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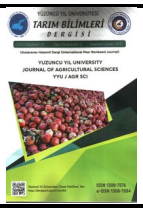

Research Article (Araştırma Makalesi)

Characterization of Iranian Olive Oils based on Biophenolic Minor Polar Compounds and their Contribution to Organoleptic Properties

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Abstract: The presence of several minor compounds, such as biophenols, are associated with the quality, health benefits and sensory characteristics of olive oil. The objectives of this study were to compare the profile of the individual phenolic compounds of major brands of olive oils produced in Iran and to correlate the minor polar biophenolic compounds with sensorial properties and finally discriminate the samples. In order to define similarities and differences between Iranian virgin olive oils, profiles of their biophenolic compounds have been investigated using HPLC, analysis of variances and principal component analysis (PCA). Samples of olive oil were notably varied in terms of individual biophenolic compounds and total phenolic content (TPC). Hydroxytyrosol, tyrosol, oleuropein, luteolin, apigenin, and ligstroside aglycone (aldehyde and hydroxylic form) were detected in all samples, whereas caffeic acid was not found in any brands. Based on the differentiating made by PCA, samples were categorized into two distinct groups (TPC<300 and TPC>300 mg tyrosol/kg of olive oil). The analysis of the main components resulted in a model that describes 86% of the total variance discriminating them from the minor biophenolic compounds of the examined olive oils. This analysis can be considered for assessing the quality and commercial needs related to preferences on olive oil.

İran Zeytinyağlarının Biyofenolik Minör Polar Bileşiklerine Göre Karakterizasyonu ve Organoleptik Özelliklere Katkıları

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Anahtar kelimeler
Kemometrik analiz,
Zeytin yağı,
Fenolik bileşikler,

Öz: Biyofenoller gibi birkaç küçük bileşiğin varlığı, zeytinyağının kalitesi, sağlık üzerine yararları ve duyusal özellikleriyle ilişkilidir. Bu çalışmanın amacı, İran'daki başlıca zeytinyağı markalarının bireysel fenolik bileşiklerinin profilini karşılaştırmak ve küçük polar biyofenolik bileşikleri duyusal özelliklerle ilişkilendirmek ve son olarak örnekleri ayırt etmekti. İran sızma zeytinyağları arasındaki benzerlik ve farklılıkları tanımlamak için, bunların biyofenolik bileşiklerinin profilleri HPLC, varyans analizi ve temel bileşen analizi (PCA) kullanılarak incelenmiştir. Zeytinyağı numuneleri, ayrı ayrı biyofenolik bileşikler ve toplam fenolik içerik (TPC) açısından önemli ölçüde farklıydı. Tüm örneklerde hidroksitirosol, tirosol, oleuropein, luteolin, apigenin ve ligstrosit aglycone (aldehit

Ana Bileşen Analizi (PCA),
Kalite,
Duyusal analiz.

ve hidroksilik form) tespit edilirken, hiçbir markada kafeik asit bulunmadı. PCA ile yapılan farklılaştırmaya dayalı olarak, numuneler iki farklı gruba ayrıldı (TPC <300 ve TPC> 300 mg tirozol / kg zeytinyağı). Ana bileşenlerin analizi, onları incelenen zeytinyağlarının küçük biyofenolik bileşiklerinden ayıran toplam varyansın % 86'sını tanımlayan bir modelle sonuçlandı. Bu analiz, zeytinyağı tercihleriyle ilgili kalite ve ticari ihtiyaçları değerlendirmek için düşünülebilir.

1.Introduction

One of the healthiest components of the Mediterranean diet is olive oil. Olive oil consumption is increasing all around the world subsequent to the growing the Mediterranean diet. Consequently, consumers should be aware of the quality aspects of the oils to have the best choice. The health benefits of olive oil not only relate to the high monounsaturated fat content, but also depends on several minor compounds such as bio phenols. Olive oil consists of 98% fatty acids, mainly oleic acid (C18:1), and 2% other minor components, including squalene, pigments, tocopherols, waxes, and the polar fraction. Phenolic compounds are located in this polar fraction which is a complex mixture of phenolic acids, simple phenols and their derivatives, lignans, and flavones (Tresserra-Rimbau and Lamuela-Raventos, 2017). Phenolic compounds of olive oils have a crucial rule, as these compounds have the health profits, effect on the organoleptic attributes such as bitterness, astringency and oxidative stability of oils (Pandey and Rizvi, 2009; García-González et al., 2010; Squeo et al., 2019, Rodríguez-López et al., 2020). Furthermore, phenolic fraction of olive and olive oil act as antioxidant and radical scavenger with anti-carcinogenic, anti-inflammatory, anti-atherogenic, and anti-microbial activities properties due to the health-promoting attributes (Lerma-García et al., 2009; Servili et al., 2014; Tresserra-Rimbau and Lamuela-Raventos, 2017).

