Physicochemical and phytonutrients Evaluation of Arbequina Extra Virgin Olive oil Cultivated Recently in Egypt

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ABSTRACT

This study was conducted in the Department of Medicinal and Aromatic Plants, Horticulture Research Institute, Agricultural Research Center, Dokki, in cooperation with the Food Science and Technology Department, Faculty of Agriculture, Al-Azhar University, Cairo, season 2016, Varieties of the olive cultivar Arbequina have recently been cultivated in Egypt. The objective of the study is to characterize and evaluate extra virgin olive oils (EVOO) produced from Arbequina grown in two places in Egypt, is Sheikh Zuid Station, the North Sinai Governorate - Center for Desert Research (NS), El Taal al kabeer area , Ismailia (IS), The aim was of the study to evaluate effective nutritive value in the extracted fruits oils, chemical and physical study of those components, 40 kg of ripe seeds were collected from two places (SN), (IS), and oil extraction, store at 4 ° C until used.the (NS) and (IS) regions of Egypt. Major and minor components such as carotenoids, squalene, phenolics and tocopherols were studied to assess their effects on product quality and health benefits. Samples were to determine fatty acids composition, sterol composition, TAG profile and squalene content. Individual phenolic fractions were analyzed by LC–MS/MS and tocopherol isomers were determined by HPLC. According to the results obtained from this study; Total phenolic content (TPC) of the samples were 454.68 and 50.86 mg as Gallic acid/kg oil for (NS) and (IS), respectively. Hydroxytyrosol and tyrosol were the main phenols. The major tocopherol isomer found in (NS) and (IS) was α-tocopherol with levels of 179.55 and 202.5 mg/kg oil, respectively. β-Carotene levels in both samples were similar at 0.2 mg/ kg. Findings of this study were compared with the literature on Arbequina olive oil produced in different countries. It was determined that Arbequina olive oil of high quality can be produced in Egypt, especially in the North Sinai Governorate (NS).

Keywords: *Extra virgin olive oil (EVOO) · Arbequina variety · Phenolics · Tocopherol · Squalene*

INTRODUCTION

The olive tree (Olea Europaea L.) is cultivated largely in Mediterranean countries (Spain, Italy, Greece, Tunisia, Turkey, Morocco and Algeria) for climatic reasons. Traditionally, olive oil has been a major part of the Mediterranean diet. In recent years, its popularity has increased worldwide due to the health benefits derived from its high levels of mono-unsaturated fatty acids, antioxidants and other minor components **(Bakhouche etal. 2013**).

The composition of extra-virgin olive oil (EVOO), which is a determining factor in its intrinsic quality, can be influenced by several geographic, agronomic and technological factors (**Bakhouche etal. 2013)**, such as olive cultivar **(Tura etal., 2007)** climate, **(Lazzez etal., 2008)**, crop season **(Rodney et al., 2014)** and the production process **(Lozano-Sánchez et al., 2010).** Temperature and rainfall may also affect olive oil composition **(Romero et al., 2003); (Ilyasoglu et al., 2010)**. However, specific characteristics of olive oil are largely determined by geographic considerations **(Petrakis et al., 2015)**. Marketing of EVOO is increasingly focused on differentiation and characterization of products from different geographical areas (**Bakhouche et al. 2013)**. Several studies have shown that climatic factors such as those mentioned above have an effect on plant physiology, and consequently on the chemical characteristics of the oil extracted from the plant fruits **(Gorinstein etal., 2003); (Pereira etal., 2006); (Ocakoglu etal. 2009).**

Arbequina is a Spanish olive cultivar, characterized by small fruits and high oil yield **Barranco et al., (1999); Torres and Maestri (2006)**. This cultivar, which owes its name to the municipal district of Arbeca (Lleida, Catalonia, Spain), where it was first grown, can be characterized by its frost resistance, low vigor, and smallsized fruit (**Bakhouche et al. 2013)**. Although there have been some studies reported by several researchers, including chemical composition of olives.

In Argentina **Carelli, (2008)**, preliminary characterization of olive oils produced, in Morocco **(Mansouri etal., 2013), and** chemical characterization of traditional varietal olive oils in Spain **(López-Cortés etal., 2013),** by there is not a great deal of data on its chemical composition, despite the large amount of Arbequina olive oil produced in Spain and other countries.

The cultivation of the Arbequina variety has recently been initiated in Egypt. Hence, the objective of this work is to characterize chemical composition of EVOO extracted from Arbequina cultivated in two different olive growing areas in Egypt, namely, the North Sinai Governorate (NS), Ismailia (IS). The task of characterizing these oils is important not only to assess their chemical qualities but also to compare them with each other and Arbequina olive oil produced in different countries. The findings of this study may provide ways for quality indication and investigation of environmental effects on olive oils from Arbequina according to their major chemical composition (fatty acid, triglycerides and sterol content), antioxidant content and minor compounds (phenolics, squalene, β-carotene and α-tocopherol content).

