ORIGINAL ARTICLE

IMPORTANCE OF THE ETHYL ESTERS ANALYSIS IN IMPROVEMENT OF THE MOROCCAN EXTRA VIRGIN OLIVE OIL QUALITY EVALUATION

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ABSTRACT

Background: The global olive oil consumption is increasing in recent years. Studies on olive oil, considering its nutritional and medical properties, have an important place in the scientific research. **Objective**: This study aims to evaluate the quality of Moroccan extra virgin olive oil, by analyzing its ethyl ester content as a new marker of olive oil quality, in comparison to the usual physico-chemical and sensory assessment markers employed in the industry. This marker becomes relevant knowing the health benefits of high quality olive oil; in nutritional prevention of cardiovascular diseases and cancer. **Methods**: This study has been performed on 23 samples of extra virgin olive oil collected, from the 2014 Agricultural Campaign held in different regions of Morocco. A physico-chemical analysis of acidity and peroxide value was conducted by spectrophotometry and titration. Then the ethyl esters analysis was done by gas chromatography after purification on chromatographic column according, to the IOC (International Olive Council) Standard 2012 (Standard COI/T.5/NC No. 3/REV.7 (2012). **Results**: The results obtained have shown that, the 23 samples meet the standards of extra virgin olive oil quality based on acidity, peroxide index and the specific UV extinction. However the results of the ethyl esters analysis have shown non-compliance in 7 samples representing 30% of all the 23 samples. The ethyl esters results correlation study with those of the sensory analysis has demonstrated that; the non-compliant samples have negative attributes due to certain fermentation. **Conclusion**: We concluded that the ethyl esters analysis has brought a considerable added value in the assessment of olive oil quality. This also shown that ethyl ester content is a powerful marker of the extra virgin olive oil quality.

Keywords: extra virgin olive oil, International Olive Council, campaign, analysis, ethyl esters analysis.

1. INTRODUCTION

The global olive oil consumption is increasing in recent years. Studies on olive oil, considering its nutritional and medical properties, have an important place in the scientific research. It is one of the oldest vegetable oils and the only one which can be consumed in its raw form without any prior treatment after the olive crushing [1]. Its global market potential focuses specifically at the Mediterranean basin, giving the countries in this region the monopoly of exports. Taking advantage of the changing needs that result in demand towards more differentiated products of biological origin, the olive oil global market is in an ascending trend both in terms of production and export prices. Therefore, olive oil has become the Mediterranean product ultimate, considered at the base of the Mediterranean diet as an essential product, just like vegetables, fruits, whole grains, herbs, spices, and oilseeds.

Several studies have demonstrated the relevant role of olive oil in the prevention of several chronic diseases. Other studies have also confirmed the association of the olive oil consumption to certain impact on cardiovascular diseases, neurological disorders and some types of cancer, taking into account its anti-oxidant proprieties. These benefits have been linked to its important fatty acid content, particularly its main component, oleic acid, as well as the minor components: vitamins and natural antioxidants [2].

In Morocco, olive oil has a strong economic and social connotation. Moreover, the national agricultural land is largely used for olive cultivation which makes it the main fruit crop of the country [3]. However, with the current exploitable agricultural capabilities estimated at 590 thousand hectares, and an olive oil production of 120 thousand T [4], it remains far from the real national olive potential. Morocco is ranked in the 5th place among all the olive oil producing countries from the European Community, Turkey, Tunisia and Syria.

Due to its peculiarities, its important nutritional value and its economic weight, olive oil is subject to well-defined quality control regulations and to strict criteria for its classification by physicochemical and sensory analyses. These tests affirm; state of freshness of the oil as well as its degree of purity or possible adulteration with seed oils or refined olive oil.