Several statistical methods were used to analyze the data of the quality attributes of olive oil. Among them, principal component analysis (PCA) is a powerful pattern recognition technique which has been used for analyzing and classifying different products (García-González et al., 2010; Shavakhi et al., 2011). Consequently, it has been successfully applied in the following areas: for data analysis of olive oil such as discriminating between olive oil produced in three Italian geographical areas based on quality parameters including phenols (Ranalli et al., 2000), for interpreting the behavior of the virgin olive oil profile of phenols and volatiles regarding olive cultivar and ripeness (García-González et al., 2010), classification of Iranian olive oils based on the fatty acids (Piravi-Vanak et al., 2012; Shavakhi et al., 2020), unsupervised grouping of individual phenolic contents of olive oil samples by crop year (Rodrigues et al., 2019), extending multivariate models to discriminate the virgin olive oils with low and high total phenolic content (Squeo et al., 2019), and discrimination of olive oils according to phenolic compounds and antioxidant potencies (Amanpour et al., 2019).

It also would be worthwhile to categorize the olive oil brands, to gain consumer insights on the importance of quality attributes, and variables which affect these properties. Thus, it is necessary to classify the different brands of olive oil based on their variables. To our knowledge, there is no data on the classification of different brands of Iranian olive oils based on biophenolic compounds. Therefore, the objectives of this study were to compare the individual phenolic compound of olive oils produced in Iran and to discriminate of different oils with various geographical origins, to correlate of these compounds to organoleptic properties, and also discrimination of samples based on the biophenolic minor polar compounds using PCA.

2. Material and Methods

Eleven Iranian virgin olive oil (VOO) samples were collected from November 2017 to March 2018 from six major producing provinces of olive oil. Characteristics of climate regions for sampling the olive oils are shown in Table 1. In terms of weather condition, Golestan, Qazvin, Gilan and Zanjan provinces have the Mediterranean climate, but Fars and Qom provinces have cold semi-arid and dry desert climate respectively. These provinces and samples were Golestan (Go_1, Go_2, and Go_3), Qazvin (Qa_1, Qa_2, and Qa_3), Zanjan (Z_1, Z_2), Gilan (G), Fars (F), and Qom (Q). Cluster random sampling was used and more samples were collected from the provinces with more factories. These oils were either

purchased or donated by the manufacturers. The suppliers guaranteed the geographical origin and quality grade of all the samples. Samples were kept in dark glass bottles at room temperature (22±2°C) prior to conducting the experiments. Tyrosol and syringic acid were purchased from (Sigma-Aldrich, Boston, USA). All reagents were analytical grade and were purchased from Merck (Berlin, Germany) and Sigma-Aldrich (Boston, USA).

Table 1. Characteristics of climate regions for sampling of the olive oil in Iran

Province	Minimum Temperature (°C)	Maximum Temperature (°C)	Longitude	Latitude	Elevation (m)
Golestan	12.8	22.7	55.38	37.23	155
Qazvin	6.9	21.2	49.99	36.31	1297
Zanjan	4	18	48.96	36..92	1638
Gilan	11.3	20.6	50.01	37.20	36.7
Fars	9.8	25.6	52.53	29.61	1519
Qom	10.2	25.9	50.88	34.65	932

2.1. Determination of acidity, peroxide and extinction coefficient

To confirm the quality grade of the samples, these analyses were conducted. All measures were determined using IOC methods (IOC, 2018b). Briefly, the titration method using potassium hydroxide solution was employed for free fatty acid (% oleic acid) quantification; the titration method using thiosulfate 0.01 N was used for peroxide value (meq O_2 kg^{-1}); a spectrophotometric method using cyclohexane as reagent was employed for extinction coefficient determination at 232 and 270 nm (k_{232}, k_{270}).

