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Enhancing Arbequina and Arbosana Olive Oils with Koroneiki Fruits and Leaves

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Abstract

This study examined the physicochemical properties, bioactive compounds, and organoleptic characteristics of olive oils from the cultivars Koroneiki, Arbosana, and Arboquina. Arboquina, a commonly cultivated olive variety, had a C18:1 content below the International Olive Council (IOC) limit of 55-83% and lower stability due to its low phenolic composition. In contrast, Koroneiki is known for its high oleic acid content, natural antioxidants, and superior oxidative stability compared to Arbosana and Arboquina oils. The study involved processing fruit mixtures and incorporating Koroneiki olive leaves during oil extraction to evaluate various parameters such as physicochemical properties, fatty acid composition, bioactive components, oxidative stability, phenolic compounds, and organoleptic characteristics. Mixing fruits, especially for Arboquina varieties, helped correct the low C18:1 content to meet IOC standards and improved oxidative stability. The oxidative stability increased by 37.55% and 40.37% when Koroneiki was mixed with Arbosana or Arboquina at a 50:50 ratio, respectively. Furthermore, adding Koroneiki olive leaves at 1% and 3% to a mixture of Koroneiki and Arboquina increased oxidative stability at 110°C by 2.2% and 4.66%, respectively, while for Arbosana, it increased by 4.7% and 26.56%. The analysis identified eighteen phenolic compounds in all olive oil samples, with oleuropein, hydroxytyrosol, phenolic alcohols, and phenolic acids being the major compounds. The results suggest that processing fruit mixtures of different cultivars can enhance the quality of olive oil, offering opportunities to adjust antioxidant content, fatty acid composition, color, nutraceutical compounds, and organoleptic properties. Additionally, incorporating olive leaves during oil extraction can yield functional olive oil with higher antioxidant levels.

Keywords**:** olive oil koroneiki, Arbosana, Arboquina varieties, olive fruit mixture, olive leaves, fatty acid composition, phenolic compounds, organoleptic attribute

1. Introduction

Olea europaea L., an evergreen tree in the Oleaceae family, is cultivated on 10.8 million hectares in 41 countries, mainly in the Mediterranean region [(1)]. Olive oil, a key component of the Mediterranean diet, is highly valued for its nutritional properties, unique aroma, and taste [(2)]. It is considered a functional food due to its high monounsaturated fatty acid and bioactive compound content [(3)], such as phenolic compounds. Olive oil produced at the beginning of the season from olives typically has superior quality, with low free acidity, peroxide value, and specific extinction coefficients at 232 and 270 nm, Delta.k. These oils also exhibit excellent sensory attributes, qualifying them as extra virgin olive oils [(4)].

The chemical and sensory quality of olive oils can deteriorate during storage due to natural oxidation caused by their high levels of unsaturated fatty acids [(5)]. Increasing their natural antioxidant content can help improve their chemical stability and enhance their nutritional and nutraceutical properties [(6)]. The nutritional quality of virgin olive oil (VOO) is linked to its composition, particularly its high concentration of oleic acid, which makes up 55-85% of the total fatty acids [(7)]. Monounsaturated fatty acids like oleic acid can help lower LDL cholesterol levels and reduce the risk of cardiovascular diseases [(8)]. VOO also contains phenols such as oleuropein derivatives and hydroxytyrosol, as well as antioxidants like vitamin E and carotenoids, which can help prevent the oxidation of LDL particles and provide various heart health benefits, including regulating cholesterol, anti-inflammatory, anti-thrombotic, antihypertensive, and vasodilator effects in both animals and humans [(9, 10)].

Arboquina olive oils generally have a lower concentration of total phenols compared to other cultivars such as Arbosana, Hojiblanca, and Koroneiki [(11, 12)]. Several studies have shown that Arboquina varieties have low total polyphenols, a low O/L ratio, low stability, low oleic acid content, and high palmitic acid content, which exceed the limits set by the International Olive Council standards. These characteristics are suboptimal and may pose challenges for marketing as a varietal oil. Previous research has shown that Arboquina olive oils typically contain oleic acid (C18:1) levels ranging from 44% to 45.37% [(14-16)].

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The fatty acid composition is a crucial factor in evaluating the quality of olive oil. Unsaturated fatty acids (UFAs) play a significant role due to their nutritional benefits and impact on the oxidative stability of oils [17]. Mixing is a valuable technique for improving oils from varieties like Arboquina, which have excellent initial quality and sensory attributes but suffer from poor stability [18].

Previous research has explored enhancing edible oils like olive oil by incorporating olive leaf extracts to improve their sensory qualities and oxidative stability. Studies have shown that olive leaves are abundant in phenolic compounds, making their extract a potent antioxidant source. Olive leaves, often viewed as a by-product, present significant potential for value addition. Extracting phytochemicals from olive leaves can lead to the production of high-value compounds for use in food products, offering added nutritional benefits [19].

Olive leaves are a common by-product of the olive oil industry, accounting for up to 10% of the total weight of olives [20, 21]. They can be utilized as a cost-effective source of valuable phenolic compounds [22]. The pharmaceutical, cosmetic, and food industries are increasingly interested in the therapeutic benefits associated with these bioactive compounds in olive leaf extract. The antibacterial and antioxidant properties of the phenolic compounds in olive leaves make them suitable for use as natural additives or supplements [19, 23].

Incorporating olive leaves during oil extraction can enhance the phenolic content and sensory quality of Arboquina olive oil, a widely cultivated Spanish olive variety known for its moderate vigor, high yield, and adaptability to high-density planting [24]. Several studies have identified a high concentration of phenolic compounds in olive leaves, including oleuropein, hydroxytyrosol, and rutin [25]. The addition of olive leaves during processing can increase the phenolic and chlorophyll content, improve the nutraceutical properties, and enhance the sensory attributes of the oil [26]. Chlorophylls and carotenoids are the primary pigments responsible for the oil's color, and there is growing interest in their potential health benefits [27].

This study examined the characteristics of oil obtained from three varieties: Koroneiki, Arbosana, and Arboquina, as well as their mixtures. The aim was to evaluate the physicochemical properties, oxidative stability, fatty acid composition, phenolic compounds, and sensory attributes of olive oils produced by blending Koroneiki with Arbosana or Arboquina fruits in a 50:50 ratio. Koroneiki varieties are known for their high natural antioxidant content, making them ideal for creating a blend with good sensory attributes, balanced fatty acid composition, and improved oxidative stability. Additionally, the study investigated the impact of adding freshly ground Koroneiki olive leaves (1% and 3%) to a mixture of Koroneiki and Arboquina fruits (50:50) and to 100% Arbosana fruits on the characteristics of the resulting olive oils.

2. Materials and Methods

2.1Materials

Olive fruit samples from three different olive varieties (Koroneiki, Arbosana, and Arboquina) and Koroneiki olive leaves were collected in November 2023 from the Horticulture Research Institute (HRI) in Giza Governorate, Egypt.

All reagents used were of analytical or HPLC grade and were sourced from Merck Millipore (Darmstadt, Germany) and Al Nasr Co., Egypt.

2.2 Methods:

2.2.1 Oil extraction

The olive fruits and their mixtures, as shown in Table 1, were prepared by grinding fresh olive leaves in a rotary mill to obtain a fine powder. Oil samples were then extracted separately using a laboratory scale. The fruits were cleaned, washed, and crushed, and the olive leaf granules were added at specified ratios. The mixture was then mixed for 30 minutes, packed in cheesecloth, and extracted using a hydraulic laboratory press (Carver press). The resulting extract (containing oil and vegetable water) was transferred to a separator funnel, where the oil layer was separated. The oil was then dried with anhydrous sodium sulfate, filtered, and stored in brown bottles at -5°C until analysis.

Table (1): Pure and mixed fruits samples with and without olive leaves.