To our knowledge, that is the first time that the chemical composition of EVOO produced from the monovarietal Arbequina variety in Egypt has been determined and compared by growing area and with olive oils produced from Arbequina in Spain, Argentina, Tunisia and Morocco with regard to quality characteristics

MATERIALS & METHODS

This investigation was carried out at medicinal and aromatic plants Department - Horticulture Research Institute - Agricultural Research Center - Dokki, In cooperation with Food Science and Technology Department, faculty of Agriculture, Al Azhar university, Cairo during the seasons 2016

Samples

Source of Arbequina Olive Cultivated in Egypt, seasons 2016,

Samples of Arbequina Olive were obtained taken from two places in Egypt season 2016, as follows:

1. Sheikh Zuid Station, North Sinai Governorate - Center for Desert Research (NS)
2. El Taal al kabeer area, (Ismailia Governorate) - Ministry of Agriculture (IS)

Collect mature fruits

Harvest was done in, October–December. Samples were divided into two groups and identified by their growing province (NS) and (IS), respectively. All of the samples were stored in dark brown bottles at 4 °C until they could be analyzed.

Get the oil from the fruits

Oil was extracted using an Abencor laboratory oil mill equipped with a crusher, a mixer and a basket centrifuge. Only healthy fruits, without any kind of infection or physical damage, were processed. After harvesting, fresh olives（40 kg）were washed and deleafed, crushed with a hammer crusher, and the paste mixed at 25℃ for 30 min, centrifuged without the addition of warm water（oil produced from each extraction was 5000 mL/kg）and then transferred into dark glass bottles, and stored at 4℃ until analysis by **(AOCS 2002)**

Chemical analysis

A fatty acid methyl ester (FAME) standard mixture (37 components), a sterol standard mix (6 components) and triacylglycerol (TAG) standard mixture were purchased from Supelco (Bellefonte, PA). All other chemicals and reagents used for analysis were of either analytical or HPLC.

Chemical Parameters

Quality Index Determination

Both sets of samples were olive fruits picked at the same stage of ripeness, and their oils were extracted with the same processing system. Determination of free acidity, peroxide value, and specific absorbance at 232 and 270 nm (K232 and K270) were

determined according to official methods. The BS EN ISO 660:2009 official method of analysis was used for determination of free acidity values and results were expressed as % oleic acid. UV absorption characteristics (K232 and K270) were determined by the analytical methods described in the Regulation EC/1989/(2003) using a *Jenway 6705 UV–visible spectro - photometer*, with cyclohexane as the blank. AOAC official method 965.33 (2000) was used to determine the peroxide value (PV) and PV was expressed as milliequivalents (meq) of active oxygem per kilogram of oil

Antioxidant Capacity

ABTS Assay

The radical scavenging power of the oils was determined by the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method **Re, etal., (1999)**. ABTS radical cation (ABTS+) stock solution was prepared by reacting 7.0 mM ABTS stock solution with 2.45 mM final concentration) potassium persulfate in the dark for 16 h. The solution was diluted with ethanol by adjusting the absorbance to 0.700 ± 0.020 at 765 nm. 100 µL of the diluted oil samples in ethanol and 2.9 mL of diluted ABTS+ solution were added. The solution was agitated with a vortex mixer for 20 s. The absorbance was measured after 6 min at 765 nm. The results were expressed as µg Trolox equivalent/100 g oil.

DPPH Assay

The DPPH (α,α-diphenyl-β-picrylhydrazyl) assay was also used to determine the radical scavenging power of the oils by method of **Bondet et al. , (1997)** . Freshly prepared DPPH solution was dissolved in a small volume of ethyl acetate and then diluted with ethyl acetate by adjusting the absorbance to 0.700 ± 0.020 at 520 nm. 20 mg oil was weighed in a test tube, then 80 µL ethyl acetate and 2.9 mL DPPH free radical solution were added. The sample was agitated with a vortex mixer for 20 s. After 30 min of incubation in darkness at room temperature, absorbance was measured at 520 nm against ethyl acetate. Trolox was used as a standard and the

results were expressed as µg Trolox equivalent/100 g oil.

ORAC Assay

Oxygen Radical Absorbance Capacity (ORAC) was determined according to the method **Huang etal. , (2002)** described, 0.5 g of the oil sample was dissolved in 20 mL acetone. An aliquot of sample mixture was properly diluted wit*h* 7 % randomly methylated β-cyclodextrin (RMCD) solvent (w/v) prepared in 50 % acetone–water mixture (v/v) and then shaken at room temperature for 1 h on an orbital shaker at 400 rpm. ORAC analysis was carried out by a *Biotek Synergy HT spectrophotometer*. All reagents were prepared with 75 mM phosphate buffer (pH 7.4), with the exception of samples and Trolox standards, which were prepared in 7 % RCMD solution. In the final assay mixture (0.4 mL total volume), fluorescence (FL) (6.3×10^{-8} M) was used as a target of free radical attack and 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) (1.28×10^{-2} M) was used as a peroxyl radical generator. Trolox (6, 25, 12.5, 25, 50 and 100 µM) was used as the control standard and 7 % RMCD was used as a blank. Final results were calculated using the differences of area under the FL decay curves between the blank and a sample. The results were expressed as µmol Trolox equivalent/100 g oil.