2.2. HPLC analysis

The official method of the International Olive Council (IOC, 2017) was used for detection and quantification of biophenolic compounds of olive oil. Extraction of the biophenolic compounds was performed using 2.0 g of olive oil that was weighed in a 10 mL test tube, and of 1 mL of internal standard (syringic acid, 0.015 mg/mL) was added to the olive oil. Then mixture was sealed with screw cap and shaken for 30 sec. Afterward, 5 mL of the methanol/water 80/20 (V/V) was added and was shaken for 1 min. The mixture was placed in the ultrasonic bath for 15 min at room temperature; subsequently it was centrifuged for 25 min (5000 rpm). An aliquot of the supernatant was filtered with a 0.45 mm PVDF filter, and subsequently injected into HPLC.

The analysis was conducted on a HPLC (Young-Lin, Acme 9000, South Korea) with Spherisorb ODS-2 column (4.6 x 250 mm, dp= 5 µ m) and UV-Vis detector at 280 nm. The chromatography column conditioned for at least 15 min with gradient elution of water 0.2 % H3PO4 (V/V), methanol and acetonitrile 96/2/2 (v/v/v) and 20 µL of the external standard solution (tyrosol 0.03 mg/mL and syringic acid 0.015 mg/mL) was injected into the HPLC system and the chromatogram was recorded at 280 nm. The value of response factor (RF) for 1µg of tyrosol, 1µg of syringic acid and the ratios between two response factors (RRF) were calculated as follows (1-3):

$$\text{RF1µg (syringic acid)} = \text{Area of syringic acid/ µg of syringic acid injected} \quad (1)$$

$$\text{RF1µg (tyrosol)} = \text{Area of tyrosol/ µg of tyrosol injected} \quad (2)$$

$$\text{RRFsyr/tyr} = \text{RF1µg (syringic acid)/ RF1µg (tyrosol)} \quad (3)$$

The injection volume was 20 µL and chromatograms were recorded at 280 nm. All analyses were carried out at room temperature. The amounts of phenolic compounds were calculated according to the following equation (4):

$$\left(\frac{mg}{kg}\right) = \frac{(\text{A}) \times 1000 \times \text{RRF}\frac{\text{syr}}{\text{tyr}} \times (\text{W syr. acid})}{(\text{A syr. acid}) \times (\text{W})} \quad (4)$$

In the equation, (A) is the peak areas of the biophenols (hydroxytyrosol, tyrosol, natural and oxidised oleuropein and ligstroside derivatives, lignans, flavonoids and phenolic acids) recorded at 280 nm; (A syr. Acid) is the area of the syringic acid internal standard; 1000 is the factor used to express the result in mg/kg; (W) is the weight of the oil used, in grams; (RRFsyr/tyr) is the multiplication coefficient for expressing the final results as tyrosol; (W syr. Acid) is the weight of the syringic acid used as internal standard in 1 ml of solution added to the sample in mg. The sum of the areas of the individual peaks (∑A) was used to calculate the total content of all individual compounds quantified.

2.3. Sensory analysis

Sensory evaluation was performed by a group of eight trained tasters and coordinated by a panel leader. Scores of positive sensory attributes (fruity, bitter, and pungent) and negative sensory attributes were given to the olive oil samples based on IOC method (IOC, 2018a).

2.4. Statistical analysis

Phenolic compounds data were analyzed using the analysis of variances (ANOVA) and principal component analysis (PCA). Data of individual phenols were subjected to ANOVA to evaluate the significant differences between the brands. The differences among the means were determined using Duncan's multiple range tests with 95% confidence interval. The correlations between variables were evaluated using Pearson's coefficients. PCA was used for interpreting the behaviour of the profiles of VOO phenols with respect to oil samples. Minitab v. 16 statistical package (Minitab Inc., Pennsylvania, USA) was used for carrying out the statistical analyses. The experiments were carried out in triplicate**.**