Sample no.	Olive varieties
1	100% koroneiki
2	100% Arbosana
3	100% Arboquina
4	50% Koroneiki+ 50% Arbosana
5	50% Koroneiki+ 50% Arbiquina
6	50% Koroneiki+ 50% Arbiquina+ 1% olive leaves
7	50% Koroneiki+ 50% Arbiquina+3% olive leaves
8	50Arbosana+1% olive leaves
9	50Arbosana+3% olive leaves

2.2.2 The oil content of fruits, Quality characteristics of olive oils, such as free fatty acids % (FFAs), peroxide value (PV), and the color (using a Lovibond tintometer model F with a 5.25-inch cell) were determined following the methodology proposed by A.O.A.C. 2019 [28]. UV spectrophotometric indices (K232, K270, and ΔK) were evaluated according to the IOC 2022 guidelines [8].

2.2.3 Fatty acid composition was analyzed using gas chromatography (GC) by preparing methyl esters and identifying them with an Agilent 6890 series gas chromatograph equipped with a DB23 column (60 m x 0.32 mm) [30].

2.2.4 The chlorophyll and carotenoid contents (mg/kg of oil) of all olive oil samples were assessed using spectrophotometric measurements following the method described by Mínguez-Mosquera et al. [29].

2.2.5 Total phenol content was determined using the method described by Gutfinger (1981). The results were expressed as milligrams of caffeic acid per kilogram of oil [31].
2.2.6 The oxidative stability of olive oils was assessed using the Rancimat model 892 with a flow rate of 20 L/h at a temperature of 110°C, following the method outlined by Mendez et al [32].
2.2.6 The antioxidant activity of phenolic extracts from all olive oil samples was analyzed using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) method as described by Blois [33].
2.2.8 Phenolic compounds in oil samples were identified and quantified using HPLC (Agilent 1260 series) following the method outlined by Goupy et al. [34].
2.2.9 Organoleptic tests were conducted on samples of olive oils following the guidelines of the International Olive Council [8]. Each oil sample (15 ml) was placed in covered blue glasses (diameter: 70 mm, capacity: 130 ml) at a temperature of 28°C. The glasses were allowed to warm up, and after removing the cover, the panelists smelled the samples and evaluated their flavor and taste. The panelists assessed various attributes of the oils and provided intensity ratings, which were then averaged to determine the overall score.

3 Results and Discussion

3.1 Oil content

The oil contents (dry weight) of the three olive varieties, Koroneiki, Arbosana, and Arboquina, were determined to be 43.29%, 34.02%, and 36.97%, respectively. These findings are consistent with those reported in a previous study[16] .

3.2 Quality parameter and oxidative stability-

The physicochemical quality indices of olive oils from the studied cultivars in Table 2 meet the standards set by the International Olive Council [8] for the extra virgin olive oil category (acidity ≤ 0.8%, peroxide value ≤ 20 meq O_2/kg oil, K232 ≤ 2.5, K270 ≤ 0.22, and Δk ≤ 0.01). All the analyzed oils exhibited very low values for the regulated physicochemical parameters, indicating high quality. The low values of these parameters in all the analyzed oils reflect the good quality of the olives harvested at optimal maturation and immediately extracted without storage. However, differences between cultivars were observed for FFA%, peroxide value, and UV absorbance, consistent with previous studies [15,19,35,78,79].
The addition of olive leaves at 1% and 3% to a mixture of 50% Koroneiki and 50% Arboquina or Arbosana did not impact the quality parameters of the extracted olive oil. Studies have shown that adding leaves during oil extraction and processing can help prevent oxidation and the formation of peroxides, thereby reducing the peroxide value[36,65].
Chlorophylls and carotenoids are the primary light-harvesting pigments found in vegetable oils. They play a crucial role in preventing auto-oxidation and photo-oxidation [37]. Carotenoids, such as β-carotene, alpha-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin, contribute yellow, orange, and red hues to foods[38]. β-Carotene is also a precursor of vitamin A[37]. Chlorophylls in olive oils give them their greenish color and are essential for their stability[37].
In the study, Table 2 shows the a* and b* values of all tested samples, with yellow fixed at 35 inches at a 5.25-inch cell. The data indicates that the koroneiki cultivar had a superior b* value of 0.6 compared to the Arbosana and Arboquana cultivars (0.1 and 0.0, respectively), while the a* values ranged around 2.0 for all three varieties.
Mixing Koroneiki fruit with Arbosana or Arboquina fruits at a 50:50 ratio produced oil samples with a recorded a* value of 0.3. Adding olive leaves at 1% and 3% resulted in higher a* and b* values, with a* values of 2.5 and 3.0 respectively when Koroneiki fruits were mixed with Arboquina fruits. The b* scale showed higher levels at 0.8 and 1.3 with 1% and 3% olive leaf additions to the same mixture. When 1% and 3% olive leaves were added to 100% Arbosana fruit, both a* and b* values were recorded at 2.4 and 2.9 for a* and 0.5 and 1.1 for b* scales.
The increase in a* and b* levels when olive leaves are added may be attributed to the higher levels of chlorophyll and carotenoids. This increase could be a result of the increased breakage of leaf cells and olive fruits during extraction, which facilitates the release of chlorophyll into the oil[39] .
Oxidative stability is a crucial parameter for assessing the quality of olive oil. The study of oxidative stability in the oils analyzed, measured by the Rancimat test at 110°C, shows that this parameter varies depending on the variety. Table 2 shows that Koroneiki olive oil has the highest induction time at 13.43 hours, followed by Arbosana olive oil at 8.92 hours and Arboquina olive oil at 8.10 hours. The differences observed among the olive oils studied are attributed to variations in their constituents. Several studies have shown that the resistance of olive oil to oxidation is linked to its monounsaturated C18:1 and polyunsaturated C18:2 fatty acids content, as well as natural antioxidants such as phenolic compounds and tocopherols [40]. Our findings on oxidative stability align with previous reports [15,16,19,78,79].
The inclusion of leaves in the industrial extraction process of Leccino and Castiglione oils resulted in a slight increase in the oxidative stability (OS) by 2%, while a decrease of 7% was observed for Dritta oils [39]. Conversely, the addition of olive leaves improved the OS of Cobranesa oil by up to 20% compared to over-mature oil[41] .
Our results showed that adding 1% and 3% of olive leaves to Koroneiki and Arboquina to the mixture increased the oxidative stability (OS) from 11.37 hours at 110°C by 2% and 4.66%, respectively. On the other hand, adding olive leaves to Arbosana varieties at 1% and 3% increased the OS from 8.92 hours by 4.7% and 26.5%, respectively. These findings support the hypothesis that the impact of the addition of olive leaves during oil extraction may depends on the extraction scale and parameters, as well as on the olive leaf cultivar [19].
Oxidative stability is a crucial factor in evaluating the quality of oils and fats. It is greatly affected by their fatty acid composition and minor compounds like tocopherols, phytosterols, and phenolic compounds[42].

Table 2: Physicochemical Properties and Oxidative Stability of Olive Oil Samples.

Sample no.	FFA % (as oleic acid)	Peroxide value (meq O_2/kg oil)	K_{232}	K_{270}	ΔK	Color a at 35 yellow		OS (hr)
						a*	b*	
1	0.45	3.05	1.38	0.047	-0.0015	2.0	0.6	13.43
2	0.41	3.42	1.39	0.063	0.0025	2.0	0.1	8.92
3	0.28	2.7	1.26	0.042	0.0020	2.0	0.0	8.1
4	0.37	4.10	1.43	0.072	-0.0065	2.1	0.3	12.27
5	0.33	2.90	1.25	0.042	-0.0025	2.0	0.3	11.37
6	0.25	3.60	1.22	0.066	-0.0015	2.5	0.8	11.6
7	0.22	2.40	1.29	0.048	-0.0020	3.0	1.3	11.9
8	0.36	3.33	1.41	0.053	-0.0015	2.4	0.5	9.34
9	0.39	3.15	1.39	0.057	-0.0010	2.9	1.1	11.29

Samples: (1) 100% Kroneiki, (2) 100% Arbosana, (3) 100% Arboquina, (4) 50% Koroneiki + 50% Arbosana, (5) 50% Koroneiki + 50% Arboquina, (6) 50% Koroneiki + 50% Arboquina + 1% olive leaves, (7) 50% Koroneiki + 50% Arboquina + 3% olive leaves, (8) 100% Arbosana + 1% olive leaves, (9) 100% Arbosana + 3% olive leaves, and OS (hr) oxidative stability at 110°C.
(Data are expressed as mean values given to represent the means of three determinations.)

