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REPORT

EVALUATION OF QUALITY PARAMETERS AND BIOLOGICAL PROPERTIES OF IL RE EXTRA VIRGIN OLIVE OIL

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INTRODUCTION

The Mediterranean diet is recommended by UNESCO as a nutritional prototype of worldwide value [1], which is characterized by the consumption of significant amounts of fruit, vegetables, legumes, cereals, fish and seafood.

Olives and olive-derived products, including extra virgin olive oil (EVOO), play a key role in the Mediterranean diet and represent a valuable source of polyphenols [3-5], natural phenolic compounds that are attracting relevant interest due to their relevant biological properties.

In order for an olive oil to be considered extra virgin, it must be obtained from the fruit of the olive trees solely by mechanical or other physical means, under conditions that do not lead to alteration in the oil and without any treatment other than washing, decantation, centrifugation or filtration [6]. As a result, EVOO contains a large number of phenolic compounds, including simple phenols, lignans, flavonoids and secoiridoids. Among them, oleuropein, hydroxytyrosol and tyrosol are the most abundant in olive oil and they are characterized by antioxidant, antimicrobial and anti-inflammatory activities [7].

Aim of the study

The fruit is characterized by a spherical shape and reaches 3-4 grams. The maturation is delayed, with a high production of fruits and a yield in oil fluctuating between 14 and 16%.
The present study aims to evaluate the biological properties of extra virgin olive oil from Il Re in terms of scavenging activity towards free radicals, such as DPPH and ABTS, total antioxidant activity and total polyphenols content. Furthermore, quality parameters including free acidity, peroxide value and ultraviolet absorption indices (K232, K270 and ΔK) were determined.

QUALITY PARAMETERS

Determination of free acidity

The “acidity” in olive oil is the result of the degree of breakdown of the triacylglycerols, due to a chemical reaction called hydrolysis or lipolysis, in which free fatty acids are formed. Oil extracted carelessly and/or from poor quality fruit suffers from a very significant breakdown of the triacylglycerides into fatty acids. Therefore, free fatty acid content represents one of the most relevant parameter featuring the quality of an olive oil, it is often assessed in order to classify the oil and reflects the care taken right from blossoming and fruit set to sale and consumption. Factors, which lead to a high free fatty acidity in an oil, include fruit fly infestation, delays between harvesting and extraction, fungal diseases in the fruit, prolonged contact between oil and vegetation water and careless extraction methods.

The acidity of the tested oil sample from Il Re was evaluated according to Reg CEE 2568/1991 11/07/1991 GU CEE L248 05/09/1991 All II.

The oil sample was dissolved in a mixture of equal parts by volume of ethyl ether and ethyl alcohol and titrated with an ethanolic solution of potassium hydroxide using phenolphthalein as indicator.

The results were expressed as grams of oleic acid per 100 grams of oil, commonly known as the free fatty acidity of the oil in percent. **The maximum level of free acidity for an extra virgin olive oil is 0.8 gram per 100 grams.**

QUALITY PARAMETERS	RESULTS
Free acidity	0.17% oleic acid
Peroxide value (PV)	3.8 meq O_2/kg
K232	1.664
K270	0.104
ΔK	0.001

The tested item has been shown a free acidity equal to ***0.17%*** (*Table 1.*), which means that the olive oil from Il Re can be classified as an ***extra virgin olive oil***.

Table 1. *Quality parameters.*

Determination of peroxide value (PV)

The determination of peroxide value (PV) represents another important quality control parameter for olive oil because it is an indicator of the primary oxidation status of the product. This parameter, indeed, measures the concentration of primary oxidation products, mainly consisting on hydroperoxides that are not stable and decompose producing secondary oxidation products, such as ketones and aldehydes, which are responsible for off-flavour development and rancidity [10, 11].

Oxidation is an inevitable and natural process involving the formation of hydroperoxides and it can occur during processing or storage through auto-oxidation and photo-oxidation [12]. However, it appears later on among the virgin olive oils that present a high percentage of oleic acid and a high polyphenols content.

In the present study, peroxide value was evaluated according to Reg CEE 2568/1991 11/07/1991 GU CEE L248 05/09/1991 All III and expressed as milliequivalents of active oxygen per kilogram (meq O_2/kg).

The obtained peroxide value was equal to 3.8 meq O_2/kg (Table 1.), which was lower than 20, the maximum level of PV for an extra virgin olive oil.

Ultraviolet absorption indices (K232, K270 and ΔK)

The aforementioned parameters, such as free acidity and peroxide value, together with spectrophotometric indices (K232, K270 and ΔK) are valuable olive oil freshness indices.

