composition was determined by using the Folin–Ciocalteu reagent [15]. A 1 ml from each sample was solubilized in 1 ml of hexane, then 3 ml of the mixture methanol/water (60/40, v/v) were added and shaken vigorously for 1 minute. Then 0.5 ml of solution were mixed with 0.5ml of Folin-Ciocalteu phenol reagent, 3 min later, 0.5 ml of Na2CO3 was added, then completed until 5 ml with deionized water. The mixture has left in the darkness for 2 hours; the optical density is measured at 760 nm against water in the UV-Vis spectrophotometer (Biotek, Power XS2, Logiciel Gen 5) [16]. The assay was calibrated against gallic acid in the 0.3125-5.0mg/100 ml range. Polyphenol concentrations were expressed as mg gallic acid equivalents Kg-1 (mgGAE, Kg-1 oil). Each sample was duplicated three times, and the mean was generated as the result.

2.4 METHYLATION OF FATTY ACIDS

The fatty acid methylation procedure was performed in a standardised way to ensure good accuracy and repeatability of the fatty acids analysis. The derivation of the fatty acid methyl esters was the most used technique for lipid analysis by GC-FID, the most based catalysed trans-esterification agents are sodium or potassium methoxide in anhydrous methanol [17]. Indeed, to the 10 mg of oil sample was added 1 ml of hexane and 500 μl of sodium methoxide, after vortexing, the whole is brought to heating between 40 and 50°C for 15 min. Then 4 ml of hexane were added and 5 ml of water saturated with NaCl was used to wash the sample. Stirred and allowed to stand until the separation of two phases. The organic phase was drawn into a Pasteur pipette containing delipidated cotton and approximately 1 cm of dry magnesium sulphate, the filtrate was collected in a test tube.

2.5 CHROMATOGRAPHIC ANALYSIS

The chromatographic separation was performed in an Auto system gas chromatograph with a split/splitless injector and an FID detector, equipped with a BPX 70 capillary column of 60 m length, 0,25 mm i.d., and 0,25 μm film thicknesses (made in the USA) [18]. The oven temperature was held at 60°C for 1 min then ramped to 190°C at the rate of 10°C/min and maintained for 15 min, before the second ramp at the rate of 5°C/min to 200°C. This was then held isothermally for 14 min. Hydrogen was used as carrier gas with a flow rate of 40 ml/min. The temperature of the injector and detector injector was set at 250°C. The total flow was 68,7 ml/min and the pressure was at 125,5/97,6 Kpa. The volume injected was 1 μL, and the time of analysis was 45 min. The results are expressed as peak area percent. All the measurements were carries out with three independent replicates.

3. RESULTS AND DISCUSSION

Olive oil is one of the Mediterranean countries' important crops and it has an integral part of the Mediterranean diet's economic role [19]. The chemical composition of the fresh virgin olive oils (organic oil) of three geographical provenances (Tunisia, Italy, and Spain) was compared with those subjected to the various heat treatments. The results provided by this study contribute to the understanding the effect of the increased temperature and duration of treatment on the stability and integrity of the biochemical composition of the studied oils (chemical transformation).

3.1 TOTAL FATTY ACIDS COMPOSITION BEFORE HEAT TREATMENT

All studied oils contain palmitic acid and stearic acid as major saturated fatty acids and oleic and linoleic acids as major unsaturated fatty acids (Table I). The Tunisian oil is the richest in saturated fatty acids, whereas the Italian and Spanish oils are the richest in total unsaturated fatty acids. However, Tunisian oil is richer in polyunsaturated fatty acids (C18: 2 and C18: 3) than other oils, the content of these fatty acids is 12.73%, whereas it is only 7.61 and 4.04% respectively for oils from Italy and Spain.
Only the Italian virgin oil contains a trans fatty acid (C18: 1 t) (Fig. 1), the Spanish oil does not contain the C17: 0. The sum of the non-identified fatty acids (NI FA) are 1.48, 1.92, and 1.53% respectively for the oils of Tunisia, Italy, and Spain.

3.2 THE EFFECT OF HEAT TREATMENT ON THE FATTY ACIDS COMPOSITION

The fatty acid composition is an essential indicator of the nutritional value of the oil (Harhar et al., 2011). The

Table I - Fatty acids composition of Tunisian, Italian and Spanish virgin olive oil before treatment.

