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Maturity Impact on Physicochemical Composition and Polyphenol Properties of Extra Virgin Olive Oils Obtained from Manzanilla, Arbequina and Koroneiki Varieties in Iran

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Abstract

In this study, the physicochemical properties and polyphenol composition of extra virgin olive oils (EVOOs) extracted from three different olive cultivars including Arbequina, Koroneiki, and Manzanilla grown in Olive Research Station located in Rudbar county, Gilan province, Iran were studied at three ripening stages. Several parameters, including peroxide value, acidity value, unsaponifiable matter, oxidative stability, total aliphatic alcohols, and fatty acids (FAs), sterols, and triacylglycerols composition were analyzed. The results showed that as maturity advanced, there was an increase of oil content, acidity value, and iodine value, while there was a decrease in peroxide value, oxidative stability, aliphatic alcohols and unsaponifiable matter ($p<0.05$). The decrease of saponification value was very small in the developing ripening process ($p>0.05$). The MUFA/PUFA ratio and total sterol content were decreased during the olive ripening stages ($p<0.05$). The triterpenes decreased for Arbequina and Koroneiki but increased for Manzanilla during the maturity stages. The results showed that oleuropein was decreased while oleuropein aglycone, oxidized aldehyde and hydroxylic form was increased for all EVOOs during maturation. Apigenin, quercetin, Ligstroside aglycone, aldehyde and hydroxylic form, ferulic acid, caffeic acid, catechin were decreased during maturation of fruits ($p<0.05$). The main triglycerides were triolein (OOO),

palmitodiolein (POO), dioleolinolein (OOL) and palmitooleolinolein (PLO) in all EVOOs. Finally, the olive cultivar and harvesting date influence the physicochemical properties and polyphenol composition of EVOOs extracted from olive varieties grown in one region. Also, the results can present useful information to determine the optimum maturity stage for each studied olive variety.

Keywords: Olive Oil; Chromatography; Maturation; Polyphenols; Extra virgin olive oil (EVOO)

Introduction

Extra virgin olive oil (EVOO) is extracted from healthy fruits using mechanical procedure, which has special health benefits leads to increase its use for preventing chronic disease. The Mediterranean diet and olive oil consumption showed reducing the risk of cardiovascular disease, atherosclerosis, and special types of cancer [1]. Olive oil consists of 98% of triacylglycerols and minor components including aliphatic alcohols, tocopherols, phenolic compounds, and phytosterols. High quality EVOO is taken into account like a natural pharm-food. This characteristic of EVOO is due to its fat composition such as high oleic acid concentration (56-84%), linoleic acid (3.5-21%) and linolenic acid lower than 1.5%, and also bioactive compounds including β-carotene, tocopherols (vitamin E), volatile compounds, sterols and phenolic compounds (PCs) [1]. Bioactive compounds of olive fruits may be affected by unaccounted parameters, including growing season, variety, temperature, soil type, light, growing environment, and processing, post-harvest storage [2]. The harvesting time of olive fruits affects the composition of extracted olive oil. Optimization of harvesting time will be useful for the olive growers' earnings. The early harvest of healthy olive fruits results in an EVOO rich in polyphenol compounds in which this oil has higher nutritional value and sensory properties and can be found as a nutraceutical or functional food. In early harvest, olive fruits have low oil content and special sensory properties, including high bitterness and excess pungency, but late harvest of olive fruits possess high oil content with less quality and show lower sensory characteristics [3].

Also, the composition of EVOO depends on the environmental growth conditions, including biotic and abiotic stresses, the genetic background, and the agronomic techniques [4]. In the maturity index (MI), both the pulp and the peel color change from deep green to black. The MI strongly depends on variety, environmental situations, crop load, and cultivation practices [5].

Normally, the harvesting time of olive fruits, in Iran, starts at the end of September and lasts about 15 days, being dependent on the weather conditions and the season. Manual harvesting is performed by 3 to 4 people per olive tree. Olive trees in Iran cover an area of 92000 ha. Olive trees are cultivated in 26 provinces of Iran, which the most of them are located in the north of Iran. 60% of the cultivars are natives and 40% of them are foreign.

The aim of this study was to investigate the physicochemical and bioactive compounds characteristics of three EVOOs extracted from three foreign varieties cultivated in the north of Iran.

Materials and Methods

Materials

Methanol, 99.99%, n-hexane, 99.99%, orthophosphoric acid, 85%, and acetonitrile, 99.9%, with the chromatography grade were purchased from the Fisher Scientific (Lisbon, Portugal). Also, Folin-Ciqaultu thiosulfate and 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) reagents were purchased from the Sigma-Aldrich (The United States). Similarly, standards of phytosterols, fatty acid methyl esters, triglycerides, vanillic acid, vanillin, caffeic acid, oleuropein, tyrosol, cinnamic acid, luteolin, catechin, gallic acid, apigenin, and ferulic acid were purchased from the Sigma-Aldrich (The United States). Other materials, including acetonitrile, acetone and diethyl ether were obtained from the Merck Company (Germany).

