

# Characterization of Egyptian Monovarietal Koroneiki Virgin Olive Oil and Its Co-Products

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#### Abstract

Due to the importance of the authenticity of Egyptian monovarietal Koroneiki virgin olive oil, its special characteristics should be studied. This study aimed to investigate certain compounds to characterize monovarietal Koroneiki virgin olive oil (KVOO) and evaluate its authenticity. The reliable markers, which include fatty acid composition, tocopherols, phytosterols, total phenolic compounds, pigments, and oxidative stability, were determined to obtain a complete picture of this oil. In addition, the characterization of its by-products, such as leaves and pomace, was also evaluated. The monounsaturated fatty acid content in the Egyptian KVOO was the highest (74.46%). The linoleic/linolenic acid ratio (11.17) could be used as proof of the purity of KVOO. It is also characterized by its higher content of  $\alpha$ -tocopherol (97.77%). B-sitosterol was the main phytosterol. This oil has unique characteristics of cycloartenol and 24-methylen cycloartenol, which are present in reasonable amounts (14.0 and 13.7%, respectively). It was found that olive pomace extract contains higher total phenolic and flavonoid content than olive leaf extract, which in turn has higher radical scavenging activity. The obtained results demonstrate the unique properties and valuable compounds of the monovarietal KVOO. The KVOO co-products can be used as value-added compounds for some food industries.

Key words: Monovarietal koroneiki virgin olive oil, olive leaves pomace, co-products, authenticity.

#### 1. Introduction

Currently, the production of olive oil (OO), which accounts for around 98% of global output, is a significant source of economic gain for Mediterranean nations. Olive oil use has increased significantly over the world due to its health advantages, even in nations where it is not produced. Virgin olive oil (VOO), an excellent natural food, can be extracted from olive fruit (Olea europaea L.) by using mechanical or physical methods. Its composition varies greatly based on a number of variables, particularly the growing region and processing methods [1, 2]. VOO has shown high stability against the oxidative deterioration depends on presence of major and minor bioactive components [1]. VOOs have a large amount of monounsaturated fatty acid (oleic acid), reducing coronary heart disease risk. It also contains more phenols, vitamin E (tocopherol), and carotenoids, which help prevent some diseases [3,4]. The European Food Safety Authority (EFSA) published that the total phenolic content (TPC) present in VOO had several benefits for human health as it demonstrated a strong correlation between the

consumption of polyphenols and the protection of humans from oxidative damage by LDL.

The purity and quality factors are related to a certain region and have increased interest in the origin of the geographical area [1,5]. High-quality VOO produced from a single variety (monovarietal) found in a particular area is in higher demand from consumers. Some designations, Protected Denomination of Origin and Protected Geographical Indication (PDO and PGI, respectively), were designated to save highquality of OO. This labeling means that the OO is prepared from a certain region with special characteristics linked to natural factors and the environment of these areas, making it more expensive in the market [1,6,7]. Benincasa et al. [8] studied OO varieties from two different areas of Egypt, Siwa Oasis and Giza City. They found that Coratina, Arbequina, and Koroneiki coming from Giza city showed a higher content of total tocopherols than that of the oils of Maraqi and Wattagen coming from Siwa Oasis. This could be explained by considering that Giza city has an arid climate with high humidity due to the River Nile's valley effects, which demonstrated excellent nutritional characteristics in terms of antioxidant compounds [9]. Due to the high

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quality of monovarietal OO, it was targeted for cheating with other cheaper oils. Consequently, there is a need to make a fingerprint for the authenticity of Egyptian monovarietal OO to control adulteration with other oils.