	Country origin		
Fatty acids (%)	**Tunisian**	**Italian**	**Spanish**
C16:0	16,17	11,22	12,07
C16:1 t	0,06	0,07	0,08
C16:1 c	1,38	0,63	0,93
C17:0	0,04	0,04	-
C17:1	0,05	0,05	0,06
C18:0	2,19	2,09	1,25
C18:1 11c	2,06	1,62	2,75
C18:2 n-6	12,07	6,98	3,47
C18:3 n-3	0,66	0,63	0,57
C20:0	0,33	0,32	0,27
C20:1	0,18	0,23	0,27
εNI FA	1,48	2,03	1,53
εSFA	18,73	13,67	13,59
εUFA	79,79	84,42	84,88

εSFA: Saturated Fatty Acids, εUFA: Unsaturated Fatty Acids,
εNI FA: Non Identified Fatty acids

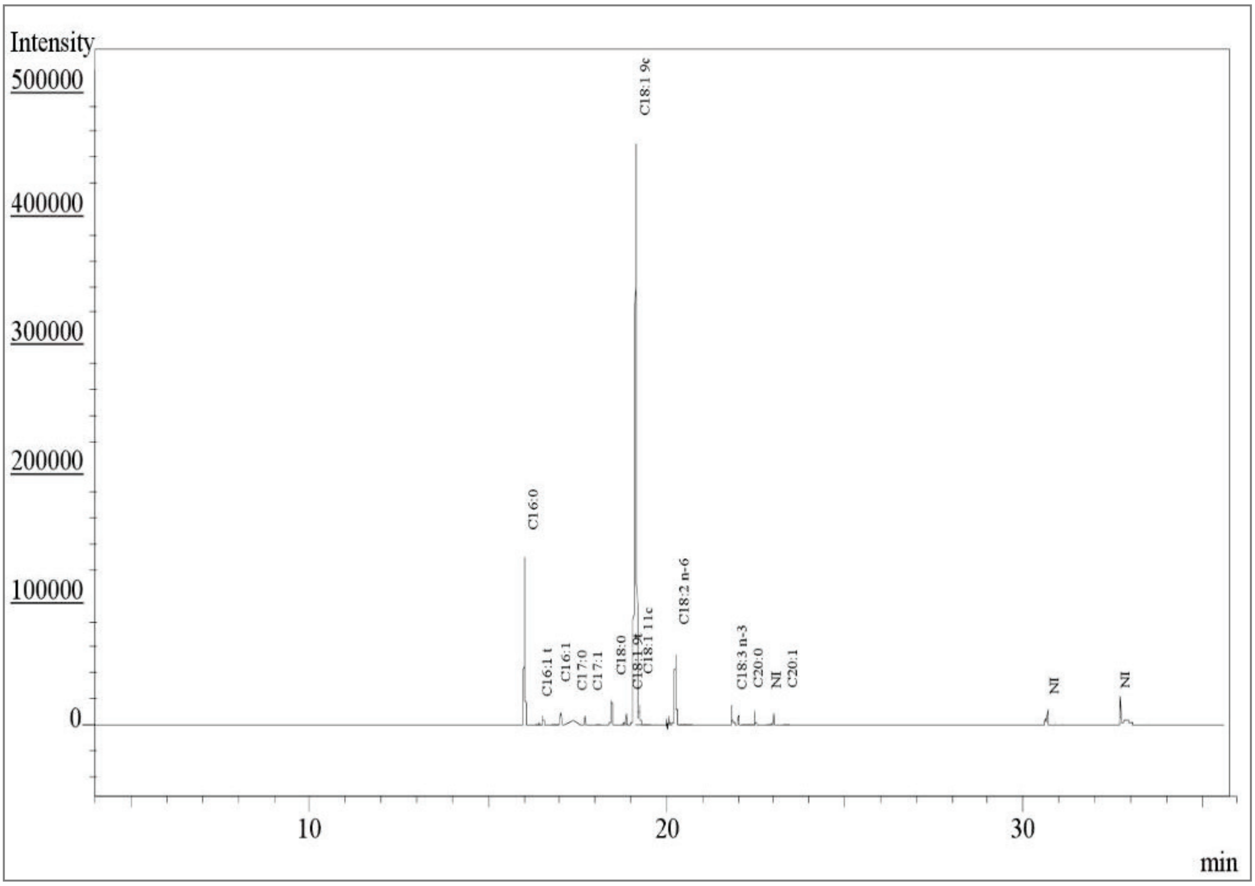

Figure 1 - Chromatogram of methyl esters fatty acids of Italian virgin olive oil obtained. NI: non- identified