Samples preparation

The research was performed during the 2021 olive harvest season. "Arbequina, Manzanilla and Koroneiki" as

foreign cultivars were harvested from an orchard as named Olive Research Station located in Rudbar county, Gilan province, Iran. This station is in the geographical coordinates 36°48'N 49°24'E. Rudbar is situated at 1050 m above sea level. Olive fruits were harvested by hand from five trees at various harvesting dates, based on fruit skin color as three stages, including green, black green, and purple. The fruits were washed with water and their pulp was separated from whole stones.

Oil recovery

The oil content of each variety was determined as the percentage of fresh olive paste by the following equation:

$$\text{Olive oil yield} = (V \times D/W) \times 100$$

Where V is the volume of EVVO (mL); D is the density of EVOO (0.915 $g.mL^{-1}$); and W is the weight of olive paste [6].

Determination of quality parameters & Physicochemical characteristics

Peroxide value, acidity value, unsaponifiable matter, oxidative stability

Peroxide value, acidity value, unsaponifiable matter and oxidative stability were determined according to the methods described in International Organization for Standardization (ISO) 3960 [7], 660 [8], 18609 [9] and 6886 [10], respectively.

Saponification value and iodine value

In addition, saponification value and iodine value were calculated according to ISO 3657 [11] and ISO 3961 [12], respectively.

Fatty acids (FAs), sterols, triacylglycerols composition

Methyl esters preparation of triglyceride FAs and quantification of FAs composition, identification, and quantification of individual and total sterol contents, and triterpenes, triacylglycerols composition by evaluation of the coherence of TAGs composition and FAs composition were conducted based on the methods described in the COI/T.20/Doc. No 33 :2017 [13], COI/T.20/Doc. No 26: 2020 [14], COI/T.20/Doc. No 20 /Rev. 4: 2017 [15] and COI/T.20/Doc. No. 25: 2018 [16], respectively.

Total aliphatic alcohols

Total alcoholic alcohols were determined according to the described method in COI/T.20/Doc. No. 26 [14].

Determination of phenolic compounds

Identification and quantification of major phenolic compounds were conducted according to the described method in the COI/T.20/DOC. 29/Rev.1. by the HPLC unit [17].

Statistical analysis

The one-way analysis of variance (one-way ANOVA) was applied for evaluation of the experimental data and statistical analyses, for a confidence interval of 95%. All tests were performed in triplicates.

Results

Peroxide value, acidity value, unsaponifiable matter, oxidative stability, saponification and iodine values

The results of measuring the oil recovery percentage, peroxide value, acidity value, unsaponifiable matter, oxidative stability, iodine value, and saponification value in the EVOOs extracted from olive fruits of Arebquina, Koroneiki, and Manzanilla varieties are shown in Table 1.

Table 1: Results of physicochemical properties determined in EVOOs, from the three olive varieties grown in the study area, collected at different stages of ripening, Data are presented as mean±SD (n=3)

Tested parameters	Sample name	September	October	November
Oil content (%)	Arbequina	13.30±0.25	15.90±0.75	17.20±0.55
	Koroneiki	12.42±0.32	14.85±0.55	16.35±0.76
	Manzanilla	9.35±0.42	13.30±0.48	15.15±0.68
Peroxide Values (meqO_2/kg oil)	Arbequina	17.56±0.42	1.65±0.10	2.85±0.16
	Koroneiki	17.32±0.23	3.05±0.25	3.00±0.12
	Manzanilla	5.08±0.0.18	1.60±0.14	1.56±0.10
Acidity values (%)	Arbequina	0.13±0.01	0.17±0.01	0.18±0.05
	Koroneiki	0.17±0.01	0.22±0.04	0.27±0.04
	Manzanilla	0.12±0.04	0.14±0.02	0.15±0.04
Unsaponifiable matter (g/kg oil)	Arbequina	1.25±0.22	1.18±0.12	0.64±0.09
	Koroneiki	1.15±0.30	0.90±0.10	0.86±0.14
	Manzanilla	1.85±0.14	1.86±0.20	1.27±0.15
Oxidative stability (h)	Arbequina	15.74±0.78	12.47±0.67	8.30±0.52
	Koroneiki	33.41±0.85	30.07±0.55	23.70±0.32
	Manzanilla	16.25±0.55	13.77±0.45	12.25±0.43
Iodine Value(g I_2/100 g oil)	Arbequina	78.99±0.55	80.97±0.25	83.07±0.22
	Koroneiki	74.83±0.15	76.27±0.20	78.05±0.40
	Manzanilla	76.45±0.30	80.81±0.20	80.25±0.18
Saponification Value (mg KOH/g oil)	Arbequina	194.60±0.25	194.10±0.15	194.00±0.20
	Koroneiki	193.60±0.11	193.00±0.24	193.10±0.30
	Manzanilla	194.40±0.45	194.30±0.25	194.00±0.20

Results showed an increase of oil recovery percentage for each variety with developing in the ripening process for the studied varieties of olive fruit. The highest oil content was observed in Arbequina ripening stage. The lowest oil content was obtained for Manzanilla cultivar (9.35±0.42%, 13.30±0.48%, and 15.15±0.68%) at all stages of maturation. In addition, the peroxide value for all EVOOs was decreased during maturation stages. Peroxide value of EVOOs extracted from Arbequina (17.56±0.42 to 2.85±0.16 meqO_2/kg oil) and Koroneiki (17.32±0.23 to 3.00±0.12 meqO_2/kg oil) cultivars was higher than that for Manzanilla (5.08±0.0.18 to 1.56±0.10 meqO_2/kg oil) EVOOs with developing in the ripening process of studied olive fruits. The acidity values obtained for EVOOs of Koroneiki cultivar (0.17±0.01% to 0.27±0.04%) were higher than those obtained for EVOOs extracted from other studied cultivars (Arbequina: 0.13±0.01% to 0.18±0.05%, Manzanilla: 0.12±0.04 to 0.15±0.04%). Results showed that there were statistically differences between acidity values during ripening of fruits of a variety or between various varieties ($p<0.05$).