The UV spectrum involves the electronic absorption of fatty acids; in particular, the 230-270 nm band shows high absorption when conjugated dienes and trienes of unsaturated fatty acids are present. For this reason, the absorbances measured at 232 nm and 270 nm, namely K232 and K270, provide an official method for olive oil quality control, which is capable of detecting product oxidation and adulteration by means of rectified oils.

In the present study, the ultraviolet absorption indices were evaluated according to Reg CEE 2568/1991 11/07/1991 GU CEE L248 05/09/1991 All IX, Reg CEE 183/1993 29/01/1993 GU CE L22 31/01/1993.

The obtained low values of K232, K270, and ΔK confirmed the good quality of the tested oil sample from Il Re.

BIOLOGICAL PROPERTIES

Evaluation of the antioxidant activity: scavenging effect on DPPH radicals

The DPPH assay is designed for the evaluation of the antioxidant activity and it is based on the measurement of the scavenging capacity of antioxidants towards DPPH (2,2'-diphenyl-1-picrylhydrazyl) radicals, which are stable organic free radicals with an absorption maximum band around 515-528 nm.

In the DPPH assay, the antioxidant molecule reduces the radical to a yellow-colored compound, diphenylpicrylhydrazine (*Figure 1.*), and the extent of the reaction will depend on the hydrogen-donating ability of the antioxidant.

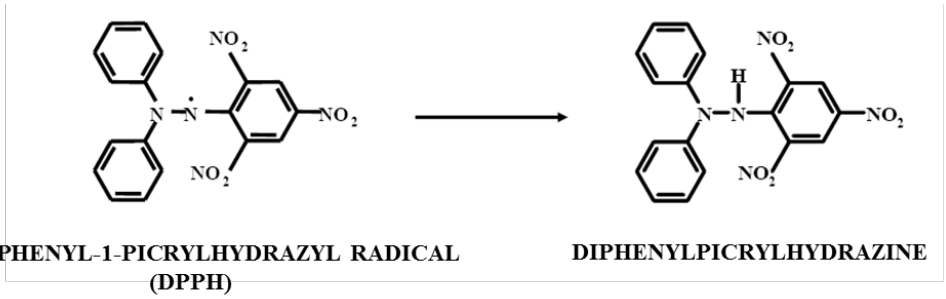

Figure 1.

In order to evaluate the antioxidant activity of the tested oil sample, its scavenging properties toward DPPH radicals were investigated according to literature with slight modification [13] and data were expressed as inhibition percent calculated according to Equation (1):

$$inhibition\% = \frac{A_0 - A_1}{A_0} \times 100 \qquad (1)$$

where A_0 is the absorbance of a standard prepared in the same conditions, but without any sample, and A_1 is the absorbance of the tested item.

The obtained results were reported in *Table 2.* and *Figure 2.*

Table 2. *DPPH inhibition expressed as percentage (mean value ± st. dev.).*

OIL SAMPLE (µL)	DPPH INHIBITION (%) mean value ± st. dev.
30	23 ± 0.9
50	43 ± 0.8
100	82 ± 0.8
150	91 ± 0.6
200	93 ± 0.7
250	95 ± 0.7

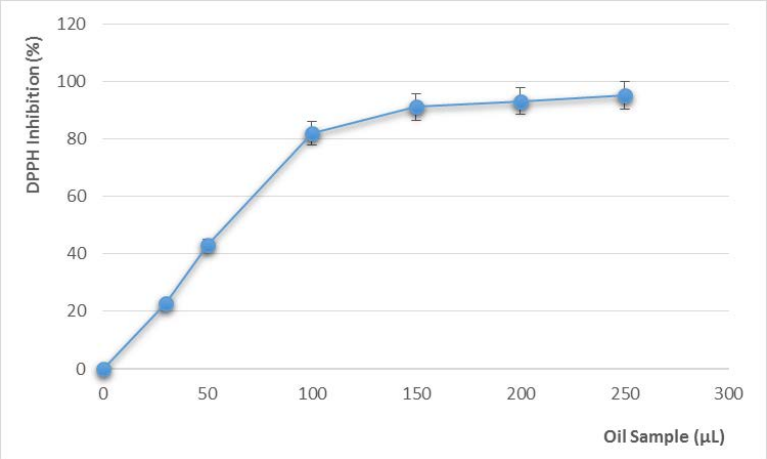

Figure 2.

According to the applied experimental protocol and the obtained results, it is possible to conclude that the oil sample from Il Re is characterized by good antioxidant properties in terms of DPPH scavenging activity.