Color Measurements, Chlorophyll‐a and β‐Carotene Determinations

CieLab coordinates (*L**, *a**, and *b**) were directly read with a spectrophotocolorimeter (Trintometer, Lovibond PFX 195 V 3.2, Amesbury, UK). In this coordinate system, the *L** value is a measure of lightness, ranging from 0 (black) to 100 (white), the *a** value ranges from −100 greenness) to +100 (redness) and the *b** value ranges from +100 (blueness) to −100 (yellowness). Chlorophylla and β-carotene content of oil samples were also directly read by the Lovibond tintometer **(Verleyen *et al*., 2002).**

Fatty Acid Determination

Fatty acid methyl esters, as converted from the corresponding fatty acids in the

seed oil according to the method, were analyzed by Philips Pye Unicam PU 4500 (Philips Electronics UK Ltd, Guildford, Surrey, UK) gas chromatography equipped with a flame ionization detector. The column (internal diameter 2 mm, length 1.5 m) (Philips Scientific, Cambridge, UK) was filled with 10% diethyl glycol succinate on a 100 to 200- (British standard sieve) mesh (Mallinckrodt Chemical Works, St. Louis, MO). Injection and detector temperatures were 230 and 250C, respectively. The column temperature was increased from 100 to 225C, with a temperature increase gradient of 4C min-1. Nitrogen gas was used as the carrier gas at a flow rate of 11.3 mL min-1. The chromatograms were recorded with Spectra Pycis 4290 integrator (Spectra Physics, Irvine, CA). The amount of each fatty acid was given as a percentage of the total fatty acid content.

Sterol Determination

Sterol content was determined according to a modified procedure of **Gutfinger, (1981); Verleyen *et al*. (2002).** , explained To prepare the unsaponifiable matter, 1.5 g oil was weighed in a flask and 2 mL of internal standard (0.1 % cholesterol in chloro-form) was added. The sample was mixed with 10 M KOH, prepared in 50 mL methanol, in flasks and shaken at 30 °C for 24 h. After incubation, 10 mL distilled water; 1 mL ethanol and 20 mL hexane were added. The flask was shaken vigorously in a separating funnel and after separation, the hexane layer was collected. The other layer (including the aqueous/alcohol phase) was extracted twice more with 20 mL hexane. The combined hexane extracts were dried over anhydrous Na_2SO_4. The solvent was completely removed under vacuum. The residue was re-dissolved in 0.5 mL pyridine and 1 mL *N,O*-bis-(trimethylsilyl)-trifluoroacetamide containing 1 % trimethyl-chlorosilane solution and derivatized at 80 °C for 30 min. The samples were analyzed using an Agilent 7890A gas chromatograph equipped with a capillary column, and a flame ionization detector at 360 °C. The oven

temperature of the GC was initially held at 285 °C for 35 min and then increased to 310 °C, with an increase of 10 °C/min, and kept for 10 min. The carrier gas was helium and the flow rate was 0.5 mL/ min. The sterol composition was identified by comparing retention times of sterols in samples with the standard sterol mixture. The internal standard method was used for quantification.

Triacylglycerol Determination

The triacylglycerol (TG) composition of the oils was determined according to the official method 0.2 g oil was weighed and dissolved in 20 mL *n*-heptane and then transferred into vials. Triacylglycerol composition was analyzed with an Agilent 7820A gas chromatograph equipped with a capillary column, and flame ionization detector at 360 °C. The oven temperature was initially 285 °C for 35 min and then increased at 10 °C/min to 310 °C and kept for 10 min. The carrier gas was helium and the flow rate 0.5 mL/min. Triacylglycerol compositions were identified by comparing their retention times with those of the standard TAG mixture **(Gutfinger, 1981)**

Total Phenolic Content

The total phenolic content was determined using the Folin–Ciocalteu method with minor modifications **by Gutfinger, (1981)**. First, 600 μL of methanolic extracts were added to test tubes containing 2.5 mL Folin-Ciocalteu reagent (diluted 1 × 10). Contents were mixed thoroughly then after 3 min, 2 mL of 7.5 % sodium carbonate was added and mixed again. After 2 h of incubation at room temperature, the absorbance of the mixtures was measured at 765 nm. The phenolic content was calculated according to a standard curve prepared with Gallic acid. The results were expressed as μg Gallic acid equivalents (GAE)/g oil.

Phenolic Compound Determination

Phenolic fractions were determined according to **Baiano et al., (2013)**. The methanolic extract was filtered through a

0.45-µm PTFE membrane filter into the vial and injected into an Agilent HPLC system (Agilent, Kyoto, Japan). A gradient solution including two solvents: (A) 2 % acetic acid in water and (B) methanol: acetonitrile (1:1, v/v) at constant flow rate of 1 mL/min was used to achieve the separation of phenolic compounds. The gradient program of solvent was the following: 100 to 70 % A in 30 min, 70 to 50 % A in 15 min, 50 to 40 % A in 10 min, 40 to 0 % A in 10 min, 0 % for 5 min, and 0 to 100 % A in 5 min.

Tocopherols Determination

Tocopherol isomers of the oils were determined according to the method **Turan, et al. , (2007)** described by Turan *et al*. Tocopherol isomers were analyzed using a HPLC system (Agilent, Kyoto, Japan) equipped with Inertsil ODS-3 normal phase column (250 × 4.6 mm, 5 µm) and SPD-M20A photodiode array detector. The tocopherol isomers were identified by comparing their retention times with authentic standards and quantified on the basis of peak areas as compared with the external standards.

Squalene Determination

Squalene content was determined according to the GC procedure explained in sterol and TG determinations by **Gutfinger, (1981)** methods. After preparation of unsaponifiable matter, the samples were analyzed with an Agilent 7820A GC system by using the same GC operating conditions given for the determination of sterols. The internal standard method was used for quantification of squalene.