The amounts of oxidative stability of all EVOOs were decreased with developing in the ripening process for the studied varieties of olive fruits. The oxidative stability values of Manzanilla, Koroneiki, and Arbequina EVOOs were ranged from 12.25 h to 16.25 h, 23.70 h to 33.41h, and 8.30 h to 15.74 h, respectively. The highest oxidative stability

was obtained in Koroneiki EVOOs.

The amounts of iodine value for all EVOOs were increased with developing in the ripening stages. Results showed that the amounts of iodine value of Arbequina, Koroneiki, and Manzanilla were ranged from 78.99±0.55 to 83.07±0.22 (gI_2/100g oil), 74.83±0.15 to 78.05±0.40 (gI_2/100g oil), and 76.45±0.30 to 80.81±0.20 (gI_2/100g oil), respectively.

The amounts of saponification value in all EVOOs showed no significant differences during maturation stages. Results showed that the amounts of saponification value in Arbequina, Koroneiki, and Manzanilla were ranged from 194.00±0.20 to 194.60±0.25 (mg KOH/g oil), 193.00±0.44 to 193.60±0.11(mg KOH/g oil), and 194.10±0.45 to 194.60±0.30 (mg KOH/g oil), respectively.

FAs composition

Results of FAs composition are presented in Table 2. The maximum values of oleic acid were belonged to the last harvest date (November) in all varieties. Also, palmitic acid levels in Koroneiki and Arbequina decreased, but slightly increased in Manzanilla during fruit maturation advances. The monitoring of FAs composition during maturation is studied due to variable of it during the stage of maturity, the place of cultivation and the crop season. The results showed the increase in PUFA level during the olive maturation advances. In addition, a decrease in MUFA level was observed in Manzanilla and very low in Arbequina, but in Koroneiki, an increase in MUFA level was observed. The MUFA/PUFA ratio was higher in the first stage or green stage, which was decreased during the olive maturation advances. This MUFA/PUFA ratio was higher in the oils extracted from the Koroneiki variety in comparison with extracted EVOOs from other varieties at different ripening stages.

Table 2: FAs composition present in EVOOs analyzed, from the three olive varieties grown in the study area, collected at different stages of ripening, Data are presented as mean±SD (n=3)

FAs composition	Arbequina			Koroneiki			Manzanilla		
	September	October	November	September	October	November	September	October	November
C12:0	0.00±0.00	0.00±0.00	0.00±0.00	0.04±0.00	0.02±0.00	0.02±0.00	0.00±0.00	0.05±0.00	0.00±0.00
C14:0	0.06±0.00	0.03±0.00	0.03±0.00	0.01±0.00	0.05±0.01	0.03±0.02	0.03±0.01	0.03±0.01	0.03±0.00
C16:0	21.29±0.08	20.48±0.05	18.94±0.08	18.14±0.06	16.13±0.09	14.72±0.04	20.99±0.03	21.00±0.05	21.35±0.04
C16:1c	2.15±0.03	2.28±0.02	2.01±0.02	1.84±0.05	1.90±0.06	1.46±0.06	2.05±0.01	2.38±0.02	2.30±0.01
C17:0	0.13±0.02	0.15±0.01	0.12±0.02	0.15±0.02	0.03±0.01	0.05±0.01	0.04±0.01	0.07±0.02	0.12±0.02
C17:1c	0.22±0.01	0.20±0.01	0.15±0.01	0.07±0.01	0.11±0.02	0.13±0.01	0.07±0.01	0.09±0.01	0.15±0.02
C18:0	2.24±0.08	2.24±0.05	2.41±0.05	2.39±0.05	2.37±0.06	2.64±0.07	2.00±0.08	2.28±0.05	2.42±0.05
C18:1c	58.24±0.40	58.08±0.25	58.55±0.60	70.12±0.60	72.17±0.55	72.88±0.45	62.65±0.40	56.63±0.45	53.24±0.60
C18:2c	14.18±0.10	15.33±0.10	16.69±0.10	5.63±0.09	5.79±0.10	6.57±0.15	10.54±0.10	15.84±0.12	16.96±0.15
C18:3c	0.81±0.03	0.60±0.04	0.83±0.02	0.90±0.03	0.83±0.05	0.99±0.03	0.82±0.03	0.87±0.04	0.97±0.02
C20:0	0.36±0.01	0.28±0.02	0.37±0.03	0.34±0.01	0.28±0.02	0.36±0.03	0.42±0.02	0.49±0.02	0.47±0.03
C20:1c	0.20±0.01	0.21±0.01	0.20±0.03	0.26±0.03	0.23±0.02	0.02±0.03	0.29±0.01	0.18±0.01	0.30±0.03
C22:0	0.00±0.00	0.00±0.00	0.12±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.10±0.00
C24:0	0.10±0.01	0.09±0.02	0.08±0.02	0.11±0.01	0.07±0.02	0.12±0.02	0.12±0.01	0.10±0.02	0.16±0.01
C24:1c	0.03±0.01	0.03±0.01	0.02±0.01	0.01±0.01	0.03±0.01	0.04±0.01	0.02±0.01	0.04±0.01	0.02±0.01
MUFA	60.84±0.50	60.80±0.70	60.42±1.01	72.24±0.65	74.43±0.95	74.49±1.02	65.06±0.40	59.28±0.70	55.99±1.00
PUFA	14.99±0.13	15.93±0.50	17.52±0.60	6.53±0.13	6.62±0.50	7.56±0.60	11.36±0.10	16.71±0.35	17.93±0.45