heat treatment shows that the virgin olive oils developed a resistance to the increase in temperature; in fact, there is a certain stability of the fatty acid composition of the oils subjected to the temperatures of 60, 90, 120 and 150°C (Table II). Only a slight change in the fatty acid composition was recorded, especially for the total unsaturated fatty acids which decreased from 74.55% to 73.08%. The stability of fatty acid composition from 60 to 150°C suggested that virgin olive oil has a higher content of active antioxidant compounds that protect the unsaturated fatty acids against deterioration during heating.
However, a significant change was recorded at 180°C. An increase in the percentage of saturated fatty acids, notably those of palmitic and stearic acids and monounsaturated fatty acids, and a decrease in the percentage of polyunsaturated fatty acids, in particular those of linoleic and linolenic acids were recorded. These observations are in agreement with a 1H nuclear magnetic resonance study, which confirmed the fact that the fatty acid degradation rate increases with the number of double bonds in the molecule [21]. The study of Gomna et al. (2019) confirmed that the duration of exposure to heat, temperature, oxygen, moisture, and other parameters can affect vegetable oil stability.
The percentage of C16: 1t remained stable throughout the heat treatment time, however, the C18: 1t

Table II - Variation of fatty acids composition during heating of Tunisian virgin olive oil at 60, 90, 120, 150, and 180ºC for 12 hours.

	Heating temperature (ºC)				
Fatty acids (%)	**60**	**90**	**120**	**150**	**180**
C16:0	22,51	22,57	22,66	22,66	23,66
C16:1 t	0,05	0,05	0,05	0,05	0,05
C16:1 c	2,59	2,60	2,58	2,58	2,59
C17:0	0,03	0,03	0,03	0,03	0,04
C17:1	0,04	0,04	0,04	0,04	0,04
C18:0	2,34	2,34	2,35	2,34	2,44
C18:1 9t	-	-	-	-	0,10
C18:1 9c	48,91	48,86	48,99	49,03	49,64
C18:1 11c	2,69	2,69	2,71	2,69	2,72
C18:2 n-6	19,56	19,48	19,33	19,34	17,47
C18:3 n-3	0,59	0,57	0,57	0,57	0,34
C20:0	0,36	0,36	0,36	0,36	0,37
C20:1	0,12	0,12	0,13	0,12	0,13
εSFA	25,24	25,29	25,40	25,40	26,61
εUFA	74,55	74,41	74,40	74,42	73,08
εNI FA	0,21	0,30	0,20	0,18	0,31

εSFA: Saturated Fatty Acids, εUFA: Unsaturated Fatty Acids, εNI FA: Identified Fatty Acids

was detected only at the temperature of 180°C. The evolution of non-identified fatty acids (NI FA) during the heat treatment compared to those of the identified fatty acids could inform about the nature of these fatty acids. These latter which are increased at 180°C can be attributed to saturated and monounsaturated fatty acids.
Overall, the results showed that the composition of the oil changes as a function of duration and of the increase in temperature, could have an impact on its stability and the law of the oxidation mechanism; this is in agreement with the results found by Cao et al. (2020).