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In 2009 the International Olive Council had established ethyl ester content as a new marker for extra virgin olive oils, the presence of ethyl esters is a strong sign of the fermentation of virgin olive oil. They are obtained by a chemical reaction between free fatty acids and the ethanol contained in the fruit of the olive tree. These markers are not removed during the refining process, and their quantification can help differentiate authentic extra virgin olive oils from fraudulent mixtures, assuring better quality products in accordance with IOC requirements. The allowable limit set is 75mg/kg for total alkyl esters content, 1.5 mg/kg for ethyl esters/methyl esters and 35mg/kg for the ethyl esters level as recommended in Campaign 2015- [4].

In this context, our study aims to determinate the content of ethyl esters in samples which have been classified as extra virgin olive oil after undergoing physico-chemical analyses such as: acidity, peroxide value and specific UV (Ultra Violet) extinction according to the methods and the standards required by the IOC. This study will permit us to confirm whether they are really of extra virgin olive oil quality and thereby promote Moroccan varieties in relation to international products even from within the IOC and the European Committee.

2. MATERIALS AND METHODS

2.1 Free acidity

2.1.1 Scope and field of application: This method describes the determination of free fatty acids in olive oils and olive pomace oils. The content of free fatty acids is expressed as acidity, calculated as the percentage of oleic acid [5].

2.1.2 Principle: The olive oil sample is dissolved in a mixture of diethyl ether and ethanol solvent with equal parts by volume which is neutralized with phenolphthalein. The free fatty acids mixture is then titrated with potassium hydroxide solution (0.1N) [5].

Acidity, as percentage of oleic acid by weight is equal to:

$$\%\,A = \frac{V \times C \times M}{10 \times m} \qquad (1)$$

Where:
V = the volume of titrated potassium hydroxide solution used, in millilitres;
c = the exact concentration in moles per litre of the titrated solution of potassium hydroxide used;
M = 282 g/mol, the molar mass in grams per mole of oleic acid;
m = the mass of the sample, in grams.

2.1.3 Equipment : 10ml burette classe 1, graduated in 0,05ml with automatic zero adjustment,
Analytical balance

2.2 Peroxide value

2.2.1 Scope and field of application: This Standard describes a method for the determination of the peroxide value of animal and vegetable oils and fats. Peroxide index is defined as the number of active oxygen in milliequivalents per kilogram fat. It expresses the oxidation rate of products present in the olive oil [5].

2.2.3 Principle: The oil is treated with acetic acid and iso-octane and then titrated with potassium iodide solution. The liberated iodine is then titrated with sodium thiosulfate solution (0.01N) [5].
The peroxide value (PV), expressed in milliequivalents of active oxygen per kilogram, is given by the formula:

$$PV = \frac{V \times T \times 1000}{m} \qquad (2)$$

Where:

V= is the number of ml of the standardized sodium thiosulphate solution used for the test, corrected to take into account the blank test
T =is the exact molarity of the sodium thiosulphate solution used, in mol/l.
m = is the weight in g, of the test portion.

2.2.4 Equipment: Burette 25-ml capacity, graduated in at least 0.05 ml, with automatic zero adjustment, Analytical balance

2.3 Spectrophotometric investigation in the ultraviolet

2.3.1 Scope and field of application: Spectrophotometric examination in the ultraviolet can provide information on the quality of a fat, its state of preservation and changes brought about by technological processes. The absorption at the wavelengths specified in the method is due to the presence of conjugated diene and triene systems resulting from olive oil oxidation processes during its storage and conservation [5].

2.3.2 Principle: Specific extinction is determined by dissolving the olive oil sample in cyclohexane and then measured by UV spectrophotometry at wavelength 270/266/274 nm [5].

Record the specific extinctions (extinction coefficients) at the various wavelengths calculated as follows:

$$K\lambda = \frac{E\lambda}{c \times s} \qquad (3)$$

Where:
K = specific extinction at wavelength;
E = extinction measured at wavelength;
c = concentration of the solution in g/100 ml;
s = path length of the quartz cell in cm; expressed to two decimal places.