MUFA/PUFA	4.06±0.11	3.82±0.20	3.45±0.18	11.06±0.18	11.24±0.20	9.88±0.11	5.73±0.20	3.55±0.15	3.12±0.15
C18:1c /C18:2c	4.11±0.10	3.79±0.13	3.48±0.10	12.46±0.30	12.45±0.25	11.09±0.20	5.94±0.12	3.58±0.11	3.14±0.25

Sterols and triterpenes composition

Sterols composition, the main triterpenic sterols and dialcohols and the total sterol content of each EVVO obtained from the various olive varieties are presented in Table 3.

Table 3: Sterols and triterpene and dialcohols composition present in EVOOs analyzed, from the three olive varieties grown in the study area, collected at different stages of ripening, Data are presented as mean±SD (n=3)

Sterol composition	Arbequina			Koroneiki			Manzanilla		
	September	October	November	September	October	November	September	October	November
Cholesterol (%)	0.03±0.00	0.01±0.00	0.00±0.00	Nd	nd	nd	0.02±0.02	0.09±0.01	0.09±0.01
Brassicasterol (%)	0.01±0.00	0.02±0.00	0.02±0.00	0.01±0.00	nd	nd	Nd	0.03±0.01	0.03±0.01
24-Methylene cholesterol(%)	0.05±0.01	0.13±0.04	0.02±0.01	0.01±0.00	0.10±0.01	0.10±0.00	0.04±0.01	0.03±0.03	0.01±0.01
Campesterol (%)	4.83±0.09	4.06±0.12	4.08±0.11	4.56±0.13	3.50±0.20	3.46±0.32	4.45±0.08	3.35±0.05	3.17±0.10
Campestanol (%)	Nd	0.46±0.02	0.07±0.02	0.05±0.01	nd	nd	0.50±0.01	0.30±0.02	0.23±0.02
Stigmasterol (%)	1.47±0.03	1.09±0.02	1.09±0.01	1.14±0.07	1.09±0.05	0.47±0.08	1.58±0.05	1.73±0.06	1.80±0.02
Δ-7-Campestrol (%)	0.09±0.01	0.40±0.05	0.20±0.02	0.22±0.05	0.33±0.07	0.41±0.05	0.11±0.08	0.23±0.03	0.13±0.02
Δ-5, 23-Stigmastadienol (%)	0.30±0.01	Nd	Nd	0.82±0.0	0.09±0.01	0.02±0.01	0.18±0.02	0.13±0.01	0.05±0.01
Clerosterol (%)	0.98±0.10	0.81±0.08	0.40±0.12	0.88±0.21	0.65±0.15	0.65±0.10	1.31±0.21	0.86±0.20	0.55±0.10
β-Sitosterol (%)	83.91±0.20	82.58±0.35	81.04±0.15	85.84±0.55	82.43±0.35	81.98±0.45	86.73±0.15	83.60±0.25	83.16±0.22
Sitostanol (%)	0.38±0.01	0.05±0.01	0.01±0.01	0.12±0.01	0.10±0.02	0.10±0.03	0.17±0.02	0.10±0.03	0.04±0.01
Δ-5-Avenasterol (%)	6.24±0.20	9.55±0.15	12.34±0.30	4.45±0.23	10.57±0.44	10.96±0.45	4.97±0.15	8.41±0.11	9.91±0.20
Δ-5, 24-Stigmastadienol (%)	1.41±0.22	0.33±0.02	0.64±0.06	0.96±0.01	0.66±0.02	0.99±0.03	0.76±0.03	0.71±0.02	0.19±0.05
Δ-7-Stigmastenol (%)	0.07±0.01	0.05±0.01	0.20±0.03	0.50±0.11	0.33±0.15	0.47±0.23	0.16±0.01	0.29±0.04	0.49±0.08
Δ-7-Avensterol (%)	0.23±0.01	0.46±0.03	0.29±0.05	0.32±0.08	0.79±0.04	0.40±0.06	0.33±0.02	0.14±0.02	0.15±0.05
Apparent β-sitosterol (%)	92.27±1.25	92.47±1.15	94.03±1.10	92.19±1.52	93.76±1.20	94.05±1.42	92.81±1.31	92.95±1.58	93.35±1.66
Total Sterol (mg/kg)	1775.9±0.51	1617.9±0.81	1513.7±0.55	1511.61±0.54	1378.57±0.31	1091.81±0.45	1936.05±1.31	1343.54±1.58	1231.95±1.66
Tri terpenes	**Arebquina**			**Koroneiki**			**Manzanilla**		
	September	**October**	**November**	**September**	**October**	**November**	**September**	**October**	**November**
Erythrodiol (%)	0.95±0.15	0.50±0.03	0.43±0.01	2.44±0.50	1.99±0.55	1.13±0.81	0.36±0.40	0.47±0.70	2.47±1.00
Uvaol (%)	0.42±0.05	0.12±0.01	0.12±0.01	0.62±0.13	0.40±0.10	0.13±0.09	0.18±0.08	0.10±0.01	0.09±0.01

