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Characterization of Overall Quality of Olive Oil from Different Lebanese Regions

Nada NEHME[1,2], Houssein BAZZI[1], Akram HIJAZI[1*], Hasan RAMMAL[1], Boushra SAYED AHMAD[1], Hanan ASSAF[1], Ziad DAMAJ[1*]
[1]Plateforme de recherche et d'analyse en sciences de l'environnement (EDST-PRASE), Beirut, Lebanon
[2]Faculty of Agricultural Engineering, UL, Lebanon, Lebanese University

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***Corresponding author**
Akram HIJAZI

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Abstract: This study aimed to determine the quality of Lebanese olive oil by analyzing their chemical composition and their physicochemical characteristics. Olive oil samples were first collected from three different Lebanese areas. Physico-chemical investigation (acidity, peroxide index, specific extinction, total polyphenol content as well as chlorophyll and carotenoid contents) of the samples associated with fatty acid analysis by GC-MS technique and NMR characterization demonstrate the compatibility of samples with the standards of International Olive Oil Council (IOC) norms. However, obtained results showed that olive oil samples exhibited a high oxidation status, revealing the necessity of improving oil extraction procedures as well as its storage conditions. Our results confirmed further that olive crushing; harvesting as well as maturity stages have a great impact on the overall quality of obtained oil.
Keywords: olive oil; Lebanese areas, physicochemical, fatty acid, NMR.

INTRODUCTION

Virgin olive oil is a product of olive fruit extraction, made simply by crushing olives and extracting the juice. It is also considered as one of the best sources of fatty acids and natural antioxidants [1]. Virgin olive oil is characterized by a high nutritional value and it is a major component of the Mediterranean diet due to its richness in important bioactive compounds.

Olive oil can be considered a functional food as its consumption is usually accompanied with several therapeutic functions including reduction of risk factors of coronary heart diseases as well as prevention of cancer and alterations of immune and inflammatory systems [2].

It has been demonstrated that olive oil quality is highly influenced by the climatic and agronomic conditions including harvesting time, rainfall, ripening stages and agricultural practices [3].

Oils obtained from olive fruit are commonly divided into two main fractions according to their chemical compositions: major and minor fractions. Major fraction, representing more than 98 % of the total content, is composed predominantly of monounsaturated, polyunsaturated, and saturated fatty acids. Minor fraction contains essential components such as sterol, terpenoids, hydrocarbons, tocopherol and important volatile compounds. As a result, olive oil constitutes a complex multi-component matrix and its analysis is not an easy task [4].

Lebanese olive is considered as original, unlike the Greek olives, Italian or Spanish; it does not come from crossing. Moreover, it is naturally rich in polyphenol and recognized to have important antioxidant properties. Lebanese history of olive tradition is as old as its cultural history: olive culture is an integral part of Lebanese cultural heritage. Olive tree is the symbol of life, eternity, victory, love, peace…[5]. Among the main different countries producing olive oil, Spanish and Italian oils are the most studied. However, limited information is available concerning the constituents of Lebanese olive oil. Although, Lebanon is a small country; its topography is diverse. It has many micro-climates and its soil is naturally fit for the cultivation of olive trees. The aim of this paper is thus to compare the chemical composition and physicochemical characteristics of Lebanese olive oil obtained from three different regions.

MATERIALS AND METHODS

Sample preparation

Three virgin olive oil samples were gathered from three different regions (Akar, Marjeyoun and Bekaa) produced in the same season (2014).

Free acidity analysis

Olive oil samples (30 g) were weighed and solubilized in 30 ml of the mixture of ethanol and ether. The color indicator (phenolphthalein) was then added and this solution was titrated against sodium hydroxide solution (0.1 N). The equivalence point is detected by the appearance of a pink coloring. The acidity is calculated and expressed as the percentage of oleic acid in grams per 100 g of oil.

Peroxide values analysis

Peroxide values are primary oxidation products that are formed when oils are exposed to oxygen, producing undesirable flavors and odors. The presence of a high level of peroxides indicates oxidized and/or poor quality oil. Olive oil samples (5g) were dissolved in a mixture of 30 ml of acetic acid and chloroform with a ratio (18:12). Then 0.5 ml of a saturated solution of potassium iodide was added, the tube was shaken for one minute, and 30 ml of distilled water were added and a titration of the iodine with sodium thiosulfate solution (0.01N) was made in the presence of starch as a dye.