The variation of the absolute value of the extinction (ΔK) is given by:

$$\Delta K = [Km - (\frac{K\lambda m - 4 + K\lambda m + 4}{2})] \qquad (4)$$

Where
Km: is the specific extinction at the wavelength for maximum absorption at 270 nm and 268nm depending on the solvent used.

2.3.4 Equipment: A spectrophotometer suitable for measurements at ultraviolet wavelengths (220 nm to 360 nm), with the capability of reading individual nanometric units. A regular check is recommended for the accuracy and reproducibility of the absorbance and wavelength scales as well as for stray light.

Rectangular quartz cuvettes, with covers, suitable for measurements at the ultraviolet wavelengths (220 to 360 nm) having an optical path-length of 10 mm. When filled with water or other suitable solvent; the cuvettes should not show differences between them of more than 0.01 extinction units [5].

2.4 Ethyl esters

2.4.1 Scope and field of application: The methodology used to determine the olive oil ethyl esters fatty acids content involves separating the ethyl esters according to the number of carbon atoms. This criterion is used as a quality parameter for extra virgin olive oils, to the extent that it allows the detection of extra virgin olive oil adulterated with other lower quality oils, or even with ordinary, lampante or deodorized virgin olive oils [4].

2.4.2 Principle: This process involves the addition of suitable internal standards and fractionation of extra virgin olive oil by chromatography on a hydrated silica gel column (15 mm internal diameter, 30 to 40 cm in height, and equipped with a tap). The fraction obtained is eluted first under the test conditions, collected, then directly analyzed by capillary gas chromatography [4].

2.4.3 Quantitative analysis of the ethyl esters: The areas under the peaks corresponding to the methyl hepta-decanoate internal standard, the ethyl esters of the C16 and C18 fatty acids are measured with the aid of an integrator.
Determine the content of each ethyl ester, in mg/Kg of fat, as follows:

$$\text{Ester, mg/Kg} = \frac{A_x \times ms \times 1000}{A_s \times m} \qquad (5)$$

Where,

A_x = area corresponding to the peak for the individual C16 and C18 ester, in computer counts
$\mathbf{A_s}$= area corresponding to the peak for the methyl heptadecanoate internal standard in computer counts
$\mathbf{m_s}$= mass of the methyl heptadecanoate internal standard added, in milligrams,
m= mass of the sample taken for determination, in grams.

2.4.4 Procedure

2.4.4.1 Preparation of the chromatographic column: Fifteen grams of silica gel is suspended in n-hexane and introduced into a chromatographic column. An electric stirrer is used to make the chromatographic layer more homogenous. Then 30 ml of n-hexane is percolated in order to remove any impurities. Once the column is ready, 500 mg of the sample is measured in a 25 ml flask. Subsequently an appropriate amount of internal standard is added depending on the presumed content of the ethyl esters. The treated sample is introduced into the chromatographic column, using two fractions of 2 ml each of n-hexane. The solvent is allowed to flow until it is 1 mm above the upper level of the absorbent before percolating 220 ml of n-hexane/ethyl ether mixture(99: 1 ratio) while maintaining a flow rate of about 15 drops every 10 seconds. (This fraction contains methyl and ethyl esters and waxes) [4].

The fraction obtained is dried in the rotary evaporator until the solvent is practically removed. The last 2 ml of the solvent are eliminated under a low stream of nitrogen. This fraction containing the methyl and ethyl esters is subsequently dissolved in 2 to 4 ml of hexane [4].

2.4.4.2 Equipment

✓ Glass column for liquid chromathography, internal diameter 15 mm, lengnt 40 cm, fitted with a suitable stopcock
✓ Gas chromatography suitable for use with a cappilary column (fused silica, lengnt 12m internal diameter 0,25 mm,internally) equipped with a system for direct, on-column injection.
✓ Rotary evaporator
✓ Analytical balance

2.5 Sensory analysis

2.5.1 Purpose: The purpose of this international method is to determine the procedure for assessing the organoleptic characteristics of virgin olive oil, and to establish the method for its classification on the basis of those characteristics [6].