Statistical Analysis

Obtained data of this study were tabulated and statistically analyzed using randomized complete block design according to **Snedecor and Cochran, (1967).**

RESULTS & DISCUSSION

Analytical Parameters of EVOO

Free Fatty Acid, UV absorption parameters (K_{232} and K_{270}) and peroxide value (PV)

are important quality factors and are generally used for classifying olive oils. Table 1 shows the parameters of the EVOO samples. EU regulation has established a value of FFA <0.8 % for "extra virgin" olive oil (Regulation EC/1989/2003).

As seen in Table 1, significant differences were observed between samples (p < 0.05), except for the K_{232} value. FFA values of both samples were below the limit. This could be because of improper fruit harvesting, transport and storage conditions. EU regulations establish a value of K_{232} < 2.5 and K_{270} < 0.22 for extra virgin olive oils (Regulations EC/1989/2003). Both of the samples analyzed showed UV absorbance values much lower than these limits. The peroxide value offers a measure of lipid oxidation. In the all samples PV values were 3.75 and 7.24 mequiv O_2/kg oil for (NS) and (IS) samples, respectively. The PV of all samples was lower than the legal limit (PV < 20 mequiv O_2/kg oil, EC/1989/2003) established for EVOO. Those quality parameters did not exceed the limits established for the best commercial quality olive oil, designated as "extra virgin olive oil category" **(IOC, 2015)**, by the International Olive Council.

Fatty Acid Compositions

Fatty acid composition has a wide range depending on genetic and environmental factors and has been used for qualitative assessment of olive oil **(Lanza, et al., 1998); (Criado, et al., 2008).** Olive oil has a high content of monounsaturated fatty acids important to good nutrition and human health, such as oleic acid **(Ozyılkan et al., 2005).** The average fatty acid composition, expressed as a percentage of the total fatty acids is summarized in Table 2. The results showed that the distribution of fatty acid composition was within the normal ranges expected for extra virgin olive oil **(IOC, 2015)**.

Significant differences were not observed between samples (p < 0.05), except for O/L (O/L: C18:1/C18:2) ratio. Oleic acid (C18:1), linoleic acid (18:2) and palmitic acid (C16:0) were the major fatty acids. Oleic acid was the primary and

dominant fatty acid in each of the samples. Oleic acid contents of (NS) and (IS)were 65.14 and 63.00 %, respectively. (NS) samples had higher oleic acid levels than (IS) samples as seen in the Table 2. Another important unsaturated fatty acid in olive oil is linoleic acid, and linoleic levels of (IS) samples were higher (13.2 %) than (NS) samples (12.3 %). Palmitic acid is the major saturated fatty acid in olive oil, with levels ranging between 16.32 and 16.96 %, according to the cultivars, respectively. Levels of other fatty acids, including palmitoleic (C16:1), margaroleic (C17:1), stearic (C18:0), linolenic (C18:3), arachidic (C20:0) and gadoleic (C20:1) acids were quite low. Fatty acid composition results were in agreement with the findings of other studies on Egypian olive oils **(Andjelkovic et al., 2009); (Arslan and Schreiner 2012).**

The amounts of total saturated (ΣSFA), mono unsaturated (ΣMUFA) and polyunsaturated FA (ΣPUFA) and the oleic/linoleic acid ratio (O/L: C18:1/C18:2) were also evaluated. The O/L ratios were 5.2 for (NS) and 4.7 for (IS) samples. This ratio can be useful to characterize olive cultivars and have a marked relationship with stability **(Mansouri etal., 2013)**. For Arbequina cultivars the ratios O/L are respectively 8.49 for cultivars grown in Morocco **(Carelli, 2008)**, between 6.02 and 6.67 for cultivars grown in Spain **(Morello et al., 2004); (López-Cortés et al., 2013) and** 4.26 in Tunisia **(Chtourou et al., 2013)**. But this ratio (O/L = 5.25) is low (2.05–3.69) for cultivars grown in Argentina **(Carelli, 2008)**, It was observed that (IS) was rich in total SFA, whereas (NS) had a higher level of total MUFA due to its higher level of oleic acid.

As compared to olive oils produced from these varieties when cultivated in their original growing area in Spain **(Tous and Romero, 2000)**, as well as in other countries such as Morocco and Argentina, the oleic acid levels are relatively similar to oils produced in Morocco **(Mansouri etal., 2013).** However, Arbequina cultivated in Egypt produced lower levels of oleic acid as compared to its original growing

area. (NS) and (IS) exhibited relatively high levels of linoleic acid and levels of palmitic acid similar to its original growing area **(Tous and Romero, 2000)**.

TG Compositions

TG is an important indicator of the quality and purity of olive oils. It is increasingly used by the food industry to confirm authenticity, because of the specificity of the triacylglycerol composition in different kinds of fats and oils **(Mansouri et al., 2013)**.

The mean values of TG for olive oils are shown in Table 3. In the study, ten TG were identified. The oils are characterized by four primaries TG: triolein (OOO), dio-leopalmitin (POO), dioleolinolein (LOO), PLO and six secondary TAG: PPO, LOL, SOO, LPL, POP and POLn which were present at a low percentage.