In previous work, Hassanein et al., [1] studied the characteristics and purity of Egyptian monovarietal coratina olive oil (CEVOO) as well as the detection of its adulteration. Models of sovbean or sunflower oils as adulterants in CEVOO at different levels of 5, 10, and 20% were investigated. Synthetic compounds are widely used as food additives, which may pose potential health risks and negatively affect consumer health. Therefore, they are not allowed for food application in many countries and have also been removed from the generally recognized as safe (GRAS) list of compounds. Consequently, natural components are needed to protect consumer health and preserve food products [10-13]. Both the cultivation of olive trees and the production of olive oil and table olives generate huge amounts of waste, including olive leaves and pomace. The food processing by-products are rich in bioactive phenolic compounds, which are variably distributed [14-17]. Much of the research focused on studying the oil specifications of mixed olive varieties. However, the characterization and evaluation of the authenticity of the Egyptian monovarietal koroneiki olive oil (KVOO) and its co-products still needs more research.

This work aimed to investigate certain compounds (fatty acids, phytosterols, tocopherols, chloroplastic pigments) present in monovarietal Koroneiki olive to discriminate this olive variety and to control adulteration of KVOO with other cheap oils. Secondly, study KVOO by-products (olive leaves and pomace) to use them as added-value compounds for food, cosmetic, and nutraceuticals industries.

#### 2. Materials and methods

#### 2.1. Materials

KVOO extracted by cold-pressing, leave, and pomace was kindly supplied by Al-Rabie Olive Farm Kilo 84-Cairo-Alexandria Desert Road, Egypt (Season 2021-2022). All of the solvents were obtained from the Egyptian company Elnassr Pharmaceutical Chemicals Co. (ADWIC) and are of analytical grade. The Folin-Ciocalteu reagent was purchased from Sisco Research Laboratories Chemicals, India. DPPH from Sigma-Aldrich (St. Louis, MO, USA).

#### 2.2. Methods

# 2.2.1. Preparation of olive pomace and leaves extracts

Ultrasonic assisted procedures were used to extract 100 g of air dried (at oven temperature,  $40^{\circ}$ C) pomace or olive leaves samples using a 50:50 v/v ratio of isopropanol to water. After bringing the pH

down to 2-3, the aqueous isopropyl solution was added at a ratio of 5:1 (v/w). The final stage was repeated twice, and then the mixture was collected and centrifuged for 20 minutes at 4000 X g. The extract was then concentrated using a rotary evaporator at 50°C after the supernatant containing it was transferred to a flask that had been previously weighed. To achieve a final concentration of 2 mg/1 l, the crude extract was weighed and diluted in a known volume of dimethyl sulphoxide [16]. The KVOO and Leaves extract as well as pomace extract were stored under refrigerator until analysis.

## 2.2.2. Proximate analysis

The KVOO proximate analyses were determined. The AOCS official procedures Cd 3d-63 and Cd 8b-90 were used to determine the levels of free fatty acids and peroxide, respectively [18]. The p-Anisidine value determination, as detailed previously [12].

# 2.2.3. Major bioactive compounds 2.2.3.1 *Fatty acids*

The AOCS Official Method Ce 1k07 was used to prepare the fatty acid methyl esters [19] and analyzed by GLC as described by Hassanien et al., [20]. The calculated oxidizability-value (COX) based on unsaturated FAs (USFAs) content was calculated using the following equation [21]:  $COX = \{1(18.1\%) + 10.3(18.2\%) + 21.6(18.3\%)\}/100$ 

# 2.2.4. Minor bioactive components 2.2.4.1. Phytosterol composition

GC determined phytosterol composition according to AOCS Official Method Ch 6–91. The procedure is described by Hassanien et al., [20].

# 2.2.4.2. Tocopherol composition

Tocopherol composition was specified, as described in details by Hassanien et al., [20].

## 2.2.4.3. Determination of carotene content

The oil (1 g) was dissolved in 10 ml of acetone, and the absorbance was measured at wavelengths of 662 nm, 645 nm, and 470 nm on Shimadzu, UV-spectrophotometer 240, according to Kotíková et al., [22]. The total content of carotenoids and chlorophyll (a & b) was calculated as in previous work [23].

# 2.2.4.4. Total phenolic content

Total phenolic content was determined by using Folin-Ciocalteu reagent in a colorimetric method using Shimadzu, UV-spectrophotometer UV-240; the method was described in previous work [23].