3.3 Fatty acid composition

The fatty acid composition is crucial for the nutritional and sensory quality of olive oil. Olive oil is renowned for its high monounsaturated fatty acid content, particularly oleic acid (C18:1), which can make up to 83% of the total composition. Various factors such as fruit ripeness, climate, and variety can influence the fatty acid composition of olive oils [43,44,45,80]. The Koroneiki variety stands out for its FAME composition, with a high proportion of monounsaturated fatty acids (72.79%) and low levels of polyunsaturated fatty acids (PUFA) (10.64%), attributed to its high oleic acid content (C18:1: 70.33%) and low linoleic acid content (C18:2: 9.89). This results in a favorable (0/L) ratio of 7.11, which is significant for the health properties of olive oil.

The Koroneiki variety produces an oil with a low level of palmitic acid (C16:0) at 15.53% and saturated fatty acids (SFA) at 16.94%. These findings are consistent with previous research [46].

The data in Table 3 show that the fatty acid composition of Kronoeik and Arbosana met the requirements of the IOC trade standard [8]. The predominant fatty acid was oleic acid, with levels of 70.33% and 57.7% per 100g of total fatty acids, respectively. However, the Arboquina sample had a lower percentage of oleic acid at 54.77% per 100g. The IOC standard for oleic acid is between 55-83%. These results for Arboquina falling below the IOC limit are consistent with findings from other authors who have noted the significant impact of variety on oleic acid composition. In Egypt, the range of C18:1 in Arboquina variety has been reported to be between 44.0% to 49.42% [14,15,16,79] .

The Kronaeiki olive oil sample had a relatively low level of palmitic acid (C16:0) at 15.53%, compared to the Arbosana and Arboquina varieties at 21.06% and 20.83%, respectively. The saturated fatty acids (SFA) content was 16.54% for the Kronaeiki sample, lower than the Arbosana and Arboquina samples at 22.72% and 22.4%, respectively. These findings are consistent with previous studies [47 ,18 ,15] .

On the other hand, the Arbosana and Arboquina olive oil samples had low levels of (O/L) as 3.97 and 3.15, attributed to their lower oleic acid content (57.21% and 54.79%) and higher linoleic acid content (14.4% and 17.33%) compared to the Kronaeiki olive oil, which had an (O/L) of 7.11, oleic acid content of 70.33%, and linoleic acid content of 9.89%. The O/L ratio of Kronaeiki, Arbosana, and Arboquina olive oil samples at 7.11, 3.97, and 3.15, respectively, correlated with their oxidative stability, with values of 13.43, 8.92, and 8.1 hours at 110°C, as shown in Table 2. This is attributed to the high level of C18:1 (oleic acid) in Kronaeiki olive oil at 70.33%, compared to 57.21% and 54.77% in Arbosana and Arboquina olive oils, respectively. These results align with previous research [48] and highlight the importance of the O/L ratio in determining the health properties of olive oils[46] .

Table 3: Fatty Acid Composition of Pure Samples and Blends and Rancimat Test

Fatty acids / Olive oil samples	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	TSFA	MUSFA	TUSFA	O⁄L	O S at 110 0c
100% Koroneiki	15.53	2.46	1.01	70.33	9.89	0.75	16.54	72.79	83.43	7.11	13.4
100% Arbosana	21.06	4.53	1.66	57.21	14.4	0.73	22.72	62.25	76.86	3.79	8.92
100% Arbiquina	20.83	4.71	1.57	54.79	17.33	0.84	22.40	59.5	77.67	3.15	8.1
50%Koroneiki+50%Arbosana	17.64	3.02	0.49	65.35	11.75	0.72	19.13	68.36	80.84	5.56	12.27
50%Koroneiki+50%Arbiquina	17.82	3.36	1.36	64.71	11.91	0.73	19.17	68.07	80.71	5.43	11.37
50%Koroneiki+50% Arbiquina+ 1% olive leaves	18.72	3.49	1.53	63.60	11.98	0.66	2.25	67.09	79.73	5.30	11.6
50%Koroneiki+50%Arbiquina +3% olive leaves	19.19	3.6	0.83	63.63	12.06	0.70	19.98	67.31	79.77	5.27	11.9
Arbosana+1% olive leaves	20.31	4.29	1.71	58.42	14.45	0.79	22.02	62,71	77.95	4.52	9.34
Arbosana+3% olive leaves	20.64	4.38	1.18	58.78	14.21	0.71	21.82	63.16	78.08	4.13	11.29
IOC limits	7-20	0.3-3.5	0.5-5	55-85	2.5-21	≤1.0	-	-	-	-	-

TSFA: Total Saturated Fatty Acids, MUSFA: Mono Unsaturated Fatty Acids, TUSFA: Total Poly Unsaturated Fatty Acids, O⁄L: Oleic Fatty Acid / Linoleic Fatty Acid, OS; oxidative stability.

3.3.1 Impact of fruit mixing on fatty acid composition:

Table 3 shows that mixing Arbosana or Arboquina fruits with Koroneiki variety in a 50:50 ratio effectively increased the levels of C18:1 and monounsaturated fatty acids (MUFA) while reducing the levels of saturated fatty acids (SFA), particularly palmitic acid (C16:0). When mixing Koroneiki with Arbosana or Arboquina at a 50:50 ratio, oleic acid increased by 8.14% and 9.92%, respectively, bringing them within the International Olive Council (IOC) limit of 55-85%. For Arboquina, which initially had 54.77% oleic acid, the mixture reached 64.71% oleic acid, within the IOC limit. The mixing also led to a reduction in linoleic acid (C18:2) from 14.40 to 11.75 and from 17.33 to 11.91. Additionally, palmitic acid decreased from 21.06 to 17.64 and from 20.83 to 17.87, respectively. These mixtures exhibited improved fatty acid compositions compared to pure Arbosana or Arboquina varieties, with higher C18:1 content (65.35 and 64.71), total unsaturated fatty acids (TUFA) content (80.84 and 80.71), and lower palmitic acid levels (17.64 and 17.27), resulting in a significant improvement in the oleic acid to linoleic acid ratio (5.56 and 5.43).

This enhancement in stability to oxidation was evident in the blends, with the oxidative stability increasing from 8.92 and 8.10 hours at 110°C in pure Arbosana and Arboquina olive oils to 12.27 and 11.37 hours, respectively, in the mixed oils. Therefore, mixing fruits proved to be an effective method for improving the oxidative stability of Arboquina variety and correcting its poor resistance to oxidation, as well as adjusting the C18:1 content [(18).]

3.3.2 The Impact of Olive Leaf Addition on Fatty Acid Composition

The fatty acid composition of olive oils extracted from mixtures of 50% Kroneiki and 50% Arboquina fruits with varying percentages of olive leaves (1% and 3%) is presented in Table 3. The fatty acid profiles obtained with both 1% and 3% olive leaves comply with the regulations set for olive oil by the International Olive Council (IOC)[(8)]. Palmitic acid content increased by approximately 1% in both mixtures with 1% or 3% olive leaves, resulting in a slight increase in total saturated fatty acids from 19.97% to 20.25% and 19.98%, respectively. Conversely, C18:1 content decreased by nearly 1%, while C18:2 showed a slight increase. The oleic/linoleic (O/L) ratio remained unchanged, but the oxidative stability increased from 11.37 hours to 11.6 hours and 11.9 hours with 1% and 3% olive leaves, respectively. These results indicate the presence of bioactive compounds in olive oil enriched with olive leaves, known for their high phenolic content [(49)].

When olive leaves were added to Arbosana, significant changes were observed in the major fatty acid fractions of the olive oil. Palmitic acid decreased by 3.5% and 2%, while saturated fatty acids decreased by 3% and 4%. Conversely, oleic acid (C18:1) increased by 2.1% and 2.7%, and linoleic acid (C18:2) showed slight changes in olive oil samples enriched with 1% and 3% olive leaves, respectively. The O/L ratio increased from 3.97% to 4.52% and 4.13%, respectively. The increase in oxidative stability from 8.12 hours at 110°C to 9.34 hours and 11.29 hours may be attributed to the increase in the O/L ratio, as well as the presence of bioactive compounds in olive oil enriched with olive leaves, known for their phenolic content.