For the studied olive oils, OOO, POO, PLO and LOO constitute the most representative TG, although their percentages vary greatly. Statistical differences ($p < 0.05$) were found between cultivars in terms of TG content. Triolein (OOO) was the major TG within all the TG and its content in the samples ranged from 25.27 to 31.69 %. The presence of a high level of 1,2,3-trioleylglycerol (OOO) in olive oil constitutes a favorable authenticity indicator. The second major TG was POO with levels of 28.2 and 31.5 for (NS) And (IS), respectively. Both olive oil samples had low levels of LLL, with 0.46 and 0.32, respectively. The level of LLL of all samples did not exceed 0.5 %, which is the maximum limit according to EU regulation for olive oils. These results indicated that TAG composition of olive oils showed great variability between different growing areas, and also showed great variability between oils, as studied **(Tous and Romero, 2000) ; (Gokcebag *et al*. 2013).**

Triolein contents of the olive oils were lower than reported for Spanish and Tunisian cultivars, while POO was higher than these compared olive oils **(Mansouri etal., 2013)**.

Sterol Compositions

A table 4, Sterols is constituents of the unsaponifiable fraction of fats and oils, including olive oil and is very important nutritional and authenticity parameters. They are also characteristic of the identity and purity of vegetable oils **(Chtourou et al., 2013)** and can be used to detect adulteration **(Ranalli et al., 2002)**.

Study showed that Arbequina grown in the Egypt had a higher value for phenolic compounds (45.68µg/kg). (NS) had TPC levels than (NS) and (IS) olive oils as well as some Spanish samples **(Carelli, 2008); (Bakhouche etal., 2013)**; **(López-Cortés etal., 2013); (Franco et al., 2014)**. However, TPC of the Ramicilla variety studied by **Carelli, (2008); Benito et al., (2010)**, had a higher value (831.33 µg/ kg) than (NS), whereas Negral variety was very close to the result of (NS) with a level of 45.68 µg/kg. Moreover, according to the study of **Pardo, et al., (2013)** Cornicabra and Picual varieties also had quite higher TPC than (NS) samples at levels of 7980 and 8030 µg/kg, respectively. TPC of olive oils from economically were studied by some researchers, **Ocakoglu et al., (2009); Ilyasoglu et al., (2010)** and TPC content of (NS) (*45.68* µ*g*/kg*)* was found to be quite higher than the findings of these researchers.

Tocopherol Content

Tocopherols are the main lipid-soluble antioxidants present in olive oil. Of the four isomers found in olive oil (α, β, γ and δ) α (vitamin E) is the most abundant (90–95 %). The concentration of these compounds in olive oil range was between 150 and 250 mg/kg **(Amelio, 2003).** In this study, the quality characterization of the different monovarietal EVOO has been studied by quantifying their tocopherol content. Table 5, was represents α, β, γ and δ -tocopherol fractions and total tocopherol content (TTC) of olive oils.

The levels of α-tocopherol and total tocopherol in the analyzed oils showed significant differences ($p < 0.05$) between the growing areas of the cultivars, as seen in Table 5.

Differences in total tocopherol content may also be related to variations in climatic conditions as known high amounts of rainfall result in higher levels of tocopherol in olives **(Ilyasoglu et al., 2010)**.

The major tocopherol was α-tocopherol with a content ranging from 179.55 to 202.50 mg/kg for (NS) and (IS), respectively (Table 5). Consistent with the α-tocopherol content, (NS) was richest in total tocopherol with 228.70 mg/kg while this value was 203.85 mg/kg for (NS). The level of α-tocopherol of olive oils from was similar to the α-tocopherol content of (NS) and (IS), **(Ilyasoglu et al., 2010)** and some olive oils from Arbequina produced in Spain [**(López-Cortés et al., 2013) ; Pardo, et al. , 2013)**]. However, these values were less than olive oil of this variety cultivated in Argentina and Tunisia [**(Torres and Maestri 2006); (Carelli, 2008); (Guerfel et al., 2012); (Chtourou et al., 2013)**].

Phenolic Content and Antioxidant Capacity

Phenolic, which are present in olive oil in appreciable quantities (50–500 mg/kg), have an important role in the stability of the oil against oxidation. The process of refining removes them almost totally **(Amelio, 2003).** Phenolic content is one of the most important parameters for evaluation of extra virgin olive oil quality, because phenols widely contribute to oil sensory properties and aroma, which are bitterness and astringency, and they prevent it from oxidation **(Bendini et al. , 2007) ; (Mansouri et al. , 2013)**. In the present study, total phenolic contents (TPC) were determined according to the Folin–Ciocalteu colorimetric method **(Gutfinger T, 1981).** Table 6 shows the total phenolic content of (NS) and (IS) samples. In addition, individual phenolic fractions were also given in Table 6.

As shown in Table 6, TPC of the analyzed oils showed significant differences ($p < 0.05$) according to the growing area of the cultivars. (NS) oils showed a higher value for TPC (454.68 µg/kg) whereas the (NS) (*50.86* µg/kg*)* was less than 100 mg/kg.

The studies of **Garcia et al., (2010) ; Pardo, et al., (2013**) reveal considerable quantitative differences in phenolic compounds content among the oils obtained from Arbequina cultivars planted in Spain which showed a total phenol level of 244 µg/kg. Results of this study showed that Arbequina grown in Egypt had a higher value for phenolic compounds (45.68µg/kg). (NS) had TPC levels than Tunisian and Argentina olive oils as well as some Spanish samples **(Carelli, 2008) ; (Bakhouche et al., 2013) ; (López-Cortés et al., 2013); (Franco et al. , 2014)**. However, TPC of the Ramicilla variety studied **Benito et al. , (2010) ; Pardo, et al. , (2013)** had a higher value (831.33 µg/ kg) than (NS), whereas Negral variety was very close to the result of (NS) with a level of 45.68 µg/kg. Moreover, according to the study of **Pardo, etal., (2013**) Cornicabra and Picual varieties also had quite higher TPC than (NS) samples at levels of 7980 and 8030 µg/kg, respectively. TPC of olive oils from economically important olive cultivars Ismailia and Sinai were studied by some researchers, **Ocakoglu et al., (2009),**. **Ilyasoglu et al., (2010)** and TPC content of (NS) (*45.68* µ*g*/kg*)* was found to be quite higher than the findings of these researchers.