## 2.2.4.5. Total Flavonoid content (TFC)

TFC content was determined spectrophotometrically as described by Navarro-González et al., [24] The TFC content was expressed as mg equivalent quirecetin / 10 g extract.

## 2.3. Antioxidant activity

# 2.3.1. β-Carotene-linoleic acid oxidation method (coupled autoxidation)

The  $\beta$ -Carotene-linoleic acid bleaching assay was measured according to Miller's [25] spectrophotometric method. The method measures the extract's ability to reduce the oxidation of  $\beta$ -carotene in  $\beta$ -carotene/linoleic acid emulsion [26].

# 2.3.2. Radical scavenging assay (RSA %) for extract

DPPH<sup>•</sup> radical scavenging assay was used to determine the antioxidant activity (R.S.A%) in both extracts and oil using methanol or toluene solution of DPPH<sup>•</sup> (0.0024mg/100mL) according to Ramadan et al. [27] as described in our previous work [15].The concentration inhibits 50% of DPPH<sup>•</sup> (EC50) and the antiradical power (ARP) were also calculated [10].

## 2.4. Measuring K232, K 270

K232 and K270 were calculated from absorption at 232 and 270 nm, respectively. Shimadzu, UV-spectrophotometer UV-240 was used with a solution of oil in cyclohexane (1%) and of 1 cm path length [28].

#### 2.5. Oxidative Stability Index

For the determination of oxidative stability of oils, AOCS Official Method Cd 12b–92 [29] was used. The test was performed on an automated Metrohm Professional Rancimat model 892 at 120  $\pm$ 0.1°C and an air flow of 20 L/h to determine the induction period (IP) of the oils.

#### 2.6. Statistical analysis

Results are presented as the mean ± standard deviation from three replicates of each experiment. A P-value 0.05 used < was to significant differences denote between mean values determined by the Microsoft Excel 2010. One-way analysis of variance (ANOVA) was used.

#### 3. Results and Discussion

#### 3.1 Proximate analysis

**FFA content**: KVOO had lower FFA content (0.57%) than that limit reported by the International Olive Council (IOC) regulations (2015), which reported that FFA% should not exceed the limit of 0.80%, the upper value limit for the "extra virgin olive oil" category (EEC, 1991). These results agreed with those reported by Elsorady [30] and Noorali et

al., [31], who studied the different varieties of olive oil (coratina, arbequina, and koroneiki). **Peroxide value**: KVOO had a moderate PV (9.83meq O2/kg) under the EEC limit of 20 meq O2/kg specified for the virgin olive oil. Also, these results agreed with Elsorady [30]. With regards to p-AV, which counts the quantity of secondary oxidation products, mainly as  $\alpha$  and  $\beta$ -alkenals and related compounds that react with p-anisidine, Table 1 shows that its value amounted to 8.10 mmol/Kg.

# 3.2. UV characteristics

K232 and K270 represent the presence of both conjugated dienes and trienes in the oil as primary oxidation products. The maximum values for K232 and K270 for extra virgin olive oils (EVOO) are 2.5 and 0.22, respectively, 2.6 and 0.25 for virgin olive oils (VOO), while they are 2.6 and 0.30 for ordinary virgin olive oils [28,32]. The results in Table 1 showed that the K232 and K270 for Egyptian KVOO were 1.76 and 0.16, respectively, indicating the low content of conjugated dienes and trienes. These results show that the low content of both primary and secondary oxidation products in EVOO, which agrees with Stefanoudaki et al., [33].

Proximate analysis	KVOO
FFA %	$0.57\pm0.03$
PV (meq/kg oil)	$9.83 \pm 0.26$
p-AV(mmol/Kg)	8.10 ±0.45
TOTOX Value	$27.76 \pm 1.02$
K <sub>232</sub>	$1.76\pm0.003$
K <sub>270</sub>	$0.16\pm0.002$

KVOO: Koroneiki virgin olive oil, AV: acid value, PV: peroxide value, P-AV: P anisidine value. Mean  $\pm$  standard deviation; Number of replicates for each analysis: 3.