3.4 Minor compound of olive oil samples:

3.4.1 Total polyphenol*s:*

The presence of phenol compounds in virgin olive oil contributes to its nutritional value, stability, food color, sensory attributes, and health-related antioxidant effects [(50)] . While olive oil was traditionally valued for its high oleic acid content, scientific research now recognizes the significant role of phenols in its health benefits[(51)]. According to Table 4, the phenol content varies among olive oil cultivars, with Koroneiki oil having the highest total phenols at 426.92 mg/kg, followed by Arbosana oil at 165.32 mg/kg, and Arboquina oil with the lowest content at 136.5 mg/kg. These findings are consistent with

previous studies[16,18,35,52]. Reported total phenolic content in mg/kg for Koroneiki, Arbosana, and Arboquina varieties were 242.35, 186.40, and 137.23 mg/kg, respectively. Higher phenol content levels were reported for the same varieties at 566.3, 454.8, and 286.51, respectively.

There is a wide range of experimental studies on olive oil phenol compounds, including in vitro and animal models, addressing various health issues. Human randomized controlled studies have provided scientific evidence of health benefits, such as reducing LDL oxidation, inflammation, and potentially increasing HDL cholesterol levels and providing antithrombotic effects[53]. The European Food Safety Authority (EFSA) released an important statement in 2011 based on scientific research, confirming the role of phenols in human health and the effectiveness of consuming olive phenols (5mg/day) in protecting LDL from oxidation [54].

Adding Koroneiki to Arbosana or Arboquina varieties in a 50:50 ratio increased the level of phenolic compounds in the olive oils by 76.24% and 111.9%, respectively. This increase in phenolic content is attributed to the high content of phenolic compounds in Koroneiki varieties.

When mixing fruit samples, the higher polyphenol content in Koroneiki oil led to an increase in total phenol concentration in the final olive oil product. It is evident that the addition of fruit enhanced the phenolic compounds in the final olive oil compared to the initial Arbosana or Arboquina varieties.

Adding 1% and 3% olive leaves to a fruit mixture of Koroneiki and Arboquina at a 50:50 ratio resulted in a significant increase in phenolic content. The total phenols in the 50:50 mixture were 288.15 mg/kg of oil, which increased to 309.54 mg/kg with 1% olive leaves (a 7.42% increase) and 347.65 mg/kg with 3% olive leaves (a 20.64% increase). Similarly, adding 1% and 3% olive leaves to 100% Arbosana increased the total polyphenol content by 20.23% and 33.69%, respectively.

The literature indicates a wide range of total polyphenol content in extra virgin olive oil (EVOO), ranging from 50 to 940 mg/kg oil. This variation is influenced by factors such as the olive cultivar, maturity index, extraction method, and environmental conditions [54] .

Another observation of TPC showed a 4% decrease for cvs Leccino and Castigliones oils [39]. On the other hand, laboratory-extracted oils showed a 2-25% increase in TPC, depending on the cultivars [55, 56, 57]. In another study [58] on the influence of adding olive leaf during extraction of oil from Buza cv. fruits, it was found that the phenolic composition of the oil depended on the amount of leaf added. Adding 1% olive leaf had no effect on phenolic composition or total phenolic content, while adding 2.5% and 5% leaf caused a decrease in both particular and total phenolic content, respectively. It is reported that phenolic contents contribute nearly 51% to Rancimat stability, fatty acid composition contributes 24%, and tocopherol contributes 11% [59].

Table 4: Minor Components and Antioxidant Activity of Olive Oil Samples

Sample no.	Total poly phenols (mg as Gallic acid/ kg oil)	Antioxidant activity of olive oil phenol %	Carotenoids (mg/kg oil)	Chlorophyll (mg/kg oil)
1	426.92	90.30	0.181	1.50
2	165.32	51.84	0.171	1.22
3	136.50	40.79	0.137	0.83
4	291.37	68.7	0.174	1.32
5	288.15	65.33	0.156	1.21
6	309.54	70.53	0.214	1.66
7	347.65	73.35	0.328	3.12
8	185.55	55.77	0.214	1.57
9	221.02	61.74	0.304	2.93

(Data are expressed as mean values given to represent the means of three determinations.)

3.4.1.1 Antioxidant activity (DPPH)

The DPPH test is a common assay used in investigating antioxidant activity and provides a quick method for determining a compound's radical scavenging activity [57]. The stable free DPPH radical serves as a useful reagent for studying the scavenging activity of bioactive compounds.[57]

The radical scavenging capacity of the examined olive varieties clearly differs: Koroneiki, Arbosana, and Arboquina show percentages of 90.30%, 51.84%, and 40.79%, respectively. A positive correlation was observed between the antioxidant activity (DPPH) and the concentration of total polyphenols. These correlations are consistent with a report by.[60]

Extra virgin olive oil from the Koroneiki cultivar exhibited higher antioxidant activity compared to oils from Arbosana and Arboquina cultivars. These values were similar to those reported by other [61] for Tunisian cultivars (78.56% and 37.23%) for Chetoui and Chemlali varieties, respectively, and also from Algeria [62] (11 cultivars) which ranged between 36.57% and 72.20%.

Another study evaluated the DPPH assay results for four samples of virgin olive oil from the Frantoio cultivar, showing a range of antioxidant activity between 56.5% and 106.0% [63]. In contrast, lower antioxidant activity was observed for three different Italian olive oil varieties, with values of 18.33%, 36.85%, and 27.37% [64].

When mixing fruit samples, it was observed that adding Koroneiki to Arbosana or Arboquina varieties increased the DPPH% assay. This is likely due to the higher polyphenol content in Koroneiki. The DPPH assay recorded values of 68.7% and 65.33% for mixtures of Koroneiki with Arbosana and Koroneiki with Arboquina at a 50:50 ratio.

The inclusion of olive leaves in the extraction process impacts the quality parameters of the fortified olive oils being studied. This is evident from the DPPH assay results when olive leaves were added at 1% or 3% to a blend of Koroneiki and Arboquina at a 50:50 ratio, as well as to 100% Arbosana. In the Koroneiki-Arboquina blend, the DPPH values increased from

65.33% to 70.53% and 73.35% with the addition of olive leaves at 1% and 3%, respectively. For 100% Arbosana, the DPPH values increased from 51.84% to 55.77% and 61.74% with the same olive leaf additions. These findings are consistent with previous research [49] indicating that the addition of leaves during extraction and processing can inhibit oxidation and reduce peroxide formation by half (IOC, 2019) [65].

3.4.2 Chlorophyll and Carotenoids:

Chlorophyll and carotenoids play crucial roles in olive oils. They contribute to the oxidative stability, acting as antioxidants in the dark and as prooxidants when exposed to light. Additionally, these compounds are responsible for the yellow-green pigmentation of olive oils, which enhances consumer acceptability [66]. Furthermore, the bioactivity of these pigments is linked to their health properties for various human organs, including the brain and nervous system [67].

Significant variations were observed in the chlorophyll content of the analyzed olive oils. Koroneiki exhibited the highest level at 1.5 mg/kg of oil, while Arboquina had the lowest at 0.83 mg/kg of oil, and Arbosana recorded 1.22 mg/kg of oil. These findings are consistent with the reported values for Arboquina variety in two regions in Turkey, which were 0.52 and 0.64 mg/kg of oil, as documented in a previous study [68]

On the other hand, Spanish Clamala olive oil contained 1.68 mg/kg of chlorophylls as reported previously [49]. Egyptian Arboquina from North Sinai and Ismailia had chlorophyll levels of 0.52 and 0.64 mg/kg of oil, respectively [69]. Additionally, higher chlorophyll contents were found in Kronaeiki, Arbosana, and Arboquina varieties, with levels of 7.20, 6.20, and 5.4 mg/kg, respectively[16] .