Total phenolic content analysis

The total phenol contents (TPC) were expressed as the Gallic acid equivalents (GAE) (mmol/l) per kilogram of olive oil. Phenolic compounds were extracted with methanol water (60:40) , 100 µl of extract were added to 2 ml of Na_2CO_3 (2%) then 0.5 ml of Folin-Ciocalteau reagent was added and allowed to stand at room temperature for 30 min. Absorbance was measured at 750 nm by U-2900 UV/VIS, spectrophotometer 200 V [7].

Chlorophyll and carotenoid contents analysis

For the determination of chlorophyll content, 5 g of olive oil were dissolved in 5 ml of carbon tetrachloride. After homogenization, the absorption was measured at wavelength of 630 nm, 670 nm and 710 nm. The content of chlorophyll was determined according to the equation:

$$\text{Chlorophyll (ppm)} = \frac{A670-(A630+A\,710)/2}{L\times 0.1086}$$

For the determination of carotenoid content, 1g of olive oil was dissolved in 20 ml of petroleum ether, then the absorption was measured at 470 nm [11].

$$\text{Carotenoid (ppm)} = \frac{\text{A470}\times\text{V}\times 1000}{\text{m}\times 2000}$$

The values of the specific extinction coefficients used were E0 =0.1086 for the chlorophyll and E0=2000 for the carotenoid.

UV spectrophotometric analysis

A 0.1 g of virgin olive oil was dissolved in 10 ml of cyclohexane. After homogenization, the absorption was measured at wavelength of 232 nm ,262 nm,-266 nm and 274 nm respectively [9]. The specific absorption value Kx corresponding to a given wavelength was obtained from the following expression [10]:

$$\text{Kx} = \frac{Ax}{C.S}$$

Where

Ax, the absorption value at a given wavelength C, the concentration of the solution in g /L S, the cuvette thickness in cm K was determined at different wavelengths. The ΔK value was determined as follow:

$$\Delta K = K_{270} - (K_{266} + K_{274})/2$$

Fatty acids analysis

Approximately 100 mg of each sample were dissolved in 2 ml of hexane, with the subsequent addition of 0.2 mL of KOH solution (2M) in methanol. The tubes were shaken for approximately 30 seconds and left to allow phase separation, then 100 µL from the upper layer were injected into the gas chromatography [6].

Nuclear magnetic resonance (NMR) analysis

Southern olive oil (150µl) is mixed with 0.6 ml of chloroform-d in a 5 mm NMR tube. NMR analysis of olive oil is usually used to identify the compounds in mixture without prior separation. This technique can further be used for quantification of constituents if necessary. The NMR spectrum obtained then helps us to qualify and determine the authenticity of the oil.

RESULTS AND DISCUSSION

Free acidity

The acidity is determined by the content of free fatty acids in olive oils resulting from the hydrolysis of triglycerides. Free acidity of different samples was determined as the percentage of oleic acid in oil. Obtained results ranged from 0.77 to 1.7% (Table 1). Based on these results and according to the standard of the commercial IOC, samples from south and North Lebanon can be considered as virgin olive oil, while Bekaa sample is considered as extra-virgin olive oil. In fact, the increase of acidity was related to fermentation of olives during the storage in olive press, the use of plastic bags for storage, and the time that elapses between the time of picking and pressing [12].

Peroxide values

Peroxides are intermediate oxidation products of oil that are formed during oil extraction and processing and when oils are exposed to oxygen and/or light. Peroxides lead to the formation of a complex mixture of volatile compounds such as aldehydes, ketones, hydrocarbons, alcohols and esters responsible producing undesirable flavors and odors. The presence of a high level of peroxides indicates oxidized and/or poor quality oil. Oil storage must be done in dark and

dry places, with temperatures not higher than 15℃ [14].

Table-1: Physicochemical analysis and TPC values of olive oil samples from different Lebanese areas

Areas	South	North	Bekaa	IOC Standard
Acidity (g oleic acid/ 100 mg oil)	1.70±0.20	1.60±0.21	0.77±0.11	≤3.3
Peroxide values (meq O_2/kg of oil)	19.46±0.31	11.92±0.18	17.2±0.20	≤20
Chlorophyll (ppm)	1.51±0.09	2.36±0.27	1.27±0.03	
Carotenoid (ppm)	12.8±1.40	7.40±0.51	11.57±1.35	
TPC (GAE mmol/l/Kg oil)	288.40±10.36	451.84±15.96	399.28±13.67	

The results obtained for the peroxide content are shown in Table 1. The results are expressed in mill-equivalents active oxygen per kilogram of oil (O2 Active mEq / kg of olive oil). The peroxide values oscillate between 11.92 and 19.46 indicating that the studied olive oil samples have good quality.