As regards the campesterol content, the highest values were found in EVOOs extracted from the Arbequina variety. In addition, the campesterol content was decreased during maturation in all EVOOs. During fruit maturation advances, stigmasterol content was decreased in EVOOs extracted from Arbequina and Koroneiki varieties, but increased in the Manzanilla variety and the highest stigmasterol content was observed in EVOOs obtained from

the Manzanilla variety. The β-sitosterol was the main sterol in all EVOOs samples, and Manzanilla had the highest content during each stage of ripening. Also, Δ-5-Avenasterol content was increased during fruit maturation advances. The total sterol content was decreased for Arbequina, Koroneiki, and Manzanilla at different ripening stages. The sum of erythrodiol and uvaol decreased for Arbequina and Koroneiki, but increased for Manzanilla during the olive maturity process.

Polyphenols composition

Ripening of olive fruit influenced on the phenol contents of the EVOOs. The value of polyphenol content of extracted oils from green olive fruits is higher than ripe olive fruits ($p<0.05$) (Table 4). Results showed that oleuropein was decreased while the oleuropein aglycone, oxidized aldehyde and hydroxylic form increased with different ripening stages for all EVOOs. In addition, decarboxymethyl oleuropein aglycone, dialdehyde form was decreased for Arebquina and Manzanilla and increased for Koroneiki. Oleuropein aglycone, aldehyde and hydroxylic form was increased for Arbequina and Manzanilla and decreased for koroneiki. The amounts of apigenin, quercetin, Ligstroside aglycone, aldehyde and hydroxylic form, ferulic acid, caffeic acid, catechin were decreased during maturation of fruits. For all EVOOs, the amounts of hydroxytyrosol and tyrosol were increased with different ripening stages of fruits.

Table 4: Individual phenolic compounds of EVOOs obtained from three olive varieties at different stages of ripening. Data are presented as mean±SD (n=3)

Major phenols composition (%)	Arbequina			Koroneiki			Manzanilla		
	September	October	November	September	October	November	September	October	November
Gallic acid	2.10±0.13	2.40±0.18	2.50±0.20	1.04±0.12	0.88±0.15	0.52±0.11	5.10±0.10	4.05±0.25	3.90±0.35
Hydroxytyrosol	0.50±0.11	1.53±0.35	3.00±0.37	1.01±0.08	2.55±0.10	3.45±0.89	0.60±0.01	0.89±0.04	1.35±0.24
Tyrosol	0.90±0.08	1.48±0.05	3.94±0.09	1.14±0.06	3.50±0.09	5.72±0.04	5.99±0.88	6.56±0.67	7.35±0.55
Catechin	1.00±0.03	0.88±0.02	0.75±0.05	1.84±0.05	1.20±0.30	1.16±0.20	3.35±0.45	2.38±0.55	1.30±0.33
Caffeic acid	1.50±0.02	1.15±0.01	1.12±0.02	2.70±0.22	1.65±0.15	1.05±0.01	3.89±0.34	2.56±0.67	1.12±0.34
Vanillin	1.22±0.01	1.20±0.05	1.00±0.01	0.87±0.02	2.11±0.22	5.56±0.33	2.07±0.44	5.09±0.55	6.15±0.78
Vanillic acid	2.24±0.08	2.24±0.05	2.41±0.05	1.39±0.44	2.66±0.23	5.64±0.77	4.00±0.23	3.28±0.55	2.42±0.25
p-Coumaric acid	1.00±0.40	2.18±0.25	3.05±0.60	1.88±0.60	1.17±0.55	1.00±0.45	1.90±0.40	1.63±0.24	1.24±0.14
Ferulic acid	4.98±0.12	2.33±0.10	2.00±0.21	1.63±0.09	1.46±0.10	0.57±0.15	3.54±0.10	3.00±0.12	2.96±0.05
Decarboxymethyl oleuropein aglycone, dialdehyde form	4.81±0.03	2.60±0.04	1.83±0.02	3.90±0.54	5.83±0.66	6.99±0.47	16.82±1.78	15.87±1.04	11.97±0.33
Oleuropein	5.80±0.89	3.28±0.45	1.37±0.23	2.34±0.23	1.28±0.12	0.98±0.15	5.42±0.45	4.49±0.49	3.47±0.78
Oleuropein aglycone, oxidized aldehyde and hydroxylic form	17.80±0.89	21.28±0.45	25.37±0.23	10.34±0.14	15.28±0.10	19 .98±0.24	14.34±2.23	17.28±1.12	21.98±2.15
Cinnamic acid	3.80±0.22	3.28±0.45	3.00±0.23	2.34±0.23	1.45±0.12	0.98±0.15	4.34±0.88	5.28±0.98	6.98±0.88
Quercetin	10.20±0.01	9.21±0.01	7.20±0.03	5.26±0.17	4.23±0.13	2.02±0.33	1.50±0.22	1.18±0.15	1.00±0.12
Luteolin	2.00±0.034	2.50±0.11	0.98±0.11	6.45±0.21	5.00±0.34	2.25±0.18	1.40±0.00	2.88±0.40	4.10±0.55