The amount and composition of the phenolic fraction are related to the olive cultivar and the agricultural method **(Ilyasoglu et al., 2010); (Bakhouche et al., 2013)**. Quantitative determination of phenolic compounds in oil is usually performed according to the Folin Ciocalteu colorimetric method. However, this method is not specific, as it gives no indication of the nature of the phenolic compounds present. For this reason separation and identification of individual phenolic compounds have been performed using an LC–MS/MS system. As shown in Table 6, three main phenolic compounds were identified from the two EVOO. The comparative phenolic profiles evaluated by LC–MS/MS of oils produced from the two different growing areas revealed that the main phenolic alcohols in samples from Arbequina were

hydroxytyrosol, tyrosol and caffeic acid. However, although *p*cumaric, syringic and ferulic acid were detected in trace amounts, they were not given in Table 6. The concentration of hydroxytyrosol was higher in (NS) (18.70 µg/100 g) than in (IS) (14.65 µg/100 g). Similarly, a higher content of tyrosol was observed in (NS) (4.35 µ*g*/100 *g)* than the was 2.51 µ*g*/100 *g* found in (IS) . Caffeic acid levels were quite similar to each other at levels of 20.92 and 20.94 µ*g*/100 *g*, respectively. Hydroxytyrosol and tyrosol contents of olive oils belong to Ismailia and Sinai cultivars have already been studied **(Ilyasoglu et al., 2010).** When comparing the studied samples with results of the Ilyasoglu, (NS) and (IS), their oils were richer in phenolic compounds than our samples.

The results of the antioxidant capacity and free radical scavenging properties of the two studied olive oils were evaluated by ABTS, DPPH and ORAC (Oxidation–Reduction Absorbance Capacity) methods and are summarized in Table 7. Statistical differences ($p < 0.05$) were found between cultivars grown in different areas in terms of antioxidant capacity tests. Three different antioxidant tests were conducted: ABTS, DPPH (for quantifying the antiradical activity) based on the abilities of the antioxidants present into extracts to scavenge the radical in comparison with that of a standard antioxidant **(Baiano et al. , 2013),** and ORAC, which measures antioxidant inhibition of peroxyl-radical induced oxidations **(Karadag et al., 2009).** Within all the antioxidant activity tests, (NS) had the highest values with levels of 551.38 µg Trolox/g oil (ABTS), 536.44 µg Trolox/g oil (DPPH) and 503.67 µmol/100 g oil (ORAC), while(IS)had lower levels of 198.94 µg Trolox/g oil, 54.69 µg Trolox/g oil and 272.49 µmol Trolox/100 g oil, respectively. The higher antioxidant capacity of (NS) can be explained by the higher content of total phenols.

Correlations between total phenolic content and antioxidant activity were evaluated. The correlation coefficient between total phenolic content and antioxidant activity measured by the ABTS,

DPPH and ORAC assays was the same (R^2 = 1, p\0.05, n = 2).

Phenolic content is an important parameter in the evaluation of the extra EVOO quality because phenolic compounds protect the TAG from oxidation and contribute to oil flavor and aroma **(Karadag et al., 2009).** In addition, it was reported that a high total phenolic content appears to be beneficial for the shelf life of the oil **(Dabbou etal., 2010); (Chtourou et al. , 2013)**.

Other Minor Compounds (Squalene, Chlorophyll, Carotenoid Content)

Squalene (C30) is an important intermediary for biological synthesis of sterols, and is present in concentrations of about 1500–2000 mg/kg in olive oil **(Amelio, 2003).** Shark and whale liver oil are the primary commercial sources of squalene **(Ghimire et al., 2009).** The other sources are olive oil, wheat germ and rice bran oils. There is limited information on the squalene value of Egyptian olives. Olive oil is an important squalene source and is evaluated from this respect. The squalene levels of the two studied olive oils evaluated by GC-FID are summarized in Table 8.

Squalene levels varied from 1526.36 to 3494.68 mg/kg for (NS) and (IS) , respectively.(IS) was quite rich in squalene content. Although, there is no reliable comparable result in the literature for squalene levels of olive oil from other Arbequina cultivars cultivated in different geographical areas, our findings on the squalene content of (IS) were in accordance with those of Egyptian olive oils studied **Dıraman, (2010),** whereas (NS) was found to have lower levels of squalene. The differences in squalene levels are thought to be due to geographic conditions.

Chlorophylls and carotenoids are the main lightharvesting pigments found in vegetable oils Also, they are important for the prevention of auto-oxidation and photo-oxidation **(Chtourou etal., 2013).** β-Carotene (C40), which imparts a characteristic yellow-orange color to the oil, is a precursor of vitamin A. Its concentration in olive oils is about 300–400 mg/kg.