#### 3.3. Fatty acids composition

Table 2 displays the findings of the fatty acid profile for Egyptian KVOOs, including total saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), and monounsaturated fatty acids (MUFA), as well as their ratios. For almost all cultivars, the fatty acid profile was strongly influenced by the growth environment. From the data reported in Table 2, it was found that oleic acid was the highest (74.46%), while linolenic acid was the lowest (0.51%) in the KVOO. These results agreed with Agriopoulou et al. [34], who found that the Koroneiki olive cultivar had a mean value of 76.70 % for C18:1 and 0.68 % for C18:3.

For KVOO, the ratios of PUFA/SFA, oleic/linolenic acid ratios, and saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) percentages were also calculated. In general, pure and blended oils could be distinguished by their PUFA to SFA ratios. It can also be considered a key factor in determining the nutritional value of specific oils. It was found to be 0.34 in KVOO. Additionally, Cox values, an indicator of oil's susceptibility to oxidation, demonstrate the purity of VOO and rule out adulteration (1.44), even if adulterated by small ratios of cheaper vegetable oils [1]. In order to determine the authenticity of olive oil, one might look at the ratio of linoleic or linolenic acids to oleic acids [35,36]. According to the information in Table 2, the ratios of linoleic and linolenic acids to oleic acids in KVOO were 0.07 and 0.006, respectively. It was 0.13 and 0.008 in compared with Egyptian monovarietal coratina VOO, respectively [1].

Moreover, a useful relationship was the summation of linoleic and linolenic acids over oleic acid [(C18:2+C18:3)/C18:1], which was found to be 0.0834 in KVOO. Almoselhy [37] found that EVOO recorded 0.1397 as the lowest value, indicating its highest stability, compared with corn and soybean oils, which recorded the highest values (1.5903 and 1.5564), indicating its lowest stability. According to the results obtained in this study, the fatty acid composition is the internal factor that has the greatest effect on the oxidative stability of oils.

According to other investigations, the key factor influencing VOO oxidative stability is the MUFA/PUFA ratio. Therefore, oxidative stability was increased by high oleic and low linolenic acids [38]. According to El Yamani et al. [39], the water regime has an impact on oxidative stability. They reported that arid places with little rainfall have greater oxidative stability values and lower PUFA levels. As a result, the ratios of the fatty acids might be utilized to assess the KEVOO's validity. According to Mendez et al. [40], the PUFA/SFA ratio can be used to gauge an oil's propensity to oxidize. KVOO was more stable than the other vegetable oils in terms of fatty acid content, with a 4.25 for sunflower oil and a 6.0 for soybean oil [1].

# **3.4. Oxidative Stability Index**

One of the most crucial quality factors for vegetable oils is oxidative stability. It speaks to the ability of oils to withstand both high temperatures and oxygen. Rancimat is a well-known test to assess the conductmetric method of oxidative stability of oils by measuring the oxidation induction time. KVOO's induction period (IP) was 12.4 hours at 120 °C (Table 2). Several authors evaluated the oxidative stability values at temperatures higher than 98, 110, and 120 °C due to the high stability of olive oils [41]. At four different isothermal temperatures (110, 120, 130, and 140 °C) using Rancimat, Almoselhy [37] found that the oxidative stability values (h) of the extra virgin olive oils were 14.9, 7.2, 3.2, and 1.8 h. Additionally, IP values for various virgin olive oils were

investigated by Mateos et al., [41] at various temperatures (98, 110, 120, 130, and 140 °C). They discovered that a wide range of Rancimat stability is present with the IP at 120 °C. (3.6–26.7 h).

Table 2: Fatty acids composition of Egyptian KVOO.

