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Research Article

EVALUATION OF TOTAL POLYPHENOLS AND ANTIOXIDANT ACTIVITY OF FRUIT AND OLIVE OIL, THROUGH EXTRACTION WITH THE ULTRASOUND METHOD.

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ABSTRACT

Virgin olive oil is derived from the olive fruit through physical or mechanical processes and its quality is closely associated with the fruit quality, harvesting methods, and extraction techniques. This study aimed to investigate the presence of polyphenols and bioactive compounds in olive fruits and compare them with the olive oil obtained from the same cultivars extracted by the ultrasound method. Multiple samples of different cultivars, namely Kalinjot, Korroneik, Unripe Korroneik, Nisjot, and Frantoio, were utilized for this study. Each cultivar underwent analysis of physicochemical parameters and bioactive components in both the fruit and oil, and the results were statistically analyzed using Statistix 9 software. The findings of this study revealed that the total polyphenol content in the olive fruit was higher than that in the oil obtained through the ultrasound extraction method. This observation highlights the influence of the extraction technique on the polyphenol and bioactive compound content in the resulting oil

1. Introduction

Olea europaea L, commonly known as the olive tree, is one of the oldest cultivated trees worldwide. It has spread across various regions and been successfully grown in modern times. The olive tree thrives in mild winters (minimum 4ºC) and warm, dry summers (maximum 40ºC) with an average annual temperature of 15-20ºC. Unlike other crops, olive culture is not significantly affected by spring frosts due to the late flowering of olives (Palliotti and Bongi, 1996).

The primary product derived from the olive tree is olive oil, which has gained popularity due to its sensory properties and beneficial health effects (Oliveras-López *et al*., 2014).

Olive oil is a crucial component of the Mediterranean diet, providing a significant source of fat. It is rich in oleic acid, an unsaturated fatty acid that helps regulate cholesterol levels. Olive oil also contains an appropriate balance of linolenic acids, which are essential fatty acids that reduce the risk of diseases such as coronary heart disease and

cancer (Karupaiah *et al*., 2007; Visioli *et al*., 2020).

The evaluation of phenolic compounds is essential in determining the quality of virgin olive oil (Servili and Montedoro, 2002). These compounds exhibit strong antioxidant activity and contribute significantly to the oxidative stability of virgin olive oils (Tura *et al*., 2007).

The assessment of phenolic compounds plays a crucial role in determining the quality of virgin olive oil (Servili and Montedoro, 2002), given their potent antioxidant properties and contribution to oxidative stability (Tura *et al*., 2007). Among the most abundant antioxidants in virgin olive oil are lipophilic and hydrophilic phenols (Bendini *et al*., 2007), which are also responsible for the flavors of olive oil (Gutierrez *et al*., 1992). The concentration of phenolic compounds in olive oils varies based on the health status of the olives and the extraction methods employed (Mikolajczak *et al*., 2021).

Olives contain high concentrations of phenolic compounds, ranging between 1-3% of the fresh pulp weight. The primary classes of phenolic compounds found in olives include phenolic acids, phenolic alcohols, flavonoids, and secoiridoids (Robards *et al*., 1999; Servili and Montedoro, 2002; Servili *et al*., 2004; De la Torre-Carbot *et al*., 2005).

The positive effects of phenolic compounds on human health, as demonstrated through various studies on olive oil, have sparked interest in investigating olive fruits and determining suitable methods for olive oil extraction in processing lines.

This study aims to investigate the total phenolic compounds present in olive fruits and compare them with the phenolic content of olive oil obtained from four cultivars using the ultrasound system for extraction. The total antioxidant activities of these samples were evaluated by assessing their ability to scavenge the radical cation ABTS and the radical DPPH. The purpose is to compare these activities with the total phenolic content of olive fruits.

2. Materials and methods

2.1. Study Plan

To conduct this study, five olive samples were utilized and assigned names based on the cultivar: Kalinjot, Nisjot, Frantoio, Korroneik, and Unripe Korroneik. These samples were obtained from the Genetic Bank of the Center for the Transfer of Agricultural Technologies in Vlora.

2.2. Olive sampling

The olive samples were collected by handpicking fruits at the operator's height, avoiding fruits located inside the tree (Rodriguez de la Borbolla *et al*., 1955). Two samples, weighing approximately 3-6 kg each, were taken from each tree.

Sampling was conducted when the fruits reached their final stage of maturity, following the method described by Uceda and Frias (1975). For the “Korroneik” cultivar, samples were collected at two maturation stages: one during the initial stage and another during final maturation.

After field identification, the samples were sent to the laboratory for physico-chemical analysis of the olive fruit and olive oil.

2.3. Determinations in the olive fruit

2.3.1. Fruit weight

The weight of the samples (100 fruits) was measured using an electronic balance (Electronic laboratory balance, BC series) with a sensitivity of 0.01 g, and the average weight per fruit was calculated.

2.3.2. Ripeness Index

The ripeness index, a phenotypic method, was determined based on changes in the color of the fruits skin and endocarp, according to Uceda and Frías (1975). Homogenized samples from 100 fruits were classified into seven categories, and the resulting ripeness index value was expressed on a scale of 0 to 7.

2.3.3. Moisture Content

The moisture content was determined by drying the pulp in an oven at 60-80°C until a constant weight was achieved, following the method described by the AOAC (2000).

2.3.4. Fat content

The fat content (oil) of the fruits was determined using the Soxhlet method with hexane as the solvent, following the procedure outlined by the AOAC (2000).

2.4. Chemical analytical determination of olive fruit and olive oil

2.4.1. Extraction of polyphenols compound from olive fruit

The extraction process followed the method described by Goldsmith *et al*., (2014), with a few modifications. For sample preparation, 2 g of olive paste was mixed with 12 mL of a methanol–water solution (80:20 v/v). The mixture was then vortexed for 2 min, followed by extraction in an ultrasonic bath for 15 min. Subsequently, centrifugation was conducted at 3000 rpm for 25 min at room temperature. The resulting extracts were filtered using No. 40 Whatman paper, and then adjusted to a total volume of 10 mL using bi-distilled water.

2.4.2. Extraction of olive oil

The ultrasound system was employed to extract oil from the olive fruits, with slight modifications to the method described by Jerman *et al*., (2010). The process involved crushing approximately 3 kg of olives in a hammer mill, adding 1% water to the resulting paste, subjecting it to ultrasound treatment for 15 minutes, and then allowing the olive paste to undergo malaxation for 45 minutes. Phase separation was achieved through centrifugation at 3500 rpm for 10 minutes.

The obtained oil was filtered to remove solids and moisture, coded, and stored in laboratories at a temperature below -15 °C.

2.4.3. Analytical determination of olive oil quality.

The quality of olive oil was assessed by determining values of free acidity, K_{232}, K_{270}, and the peroxide index, following the official methods described by the Commission Regulation EC No. 1989/2003 and its amendments (EEC, No, 2568/91).