Regarding carotenoids, the data in Table 4 shows slight carotenoid contents for the three varieties: 0.181 mg/kg for Koroneiki, 0.171 mg/kg for Arbosana, and 0.137 mg/kg for Arboquina.

3.4. Impact of Fruit Mixing on Chlorophyll and Carotenoid Contents

The fruit mixing process significantly affected the amount of pigments, depending on the proportion of cultivar in the mixture. Due to its high pigment content, mixing fruit with the Koroneiki variety led to an increase in pigment concentrations compared to other varieties initially present.

When Koroneiki fruits are mixed with Arbosana or Arboquina fruits in a 50:50 ratio, the chlorophyll content increased from 1.22 to 1.32 and from 0.83 to 1.21, representing an 8.19% and 45.78% increase compared to the initial chlorophyll content of Arbosana and Arboquina, respectively. For carotenoids, the mixed fruit cases showed an increase from 0.171 to 0.174 and from 0.137 to 0.156 mg/kg of oil, representing a 1.75% and 13.86% increase, respectively.

The observed increases may be attributed to the higher content of chlorophyll and carotenoids in the koroneiki variety, with levels of 1.50 and 0.181 mg/kg of oil, respectively. Previous studies have shown that pigment concentrations can vary significantly based on the variety [70], fruit ripeness [71], climate [72], and growing conditions [73].

Other results suggest that processing fruit mixtures of different cultivars resulted in better oil quality compared to oils obtained through traditional oil blending methods[47] .

3.4.2.1 Impact of Olive Leaf Addition on Chlorophyll and Carotenoid Levels

The addition of olive leaves at 1% and 3% to a mixture of fruits in a 50:50 ratio of Kroneiki and Arboquina resulted in varying chlorophyll content increases in the oil. Specifically, the chlorophyll content increased by 37.19% and 157.85% compared to the initial mixture, respectively. These findings are consistent with other studies that have also reported an increase in chlorophyll levels [58 ,57 ,55 ,41] .

When olive leaves were added at 1% and 3% to 100 Arbosana fruits, the chlorophyll content increased by 28.68% and 140.16%, respectively, compared to Arbosana oil without olive leaves.

A study [58] found that there was an increase in chlorophyll content during the extraction of Bjazacv oil when the amount of olive leaves was increased to 1%, 2.5%, and 15%. The increase in chlorophyll content was 38%, 375%, and 1180%, respectively. This increase may be attributed to the higher degree of leaf cell breakage during the milling of leaves and olive fruits, which allowed for the release of chlorophyll into the oil [39] .

The increase in chlorophyll content can enhance the antioxidant properties of oil when stored in the dark, thereby prolonging its shelf life [55]. However, chlorophyll can act as a prooxidant in the presence of light [66]. Therefore, special care should be taken when storing oil enriched with olive leaf chlorophylls. It is highly recommended to preserve such products in dark, non-transparent bottles.

The addition of 1% and 3% olive leaf increased the carotenoid content by 37.17% and 110.25%, respectively, in a 50:50 mixture of Koroneiki and Arboquina oils. Similarly, adding 1% and 3% olive leaves to Arbosana fruits resulted in carotenoid increases of 25.14% and 136.25% compared to pure Arbosana oil. These additions also enhanced the green color of the olive oils due to the antioxidant activity of chlorophyll, which can help prevent cancer-causing agents. This increase in chlorophyll concentration makes the fortified oils more nutritionally appealing.[21,36]

3.5 Identification of Phenolic compounds-

Phenolics are crucial components that contribute to the quality and sensory characteristics of olive oil. They serve as powerful antioxidants with significant benefits for human health and diet [74]. Research strongly indicates that phenols play a key role in preventing cancer, cardiovascular diseases, and neurodegenerative conditions. The natural antioxidant content of oils is directly linked to their shelf life [75]. Phenolics effectively delay the oxidative degradation process, thereby extending the product's shelf life [74,76].

The molecular compounds of phenolic compounds in the olive oil samples were identified and quantified in all hydro-methanolic extracts. Table 5 shows variations in the content of phenolic compounds based on the cultivar. Oleuropein and hydroxytyrosol were the predominant compounds in all analyzed oils, with oleuropein being the most abundant, followed by phenolic alcohols and phenolic acids. A total of eighteen phenolic compounds were identified in all olive oil samples. The

Koroneiki variety exhibited the highest oleuropein content at 147.4 mg/kg oil, followed by Arboquina and Arbosana at 80.5 and 73.1 mg/kg oil, respectively.

Our results show that phenolic alcohols were present in higher levels than phenolic acids in the three varieties of olive oil studied. Koroneiki had the highest concentration at 141.7 mg/kg, followed by Arbosana at 54.5 mg/kg, and the lowest was Arboquina at 33.1 mg/kg. In contrast, phenolic acids were most abundant in Koroneiki oil at 89.7 mg/kg, while Arbosana and Arboquina had lower levels at 20.4 mg/kg and 14.85 mg/kg, respectively. The phenolic alcohols identified were hydroxytyrosol and kaempferol. The analysis also revealed the presence of various phenolic acids, including gallic, chlorogenic, caffeic, syringic, ellagic, coumaric, ferulic, and cinnamic acids. The levels ranged from 0.2 mg/kg of coumaric acid in Arbosana and Arboquina oils to 11.1 mg/kg of cinnamic acid in Koroneiki oil.

The variation in the levels of Oleuropen, phenolic alcohols, and phenolic acids in olive oil depends on the variety, as shown in Table 5. These results align with previous studies on phenolics in olive oil [16,35,57,58,64].

The addition of olive leaves at 3% to a mixture of Koroneiki and Arboquina fruits in a 50:50 ratio, as well as to 100% Arbosana fruits, significantly impacted the phenolic compounds present in the extracted olive oils. Specifically, the incorporation of Koroneiki olive leaves at 3% to Arbosana fruits led to a 38.16% increase in oleuropein content. Additionally, the addition of 10% Arboquina leaves resulted in a 13% increase in oleuropein levels. Phenolic alcohols also saw a substantial increase of 124.77%, while phenolic acids increased by 28.18% in the oils extracted with olive leaves [19].

Another study found that Oueslati olive leaves had a higher phenolic concentration compared to Nebjnel leaves. The study revealed that adding leaves at a 3% concentration increased the total phenolic content by 44% in Oueslati oil and by 10% in Nebjnel oil. These results were consistent with findings from other studies [23,39,55,57].

A study [19,58] demonstrated that adding olive leaves during the olive oil extraction process resulted in a significant reduction in total phenolic content.

In recent years, there has been extensive research on the biological activities of olive oil phenolics, particularly Oleuropein derivatives. Numerous studies have investigated the antioxidant properties of oleuropein derivatives, as excessive reactive oxygen species have been linked to the development of various diseases [77]. The importance of powerful antioxidants to counteract free-radical damage has been emphasized. The antioxidant effects of oleuropein are primarily attributed to its ability to scavenge free radicals. Overall, incorporating olive leaves during the oil extraction process may offer a way to enhance the quality of the oil.

Table 5: Phenolic compounds (mg/Kg oil) of olive oil varieties and their mixture extracted with addition of olive leaves at (3%)

Compound	Olive variety			% olive leaves 3 %	
	koroneiki	Arbosana	Arboquina	koro.;Arbeq. 50;50	Arbosana 100 %
Gallic A	15.1	4.7	1.5	10.5	5.9
Chlorogenic A	12.2	3.6	2.0	9.1	4.5
Catechin	3.3	0.3	0.3	1.4	0.35
Tyresol3- oH	131.4	52.5	28.7	106.0	71.0
Caffeic A	17.5	0.00	4.0	14.5	0.0
Syringic A	11.3	2.6	1.4	8.5	2.6
Rutin	13.1	2.1	2.3	10.3	2.5
Ellagic A	9.5	1.9	0.85	6.5	0.9
Coumaric A	3.3	0.2	0.20	1.3	0.25
Vanillen	19.2	3.2	0.50	12.6	3.00
Feruleic A	9.7	4.4	1.7	9.0	6.01
Noringenin	4.3	2.2	1.8	2.0	1.5
Oleuropin	147.4	37.1	80.5	133.9	101.0
Daidzein	3.7	1.5	1.0	3.0	2.35
Querctia	3.3	0.5	0.50	0.4	0.9
Cinnamic A	11.1	3.0	2.6	9.00	6.0
Kaempferol	10.3	2.0	4.4	8.0	3.4
Hespertin	1.2	7.5	2.0	1.6	8.9
Totelphenok	426.92	165.32	136.5	347.65	221.02
phenolic acds	89.7	20.4	14.25	68.4	26.15
phenolic alcohols	141.7	54.5	33.1	114.0	74.4

(Data are expressed as mean values given to represent the means of three determinations.)