Oleuropein aglycone, aldehyde and hydroxylic form	10.10±0.13	14.09±0.02	17.08±0.02	24.11±1.9	22.88±0.66	20.12±0.89	0.99±0.33	2.99±0.22	4.16±0.45
Apigenin	2.55±0.21	1.88±0.15	1.02±0.22	4.01±0.34	3.55±0.44	2.04±0.65	3.50±0.87	2.54±0.67	2.02±0.66
Methyl-luteolin	5.84±0.50	5.60±0.70	3.42±1.01	4.24±0.65	3.43±0.95	1.49±0.99	0.67±0.12	0.28±0.15	0.12±0.10
Ligstroside aglycone, aldehyde and hydroxylic form	9.99±0.13	5.93±0.50	3.52±0.60	6.53±0.13	5.62±0.50	2.56±0.60	5.67±0.40	4.28±0.70	2.99±0.89
Others	14.67±1.13	14.96±2.21	15.44±2.43	16.98±2.13	14.27±2.21	15.92±1.43	14.91±1.45	13.49±2.00	13.44±1.66

Triglycerides composition

The determination of triacylglycerol composition is a significant agent for classification and characterization of monovarietal olive oils. The triglyceride distribution of EVOOs is presented in Table 5. According to the results, major triglycerides were triolein (OOO) (14.082%-36.394%), palmitodiolein (POO) (22.187%-31.130%), dioleolinolein (OOL) (7.706%-15.524%), palmitooleolinolein (PLO) (4.705%-14.242%), dipalmitoolein (POP) (4.893%-9.67%), stearodiolein (SOO) (2.035%-4.051%). In addition, other triglycerids were palmitolinolenin (PLLn) (0.055-0.263), trilinolein (LLL) (0.017-0.466), palmitodilinolein (PLL) (0.190- 2.285), oleolinoleolinolein (OLLn) (0.198%-0.501%), dioleolinolenin (OOLn) (0.553%-1.505%), oleodilinolein (OLL) (0.623%-4.497%), palmitoolein (POLn) (0.746%-1.215%), dipalmitoolein (POP) (4.893%-8.755%), and dipalmitoolein (PoOP) (1.205% - 2.129%). During maturation, the OOO percentage for Arbequina and Koroneiki was increased but for Manzanilla decreased, compatible with fatty acid results. In addition, OOL, PLL, OLL, PLO and SOL percentages were increased during growth of olive fruits, while, POO, SOO, POP decreased with maturation stages.

Table 5: Triglycerides composition present in EVOOs analyzed, from the three olive varieties grown in the study area, collected at different stages of ripening

TAGs	Arbequina			Koroneiki			Manzanilla		
	September	**October**	**November**	**September**	**October**	**November**	**September**	**October**	**November**
LLL	0.261	0.331	0.434	0.017	0.019	0.027	0.107	0.363	0.466
PoLL	0.131	0.163	0.173	0.018	0.020	0.020	0.069	0.180	0.209
OLLn	0.367	0.294	0.450	0.201	0.198	0.273	0.297	0.427	0.501
PoOLn	0.061	0.048	0.059	0.072	0.072	0.067	0.064	0.071	0.075
PLLn	0.174	0.134	0.187	0.066	0.055	0.068	0.129	0.206	0.263
PPoLn	0.029	0.022	0.025	0.024	0.020	0.017	0.028	0.034	0.039
PoPoL	0.022	0.027	0.023	0.007	0.007	0.005	0.015	0.030	0.031
PoPoPo	0.001	0.002	0.001	0.001	0.001	0.000	0.001	0.002	0.002
SLnLn	0.001	0.000	0.000	0.001	0.001	0.001	0.000	0.001	0.001
OLL	3.186	3.734	4.497	0.623	0.686	0.901	1.897	3.862	4.352
PoOL	1.065	1.224	1.938	0.449	0.496	0.441	0.813	1.279	1.301
OOLn	0.747	0.553	0.777	1.241	1.225	1.505	0.877	0.758	0.781
PLL	1.515	1.700	1.868	0.204	0.190	0.223	0.822	1.858	2.285