2.4.4. Total polyphenols content.

The content of polyphenols in the olive oil and olive fruit extractions was analysed using the Folin-Ciocalteu reagent and measuring the absorbance at 726 nm (with Biochrom Libra S22 UV/Vis Spectrophotometer), as described by Dini *et al*., 2020. The results were expressed as mg/kg of Gallic acid.

2.4.5. Determination of pigments.

The determination of olive oil pigments was carried out with the method described by Mínguez-Mosquera *et al*., (1991), which provides the total amount of carotenoids and chlorophyll derivatives, expressed in mg/kg. This method is based on the calculation of two indexes, namely K_{670} and K_{470}, related to absorbance values of the olive oil, diluted in cyclohexane, at a wavelength of 670 nm and 470 nm, respectively.

2.4.6. Determination of index of Bitterness.

The determination of bitterness defined as K_{225} is performed according to the method described by Gutiérrez *et al*., (1992). For the chemical analysis of the bitterness of olive oil, a SPE C_{18} solid phase extraction column with a volume of 6 mL and a stationary phase weight of 500 mg is used. The bitter components present in the SPE column are eluted with methanol:water (50:50) and the eluted mass is collected in a 25 mL volumetric flask. The determination of bitterness is completed by reading the mass obtained at 225 nm by UV-VIS spectrophotometer. The results were expressed as the absorbance of 1 g in 100 g.

2.5. Determination of antioxidant activity.

2.5.1. DPPH method.

The analysis was performed according to the modified method of Blois (1958). 1 mL of 0.1 mM solution of DPPH in methanol was mixed with 2 mL of extract at different concentrations (0.5-5.0 mg/mL). The mixture was then incubated at room temperature for 30 min in the dark. Absorbance measurement was performed at 517 nm. Ascorbic acid was used as a standard. The percentage of activity of each extract on the DPPH radical was calculated as % DPPH inhibition (I %) using the following equation:

$$I\ \% = (A_0 - As)/A_0 \times 100 \quad (1)$$

Where: A_0 – absorbance value of the control, A_S – absorbance value of the analyzed extracts.

2.5.2. Method with ABTS.

This analysis was performed using the modified method of Re *et al*., (1999). ABTS stock solution was prepared by mixing equal amounts of 7 mM ABTS aqueous solution with 2.45 mM potassium persulfate aqueous solution; this mixture should stand in the dark at room temperature for 12-16 hours before use. The working solution of ABTS was prepared by diluting the stock solution with methanol until an absorbance of 0.70 ± 0.02 at 734 nm was obtained. Then, 2.0 mL of this solution was mixed with 1 mL of extract at different concentrations (0.5-5.0 mg/mL). The mixture was then incubated at room temperature for exactly 10 min in the dark. Absorbance measurement was performed at 734 nm. Trolox was used as standard. The percentage of the activity of the action of each extract on ABTS was calculated as % inhibition of ABTS (I %) using the following equation:

$$I\ \% = (A_0 - A_S)/A_0 \times 100 \quad (2)$$

Where: A_0 – absorbance value of the control, A_S – absorbance value of the analyzed extracts.

2.6. Statistical analysis.

All analysis were performed in triplicate. Mean and SD were performed using Statistics 9.0.

3. Results and discussions

3.1. Physico-chemical parameters of the fruit.

The assessment of olive fruit ripeness is determined through the use of a ripeness index. This index is based on changes in pigmentation that occur in both the fruit's epidermis and pulp, as outlined by Uceda and Frías (1975). The purpose of this parameter is to identify the optimal time for harvesting olives, as the ripening period is influenced by factors such as climatic conditions and varietal characteristics (Barranco *et al*., 2000). When the ripeness index approaches a value of 3.5, most fruits exhibit a light color (grade 2 or 3), while some have a black skin (grade 4 or higher), and a few display a yellow-green color (class 1). At this stage, the oil content and chemical composition of the fruits are present in high quantities.

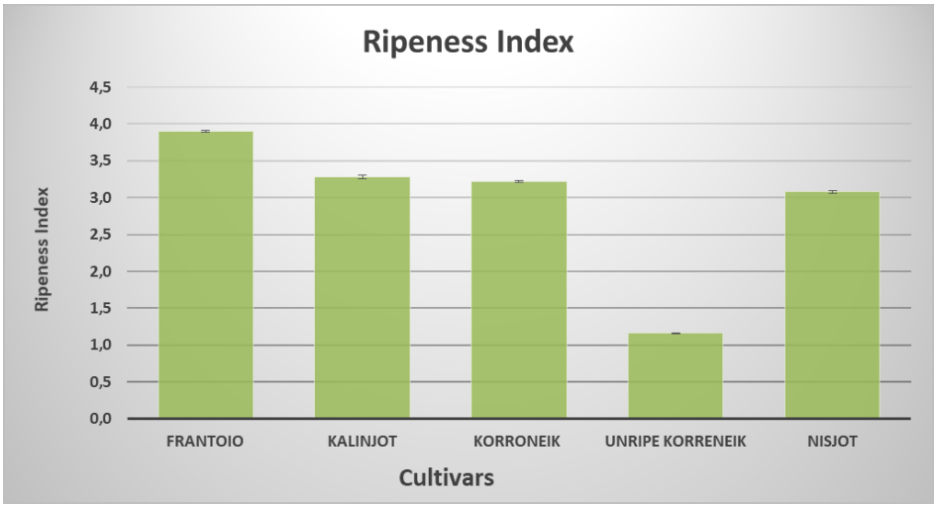

Figure 1. Ripeness Index of olive cultivars (Mean ± SD).

As illustrated in Figure 1, the fruit ripeness index exhibited values around 3, except for the Unripe Korroneik sample which displayed a lower ripening index (IP=1.16).

The ripeness index plays an important role in the biosynthesis of chemical compounds within the olive fruit, many of which are transferred to the olive oil during extraction.

Phenolic composition, in particular, undergoes significant changes during maturation (Fernández-Poyatos *et al*., 2021), primarily due to enzymatic activity (Youssef *et al*., 2010). Oleuropein, the predominant polyphenol in olive fruit, experiences a notable decrease in quantity as the fruit matures (Yorulmaz *et al*., 2013; Mena *et al*., 2018; Qarnifa *et al*., 2019). Consequently, selecting the appropriate harvest date is paramount to ensure that the resulting olive oil possesses desirable sensory characteristics, oxidative stability, and nutritional value.

Another parameter for fruit harvesting is its weight. Table 1 provides the average fruit weight, which varies depending on the cultivar. The olive is a small ellipsoidal fruit with dimensions ranging from 1 to 4 cm in length and 0.6 to 2 cm in diameter. At full development, the pulp constitutes approximately 70-90% of the total fruit weight, while the pit accounts for 9-27%, and the seed represents around 2-3% of the overall fruit weight.