Total phenol content

Polyphenols are antioxidants found in virgin olive oil and have proven to be important because of their correlation with the pungent and bitter taste of oil. Phenolic compounds play a very important role in the characterization and nutritional value of oils. They can act as antioxidants by helping the body strengthen its defense system against oxidative stress-related abnormalities such as cardiovascular disease, cancer and the inflammatory process [8].

TPC values of our samples are shown in the table 1. Obtained results showed that the studied olive oil samples contain an appreciable amount of phenolic compounds. These amounts varied between 288 and 451 ppm.

The variations observed in polyphenol levels may be due to the difference in the degree of maturity and the geographical area [13].

Chlorophyll and carotenoid contents

Olive oil contains minor compounds that give it its organoleptic and nutritional qualities. Among these minor compounds, mention may be made of pigments such as chlorophyll and carotenoid, which play an important role. The chlorophyll and carotenoid contents for the samples studied in ppm are shown in Table 1. Chlorophyll contents for both South and Bekaa samples are strictly less than 2 mg / Kg. The low levels of chlorophyll help preventing the action of pro-oxidation of pigments and thus ensure good conservation and storage of oil [15]. In olive oil, a carotenoid, acts as protective deactivating free oxygen produced by chlorophyll, and is thus an inhibitor of photo-oxidation [16]. Our results show that southern olive oil is richer in carotene (12.8 ppm) than Bekaa (11.57 ppm) and northern (7.40ppm). Carotenes are natural chemicals involved in the oxidation mechanisms of the oil, their presence in sufficient quantity in the oil can delay the phenomenon of photo oxidation and preserve the quality parameters of the oil during storage[17].

UV-spectrophotometric analysis

The olive oil quality is determined by UV-spectrophotometric analysis; this is due to the absorption of light by fatty acids. Refining causes a change in the configuration of fatty acid and the formation of conjugated dienes and trienes. Increased values of K232 and K268 in olive oil indicate the presence of refined oils. Autoxidation reactions are also associated with conjugation, due to the formation of either carbon-carbon bonds or carbon-oxygen bonds which cause an increase of absorption in the region between 225 and 325nm.

Referring to the IOC standards, K232 should not exceed 2.5 for extra virgin oil as the K270 it has a 0.22 as limit. The values of specific extinctions ultraviolet K232 and K270 obtained for samples, shown in Figure 1, indicate that they do not exceed the limits set by the IOC for virgin olive oils. As regards to the variation of the specific extinction, it slightly differs from one sample to another, but its value is comparable to the one established by the IOC ($\Delta K < 0.01$).

The increase in the coefficient of extinction can be explained by the late harvest of olives, warming of the olive paste during the extraction process as well as the olives exposure to oxygen and light. Similarly, the higher value of K232 explains that the oil studied is peroxided, and the increase of K272 value indicates the richness of oil in secondary oxidation products resulting in low ability preservation.

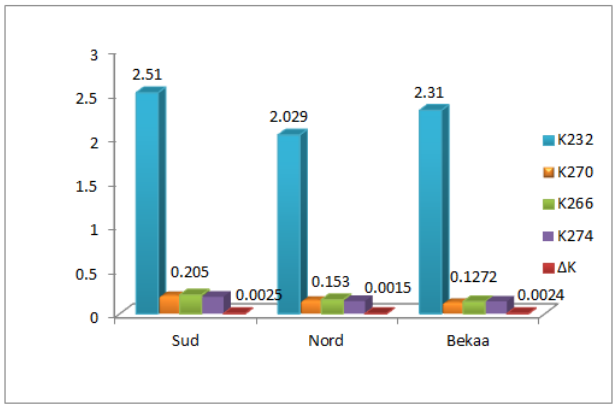

Fig-1: Specific UV extinction at different wavelengths and ΔK of studied samples

Fatty acid composition

Fatty acid profiles of different samples are presented in the table 2. The presence of monounsaturated fatty acid: linoleic acid (C18: 2) with a significant percentage compared to other unsaturated fatty acids can be explained by the presence of an enzyme, oleate desaturase which converts oleic acid (C18:1) to linoleic acid (C18: 2) during fruit ripening.

The amount of oleic acid is the most important in South sample and that of the Bekaa and the North eventually. The percentages of fatty acids are obtained between the IOC limits for the three samples analyzed [10].

The fatty acid composition of olive oil is an important factor in determining the length of the shelf life that is quantitatively affected by two main factors: olive variety used in the manufacture of oil and maturation step during which the olives are harvested. The first stage of ripening fruit is rich in phenolic compounds and linoleic and oleic acid and this can lead to a good shelf life [18].