3.6 Organoleptic attributes

The results in Table 6 showed that Koroneiki olive oil exhibited higher fruity, bitter, and pungent sensory attributes (positive attributes) compared to Arbosana and Arboquina olive oil. These positive sensory attributes were further enhanced by mixing Koroneiki fruits with both Arbosana and Arboquina fruits and adding olive leaves during the oil extraction process.

Tasters observed higher intensities of green fruity, pungent, and bitter flavors with an increase in the percentage of olive leaves from 1% to 3%. These increases may be attributed to the higher total phenolic content in olive oil when olive leaves are added during the oil extraction process (Table 4). These findings are consistent with previous studies [23, 41, 40].

The data in Table 6 shows that the median number of defects in all oil samples was zero, while the median values for fruity, pungent, and bitter (positive attributes) were greater than zero. These results indicate that all oil samples met the criteria for extra virgin olive oil classification as defined by the International Olive Council [8,80].

Table 6: Organoleptic assay of olive oil samples

Samples	Fruity	Bitterness	pungent
100% koroneikii	5.4	3.7	4
100% Arbosana	2.9	1.5	1.5
100% Arbiquina	2.2	1	1.0
50%Koroneiki+50%Arbosana	4.1	2.6	2.5
50%Koroneiki+50%Arbiquina	4.3	2.6	3.0
50%Koroneiki+50% Arbiquina+ 1% olive leaves	4.9	3.2	2.8
50%Koroneiki+50%Arbiquina +3% olive leaves	5.7	4.0	3.5
Arbosana+1% olive leaves	4.5	3.0	2.5
Arbosana+3% olive leaves	5.3	3.5	3.0

(Data are expressed as mean values given to represent the means of three determinations.)

Conclusion

The physicochemical quality of olive oils from the studied cultivars (Koroneiki, Arbosana, and Arboquina) met the standards set by the International Olive Council for extra virgin oils .

Our study results indicate that the Arboquina variety has a lower percentage of C18:1 at 54.77%, which falls below the IOC limit of 55-83%. Additionally, it is less stable in terms of oxidation compared to the oils of the Koroneiki and Arbosana varieties.

The Koroneiki variety stands out for its high monounsaturated fatty acids content, richness in total polyphenolic constituents, and high levels of oleuropein, phenolic alcohol, and phenolic acids compared to the Arbosana and Arboquina varieties.

The data from this investigation show that oils produced by blending equal parts of Koroneiki and Arbosana or Koroneiki and Arboquina fruits result in higher oil quality and increased levels of bioactive compounds. The mixing process allows for the adjustment of antioxidant, fatty acid, and sensory characteristics. When Koroneiki is mixed with Arboquina fruits in a 50:50 ratio, the resulting oil meets the International Olive Council standards for C18:1, suggesting that blending fruits can improve oxidative stability and oleic acid content, particularly for the Arboquina variety. This blending technique offers a solution to enhance resistance to oxidation and correct fatty acid composition.

The addition of up to 3% Koroneiki olive leaves to a mixture of Koroneiki and Arboquina fruits in a 50:50 ratio and to 100% Arboquina fruits had a significant impact on the quality and chemical composition of the olive oil. This resulted in increased resistance to oxidation, improved nutritional qualities, and enhanced positive organoleptic attributes such as fruity, bitterness, and pungency. There was also a notable increase in total polyphenols, oleuropein, phenolic alcohols, and phenolic acids, leading to higher levels of total phenolic compounds, chlorophyll, and carotenoids. In conclusion, the addition of small percentages of Koroneiki olive leaves can enhance the nutraceutical properties of extra virgin olive oil by boosting the content of phenolic compounds, which provide protection against oxidative stress and extend the shelf-life of the oil due to their antioxidant properties.

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REFERENCES

1-Cavaca, L. A. S.,López-Coca, I. M., Silvero, G., Afonso, C. A. M. (2020).The olive-tree leaves as a source of high-added value molecules: Oleuropein stud. Natural Products Chemistry, 64, 131:180.

2- Kiritsakis, k., Rodríguez-Pérez, C., Gerasopoulos, D. and Carretero, A.S. (2017).Olive oil enrichment in phenolic compounds during malaxation in the presence of olive leaves or olive mill wastewater extracts: Use of olive oil by-products for olive oil phenol-enrichment.European J. of Lipid Sci. and Technol., 119(9)-1:13.

3-Perona, J. S. and Botham, K.M. (2013). Olive Oil as a Functional Food: Nutritional and Health Benefits.Handbook of Olive Oil USA (pp.677-714).