Table 1. Average values of grain weight, pulp/pit ratio of the cultivars taken in the study.

Cultivars	Weight of 100 fruits (g)	Fruit weight (g)
Frantoio	228.7± 15.5 [a]	3.22 ± 0.7
Kalinjot	411.8 ± 6.5	4.27 ± 0.0
Korroneik	163.9 ± 5.1	2.23 ± 1.1
Unripe Korroneik	189.9 ± 9.6	2.47 ± 0.1
Nisjot	378.6 ±18.1	4.18 ± 0.0
Mean	298.90	6.101
SD	97.43	1.694
Variance	9492.1	2.870
C.V	32.60	27.767
Minimum	163.21	3.960
Maximum	416.16	8.100

a: Mean ± SD; Statistics 9.0 software

Based on the obtained data, it is evident that the Kalinjot cultivar exhibits a higher average fruit weight, while the Korroneik cultivar has smaller fruits. The weight of olive fruit is influenced not only by genetic factors (cultivar) but also by external factors, particularly tree productivity and weather conditions (Del Río *et al*., 2005).

Moisture content is a parameter that reflects the water content in the fruit. This parameter is correlated with the ripeness index, wherein an increase in ripeness index corresponds to a decrease in moisture percentage. Additionally, a decline in certain components, particularly volatile compounds present in olive pulp, is observed (Gigliotti *et al*., 1992). The moisture content of olives at harvest is a significant parameter as it affects fat content and the final quality of the obtained oil. Figure 2 illustrates the moisture values of fruits from the different cultivars under study.

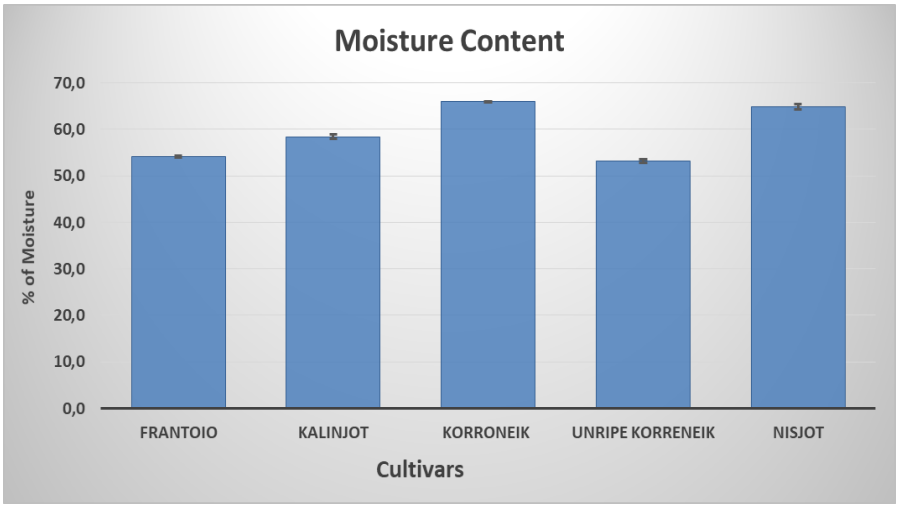

Figure 2. Moisture percentage of the cultivars taken in the study (Mean ± SD).

The results indicate that the Korroneik cultivar exhibits a significantly higher average moisture percentage compared to the other analyzed cultivars. In contrast, the moisture percentages for the Frantoio, Kalinjot, and Nisjot cultivars fall within the range of 54% to 64%.

Fat content is an important parameter that reflects the stage of oil formation in the fruit. It is influenced by the specific cultivar and the developmental characteristics of the fruit. Once lipid synthesis ceases, the fat content remains relatively constant (Aguilera *et al*., 2005).

Figure 3 presents the average values of fat content for the cultivars studied in this analysis.

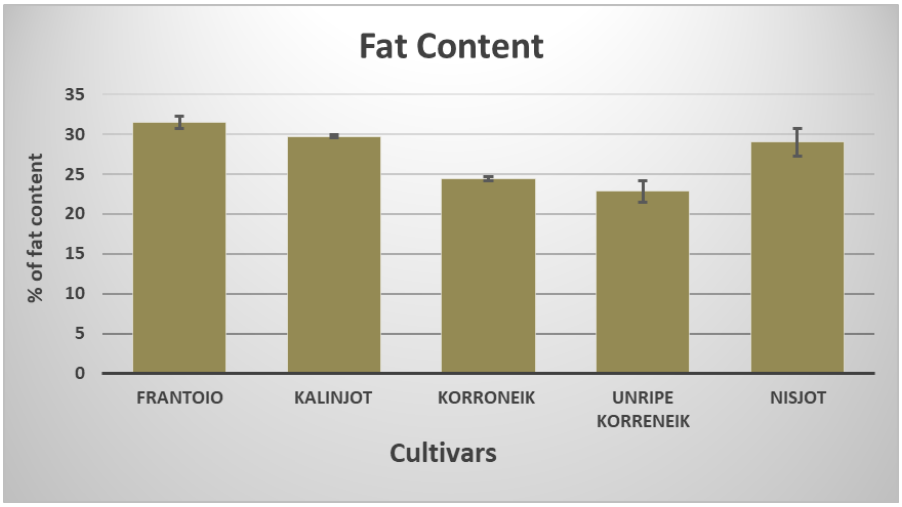

Figure 3. Fat percentage of cultivars taken in the study (Mean ± SD).

As shown in Figure 3, the cultivar Frantoio exhibits the highest average fat content, while the cultivars Kalinjot and Nisjot show similar average values, around 29%.

The fat content is a parameter characterized by small fluctuations and is influenced by the pulp/pit ratio (Khdair *et al*., 2015). For example, the cultivars Kalinjot and Nisjot have higher fat content and also they have a higher pulp/pit ratio compared to the other cultivars studied.

3.2. Chemical parameters of the oil.

Virgin olive oil is primarily composed of triglycerides, which account for approximately 96% of its composition and are commonly referred to as the saponifiable fraction due to

their ability to form soaps in the presence of alkaline substances. The remaining 1% to 3% consists of a diverse group of complex substances that constitute the unsaponifiable fraction. It should be noted that the composition of virgin olive oil is influenced by various biotic and abiotic factors, resulting in quantitative and compositional changes. Additionally, the biosynthesis reactions involved in both major and minor components may be incomplete or exhibit collateral deviations, leading to significant variability (Piscopo *et al*., 2021) influenced by climatic conditions and varietal characteristics. Table 2 presents the quality parameter values of the olive oil extracted from the studied cultivars.