Fatty acids	Percentage	Ratios	
C16:0 (palmitic acid)	14.95±0.03	USFA/ SFA	4.54
C16:1 (palmitoleic	1.6±0.09	PUFA/ SFA	0.34
C18:0 (stearic acid)	2.73±0.05	L+ Ln/O	0.0834
C18:1 (oleic acid)ω9	74.46±0.12	L / O	0.07
C18:2 (linoleic acid)ω6	$5.7{\pm}0.08$	Ln / O	0.006
C18:3 (linolenic	0.51±0.01	MUFA/PUFA	13.06
C20:0 (arachidic acid)	0.41±0.01	ΜΟΓΑ/ΓΟΓΑ	
USFA	82.21	Cox value	1.44
SFA	18.09	Cox value	
MUFA	76.06	Induction period	12.4 h
PUFA	6.2	@ 120°C	12.41

PUFA = polyunsaturated fatty acids R- ratio=  $\beta$ -sitosterol/ Campasterol+ Stigmasterol,

AF factor= [100 – (Campasterol+ Stigmasterol)]/ Campasterol+ Stigmasterol

Mean  $\pm$  standard deviation; Number of replicates for each analysis: 3.

# **3.5.** Minor bioactive components (Tocopherols and Phytosterols)

# 3.5.1. Tocopherols

Tocopherols, or vitamin E, are very significant nutritional ingredients. They possess antioxidant qualities that guard the body's tissues from the harm that free radicals can do. The highest biological potency of all tocopherol homologs is that of  $\alpha$ tocopherol [35,36]. In VOO, it makes up the majority of the vitamin E. For high-quality VOOs, the literature reports that the concentration of  $\alpha$ tocopherol is typically between 100 and 800 mg kg-1,  $\beta$ - and  $\gamma$ -tocopherols were only found in trace amounts [42]. The outcomes in relation to the tocopherol homologs identified in the Egyptian monovarietal KVOO are shown in Table 3.

According to the findings,  $\alpha$ -tocopherol predominates in the OO sample, followed by  $\gamma$ -, and  $\beta$ -tocopherol, respectively, with no evidence of  $\delta$ -tocopherol. These findings appear to be consistent with those of Benincasa et al., [8] who demonstrated that highquality oils typically contain  $\alpha$ -tocopherols in concentrations greater than 100 mg kg-1, with  $\alpha$ tocopherol making up over 95% of the total. In comparison to Egyptian mono-varietal coratina virgin olive oil (CEVOO), it was discovered that KVOO had a total tocopherol concentration that was twice as high (44.33 mg/100g) [1]. In general, OOs from Giza's Coratina, Arbequina, and Koroneiki displayed a higher amount of total tocopherols [30,31]. It is well known that each pure oil has its own ratios of PUFA/ $\alpha$ -T and  $\alpha$ -T/ $\gamma$ -T, which were 0.14 and 56.88 in KVOO and 0.49 and 103.5 in Egyptian monovarietal CEVOO, respectively, in comparison [1].

# 3.5.2. Phytosterol composition

According to the findings in Table 3,  $\Delta$ 5-avena and campesterols made up 4.1 and 2.27% of KVOO, respectively, whereas  $\beta$ -sitosterol made up the majority of phytosterols (59.17%). In contrast,

Agriopoulou et al., [34] discovered that  $\beta$ -sitosterol,  $\Delta$ -5-avenasterol, and campesterol, with mean values of 80.73%, 12.28%, and 3.71%, respectively, were the predominant phytosterols in koroneiki olive cultivar samples. It was observed that Egyptian KVOO has high concentrations of 24-methylene cyclosterol and cyclosteroal (14.0 and 13.7%, respectively). It was 1.15% for 5- stigmasterol. It is generally known that 5-stigmasterol always remains less than campesterol as determined by Commission Regulation (EEC) No. 2568/1991, and campesterol does not exceed 4% in EVOO [43].