4-Rotondi, A., Bendini, A., Cerretani, L., Mari, M., Lercker, G. and Toschi, T.G.(2004).Effect of olive ripening degree on the oxidative stability and organoleptic properties of cv. Nostrana di Brisighella extra virgin olive oil.JAgric Food Chem. 2;52(11):3649-54.
5-Sanmartin, C., Venturi., F., Sgherri, C., Taglieri, I., Andrich, G. and Zinnai, A. (2018). The effects of packaging and storage temperature on the shelf-life of extra virgin olive oil.Heliyon. 4, 11, e00888.
6-Taghvaei, M. and Jafar S. M. (2015). Application and stability of natural antioxidants in edible oils in order to substitute synthetic additives.J Food Sci Technol. 52(3): 1272–1282.
7-Codex alimentarius commission. (2024).
8-IOC. (2022).Trade standard applying to olive oilsand olive pomaceoils.COI/T.15/NC No 3/Rev. 19.
9-Bouaziz, M., Jemai, H., Khabou, W. and Sayadi, S. (2010). Oil content, phenolic profiling and antioxidant potential of Tunisian olive drupes. J Sci Food Agric. 15;90(10):1750-1758.
10-Psomiadou, E., Tsimidou,M. and Boskou, D. (2000).Alpha-tocopherol content of Greek virgin olive oils.JAgric Food Chem. 48(5):1770-1775.
11- Borges, T. H., Serna, A., López, L. C., Lara, L., Nieto, R. and Seiquer, I. (2019). Composition and Antioxidant Properties of Spanish Extra Virgin Olive Oil Regarding Cultivar, Harvest Year and Crop Stage.Antioxidants. 11;8(7):217.
12-Mansouri, F., Benmoumen, A., Richard, G., Fauconnier, M.L., Sindic, M.,Serghini-Caid, H. and Elamrani, A.(2016). Characterization of monovarietal virgin olive oils from introduced cultivars in eastern Morocco. La rivistaitalianadellesostanzegrasse – 93, 21-30.
13- Molina, M.S., Matías, A.C. and Maldonado, M. (2014). Improvement of the 'Arboquina' olive oil quality from central valley of Catamarca, Argentina, by preparation of blends with olive oils varietals. ActaHorticulturae. 1057(1057):685-692.
14- Benincasa, C., Russo, A., Romano, E., Muhammad E. E., Perri, E. and Muzzalupo, I. (2011). Chemical and Sensory Analysis of Some Egyptian Virgin Olive Oils.Journal of Nutrition & Food Sciences.1:5.
15- Eid, M. M. and EL-sayed, M. M. (2013). Characterization of some new olive oil genotypes growing in El-khatatba zone – Egypt.Egyptian Journal of Agricultural Research . 91, 2, 611-626.
16- Arafat , S.M., Abd El-Baset, W.A. and ElLabban , A. (2022). The quality of olive oil extracted from some olive varieties cultivated by highly intensive in Egypt. Egypt. J. Chem. 65, 8, 407- 417.
17- IOC (2007). Sensory analysis of olive oil.methods for the organoleptic assessment of virgin olive oil. COI/T 20/DOC 15/REV.2.
18- Mansouri, F., Ben Moumen, A.,Houmy, N. Fauconnie, M., Sindic, M., Caid, H.S. and Elamrani, A.(2014). Evaluation of the oxidative stability of blends of 'Arboquina' olive oils with other monovarietal olive oils.OLIVA NO.120.
19-Marx, Í. M. G., Casal, S., Rodrigues, N., Cruz, R., Veloso, A. C. A., José, A. P.and Peres, A. M. (2022). Impact of incorporating olive leaves during the industrial extraction of cv. Arboquina oils on the physicochemical–sensory quality and health claim fulfillment.European Food Research and Technology.248, 171–183.
20 – Bouaziz, M., Fki, I., Jemai, H., Ayadi, M. and Sayadi, S. (2008).Effect of storage on refined and husk olive oils composition: Stabilization by addition of natural antioxidants from Chemlali olive leaves. Food Chemistry 108, 1, 253-262.
21- Vidal, A.M., Alcalá,S., Ocaña, M. T., De Torres, A., Espínola, F. and Moya, M. (2019). Elaboration of extra-virgin olive oils rich in oleocanthal and oleacein: pilot plant's proposal. European Food Research and Technology, 246, 1459–1468.
22- Caponio, F., Difonzo, G., Calasso, M., Cosmai, L. and De Angelis, M. (2019).Effects of olive leaf extract addition on fermentative and oxidative processes of table olives and their nutritional properties. Food Res Int, 116:1306-1317.
23- Ammar, S., Kelebek, H. Abichou, M., Selli, S. and Bouaziz, M.(2017). LC-DAD/ESI-MS/MS characterization of phenolic constituents in Tunisian extra-virgin olive oils: Effect of olive leaves addition on chemical composition.Food Research International, 100:477-485.
24-Tous, J. (2018). High planting density trial with olive cultivar 'Arboquina'. ActaHorticulturae, 1199:285-290.
25-Ryan, D., Antolovich, M., Prenzler, P., Robards, K. and Lavee, S. (2002). Biotransformations of phenolic compounds in Oleaeuropaea L. ScientiaHorticulturae, 92, 2, 147-176.
26-Nenadis, N., Wang, L., Tsimidou, M.Z. and Zhang, H. (2005). Radical scavenging potential of phenolic compounds encountered in O. europaea products as indicated by calculation of bond dissociation enthalpy and ionization potential values. J Agric Food Chem, 26;53(2):295-299.
27-Moyano, M.J. , Heredia, F.J. and Meléndez-Martínez, A.J. (2010).The Color of Olive Oils: The Pigments and their likely health benefits and visual and instrumental methods of analysis. Compr Rev Food Sci Food Saf, 9(3):278-291.
28-AOAC Official Methods of Analysis of AOAC International 20th Ed. Association of Official Analytical Chemists, Gaithersburg, Maryland.U.S.A (2019).
29- M´ınguez-Mosquera, M.I.,Rejano, L.,Gandul, A., Sanchez, B.H. andGarrido, J. (1991).Color-pigment correlation in virgin olive oil. J. Am. Oil. Chem. Soc. 68:322-337.
30-ISO (12966-2). International standard.Animal and vegetables fats and oil preparation of methyl esters of fatty acids (2017).
31-Gutfinger, T. (1981).Polyphenols in olive oils. J. Am. Oil Chem. Soc. 58:966-968.
32-Mendez, E.; Sanhveza, J.; Spoisky, H. and Volenzueln, A. (1996).Validation of the rancimate test for the assessment of the relative stability of fish oil. Journal of the American Oil Chemists Society, 73:1033-1037.
33-Blois, M. S. (2002). Antioxidant determination by the use of a stable free radical.Nature. 26:1119-1200.
34-Goupy, P.,Hugues, M.,Biovin, P. &Amiot, M. J. (1999). Antioxidant composition and activity of barley (Hordeumvulgare) and malt extracts and of isolated phenolic compounds. J. Sci. Food Agric. 79:1625-1634.
35-Mansouri, F., Ben Moumen, A., Aazza, S., Belhaj, K., Fauconnier, M. L., Sindic, M., Serghini, H. C. andElamrani, A. (2019).Quality and chemical profiles of virgin olive oils of three European cultivars suitable for super-high-density planting conditions in eastern Morocco. Materials Today: Proceedings, 13 998–1007.