The amount of free acids in olive oil is crucial as it determines the quality and classification of the oil. Extra virgin and virgin olive oils should have free acidity values not exceeding 1-0.8% oleic acid (EEC, 2003). Table 2 displays the total free acidity values of the studied cultivars.

Table 2. Olive oil quality parameters of cultivars taken in the study.

Cultivars	Total Acidity (% oleic acid)	K_{232}	K_{270}	Index of peroxides (meq O_2/kg oil)
Frantoio	0.320 ± 0.03[a]	1.674 ± 0.00	0.127 ±0.00	9.00 ±0.60
Kalinjot	1.200 ± 0.11	2.012 ± 0.00	0.133 ± 0.00	14.03 ± 0.66
Korroneik	0.350 ± 0.03	1.674 ± 0.00	0.123 ± 0.00	9.03 ± 0.20
Unripe Korroneik	0.313 ± 0.03	2.041 ± 0.00	0.152 ± 0.00	9.26 ± 0.41
Nisjot	0.650 ± 0.06	1.970 ± 0.00	0.109 ± 0.00	9.40 ± 0.20
Mean	0.568	1.874	0.129	10.147
SD	0.357	0.015	0.170	2.050
Variance	0.128	2.378	0.0292	4.221
C.V	62.858	9.113	11.943	20.249
Minimum	0.280	0.104	1.668	8.400
Maximum	1.290	2.050	0.157	14.800

a: Mean ± SD; Statistics 9.0 software

As indicated in Table 2, the olive oils extracted from the studied cultivars fall into the category of extra virgin olive oil, except for the oil obtained from the Kalinjot cultivar, which belongs to the virgin category. The evaluation and classification of the oils were conducted according to the standards set by the Republic of Albania, the European Community Regulation (2015), and the IOOC standards of 2022.

Table 2 provides the values of K_{232} and K_{270} for the olive oils of the analyzed cultivars. These spectrophotometric values are important indicators of olive oil quality, storage conditions, and technological changes that occurred during extraction. Absorption at these wavelengths is attributed to the presence of conjugated dienes and trienes.

The values of K_{232} for the analyzed olive oils range from 1.67 to 2.04, falling within the established limits, as well as the obtained values of total acidity, classifying these oils as virgin oils.

Regarding K_{270}, the values for the oils in the study range from 0.10 to 0.15 (Table 2). For the extra virgin olive oil category, the limits established by IOOC (2022) and CEE (2003) are 0.22. Therefore, the results obtained for the oils in the study classify them as extra virgin oils.

The peroxide index is a parameter that indicates the initial oxidation state of olive oil, expressed in milliequivalents of active oxygen per kilogram of fat. Higher peroxide values indicate incorrect extraction practices or inadequate protection and storage of the oil from light and heat, resulting in decreased antioxidant activity.

To maintain the high organoleptic value of olive oil, the European Community has

established standard 2568/91 (EEC, 2003), which sets the peroxide limit for virgin oils at 20 meq O_2/kg.

3.3. Secondary components of oil

The majority of antioxidant compounds found in olive oil are phenolic in nature. These phenolic compounds not only contribute to the oil's stability but also influence its organoleptic properties (Beltrán *et al*., 2010). The levels of polyphenols in the oil decrease during the fruit ripening process, with variations depending on the variety and cultivation area (Gutiérrez *et al.*, 1999; Beltrán, 2000).

Moreover, the content of polyphenols in olive oil is related to the ripeness index and moisture content of the fruit. Figure 4 illustrates the polyphenol values in the oil obtained from the studied cultivars.

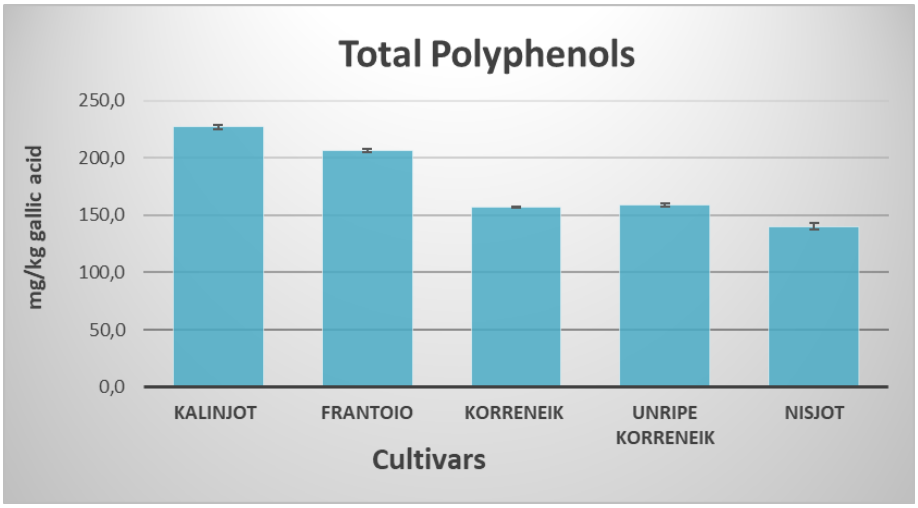

Figure 4. The content of total polyphenols of olive oil extracted from the cultivars taken in the study (Mean ± SD).

The olive oil extracted from the cultivars analyzed in this study exhibited varying levels of total polyphenols, ranging from 46.78 to 75.74 mg/kg of gallic acid. According to previous studies by Cimato *et al*., (1990), and Beltrán *et al*., (2010), differences in phenolic compound levels within the same variety can be attributed to factors such as agronomic practices, technical factors, and genetic variations. Based on these findings, it can be concluded that the disparities in total polyphenol content among the olive oils from the studied cultivars may be influenced by both genetic factors and agronomic practices.

3.3.1. Total polyphenols in the fruit.

The content of total polyphenols in the olive fruit itself was also investigated in this study. Extraction of these compounds was carried out directly from the fruit using the ultrasound technique, as most polyphenols are soluble in the aqueous phase of the fruit.

During the extraction process, a significant amount of these compounds was removed along with the vegetative water.

The results obtained are presented in Figure 5. It can be observed from the figure that the Unripe Korroneik cultivar had higher levels of total polyphenols, which may be attributed to its ripeness index.

On the other hand, the Nisjot cultivar exhibited the lowest values, with an average of 262.11±0.48 mg/kg of gallic acid.

Phenolic compounds play a crucial role as bioactive components in olive oil, providing protection against oxidation (Servili and Montedoro, 1992). The amount of polyphenols directly extracted from the fruit varies between 262.11 and 496.17 mg/kg. Notably, the Korroneik cultivar, especially the Unripe Korroneik, exhibited higher levels of total polyphenols compared to other cultivars.