Table 3: Minor components (Tocopherols ad phytosterols) of Egyptian KVOO

Minor components				
Tocopherol composition		Phytosterol composition		
components	(mg/100g)	Phytosterol	%	µg/g (ppm)
α-Toc	$43.23 \pm 0.26$	Campasterol	2.74	$29.60 \pm 2.01$
β- Toc	$0.34\pm0.09$	5-Stigmasterol	1.15	$12.46 \pm 0.56$
γ- Toc	$0.76 \pm 0.08$	β-sitosterol	59.17	639.37±11.63
δ- Toc	ND	5-avenasterol	4.1	$44.27{\pm}2.8$
Total-Toc.	44.33	Cycloartenol	14.0	$151.35 \pm 5.13$
(mg/100g)		7- stigmasterol	1.73	$18.72 \pm 1.63$
$\alpha$ -Toc/ $\gamma$ -Toc	56.88	24-Methylen Cycloartenol	13.7	$148.11 \pm 2.76$
		Citrostadienol	3.42	36.94± 3.66
PUFA/ α-Toc	0.14	Total sterols	100	1080.82
		β-sitosterol/ Campasterol	21.59	
		R- ratio	15.21	
		AF Factor	24.7	

PUFA = polyunsaturated fatty acids R- ratio= $\beta$ -sitosterol/ Campasterol+ Stigmasterol,

AF factor= [100 - (Campasterol+ Stigmasterol)]/ Campasterol+ Stigmasterol Mean + standard deviation: Number of raplicates for each analysis; 2

Mean  $\pm$  standard deviation; Number of replicates for each analysis: 3.

A species of Cornicaba OO has campsterol over the 4% limit specified by EC Regulation, according to research by Salvador et al. [44]. Al-Ismail et al. [45] said that campesterol and stigmasterols might be used to demonstrate the purity of OO by calculating the authenticity factor (AF) in the manner described below:

AF = [100-(campsterols% + stigmasterols %)]/(campsterols% + stigmasterols %)

Egyptian KVOO had an AF value of 24.7, while CEVOO had an AF value of 1.34. Therefore, it would be possible to demonstrate the purity of OO using the AF factor [1]. It was observed that each pure oil has a AF factor. unique ratio for the βsitosterol/campsterol, and R-ratio (βsitosterol/Campasterol+ Stigmasterol) [1]. Additionally, the campesterol/stigmasterol ratio has been mentioned as an indicator of an oil's quality [46].

The total amount of phytosterols found in KVOO was 1080.82 ppm. This finding was in line with that of Agriopoulou et al., [34], who said that the total

amount of phytosterols in the Messinian region's cv Koroneiki was 1033.3 ppm.

The purity of VOO can almost entirely be predicted by the phytosterol profile. Since it can be thought of as an actual fingerprint, the phytosterol composition of OO is a highly helpful metric to check authenticity or detect adulterations [47]. These metrics are impacted by a number of variables, including the quantity and quality of the oil and the growing regions [48].

# **3.6.** Another principal bioactive components in koroneiki olive oil and its co-products

3.6.1. Chloroplast pigments:

Chloroplast pigments have received extra study since they are crucial to the stability of VOO. The findings of the analysis of the chloroplast pigments in KVOO are shown in Table 4. KVOO had 11.19 and 17.34 g/g of chlorophyll a and b, respectively.

Concerning the KVOO by-products, namely leave and pomace extracts, higher amounts of chlorophyll a and b in leave extract were 618.5 and 455.42  $\mu$ g/g,

while they were 202.47 and 265.84  $\mu$ g/g for pomace (TCC) was found to be 2.93  $\mu$ g/g for KVOO. The higher content of TCC was 120.66  $\mu$ g/g in leave extract, followed by pomace extracts, 33.42  $\mu$ g/g. Fakourelis, et. al., [49] reported that chlorophylls and carotenoids play an important role in the oxidative activity of OO, especially in the dark, due to their antioxidant nature. Also, they found that those chlorophylls (and their derivatives) can act as photosensitizers in VOO. The chloroplast pigments have been postulated to act as protectors, capturing free radicals similar to  $\alpha$ -tocopherol [50]. Carotenoids, along with polyphenols and tocopherols, support the oxidative stability of VOO, according to the International Olive Oil Council.