Oil sample pre-treatment

In order to perform the following assays, the oil sample from Il Re was pretreated obtaining an aqueous extract.

Evaluation of the antioxidant activity: scavenging effect on ABTS radicals

The ABTS assay is designed for the evaluation of the antioxidant activity and it is based on the measurement of the scavenging capacity of antioxidants towards ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) radicals, which are stable organic free radicals with an absorption maximum band around 734 nm.

In order to evaluate the antioxidant activity of the tested oil sample, its scavenging properties toward ABTS radicals were investigated according to literature with slight modification [14] and data were expressed as inhibition percent calculated according to Equation (1).

The obtained results were reported in *Table 3.* and *Figure 3.*

Table 3. *ABTS inhibition expressed as percentage (mean value ± st. dev.).*

OIL SAMPLE (µL)	ABTS INHIBITION (%) mean value ± st. dev.
25	41
50	65
75	81
100	92
125	96
150	98

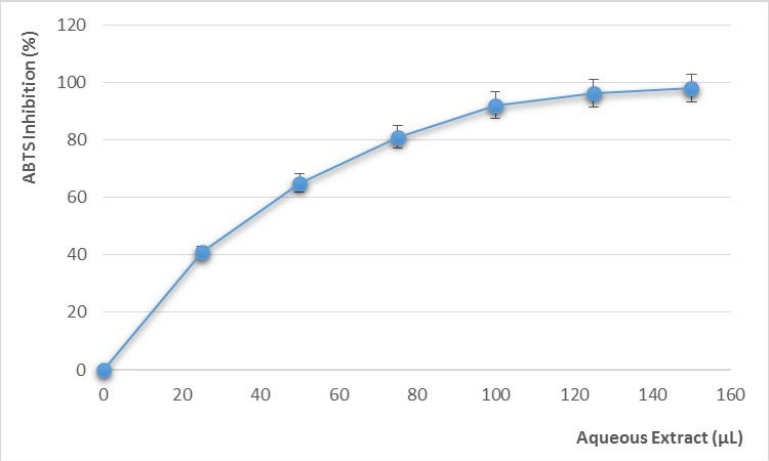

Figure 3.

According to the applied experimental protocol and the obtained results, it is possible to conclude that the oil sample from Il Re is characterized by good antioxidant properties in terms of ABTS scavenging activity.

Determination of Total Antioxidant Activity

The total antioxidant activity of the tested oil sample from Il Re was evaluated according to the method reported in literature [15].

The assay is based on the reduction of Mo(VI) to Mo(V) by antioxidant compounds and subsequent formation of a green phosphate/Mo(V) complex at acid pH.

The total antioxidant activity was expressed as mg equivalent of ferulic acid per gram of olive oil (mg eq FA/g) by using the equation obtained from the calibration curve of the antioxidant. This one was recorded by employing five different ferulic acid standard solutions.

The high absorbance values indicated that the oil sample possessed significant antioxidant activity, which was found to be 25.4 ± 1.0 mg eq FA/g.

Evaluation of the Total Polyphenols Content: Folin-Ciocalteu assay

The Folin-Ciocalteu assay represents a conventional method for the quantification of the amount of disposable phenolic groups [16].

Phenolic compounds undergo a complex redox reaction with phosphotungstic and phosphomolybdic acids present in the Folin-Ciocalteu reagent (*Figure 4.*) to form a blue complex that can be quantified by visible-light spectrophotometry.

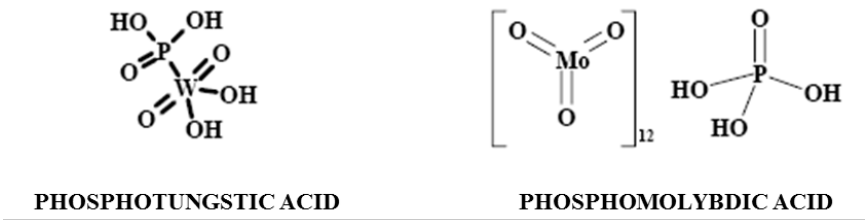

Figure 4.

The color development is due to the transfer of electrons at basic pH to reduce the phosphomolybdic/phosphotungstic acid complexes to form chromogens in which the metals have lower valence.

This method is based on the reducing ability of polyphenols, therefore, it is also considered an antioxidant capacity assay.

The amount of total phenolic groups was expressed as mg equivalent of catechin per gram of olive oil (mg eq CA/g) by using the equation obtained from the calibration curve of the antioxidant. This one was recorded by employing five different catechin standard solutions.

The obtained total polyphenols content was equal to 1.65 ± 0.3 mg eq CA/g

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