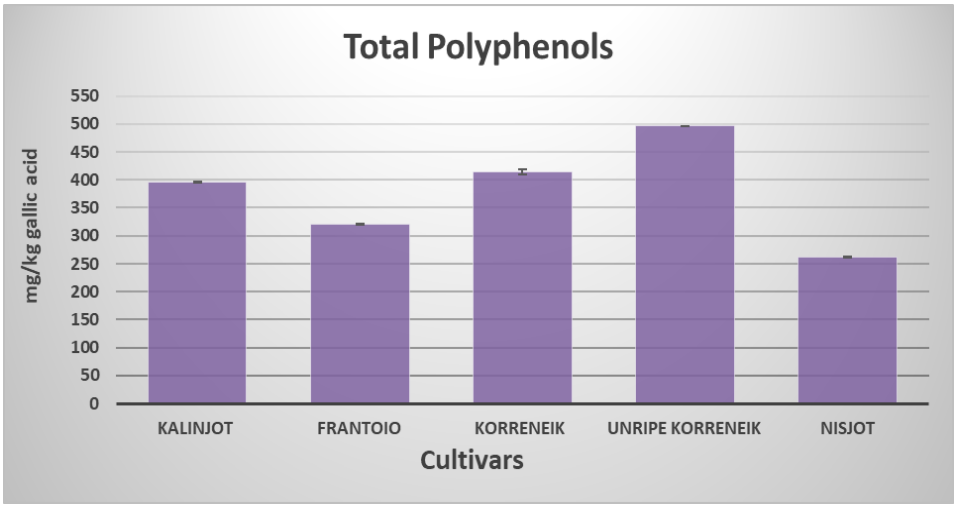

Figure 5. Quantity of total polyphenols extracted directly from the olive fruit (Mean ± SD).

When comparing the polyphenol values in the oil with those in the fruit, it is evident that the total polyphenols extracted directly from the fruit are higher than those found in the oil. This indicates that technological factors impact the amount of total polyphenols in the final oil product, as noted by authors such as Cimato *et al*., (1990), Uceda *et al*., (1999), and Beltrán *et al*., (2000). The results demonstrate that only a small fraction of the fat-soluble polyphenols can transfer into the oil during the extraction process (Boskou, 2006).

Observing these results underscores the importance of further research aimed at enhancing olive oil extraction techniques to maximize the yield of polyphenols present in the fruit. Polyphenols play an important role in maintaining the oxidative stability of the oil and enhancing its organoleptic qualities. Moreover, they offer significant health benefits to consumers. Therefore, prioritizing efforts to optimize polyphenol extraction could lead to improved quality and health-promoting properties of olive oil.

3.3.2. Bitterness index of olive oil.

Bitterness is considered a desirable sensory attribute in virgin olive oils, as it contributes to the overall aroma associated with green olive fruit. Consumers are increasingly embracing olive oils with pronounced bitterness. Consequently, the evaluation of bitterness has gained significance in olive oil research (Vitaglione *et al*., 2015). Table 3 presents the average bitterness index values of oils from different cultivars cultivated in Albania. The oil extracted from the "Kalinjot" cultivar exhibited the highest bitterness index value of 0.32, while the oil from the "Nisjot" cultivar displayed the lowest value. The oils of the varieties 'Kalinjot', 'Frantoio', 'Korroneik' and 'Unripe Korroneik' according to the categories established by Gutiérrez *et al*. (1992), they are considered lightly bitter oils because they have a K_{225} value between 0.20 - 0.32. The oil of the 'Nisjot' variety can be classified as a non-bitter oil, as it has a K_{225} value of less than 0.20.

Table 3. Bitterness index of olive oil from cultivars taken in the study.

Cultivars	Bitterness Index
Frantoio	0,23 ± 0,01[a]
Kalinjot	0,32 ± 0,02
Korroneik	0,26 ± 0,02
Unripe Korroneik	0,23 ± 0,00
Nisjot	0,16 ± 0,00

a - Mean ± SD

Based on the study findings, it is evident that the oil derived from the Kalinjot cultivar exhibits similar bitterness index values to those reported in the study conducted by Kyçyk *et al*., (2020).

3.3.3. Oil pigments

Chlorophyll and carotenoid pigments are present in the fruit throughout its maturation process. These fat-soluble compounds are transferred into the oil during extraction, thus influencing its color (Mínguez-Mosquera and Garrido Fernandez, 1989; Beltrán *et al*., 2000).

These pigments also possess antioxidant activity, particularly when the oil is protected from light (Beltrán *et al*., 2000). The maturity stage of the fruit plays a significant role in the content of these pigments. It has been documented that early-ripe fruits exhibit higher levels of chlorophyll and carotenoids (Mínguez-Mosquera *et al*., 1991).

Chlorophyll compounds comprise a group of components responsible for the green coloration of the oil, with chlorophyll being the primary constituent (Mínguez-Mosquera *et al*., 1991). Figure 6 presents the average chlorophyll values in the oil extracted from the studied cultivars.

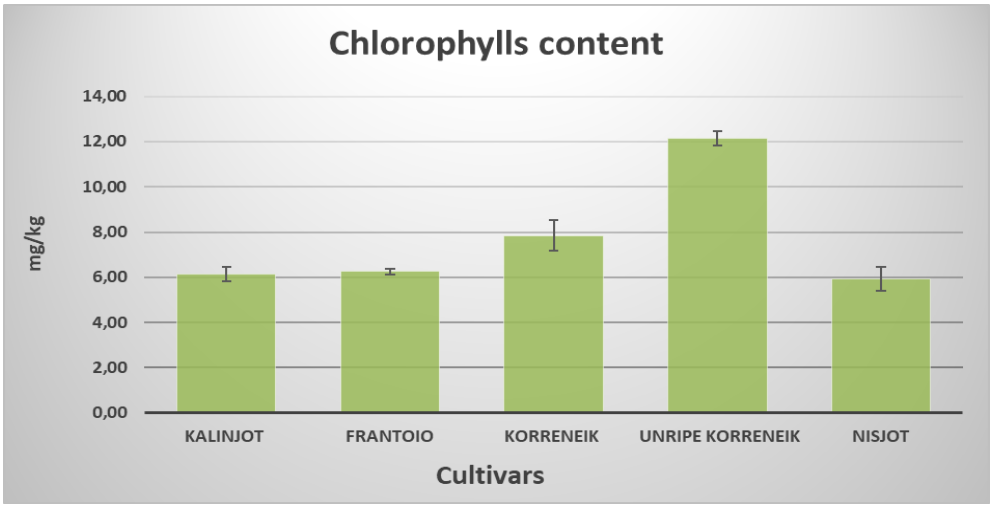

Figure 6. Chlorophyll content in olive oil extracted from the cultivars studied (Mean ± SD).