3.3 THE EFFECT OF HEAT TREATMENT ON THE POLYPHENOLS COMPOSITION

During heat treatment, the total polyphenols ranged from 67.18 to 180.42 mg/kg; from 55.36 to 103.09 mg/kg and from 56.69 to 112.87 mg/kg for Tunisian, Italian and Spain olive oil, respectively. The general trend shows a reduction of total polyphenols content as a function of the increase in heat treatment.
According to Fig. 2, there are three major changes during the heat treatment of these oils: a decrease of the total polyphenols at T = 60°C for the Spanish and Italian oils, this decrease is rather recorded at T = 90°C in Tunisian oil. An increase in total polyphenols is also marked at T = 90°C in Spanish and Italian oils, however, this increase is noted at T = 60°C in Tunisian oil. The third change is similar in the three types of oil, which is a gradual decrease of the total polyphenols from T = 150°C. The decrease becomes considerable from T = 180°C.
This study shows that Tunisian olive oil contains much more polyphenols than the other two types of olive oil. This gives information on the classification in ascending order of the antioxidant capacity of these oils, which can be indicated as follows: Tunisian, Italian, and then Spanish olive oil. The variation in the contents of the total polyphenols depending on the temperature increase represented by the above curves shows ups and downs for the three types of oil. This suggests that during the heat treatment there is a transformation of the minor compounds of the unsaponifiable fraction into polyphenols, however, some phenolic compounds are transformed or degraded under the action of temperature, this is in agreement with the results found by Alean et al. (2016) who reported a reduction of 45% of polyphenol in dried fruit at a temperature of 40°C, while the higher degradation of polyphenols was presented at a temperature of 60°C. It was concluded that the degradation depends on temperature, moisture, and dry times. On the other hand Abhay et al. (2016) proved that high temperature and heating time harm cocoa polyphenols. The studies reported by Hii et al. (2009) and Ndukwu (2009) show that there is a thermal degradation of volatile phenolic constituents due to high temperature.

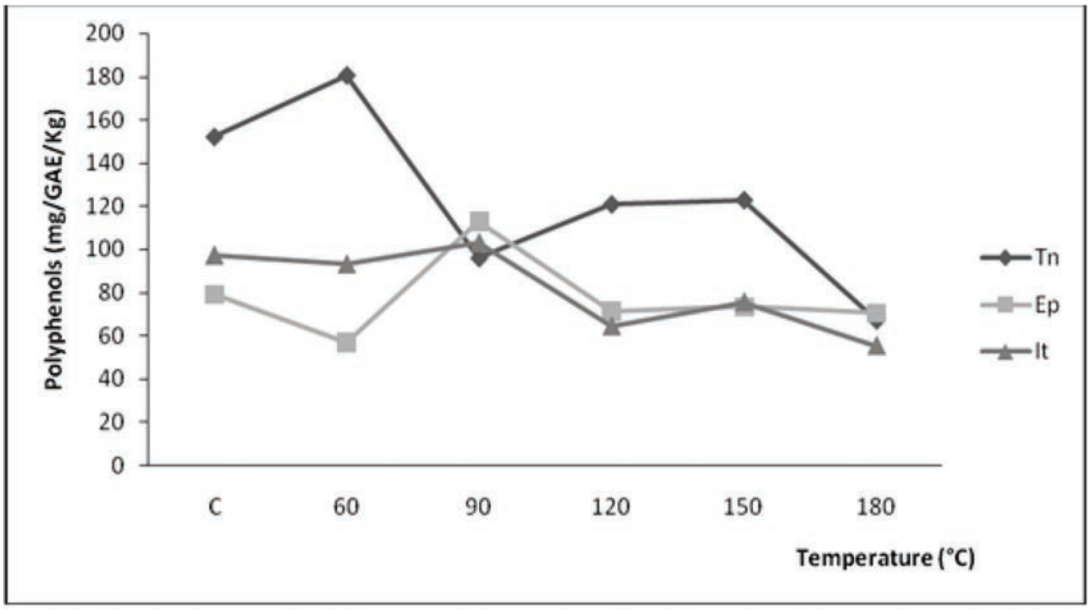

Figure 2 - Variation of total polyphenols during heating processes of Tunisian, Spanish, and Italian virgin olive oil. Tn: Tunisian, Sp: Spanish, It: Italien, C: control

The stability of the oils against oxidation depends not only on the degree of unsaturation but also on the amount of antioxidants present in the unsaponifiable fraction [3]. Other studies Tabee et al., 2008) recorded different results for the stability of oils with similar monounsaturated fatty acids contents during heating depending on the amounts of α-tocopherol and phytosterols present in the oils. The results found by Yi et al. (2020) showed that the antioxidant capacity of corn oils can be increased by enrichment with other tocopherols (γ-oryzanol). According to our results, it can be assumed that the resistance to thermal effects is proportional to the contents of polyphenols, in fact, there is a considerable decline in the amount of total polyphenols when the olive oil was exposed to high temperature (more than 150°C). Therefore, more attention should be paid to the heat treatment used and the heating period to preserve the quality of edible oils [30].