36-Lorini, A., Aranha, B. C., Antunes, B. D., Otero, D. M., Jacques , A. C.andZambiaz, R. S. (2021).Metabolic profile of olive leaves of different cultivars and collection times. Food Chem., 30:345:128758.
37-Chtourrou M, Gorgourib, JaberH,Abdelhedir, BouazizM(2013) Comparative study of olive oil quality from chemlolisfaxvarisusArboquina cultivated in Tunisia. Eurj Lipid scitechnal 115:631-640.
38-DE MEJJA, EG, ZHANG Q, PENTA, K, EROGLU A, LILA. MA,(2020). The colors of health: Chemistery, bioactivity and market demand for colorful foods and natural food sources of colorants. Annual Review of food Sci. and Tch.11: 145-182
39- Di Giovacchino,L., Angerosa,F. and Di Giacinto,L. (1996).Effect of mixing leaves with olives on organoleptic quality of oil obtained by centrifugation. Journal of the American Oil Chemists' Society, 73, 371–374.
40- Aguilera, M. P., Beltrán , G.,Ortega, D.Fernández, A., Jiménez, A., and Uceda, M.(2005). Characterisation of virgin olive oil of Italian olive cultivars: "Frantoio" and "Leccino", grown in Andalusia. Food Chemistry, 89: 387-391.
41-Malheiro, R., Casal, S., Teixeira, H., Bento, A. and Pereira, J.A. (2013). Effect of olive leaves addition during the extraction process of over mature fruits on olive oil quality. Food and Bioprocess Technology, 6, 509–521.
42- Santos, K. A., Filho, O. B. A., Aguiar, C. M.,Milinsk, M. C., Sampaio b , Palú, S. C. F. and da Silva, E. A. (2017). Chemical composition, antioxidant activity and thermal analysis of oil extracted from favela (Cnidoscolusquercifolius) seeds. Industrial Crops and Products, 97 368–373.
43- García, J. M., Seller, S. and Pérez-Camino, M. C. (1996). Influence of fruit ripening on olive oil quality.Journal of Agricultural and Food Chemistry.44, 11.
44-Judde, A. (2004).Prévention de l'oxydation des acidesgrasdans un produitcosmétique :mécanismes, conséquences, moyens de mesure, quelsantioxydants pour quellesapplications?.OCL, 11, 6, 414: 418.
45-Pardo, J.E., Cuesta, M.A. and Alvarruiz, A. (2007). Evaluation of potential and real quality of virgin olive oil from the designation of origin "Aceite Campo de Montiel" (Ciudad Real, Spain). Food Chemistry, 100, 3, 977:984.
46- Dag, A.,Kerem, Z.,Yogev, N., Zipori, I.,Lavee, S. and Ben-David, E. (2011). Influence of time of harvest and maturity index on olive oil yield and quality. ScientiaHorticulturae, 127, 3, 358:366.
47- Tekaya1, M., Amel, M. A., Mechri, B., Ayadi, M., Aouina4, JalelMkada, M. B, Belkhiria, H., Guesmi, A., Ben Hamadi, N., Hammami, M., Flamini, G.andMezghani, M. A. (2022). Biochemical characterization of olive oil samples obtained from fruit mixtures and from oil blends of four cultivars grown in Central Tunisia. OCL, , 29, 5.
48- Gomez-del-Campo, M., Pérez, A. G. and García, J. M. (2023).Olive Oil Quality of Cultivars Cultivated in Super-High-Density Orchard under Cold Weather Conditions. Horticulturae, 9(7), 824.
49-Baccouri, B., Rajhi, I., Theresa, S., Najjar, Y., Mohamed, S. N. and Willenberg, I. (2022). The potential of wild olive leaves (Oleaeuropaea L. subsp. oleaster) addition as a functional additive in olive oil production: the effects on bioactive and nutraceutical compounds using LC–ESI–QTOF/MS. European Food Research and Technology, 248, 2809–2823.
50-Nenadis, N., Mastralexi, A. and Tsimidou. M. Z.(2019). Physicochemical Characteristics and Antioxidant Potential of the Greek PDO and PGI Virgin Olive Oils (VOOs).The European Journal of Lipid Science and Technology, 16, 4.
51-Solinas, M.(2010).Olive oil quality and its determination factors proceeding of problems on olive oil quality, congress, sassavi, Italy, 23-55.
52-Reboredo-Rodríguez, P., González-Barreiro, C., Cancho-Grande, B., Fregapane, G., Salvador, M.D. and Simal-Gándara, J. (2015). Blending local olive oils with Arboquina or Picual oils produces high quality, distinctive EVOOs.The European Journal of Lipid Science and Technology. 17,8, 1238-1247.
53- Martín-Peláez, S., Covas, M., I., Fitó, M., Kušar, A. and Pravst, I. (2013). Health effects of olive oil polyphenols: recent advances and possibilities for the use of health claims.Mol.Nutr.Food Res, 57(5):760-71.
54- Servili, M., Sordini, B., Esposto, S., Urbani, S., Veneziani, G., Maio, I. D., Selvaggini, R. and Taticchi, A. (2014).Biological Activities of Phenolic Compounds of Extra Virgin Olive Oil. Antioxidants (Basel), 3(1): 1–23.
55-Sevim, D. and Tuncay, O. C. (2013). Effect of olive leaves addition before extraction of Turkish olive cultivars on olive oil minor components and antioxidant activity. Open Access Scientific Reports, 3, 2:1-8.
56-Sanmartin, C., Taglieri, I., Macaluso, M., Sgherri, C., Ascrizzi, R., Flamini, G., Venturi, F., Quartacci, M. F.,Luro, F., Curk, F., Pistelli, L. and Zinnai, A. (2019). Cold-pressing olive oil in the presence ofcryomacerated leaves of olea or citrus:nutraceutical and sensorial features.Molecules, 24, 2625.
57-Tarchoune, I.,Sgherri, C., Eddouzi, J.,Zinnai, A.,Quartacci, M. F. and Zarrouk, M. (2019). Olive leaf addition increases olive oil nutraceutical properties. Molecules, 24(3): 545.
58- Novoseli´c, A., Klisovi´c , D., Luki´c , I., Luki´c, M. and Bubola, K. B. (2021). The use of olive leaves in Buža olive cultivar oil production: exploring the impact on oil yield and chemical composition. Agriculture, 11, 917.
59-Aparicio, R., Roda, L., Albi, M. A. and Gutiérrez, F. (1999). Effect of Various Compounds on Virgin Olive Oil Stability Measured by Rancimat. J. Agric. Food Chem., 47, 10, 4150:4155.
60-Somaniego-Sanchez C ,Trancosa Gonzalez AM. Garcia- Parrilla Mc, Quesada Granados JJ, Lopez Garcia de La Serrano H, Lopez Martinez (2007) Different radical scavenging tests in Virgin olive oil and their relation to the total phenol content. Anal. Chim. Acta 593, 103-107.
61- Nakbi, A., Issaoui, M., Dabbou, S. and Nadia, K. (2010).Evaluation of antioxidant activities of phenolic compounds from two extra virgin olive oils. Journal of Food Composition and Analysis, 23(7):711–715.
62-Laincer,F., Laribi, R.,Tamendjari, A.,Arrar, L.,Rovellini, P. and Venturini, S. (2014). Olive oils from Algeria: Phenolic compounds, antioxidant and antibacterial activities.Grasas y Aceites, 65(1).
63-Loizzo, M. R., Di Lecce, G.,Boselli, E. and Menichini, F. (2012).Radical Scavenging, Total Antioxidant Capacity, and Antiproliferative Activity of Phenolic Extracts from Extra Virgin Olive Oil by Cultivar 'Frantoio'.nternational Journal of Food Properties, 15(6):1345-1357.
64-Sicari, V. (2017). Antioxidant potential of extra virgin olive oils extracted from three different varieties cultivated in the Italian province of Reggio Calabria. Journal of Applied Botany and Food Quality, 90, 76 – 82.
65- IOC. (2019). trade standard applying to olive oilsand olive pomaceoils.COI/T.15/NC No 3/Rev. 14.

66- Gutiérrez-Rosales, F., Garrido-Fernández, G., Gallardo-Guerrero, L., Gandul-Rojas, B. andMinguez-Mosquera, M. I. (1992). Action of chlorophylls on the stability of virgin olive oil.J. Am. Oil Chem. Soc.,69, 866–871.
67- Lazzerini, C and Domenici, V. (2017). Pigments in extra-virgin olive oils produced in Tuscany (Italy) in different years. Foods, 6(4): 25.
68- Uluata, S., Altuntaş, Ü. and Özçelik, B. (2016). Biochemical characterization of Arboquina extra virgin olive oil produced in Turkey. 93, 5, 617: 626.
69- Mahmud, F., ElGhadban, E.,Shahat, S. and El Sharnouby, G. (2017).physicochemical and phytonutrients evaluation of Arboquina extra virgin olive oil cultivated recently in Egypt. Bulletin of the National Nutrition Institute of the Arab Republic of Egypt, (50)125.
70-Omri, A., Abdelhamid, S. andBenincasa, C. (2020). The investigation of minor and rare Tunisian olive cultivars to enrich and diversify the olive genetic resources of the country.Journal of Food Composition and Analysis 95, 103657.
71.Hassine, K. B., Mnif, W., Smaoui4, S., Dhaouadi, S., Aichi, H., Ben Chikha, M. And Kammoun, G. (2021).Virgin olive oil quality in relation to olive ripening stage and malaxation temperature. Agriculture and Biotechnology, 82 (6), 4781-4788.
72-Criado, M. N., Romero, M., Casanovas, M. andMotilva, M. J. (2008). Pigment profile and colour of monovarietal virgin olive oils from Arboquina cultivar obtained during two consecutive crop seasons. Food Chem, 15;110(4):873-80.
73-Bedbabis, S., Trigui, D., Ben Ahmed, C., Clodoveo, M. L., Camposeo, S., Vivaldi, G. A. and Ben Rouina, B. (2015).Long-terms effects of irrigation with treated municipal wastewater on soil, yield and olive oil quality. Agricultural Water Management, 160, 14-21
74 - Bouaziz M., Chon Kho , M., Sayadi ,S , (2004) Comparative study on phende concentration and antioxidant activity during maturation of olive cultiverChemlali from Tunisia. Agrie. Food Chem. 52, 5476-5481.
75- Boss A., Bishop k:S., Marlow G, Barnett M.PG., Ferguson L.,R, (2016) Evidence to Support the Anti-Cancer, Effect of olive leaf extract and future Directions. Nutrients 8,513.
76- Gambacorta, G.; Faccia, M., Prvitali M, MA.,Fati,S. La Notte, E ., Biono, A . Effects of olive maturation stoning on quality indices and antioxidants concentration of Extri virgin oils (cv.Coratina) during storage J. Food Sci.2010, 75, 229-235.
77-Chin, k.y, Ima-Nirwana, S. (2018) The Roll of Vitamin E in preventing and treating Osteoarthritis - A Review of the Current Evidence. Front pharmacol 9,946.
78 - Khalil M.F. (1994): Polyphenol content and stability of Virgin olive oils, separation of Naturel antioxidant. From vegetation water of olives. Egypt J. Agric 72 (2).
79 - Khalil MF, Badawy. H. A AawdAbd El-Mula K G.S (1990) Identification and Quality characteristics of Local and Imported olive oils. Zagazig J. Agric Res • 17 (4) A.
80 - Khalil MF and Badaway H.AA (1996) Changes occurring in oils of different olive fruits varieties during maturation # : Quality characteristics, oxidative Stability and Organoleptic evaluates of virgin olive oils. Res. Bull.HomeEcom. MonoufiaUniv 6(1):75-93