Figure 6, describes the chlorophyll content in olive oil extracted from the cultivar Unripe Korroneik, which displays the highest pigment content at approximately 12.4 mg/kg of gallic acid, while the Nisjot cultivar yields the lowest values. These disparities may arise from both genetic factors and the maturity stage of the fruit. Notably, the oil obtained from the Unripe Korroneik cultivar, characterized by a lower ripeness index compared to other cultivars, exhibits variations in chlorophyll pigment content. Previous studies by Mínguez-Mosquera *et al*., (1991) have established that the ripeness of the fruit and the specific variety influence the levels of chlorophyll pigments, thereby causing variations in their content within olive oil.

Furthermore, a comparison with the findings reported by Kyçyk *et al*., (2020) reveals that the average chlorophyll pigment values in the oil obtained from the Kalinjot cultivar are lower. This discrepancy could be ascribed to the impact of climatic conditions on the specific olive cultivar.

Figure 7, provides a summary of the average carotenoid pigment content in the analysed cultivars. The oils derived from the Unripe Korroneik cultivar exhibit the highest concentration of carotenoid pigments, reaching levels of up to 6.84 mg/kg, while the oils obtained from the Nisjot cultivar exhibit the lowest value of 4.20 mg/kg.

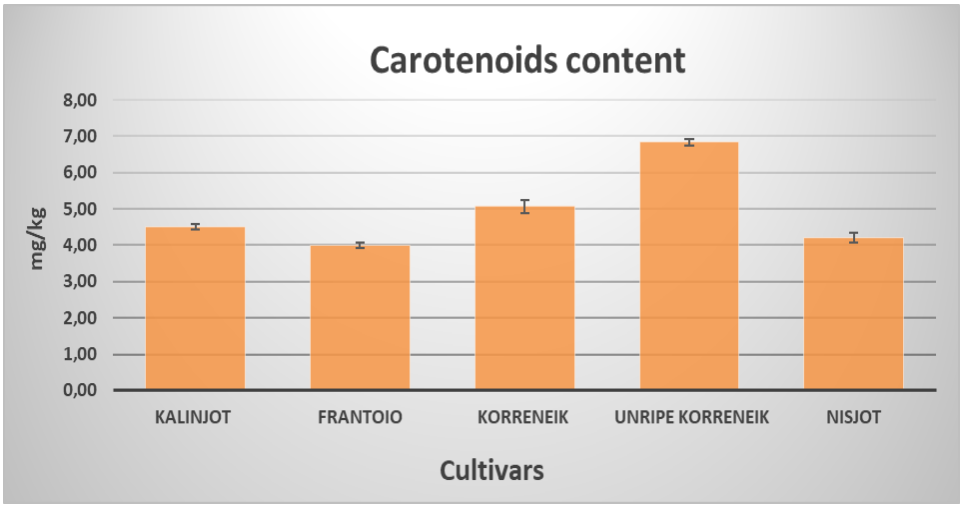

Figure 7. Carotenoids content in olive oil extracted from in four cultivars (Mean ± SD).

The average values of carotenoid pigments in the oil extracted from the Kalinjot cultivar are found to be lower compared to the results reported by Kyçyk *et al*., (2020). These variations may be attributed to climatic influences on the olive trees, including the production of chlorophyll pigments.

3.4. Antioxidant activity of olive fruit extracts.

According to several studies, the assessment of antioxidant activity requires the utilization of multiple methods to evaluate the antioxidant activity of plant extracts. This is because antioxidants can exert their effects through diverse mechanisms, depending on the specific reaction system or radical source (Yu *et al*., 2002).

Based on the findings depicted in Figure 8, it is evident that the extract derived from the fruits of the Korroneik cultivar exhibits a high activity for reducing the DPPH radical.

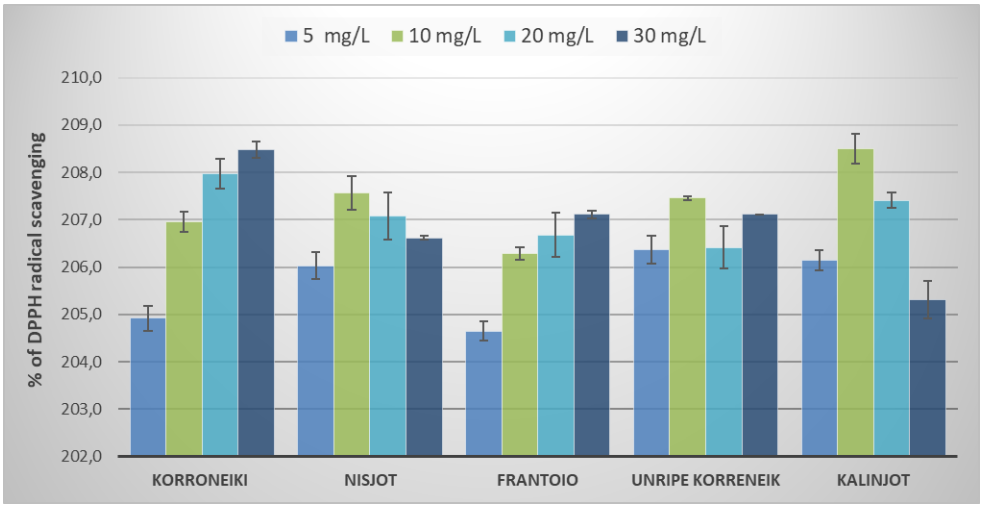

Figure 8. % of free radical scavenging activity of extracts of olive varieties measured by reducing the DPPH radical (Mean ± SD).

The antioxidant activity of the tested antioxidants using the ABTS assay is illustrated in Figure 9. It is evident from the figure that the extract obtained from the Nisjot cultivar exhibits

lower antioxidant activity compared to the other cultivars investigated in this study.

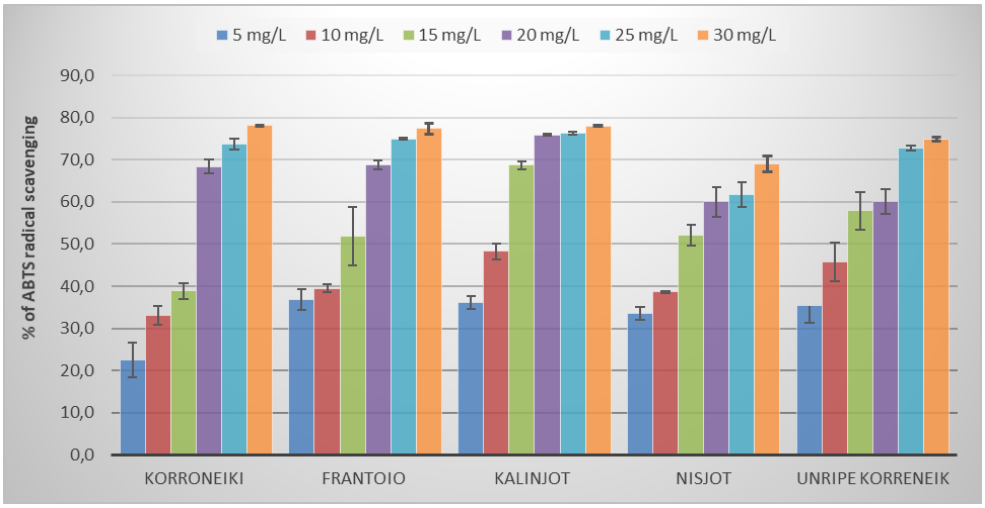

Figure 9. % of the free radical scavenging activity of the extracts of the olive varieties studied with the ABTS method (Mean ± SD).