2.5.2 Scope and field of application

The method described is only applicable to extra virgin olive oils, and to the classification of such oils according to the intensity of the defects perceived and of the fruitiness [6].

2.5.2.1 Principle

The examination of the organoleptic properties of olive oil is done through sensory assessment. A selected group of tasters, trained to IOC standards, conduct and monitor the sensory tests as a jury; smelling and tasting the product to evaluate and classify it according to perceived characteristics and attributes [4].

2.5.2.2 Equipment

Test room:

✓ glasses (standardised) containing the samples, code numbered, covered with a watch-glass and kept at 28 ºC ± 2 ºC;
✓ profile sheet on hard copy, or on soft copy provided that the conditions of the profile sheet are met, together with the instructions for its use if necessary;
✓ trays with slices of apple and/or water, carbonated water and/or rusks
✓ glass of water at ambient temperature;
✓ spittoons.

3. RESULTS

The physico-chemical analysis which includes free acidity, peroxide value and specific UV extinction was done on the 23 samples of extra virgin olive oil. Results show that all the samples meet the criteria set for extra virgin olive oil except those that have acidity level higher than 0.8 (according to the IOC standard) [7, 8].

Table 1: Physico-chemical analysis of the 23 samples of extra virgin olive oil.

	2014 Campaign			
	Average	**Min**	**Max**	**IOC standards**
Free acidity	0.43	0.12	1.3	0.8 %
Peroxide value	5.32	1.6	13,4	20 meq/Kg
Specific UV extinction at 270 nm	0.15	0.09	0.22	0.22

As shown in Table 1, the peroxide value and the coefficient of the specific UV extinction at 270 nm meet the IOC standards(a peroxide level less than 20 meq/kg, values obtained for coefficient of the specific UV extinction falls within the range of 0.09 – 0.22)

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The ethyl esters content analysis was performed on all the samples. It identifies non-compliance for 30% of the samples as shown on Figure 1.

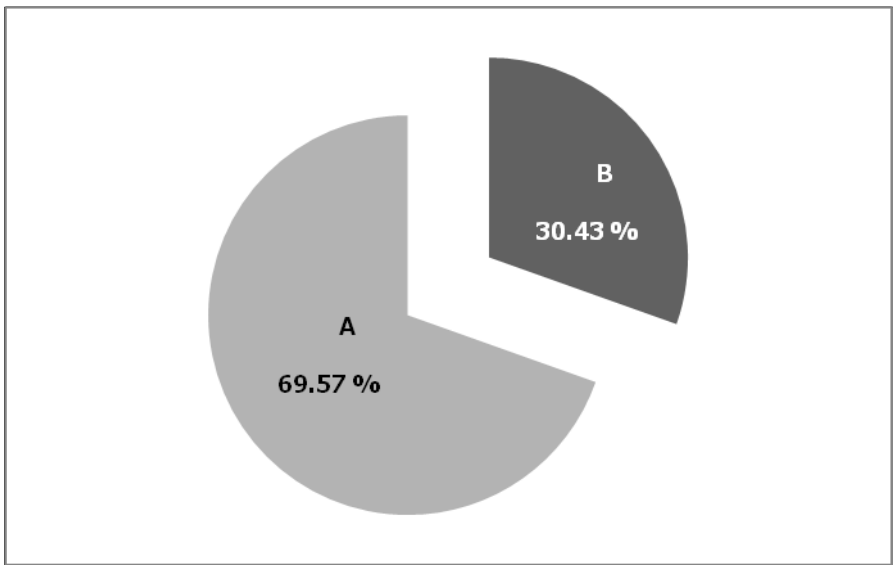

Figure 1: The figure presents the percentage results of ethyl esters content in extra virgin olive oil.