POLn	1.053	0.746	0.956	1.199	1.004	1.104	1.126	1.080	1.215
PPoPo	1.421	1.475	0.984	0.757	0.634	0.312	1.250	1.686	1.748
PoOO	2.170	2.300	2.061	2.776	3.070	2.429	2.399	2.269	2.027
PoPoO	0.089	0.100	0.079	0.081	0.090	0.054	0.087	0.106	0.097
PPoL	0.506	0.557	0.496	0.147	0.138	0.109	0.353	0.616	0.683
OOL	12.988	14.032	15.524	7.706	8.487	9.916	11.193	13.706	13.559
PoOO	2.170	2.300	2.061	2.776	3.069	2.429	2.399	2.269	2.027
SLL	0.144	0.168	0.214	0.024	0.025	0.036	0.071	0.182	0.233
PLO	12.355	12.777	12.898	5.035	4.705	4.920	9.704	13.188	14.242
PLP	2.148	2.125	1.955	0.600	0.475	0.444	1.537	2.318	2.734
PoPP	0.378	0.367	0.274	0.228	0.182	0.115	0.347	0.404	0.429
PoOP	2.065	2.094	1.712	1.814	1.702	1.205	2.080	2.184	2.129
LnPP	2.130	0.088	0.103	0.102	0.073	0.072	0.127	0.135	0.166
SPoL	0.048	0.055	0.057	0.017	0.018	0.018	0.030	0.060	0.070
SOLn	0.067	0.050	0.074	0.095	0.091	0.121	0.065	0.071	0.083
OOO	17.648	17.577	18.864	31.762	35.008	36.394	22.011	16.212	14.082
POO	25.183	24.007	22.261	31.130	29.112	27.088	28.627	23.399	22.187
POP	8.755	7.984	6.749	7.419	5.879	4.893	9.670	8.227	8.519
SLL	0.144	0.168	0.214	0.024	0.025	0.036	0.071	0.182	0.234
PPoO	2.065	2.095	1.712	1.814	1.702	1.205	2.080	2.184	2.129
PLS	0.825	0.849	0.909	0.287	0.258	0.292	0.535	0.919	1.132
PoPP	0.378	0.370	0.274	0.228	0.182	0.115	0.347	0.404	0.429
SOL	1.157	1.240	1.459	0.585	0.619	0.784	0.823	1.274	1.436
SOO	3.031	2.340	2.035	4.051	4.050	3.065	4.032	3.380	3.029
POS	1.747	1.658	1.633	1.845	1.662	1.676	1.639	1.694	1.830
SLS	0.204	0.209	0.224	0.071	0.063	0.072	0.132	0.227	0.279

Total aliphatic alcohols

Results showed that the total aliphatic alcohols content of EVOO varied significantly based on the variety ($p<0.05$) (Table 6). The total aliphatic alcohols content from 780.07±0.20 mg/kg to 411.24±0.45 mg/kg for Arbequina, from 937.70±0.44 mg/kg to 386.15±0.44 mg/kg for Koroneiki, and from 1097.67±0.18 mg/kg to 286.10±0.50 mg/kg for Manzanilla, was significantly decreased with developing of the ripening process of olive fruit.

Table 6: Total aliphatic alcohols content of EVOOs from three olive varieties at different stages of ripening. Data are presented as mean±SD (n=3)

Sample Name	September	October	November
Arbequina (mg/kg oil)	1780.07±0.20	1136.18±0.25	411.24±0.45
Koroneiki (mg/kg oil)	937.70±0.44	711.87±0.55	386.15±0.44
Manzanilla (mg/kg oil)	1097.67±0.18	743.06±0.33	286.10±0.50

The total aliphatic alcohols contents of studied EVOOs were significantly affected by ripening stages ($p<0.05$).

Discussion

It is so important to specify the optimal harvesting time for obtaining high yield and high-quality EVOO. The maturation of olives is associated with several physiological and chemical changes happening in olive fruit. Therefore, the mature stage is an important factor affecting olive oil composition. The level of oil extractability from olive fruit is an important factor which influences on determination of its optimum harvesting time. The rise of oil content in November might be due to the decrease of moisture level of the extracted oil during the late maturation stage. Result of the oil content of the Manzanilla variety in different stages of maturity cultivated in Egypt, was ranged from 15.84±0.36% to 25.76±0.49% [18]. It was found that the wet oil content enhanced from green to spotted stages of olive fruit maturation for all studied varieties of the southwest of Spain (Arbequina, Corniche, Morisca, Cacerena, Carrasquena, Manzanilla, Picual, Morisca, and Verdial de Badojoz) [19].

The decrease in peroxide values was observed during olive ripening, therefore, EVVOs obtained from olive fruits at the more advanced stage of maturity indicated lower peroxide values due to a decrease in lipoxygenase activity. These results agree with those of Youssef et al., (2010) [20]. In addition, none of the olive oil samples exceeded the maximum peroxide value specified (20 meq O_2 kg^{-1}) for EVOO in the international standard [21]. There were statistically differences between peroxide values during ripening of fruits ($p<0.05$).

Results showed that the acidity values of none of the obtained EVOOs from three studied cultivars exceeded the specified value of 0.8 (%m/m expressed in oleic acid) for EVOO in international standard COI/T.15/NC No 3/Rev. 17 [21].

Olive fruits at a later stage of maturity yield EVOOs with higher amounts of free acidity because they undergo an increase in enzymatic activity, particularly by lipolytic enzymes. Differences between acidity values of various EVVOs might be described by differences in olive fruit maturity. Therefore, a high level of acidity value can be related to the advanced state of fruit ripeness or the action of lipase enzyme on the olive oil triglycerides leads to increase of free fatty acids content [22]. Boussahel et al. (2020) analyzed the peroxide value and acidity value in five olive oil varieties from northeast Algeria which obtained 12.75 to 15.50 meq O_2/kg oil and 0.48±0.03% 1.25±0.11%, respectively [23].