4. Conclusion

In conclusion, the findings of this study demonstrate that the weight of the fruit, moisture content, oil content, and polyphenol content of the olive samples meet the optimal values defined by AOAC standards.

The quality characteristics of the extra virgin olive oil (EVOO) extracted from the studied cultivars fall within the category of extra virgin and virgin olive oils.

Furthermore, the analysis reveals that the total polyphenol content is higher in fresh olives compared to the polyphenol content in olive oil. These variations in total polyphenol content can be attributed to both genetic factors and the extraction process employed to obtain the oil from the olives.

Direct extracts obtained from the fruit display significant antioxidant activity using both assay methods. The present study indicates that the fruit extracts exhibit higher antioxidant activity, which correlates with their higher total polyphenol content.

5. References

Aguilera, M.P., Beltrán, G., Ortega, D., Fernández, A., Jiménez, A., Uceda, M. (2005). Characterisation of virgin olive oil of Italian olive cultivars: 'Frantoio' and 'Leccino', grown in Andalucia. *Food Chemistry*, 89(3), 387-391 https://doi.org/10.1016/j.foodchem.2004.02.046

AOAC (2000). Official Methods of Analysis, 17th ed. Washington, DC: Association of Official Analytical Chemists.

Barranco, D., Cimato, A., Fiorino, P., Rallo, L., Touzani, A., Castañeda, C., Serafín, F. and Trujillo, I. (2000). World catalogue of olive varieties. *International Olive Oil Council*, Madrid, 360.

Beltrán, G. (2000). "Influencia del proceso de maduración del fruto de *Olea europaea L* sobre las características fisico-químicas de los aceites" PhD thesis, Facultad de CC, Experimentales, Universidad de Jaén. (in Spanish).

Beltrán, G., Jiménez, A., Aguilera, M.P., Uceda, M. (2000). Phenolic fraction analysis by HPLC of 'Arbequina' virgin olive oils. Relationship with bitterness K_{225} and oil stability. *Grasas y Aceites*, 51(5), 320-324. https://doi.org/10.3989/gya.2000.v51.i5.432

Beltrán, G., Jiménez, A., Del Río, C., Sánchez, A., Martínez, L., Uceda, M., Aguilera, M.P.

(2010). Variability of vitamin E in virgin olive oil by agronomical and genetic factors. *Journal of Food Composition and Analysis*, 23(6), 633-639. http://dx.doi.org/10.1016/j.jfca.2010.03.003

Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gómez-Caravaca, AM., Segura-Carretero, A., Fernández-Gutiérrez, A., Lercker, G. (2007). Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. *Molecules*, 12(8), 1679-1719. https://doi.org/10.3390/12081679

Blois, M.S. (1958). Antioxidant Determinations by the Use of a Stable Free Radical. *Nature,* 181, 1199-1200. http://dx.doi.org/10.1038/1811199a0

Boskou, D., ed. (2006). Olive Oil: Chemistry and Technology, 2nd edition. *AOCS Publishing,* New York, 2006, 288. https://doi.org/10.4324/9781003040217

Cimato, A.; Mattei, A.; Osti, M. (1990) Variation of polyphenol composition with harvesting period. *Acta Horticulturae*, 286, 453-456. https://doi.org/10.17660/ActaHortic.1990.286.92

Commission Regulation No 1989/2003 of 6 November 2003 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-pomace oil and on the relevant methods of analysis.

De la Torre-Carbot, K., Jauregui, O., Gimeno, E., Castellote, A.I., Lamuela-Raventós, R.M., López-Sabater, M.C. (2005). Characterization and quantification of phenolic compounds in olive oils by solid-phase extraction, HPLC-DAD and HPLC-MS/MS. *Journal of Agricultural and Food Chemistry*, 53(11), 4331-4340. https://doi.org/10.1021/jf0501948

Del Río, C., Caballero, J., García–Fernández, M. (2005). Rendimiento graso de la aceituna. Variedades de Olivo en España. Junta de Andalucía. *Grupo Mundi–Prensa,* 347-356. (in Spanish)

Dini, I., Seccia, S., Senatore, A. (2020). Development and Validation of an Analytical Method for Total Polyphenols Quantification in Extra Virgin Olive Oils. *Food Analytical Methods,* 13, 457–464. https://doi.org/10.1007/s12161-019-01657-7

Fernández-Poyatos, M.d.P.; Llorent-Martínez, E.J.; Ruiz-Medina, A. (2021). Effect of Ripening on the Phenolic Composition and Mineral Content of Three Varieties of Olive Fruits. *Foods,* 10(2), 380. https://doi.org/10.3390/foods10020380

Gigliotti, C., Daghetta, A., Maspero, C. (1992). Caratterizzazione della produzione oleicola ottenuta nell'annata 1990 in oliveti sperimentali allestiti sulla riviera gardesana. Nota I: caratteristiche morfologiche e compositive delle olive ottenute dalle diverse cultivar. *Rivista Italiana delle Sostanze Grasse*, 69, 347-351 (in Italian).

Goldsmith, C.D.; Stathopoulos, C.E.; Golding, J.B.; Roach, P.D. (2014). Fate of the paorlic compounds during olive oil production with the traditional press method. International *Food Research Journal*, 21, 101–109.

Gutiérrez Rosales, F., Perdiguero, S., Gutiérrez, R. (1992). Evaluation of the bitter taste in virgin olive oil. *Journal of the American Oil Chemists' Society*, 69(4), 394–395. https://doi.org/10.1007/BF02636076/

Gutiérrez, F., Arnaud, T. & Albi, M.A. (1999). Influence of ecological cultivation on virgin olive oil quality. *Journal of the American Oil Chemists' Society,* 76, 617–621. https://doi.org/10.1007/s11746-999-0012-8

IOOC (International Olive Oil Council) (2015). Trade standard applying to olive oils and olive-pomace oils. COI/T.15/NC No 3/Rev. 19 November 2022

Jerman, T., Trebše, P., Vodopivec, B.M. (2010). Ultrasound-assisted solid liquid extraction (USLE) of olive fruit (Olea europaea) phenolic compounds. *Food Chemistry,* 123 (1), 175-182. https://doi.org/10.1016/j.foodchem.2010.04.006

Karupaiah, T., & Sundram, K. (2007). Effects of stereospecific positioning of fatty acids in

triacylglycerol structures in native and randomized fats: a review of their nutritional implications. *Nutrition & metabolism*, 4, 16. https://doi.org/10.1186/1743-7075-4-16

Khdair, A., Ayoub, S., Abu-Rumman, G. (2015). Effect of processing techniques on olive oil quality. *American Journal of Food Technology*, 10(4), 175-182. http://dx.doi.org/10.3923/ajft.2015.176.183

Kyçyk, O., Peculi A., Xhaferaj, N., Brahollari, A., Lamce, F., Kopali, A. (2020). Comparison of Polyphenol Content in Olive Oil cv Kalinjot Obtained in Two Different Extraction System. *Albanian Journal of Agricultural Sciences*, 19 (4), 55-60.