## 3.6.2. Phenolics and flavonoids contents

From the results recorded in Table 4, it is noticeable that higher amounts of total phenolics (TPC) and flavonoid content (TFC) were determined in the byproducts from KVOO (leaves and pomace). It was found that the pomace extract has higher TFC than the leaf extract, and both are still higher than that in KVOO (Table 4). Due to the synergy of flavonoids, oleuropein, and phenols, olive pomace and leaf extract exhibit greater antioxidant activity than vitamin C and E. This is perhaps the most startling extract, respectively. The total carotenoid content finding [51]. Compared to vitamins C and E, flavonoids, rutin, catechin, and luteolin have nearly 2.5 times greater antioxidant activity [52] which have an important role in health-promoting abilities and shelf-life stability. Mohamed et al., [53] found that phenolic compounds from olive pomace possess antimicrobial and antioxidant activities when added to yoghurt fortified with probiotics and may be used to attenuate complications in type-2 diabetic patients.

# 3.6.3. Antioxidant activity (AOA)

Olive pomace extract showed higher AOA (26.51%) than all other extracts with followed by leaves extract (17.33%) and KVOO (10.4%). It is well known that the EC<sub>50</sub> value represents the effective concentration needed to reduce the initial concentration of DPPH radicals by 50%, and a lower EC<sub>50</sub> value indicates a more effective protective effect [54]. The antioxidant capacity, expressed as EC<sub>50</sub> values, amounted to 15.5 mg/ml for olive pomace extract, 23.5 mg/ml for leave extract, and 42.46 mg of oil in KVOO. A substantial correlation between AOA, EC<sub>50</sub>, and TPC is shown in Table 4, increasing the antioxidant capacity [3]. Consequently, pomace and olive leaves extracts and KVOO have very good antioxidant potential at lower concentrations.

Table 4: Bioactive components and antioxidant activity of Egyptian KVOO

Bioactive compo	onents	KVOO	Pomace extract	Leaves extract		
Bio-active components						
TFC (mg/ 10 g)		$1.56 \pm 0.05$	$166.22 \pm 4.01$	127.73 ±1.38		
TPC (mg /10g)		$6.879 \pm 0.14$	733.61 ±37.3	$639.26\pm18.03$		
TCC (µg/g)		$2.93{\pm}0.67$	$33.42{\pm}3.96$	120.66±14.17		
content of chlorophyll (µg/g)	а	$11.19 \pm 1.00$	$202.47{\pm}~8.81$	618.56±46.62		
	b	17.43±1.15	$265.84{\pm}26.27$	455.42±46.62		
Antioxidant activity						
EC <sub>50</sub>		42.46	15.5	23.25		
1/EC <sub>50</sub>		0.024	0.065	0.033		
AOA %		10.4	26.51	17.33		

TFC: total flavonoid content, TPC: Total phenolic content ( $\mu$ g gallic acid/ 1g oil), TCC: total carotenoid content, EC50: Concentration of extract that causes a 50% decrease in DPPH absorbance, 1/EC50 antiradical power. AOA%: Antioxidant activity (value represent the percent inhibition of oxidation of the linoleic acid/ $\beta$ -carotene emulation). Mean  $\pm$  standard deviation; Number of replicates for each analysis: 3.

# **Conclusion:**

This study is focused on the Egyptian monovarietal KVOO to investigate certain compounds present in this OO. The major and minor components, as well as the relationships mentioned above, help to throw light on the properties of Egyptian monovarietal KVOO. This oil is characterized by high amounts of oleic acid, and the  $\beta$ - sitosterol is the major phytosterol. It has a unique characterization by the presence of

cycloartenol and 24-methylene cycloartenol, which can be suggested as possible authenticity indicators. The KVOO by-product extracts (olive leaf and pomace) contain high amounts of chlorophyll a and b, TFC, TCC, and TPC. So, it can be used as an added-value material for food, cosmetics, and pharmaceutical applications. The results confirmed that the major, minor components and some ratios might be used as a fingerprint of monovarietal KVOO. The investigation of certain components, and ratios present in this OO as novel markers could be a promising purity and authenticity indicator of this oil. **Conflicts of Interest** 

The authors declare that they have no conflict of interest in this article.

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