A: 30, 43 % of samples having an ethyl esters percentage above the IOC standard
B: 69, 57 % of samples having an ethyl esters percentage lower the IOC standard

After the ethyl esters content analysis, a sensory analysis was also performed on the corresponding samples.

Table 2: Sensory analysis results of extra virgin olive oil samples.

Sensory analysis	**Robust fruitiness**	**Medium green fruitiness**	**Ripe fruitiness**	**Winey**	**Fusty**
Number of samples	2	5	8	7	7
%	9.10	22.73	36.36	31.81	31.81

While samples that have lower values in ethyl esters show a compliant organoleptic. Our results show that the majority of samples have a compliant organoleptic assessment with a percentage of almost 70 %, whereas except 30 % of samples identified as non- compliant, with certain tasting defects such as: fusty, winey.

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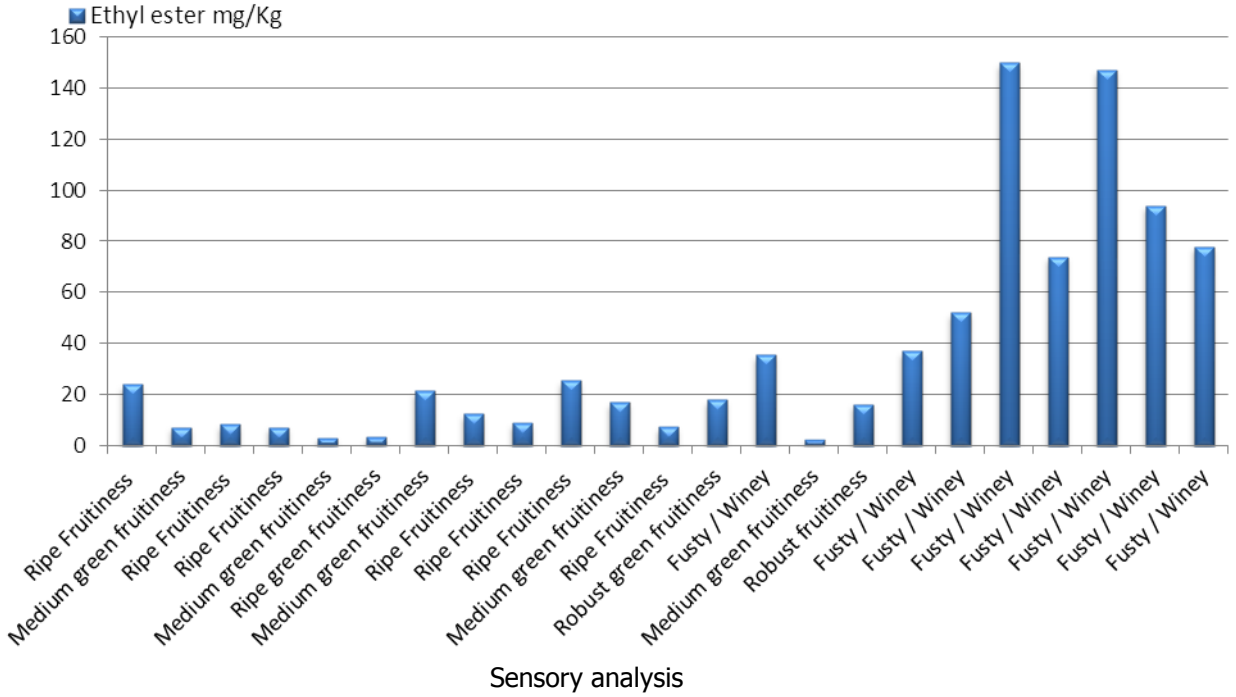

Figure 2: Correlation between ethyl esters content and sensory analysis of extra virgin olive oil samples.

According to the sensory analysis results and ethyl esters content, we found that samples with high levels in ethyl esters have identified a non-compliant organoleptic assessment; with certain tasting defects such as: fusty, winey.