Mena, C., González, A. Z., Olivero-David, R., & Pérez-Jiménez, M. A. (2018). Characterization of "Castellana" virgin olive oils with regard to olive ripening. *Horticulture Technology*, 28, 48–57. https://doi.org/10.21273/HORTTECH03845-17

Mikołajczak, N., Tańska, M., Ogrodowska, D. (2021). Phenolic compounds in plant oils: A review of composition, analytical methods, and effect on oxidative stability *Trends in Food Science & Technology*, 113, 110-138. https://doi.org/10.1016/j.tifs.2021.04.046/

Mínguez-Mosquera, M. I., Garrido-Fernández, J. (1989). Chlorophyll and carotenoid presence in olive fruit (Olea europaea). *Journal of Agricultural and Food Chemistry*, 37, 1-7. https://doi.org/10.1021/JF00085A001

Mínguez-Mosquera, M.I., Gandul-Rojas, B., Montano-Asquerino, A. and Garrido-Fernández, J. (1991). Determination of Chlorophylls and Carotenoids by High-Performance Liquid Chromatography during Olive Lactic Fermentation. *Journal of Chromatography A*, 585(2), 259-266. http://dx.doi.org/10.1016/00219673(91)85086-U/

Oliveras-López, M., Berná, G., Jurado-Ruíz, E., Serrana, H.L., & Martín, F. (2014). Consumption of extra-virgin olive oil rich in phenolic compounds has beneficial antioxidant effects in healthy human adults. *Journal of Functional Foods,* 10, 475-484. https://doi.org/10.1016/J.JFF.2014.07.013

Palliotti, A., & Bongi, G. (1996). Freezing injury in the olive leaf and effects of mefluidide treatment. *Journal of Horticultural Science* 71(1), 57–63. https://doi.org/10.1080/14620316.1996.11515382

Piscopo, A., Mafrica, R., De Bruno, A., Romeo, R., Santacaterina, S., & Poiana, M. (2021). Characterization of Olive Oils Obtained from Minor Accessions in Calabria (Southern Italy). *Foods* (Basel, Switzerland), 10(2), 305. https://doi.org/10.3390/foods10020305

Qarnifa, S. E., Antari, A. E., & Hafidi, A. (2019). Effect of maturity and environmental conditions on chemical composition of olive oils of introduced cultivars in Morocco. *Hindawi Journal of Food Quality*, 14, 1854539.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology & medicine,* 26(9-10), 1231–1237. https://doi.org/10.1016/s0891-5849(98)00315-3

Robards, K., Prenzler, P.D., Tucker, G., Swatsitang, P. and Glover, W. (1999). Phenolic Compounds and Their Role in Oxidative Processes in Fruits. *Food Chemistry*, 66(4), 401-436. https://doi.org/10.1016/S03088146(99)00093-X

Rodriguez de la Borbolla, J.M.; Fernández, M.J., González, F. (1955). Cambios en la composición de la aceituna durante su desarrollo. *Grasas y Aceites*, 6, 5-20 (in Spanish).

Servili, M., Selvaggini, R., Esposto, S. (2004). Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. *Journal of chromatography A*, 1054(1-2), 113-127. https://doi.org/10.1016/J.CHROMA.2004.08.070

Servili, M. and Montedoro, G. (2002). Contribution of phenolic compounds to virgin olive oil quality. *European Journal of Lipid Science and Technology*, 104(9-10), 602-613. https://doi.org/10.1002/1438-9312(200210)104:9/10%3C602::AID-EJLT602%3E3.0.CO;2-X

Tura, D., Gigliotti, C., Pedo, S., Failla, O., Bassi, D., Serraiocco, A. (2007). Influence of cultivar and site of cultivation on levels of lipophilic and hydrophilic antioxidants in virgin olive oils (Olea Europea L.) and correlations with oxidative stability. *Scientia Horticulturae*, 112(1), 108-119. https://doi.org/10.1016/J.SCIENTA.2006.12.036.

Uceda, M. and Frias, L., (1975). Harvest dates. Evolution of the fruit oil content, oil composition and oil quality. In: Proceedings of the II Seminario Oleícola International. International Olive Oil Council. Córdoba, Spain. 125-130.

Uceda, M., Hermoso, M., Garcia-Ortiz, A., Jimenez, A., Beltran, G. (1999). Intraspecific variation of oil contents and the characteristics of oils in olive cultivars. *Acta Horticulturae,* 474, 659–662. https://doi.org/10.17660/ACTAHORTIC.1999.474.136

Visioli, F., Davalos, A., de las Hazas, M-C.L., Crespo, M.C., Tomé-Carneiro, J. (2020). An overview of the pharmacology of olive oil and its active ingredients. *British Journal of Pharmacology*, 177(6), 1316–1330. https://doi.org/10.1111/bph.14782.\

Vitaglione, P., Savarese, M., Paduano, A., Scalfi, L., Fogliano, V., & Sacchi, R. (2015). Healthy virgin olive oil: a matter of bitterness. *Critical reviews in food science and nutrition,* 55(13), 1808–1818. https://doi.org/10.1080/10408398.2012.708685ç

Yorulmaz, A., Erinc, H., & Tekin, A. (2013). Changes in olive and olive oil characteristics during maturation. *Journal of the American Oil Chemists' Society*, 90 (5), 647-658. https://doi.org/10.1007/s11746-013-2210-7

Youssef, N. B., Zarrouk, W., Carrasco-Pancorbo, A., Ouni, Y., Segura-Carretero, A., Fernandez-Gutierrez, A. , Daouda, D., & Zarrouk, M. (2010). Effect of olive ripeness on chemical properties and phenolic composition of Chetoui virgin olive oil. *Journal of Science and Food Agriculture*, 90(2), 199–204. https://doi.org/10.1002/jsfa.3784

Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J., Qian, M. (2002) Free radical scavenging properties of wheat extracts. *Journal of Agricultural and Food Chemistry*, 50(6), 1619–1624. https://doi.org/10.1021/jf010964p