Chlorophylls are present in olive oils and are responsible for the greenish coloration of certain olive oils. These pigments are also important factors in olive oil stability **(Chtourou et al., 2013).** Table 8 represents β-carotene and chlorophylla levels of olive oils. Significant differences in the levels of chlorophylla in the analyzed oils were observed ($p < 0.05$), while no significant difference was observed in the β-carotene content. Levels of chlorophylla pigment varied between 0.52 mg/ kg, in (NS) and 0.64 mg/kg in the (IS). The content of β-carotene was similar with levels of about 0.20 mg/kg in both the samples. When comparing our results with the studies of **Chtourou *etal*. (2013) and Lopez-Cortes *etal* (2013)**. Olive oils from this cultivar in Tunisia and Spain had higher chlorophylla and β-carotene content.

CONCLUSION

Oil produced from the Spanish Arbequina olive cultivar, when grown in Egypt under climatic and environmental conditions similar to Spain, has some compositional differences than when grown in its native Spain and other parts of the world. In particular, Arbequina EVOO produced in Egypt showed considerable deviations when compared to same cultivars cultivated in different parts of the world with respect to compositions of TAG, phenolics, tocopherols and sterols. The geographic area appears to have a significant effect on the qualitative characteristics and the chemical composition of olive oil. Oil from Arbequina has a composition in fatty acid close to that of economically important varieties of Egypt, Ismailia and Sinaicultivars, but at the same time there was great statistically significant difference in TAG composition, phenolic content and antioxidant activity.

Egypt, especially the North Sinai Governorate (NS) of Egypt, could be a quite adaptable area to yield good quality Arbequina olives and thus to produce olive oils from Arbequina varieties. The knowledge about the oil composition from the olives

grown in areas near the Mediterranean region will contribute to the updating of market conditions and the review of international rules affecting trade in olive oils.

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Table 1 : Some chemical characteristics of Arbequina EVOO

Physicochemical parameter	(NS)	(IS)
UV270	0.14 ± 0.00^{a}	0.11 ± 0.00^{b}
UV232	0.73 ± 0.04^{ns}	0.76 ± 0.00^{ns}
PV (meq O_2/kg)	3.75 ± 0.03^{a}	7.24 ± 0.05^{b}
Free Acidity (% oleic acid)	0.75 ± 0.05^{a}	0.34 ± 0.03^{b}
L	$3.88 \pm 0,01^{a}$	14.69 ± 1.13^{a}
a	0.35 ± 0.00^{a}	2.98 ± 0.70^{a}
b	7.46 ± 0.24^{a}	24.8 ± 2.25^{a}

Significant differences in the same row are shown by symbol (a–b) ($p < 0.05$)
PV peroxide number, *FFA* free fatty acidity as % oleic acid *ns* no significance

Table 2 : **Fatty acid composition of Arbequina EVOO samples**

Fatty acids	(NS) (%)	(IS) (%)
Palmitic (C16:0)	16.32 ± 0.66	16.96 ± 0.02
Palmitoleic (C16:1)	2.17 ± 0.48	2.57 ± 0.00
Stearic (C18:0)	1.85 ± 0.01	1.87 ± 0.00
Oleic (C18:1)	65.14 ± 2.16	63.00 ± 0.02
Linoleic (C18:2)	12.36 ± 0.92	13.28 ± 0.00
Linolenic (C18:3)	0.35 ± 0.01	0.36 ± 0.00
Arachidonic (C20:0)	0.59 ± 0.02	0.61 ± 0.00
Eicosenoic (C20:1)	0.27 ± 0.01	0.27 ± 0.00
Behenic (C22:0)	0.30 ± 0.05	0.35 ± 0.00
Σ SFA	19.08^{a}	20.32^{b}
Σ MUFA	67.5^{a}	65.58^{b}
Σ PUFA	12.95^{a}	13.89^{b}
O/L ratio	5.2^{a}	4.7^{b}

Significant differences in the same row are shown by different letters (a–b) varieties ($p < 0.05$)
SFA saturated fatty acid, MUFA monounsaturated fatty acid, PUFA polyunsaturated fatty acid, O/L oleic/linoleic ratio ns non significant

Table 3 : **TAG composition of Arbequina EVOO samples**

TAG	(NS) (%)	(IS) (%)
PPO	6.58± 0.12[a]	8.66± 0.71[b]
PPL	3.42± 0.07[a]	5.30± 0.12[b]
POS	1.18± 0.01	1.18± 0.16
POO	28.21± 0.12[a]	31.54± 1.54[b]
PLO	11.61± 0.08[a]	14.14± 0.77[b]
SOO	2.82± 0.04	2.00± 0.24
OOO	31.69± 0.64[a]	25.27± 0.50[b]
OLO	11.45± 0.28	9.20± 1.37
LLO	1.57± 0.25	1.84± 0.29
LLL	0.46± 0.05[a]	0.32± 0.00[b]
Others	1.03± 0.99[a]	0.58± 0.54[b]

Significant differences in the same row are shown by symbol (a–b) ($p < 0.05$)
Ns= no significance

Table 4: Sterol composition and some chemical characteristics of Arbequina EVOO

Sterols	(NS) (%)	(IS) (%)
Campesterol	3.15± 0.02a	1.95± 0.04b
Stigmasterol	0.58± 0.02a	1.75± 0.04b
β-Sitosterol	81.30± 0.70a	76.46± 0.65b
Δ5-Avenasterol	13.72± 0.17a	18.57± 0.58b

Significant differences in the same row are shown by different lower case letters (a–b) ($p < 0.05$) *ns =no significance*