While samples that had lower values in ethyl esters showed; a compliant organoleptic analysis conforms to the IOC standard with positive attributes such as robust fruitiness, green fruitiness.

4. DISCUSSION

In order to guarantee the quality of Moroccan extra virgin olive oils, producers and consumers countries tend to integrate new physicochemical criteria, which aim to limit the production of poor quality oils and of oils adulterated with defective lots or old olive oil product [1].

For that purpose, the International Olive Council has imposed the application of ethyl esters assay as a new quality criterion and consequently, it has fixed in the commercial standard the statutory limits for it [9]. At present the chemists of IOC have preferentially suggested at first to limit the ethyl esters content to 35mg/Kg then to gradually lower it to 30 mg/Kg beyond 2016 [6].

Olives contain a certain amount of sugars which will be transformed into ethyl alcohol and methyl alcohol, through fermentation. Generally olive oil contains free fatty acids at varying levels which give an idea about the quality of the olives as well as the aging of the oils. Free fatty acids (R-COOH) and the methyl and ethyl alcohols (R'-OH) react by the esterification process to form ethyl esters FAEE and methyl esters FAME. [10].

In this study, the analyses of the samples are done in 3 parts:

The first step is to classify the oil samples as an extra virgin olive oil according to the IOC standard: the free acidity must not exceed 0.8 per cent; the peroxide index must be less than 20 meqO2/kg; and the specific UV extinction at 270 nm has a maximum value of 0.22. Our results show that most of the samples can be considered as extra virgin olive oils except for those that have higher acidity level than 0.8.

This means that, the olive oil samples did not undergo a strong alteration of fatty acids by atmospheric oxygen during the trituration steps and; thus contain very little oxidation products. However, these criteria remain incomplete to confirm the quality extra virgin olive oil. The second step, an ethyl esters content analysis was therefore necessary. For that, the fatty acid ethyl esters content (FAEE) in the extra virgin olive oil was calculated after its purification on chromatographic column and separation by gas chromatography.

In contrast to the classic physicochemical criteria employed that classify all the samples to be of extra virgin olive oil quality, the ethyl esters analysis reveals a non-compliance in 7 samples. This indicates that 30% of the samples analyzed have more than 35 mg/kg ethyl esters content, the limit set by the IOC [4]. Such a threshold limit is necessary to ensure that the extra virgin olive oils are not altered and meet the IOC requirements for the said products classification [11].

These results confirm the inadequacy of the classic physico-chemical criteria for an accurate determination of extra virgin olive oils quality.
The third step, the sensory analysis was conducted to compare and confirm the importance of the ethyl esters analysis in the detection of the presence of adulterants in olive oil [12].

The results of sensory analysis allowed us to note some defects in samples containing high level of ethyl esters such as: fusty, and venous. This can be due to the fermentation occurring in the olives prior to crushing or to a contamination on storage period. These results agree with the results obtained in some other studies [13].

Meanwhile, the samples with lower levels (even down to zero) than the limit (35mg/kg) have revealed a compliant analysis illustrating the positive attributes such as: ripe fruitiness, intense fruity. These results confirm the importance of the correlation and of the strong concordance between sensory analysis and the ethyl esters content in order to guarantee a complete evaluation thereby improving the quality of the olive oils in the market [14].

5. CONCLUSION

We conclude that the classic physico-chemical criteria (free acidity, peroxide value and specific UV extinction) appear insufficient in determining extra virgin olive oil quality; the results of the sensory assessment are in concordance with the results of ethyl esters content analysis; and that the ethyl esters content analysis is an important and powerful tool in the assessment of extra virgin olive oil quality.

Our finding will help us promote the different varieties of Moroccan olive oil at the international forums, for the consumers to appreciate its quality and would lead to an increase in demand and in the preservation of the nobility of a traditional product that we consider an essential ingredient in our daily nutrition.

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Conflicts of interest

This work has been dedicated to enrich the scientific research and has no conflicts of interest.

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