Table-2: Fatty acid profiles of olive oil samples from different Lebanese areas

Areas Fatty acid (%)	South	North	Bekaa
Myristic acid	0.03	0.01	0.01
Palmitic acid	9.63	12.46	12.08
Palmitoleic acid	0.34	0.32	0.53
Margaric acid	0.01	0.25	0.09
Margaroleic acid	0.19	0.21	0.20
Stearic acid	4.14	3.29	3.42
Oleic acid	81.12	77.43	79.26
Vaccinic acid	0.07	0.07	0.07
Linoleic acid	3.30	4.76	2.95
ã-linoleic acid	0.14	0.12	0.12
Arachidonic acid	0.49	0.54	0.42
Gadoleic acid	0.37	0.36	0.66
Behemic acid	0.11	0.12	0.14

NMR spectrum

According to the following 1H NMR spectrum shown in the figure 2, and referring to the work of Dais and Hatzakis [19], the compounds found in our sample were identified with respect to their chemical shift.

The peak obtained at 5.305 ppm corresponds to hydrogen (C**H** = CH) located next to the double bond of all the unsaturated fatty acids.

The δH signal equal to 5.164 ppm corresponds to the hydrogen (C**H**-OCOR) in triglycerides. Similarly, the hydrogen of C**H$_2$** in triglycerides has a characteristic signal at δH = 4.19 ppm.

The hydrogens CH_2 of the following CH = CH-C**H$_2$**-CH = CH chain of linoleic and linolenic acids have a characteristic signal around 2.780 ppm.

The shift around 2.230 ppm corresponds to the CH_2 hydrogens of all C**H$_2$**-COOH acyl chains.

The linolenic chains as well as the linoleyl and oleoyl chains are characterized by the hydrogens of CH_2 at 2.048 ppm (C**H$_2$** -CH = CH).

A signal of δH = 1.621 corresponds to the hydrogens of all the C**H**$_2$-CH$_2$-COOH acyl chains.

The hydrogens of the acyl chains (C**H**$_2$) n are around 1.2 ppm.

The chemical shift at 0.959 is attributed to the hydrogens of the linolenic chain CH = CH-CH$_2$-C**H**$_3$.

The signal at 0.85 ppm corresponds to the CH$_3$ hydrogens of all the acyl chains with the exception of the linolenic CH$_2$-CH$_2$-CH$_2$-C**H**$_3$.

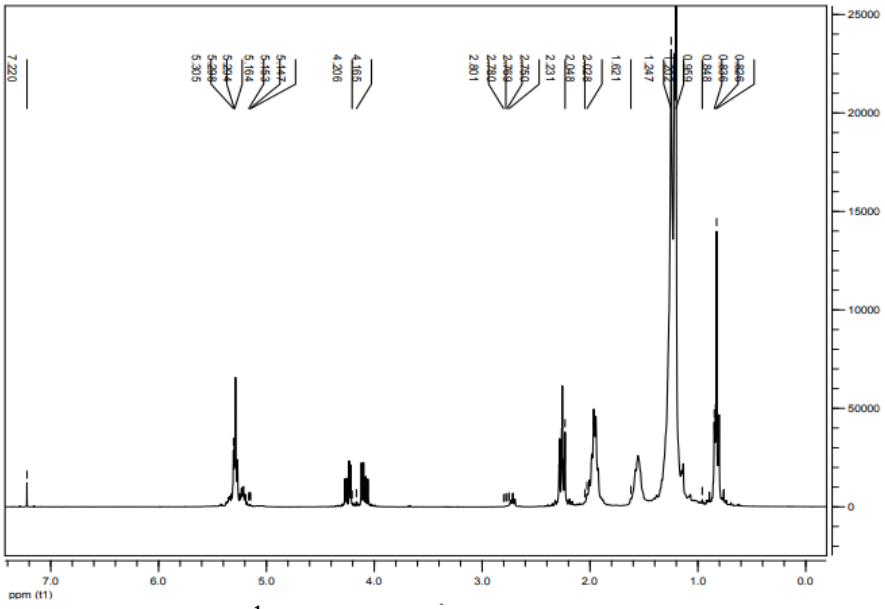

Fig-2: ^{1}H NMR spectrum in olive oil in CDCl$_3$

The identity of these compounds allows us to qualify this oil as oil not mixed with other fat and to ensure its authenticity.

CONCLUSION

A broader sampling series will be conducted to judge the results, representative samples of all regions and cultivation method. As a suggestion, efforts must be deployed in the Ministry of Agriculture and other authorities to disclose the best methods of collecting and storage as a guide distributed to farmers and contractors in order to achieve a convenience meets international standards and can be exported to the outside.

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