Table 5 : Tocopherol content of Arbequina EVOO

Tocopherol fractions and contents	(NS) (mg/kg)	(IS) (mg/kg)
Tocopherols		
α-Tocopherol	179.55± 4.69a	202.50± 11.46b
β-Tocopherol	9.49± 0.10a	9.84± 0.59a
γ-Tocopherol	11.60± 2.14a	13.14± 1.98a
δ-Tocopherol	3.18± 0.60a	3.19± 0.60a
TTC	203.85± 1.26a	228.70± 5.51b

** Significant differences in the same row are shown by different lower case letters (a–b) ($p < 0.05$) ns-= no significance*

Table 6: Phenolic composition, of Arbequina EVOO

Phenolics	(NS) (µg/100 g oil)	(IS) (µg/100 g oil)
Hydroxytyrosol	18.70± 0.12[a]	14.65± 0.03[b]
Tyrosol	4.35± 0.41[a]	2.51± 0.05[b]
Caffeic acid	20.92± 0.00[ns]	20.94± 0.00[ns]
TPC (µg gallic acid	45.68± 3.10[a]	50.86± 4.13[b]

Significant differences in the same row are shown by different lower case letters (a–b) ($p < 0.05$)
TPC total phenolic content, ns no significance

Table 7: Antioxidant capacity of Arbequina EVOO samples

Antioxidant capacity	(NS)	(IS)
ABTS (µg Trolox/g oil)	551.38± 32.91[a]	198.94± 5.31[b]
DPPH (µg Trolox/g oil)	536.44± 17.13[a]	56.69± 7.78[b]
ORAC (µmol/100 g oil)	503.67± 0.00[a]	272.49± 31.31[b]

Significant differences in the same row are shown by different lower case letters (a–b) ($p < 0.05$)
Ns= no significance

Table 8: Squalene, β-carotene and chlorophyll- a content of Arbequina EVOO

Other minor components	(NS) (mg/kg)	(IS) (mg/kg)
Squalene	1526.36± 0.98[a]	3494.68±22.15[b]
β-Carotene	0.20± 0.00[ns]	0.19± 0.02[ns]
Chlorophyll-a	0.52± 0.00[a]	0.64± 0.00[b]

Significant differences in the same row are shown by different lower case letters (a–b) ($p < 0.05$)
Ns= no significance

تقييم الخواص الفيزيوكيميائية والمغذيات النباتية لزيت الزيتون أربيكينا البكر المنزرع حديثاً في مصر

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الملخص العربي

أجريت هذه الدراسة في قسم النباتات الطبية والعطرية – معهد بحوث البساتين – مركز البحوث الزراعية – الدقي ، بالتعاون مع قسم علوم وتكنولوجيا الأغذية – كلية الزراعة – جامعة الأزهر – القاهرة خلال موسم 2016 على الزيوت المستخلصة من زيتون أربيكينا (Arbequina) البكر والمنزرعة حديثاً تحت ظروف الأراضي المصرية في منطقتين موضع الدراسة و هي محطة الشيخ زويد – مركز بحوث الصحراء - محافظة شمال سيناء (NS) ومنطقة التل الكبير - محافظة الإسماعيلية (IS) ويعتبر صنف زيتون أربيكينا (Arbequina) من الأصناف الزيوت التي دخلت مصر حديثاً ، وكان الهدف من هذه الدراسة هو تقييم زيت الزيتون البكر (EVOO) كيميائياً و تغذوياً ودراسة نسب المغذيات الدقيقة ، وقد تم جمع 40 كجم من الثمار الناضجة من المنطقتين (NS)، (IS)، واستخراج الزيت ، وتخزينها في 4 درجات مئوية حتى التقييم .. وتمت دراسة المكونات الرئيسية والثانوية مثل الكاروتينات (carotenoids) والسكوالين (squalene) والفينوليك (phenolics) والتوكوفيرول (tocopherols) لتقييم آثارها على جودة الزيت والفوائد الصحية ، وتم تحديد تكوين الأحماض الدهنية ، وتكوين ستيرول (sterol) ، والمحتوى السكوالين (squalene content) . و تم تحليل وتجزئة الفينولات الفردية بواسطة LC–MS/MS وتم تقدير Tocopherols بواسطة HPLC. وفقا للنتائج التي تم الحصول عليها من هذه الدراسة. وكان مجموع محتوى الفينول من العينات 454.68 و 50.86 ملجرام حمض Gallic / كجرام زيت للمنطقتين موضع الدراسة (NS) ، (IS) ، على التوالي. و تم تحديد هيدروكسيتيروسول (Hydroxytyrosol) و تيروسول (Tyrosol) لتكون الفينول. و تم العثور على Tocopherolsالرئيسي في (NS)، (IS) Tocopherol-α مع مستويات 179.55 و 202.5 ملجرام / كجرام من الزيت، على التوالي. β-Carotene مستويات في كلا العينات كانت مماثلة في 0.2 ملجرام / كجرام . وتمت مقارنة نتائج تلك الدراسة وما تحتويه من مواد فعالة في زيت الزيتون أربيكينا (Arbequina) المنتجة في بلدان مختلفة. وقد تقرر أن زيت الزيتون أربيكينا (Arbequina) المزروع تحت ظروف الاراضى المصرية ذات جودة عالية يمكن أن تنتج في مصر، وخاصة في محافظة شمال سيناء (NS).

الكلمات المفتاحية: زيت الزيتون البكر الممتاز (إيفو) · أربيكينا متنوعة · الفينوليكس · توكوفيرول · سكوالين