3.4 CORRELATION BETWEEN FATTY ACIDS AND POLYPHENOLS CONTENT

Most results of fatty acids analysis of the Italian olive oil are in agreement with those mentioned by Piscopo et al. (2016); however, the percentages of polyphenols found by those authors were three times higher

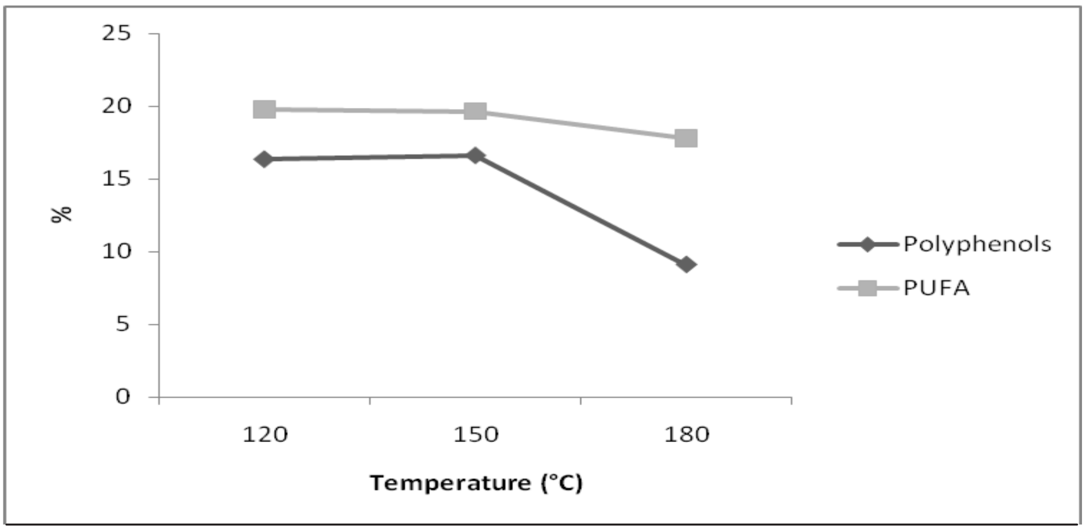

Figure 3 - Relationship between the evolution of polyphenols and polyunsaturated fatty acids (PUFA) of Tunisian virgin olive oil during the heating process. PUFA: Polyunsaturated fatty acids

than those reported by this study.

By examining tables I and II, it can be suggested that the variation in the polyphenol content is proportional to that of the polyunsaturated fatty acids, in particular linoleic and linolenic acid, both of which decrease from T = 150°C (Fig. 3). This could be explained by the sensitivity of these chemical compounds to the temperature increase. Therefore, we should pay more attention to the processing techniques that can be used to preserve the quality of oil and enhance their benefits [32].

4. CONCLUSION

The investigation shows only a slight effect of heating on the fatty acid composition of virgin olive oil at a temperature between 60°C and 150°C; in this condition, the differences between the fatty acid composition before and after heating were not significant. The changes in the fatty acid composition provide insight into the kinetics of the fatty acid oxidation reactions. In fact, the reduction of the content of linoleic and linolenic acid was higher in comparison to the other fatty acids of oil submitted at 180°C.

Olive oil is rich in phenolic compounds, which have a strong antioxidant activity, so, the content and the composition of the polyphenols are further important criteria for the assessment of the quality of the oil.

During heat treatment, there is a slight change in polyphenol content at temperatures of 60°C to 120°C, while the decrease becomes important from 150°C to 180°C, and the effect of the high temperature on polyphenols content was significant. The positive correlation between total polyphenols and polyunsaturated fatty acids, thus begun at 150°C.

The diminution of polyunsaturated fatty acids and polyphenols caused by heat treatment reduces the nutritional value of virgin olive oil; it is advisable to consume this oil in its raw state.

Acknowledgment

We are thankful for the technical support of Mr. Ronan Corcuff

Conflict of interest

The authors confirm that there is no conflicts of interest.

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