There is no specified limitation for oxidative stability in the international standard COI/T.15/NC No 3/Rev. 17 [21], but results showed that EVOOs had the suitable oxidative stability. The highest oxidative stability was observed in the Koroneiki variety EVOOs and was higher than many vegetable oils. It might be due to the highest amounts of polyphenol compounds in the EVOOs of Koroneiki variety. Also, higher iodine value shows a higher degree of unsaturation in fat or vegetable oil, which also indicates oil´s stability to oxidation [24]. In a study, the iodine value of Manzanilla oil was obtained from 74.32±1.4 to 90.04±1.33 mgI_2/g oil [18]. The saponification value depends on several factors, including pressing conditions, cultivar, climate, altitude, and geographical variations [18].

The maximum values of oleic acid belonged to the last harvest date (November) in all varieties which were reported by other research [25,26]. The FA composition of EVOO is affected by various parameters which depend

mainly on the olive variety as well as climate, the olive-growing area, and the ripening stage, at which the fruit olives are harvested [27]. The main FAs were oleic, palmitic, and linoleic acids, respectively [28]. The high oleic acid content of EVOO has found properties like an increase in oxidative stability, decrease in low-density lipoprotein (LDL), and antihypertensive activity [27].

Sterols are the main constituents of unsaponifiable matter of EVOOs, which their determination is necessary for detecting EVOO adulterations and checking authenticity. The sterol composition determination can be applied for classification of virgin olive oils based on their fruit variety. The total sterol content of EVOO affected by crop year, geographic factors, fruit ripeness, variety, fruit storage time before oil extraction. Yorulmaz et al. (2013) reported that the total sterol content of Memecik VOO was decreased from 1747.47 to 1479.28 mg/Kg during ripening stage and β-sitosterol had a same trend with total sterols [6].

The triterpene alcohols like erythrodiol and uvaol are part of unsaponifiable matter of olive oil. These triterpenes are usually determined together with the sterol fraction and mostly existed in the exocarp of olive fruit [29]. The EU regulatory limit for the sum of erythrodiol and uvaol content was 4.5% for the category of EVOO, which for all mentioned EVOOs, erythrodiol + uvaol were lower than 4.5% [21].

Oleuropein content of olive fruits is attributed to the pathway of β-glucosidase activity transforming oleuropein to aglycones Which may be affected by the olive fruit cultivar [30].

Yorulmaz et al., (2013) reported that differences between phenolic composition of varieties during the maturity stage indicating various phenol metabolism for distinct cultivars and/or geographical origination.

Yorulmaz et al. (2013) reported that the OOO was the main triglyceride, between 36.58% - 39.06% for Memecik and 37.06% - 38.12% for Edremit [6]. In addition, the amount of OOO value was decreased as the ripening proceeded for Memecik and Edremit. Also, it was reported by Baccouri et al. (2008) for Tunisian VOOs during ripening. The OOP decreased while OOL and PLO increased with maturation proceeded for Memecik and Edremit varieties [6] which was the same of our results for three varieties.

Pardo et al., (2020) reported that the highest stability was found in virgin olive oils containing the highest polyphenol content, therefore, the oxidative stability of virgin olive oils is directly depending on the total polyphenol content [32]. Kafkaletou et al. (2021) reported that phenolic compounds were decreased during olive fruit development on the tree [5]. Also, tyrosol is present at lower levels than hydroxytyrosol [5]. During the ol32ive maturation process, qualitative and quantitative modifications in phenolic content happen and these changes are attributed to a series of enzymatic and chemical alterations of some phenolic compounds [5]. In addition, environmental factors, and cultivation practices could influence the phenolic compounds in olives and olive products [33].

The high content of total aliphatic alcohols in olive oils is attributed to the presence of the free form of aliphatic alcohols rather than as waxes, which is related to adverse climatic conditions [34].

Conclusions

The present study provides information about the maturation process of three cultivars including Arbequina, Koroneiki, Manzanilla cultivated in Rudbar county, Gilan province, Iran, how their extracted extra virgin olive oils physicochemical properties and polyphenol contents are influenced by the maturation advances of the olives.

Results showed that progress in maturity increased oil content, acidity value and iodine value, while peroxide value, oxidative stability, saponification value, total aliphatic alcohols and unsaponifiable matter were decreased. In addition, PUFA was increased and MUFA/PUFA ratio decreased during the olive maturation advances in all EVOOs extracted from three examined varieties. Total sterol content and total aliphatic alcohols content were decreased with maturity stages proceeded. Also, as the ripening stages, triolein (OOO), palmitodiolein (POO), dioleolinolein (OOL) and palmitooleolinolein (PLO) were the predominant triglycerids in all studied EVOOs. Oleuropein aglycone, oxidized aldehyde and hydroxylic form, hydroxytyrosol and tyrosol were increased but oleuropein, apigenin, quercetin,

Ligstroside aglycone, aldehyde and hydroxylic form, ferulic acid, caffeic acid, catechin decreased with different ripening stages for all EVOOs. This research evidenced the maturation advances influence significantly the chemical composition and polyphenol contents of EVOOs.

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Statements & Declarations

Conflict of Interest: There are no conflicts of interest with respect to the research described in this manuscript

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Data availability

The current study is available from the corresponding author on reasonable request.

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