3. Results and Discussion

According to the international standard of IOC (2018b), acidity of all samples was within the range of extra virgin olive oil (less than 0.8%) except for Fars and Golestan (F, Go_3) as ordinary and virgin olive oil with the acidity of 2.59±0.3 and 1.75±0.3 respectively. Also, peroxide values and delta extinction coefficients (Δk) of all samples were in accordance with the IOC standard (less than 20 meqO2/kg and ≤0.01 of oil respectively). According to the results of sensory analysis (Table 2), all examined olive oils were in extra VOO (EVOO) category with no defect and fruity score of more than zero, except for Q and F samples which showed fusty and musty defects, respectively. Q sample with fusty defect median of 0.6, and median of fruity above zero and F sample with musty defect median of 1.1 categorized as virgin olive oil (VOO). Fusty and musty are defects of olive oil and define as characteristic flavor of olive oil as a result of anaerobic fermentation and also long storage in humid condition or earth or mud with high amounts of fungi and yeasts, respectively (IOC, 2018a).

Table 2. Results of panel test of Iranian olive oil samples*

Sample Code	Fruity	Pungent	Bitter	Defect	Defect Specification
Q	2.2	2	1.7	0.6	Fusty
G	2.6	0.7	0.5	0	-
Go_1	2	1	1.8	0	-
Go_2	3.5	1.5	0	0	-
Go_3	1	1.5	1.4	0	-
Z_1	1.9	1.4	1	0	-
Z_2	2.65	2.9	2.5	0	-
Qa_1	1.5	1	0.8	0	-
Qa_2	2.1	1.7	0.25	0	-
Qa_3	3.3	1.7	1.25	0	-
F	0	0.7	0.1	1.1	Musty

*Data expressed as median value.

Table 3-Phenolic compounds of different samples of Iranian Olive oil-mean (sd) (mg/kg)

Sample code / Phenolic compound	Go_1	Go_2	Go_3	Qa_1	Qa_2	Qa_3	Z_1	Z_2	G	Q	F
Hydroxytyrosol	11.82(1.51) de	9.43(0.81) c	9.86(1.61) cd	14.77 (0.99) f	1.72(0.51) a	12.70(1.2) ef	17.61(1.82) g	12.83(1.91) ef	3.48(1.55) ab	15.01(1.01) f	4.41(0.6) b
Tyrosol	19.05(1.54) e	17.92(1.1) e	9.82(1.55) bc	11.19 (0.93) cd	2.05(0.16) a	7.82(1.54) b	13.34 (1.60) d	7.73(1.14) b	2.11 (0.95) a	12.92(0.96) d	3.09(0.96) a
Vanilic acid	9.75(0.87) e	3.45(0.47) c	ND	ND	7.41(0.68) d	ND	ND	ND	0.5(0.06) a	1.74 (0.52) b	ND
Caffeic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Vanillin	1.37 (0.27) b	ND	6.54(0.49) d	ND	5.53(0.5) c	ND	9.41(0.67) f	ND	ND	7.437(0.70) e	ND
Para-Cumaric acid	2.37(0.14) b	ND	ND	4.55(0.08) c	ND	ND	ND	14.56 (0.53) e	2.29(0.24) b	ND	8.53(0.06) d
Hydroxytyrosol acetate	4.16(0.73) d	ND	ND	ND	6.26 (0.29) e	7.32 (0.23) f	5.81 (1.09) e	11.21(0.78) g	1.61(0.16) b	2.91(0.01) c	ND
Ferrulic acid	1.52(0.04) b	2.67(0.67) b	5.29(0.06) c	ND	13.38(0.73) f	ND	10.53(0.63) e	16.41(0.94) g	ND	8.04(0.66) d	ND
Ortho-Cumaric acid	2.71 (0.63) b	5.78 (0.15) c	11.93(0.61) e	6.41(0.54) c	ND	2.19 (0.87) b	ND	17.47(0.70) f	10.96(0.87) e	ND	9.55(1.07) d
Decarboxymethyl Oleuropein aglycone [1]	6.99 (0.63) b	ND	6.67(0.18) b	25.59(1.79) d	ND	13.87(1.06) c	30.41(2.17) e	50.21(3.18) f	ND	28.73(0.88) e	7.39(0.61) b
Oleuropein	2.42(0.64) a	27.56 (1.06) f	1.71(0.43) a	6.65(1.14) cd	6.22(0.06) cd	5.06 (0.35) bc	4.55(0.49) b	2.50(0.04) a	33.08(1.04) g	6.15(0.14) cd	9.64(1.07) e
Oleuropein aglycone [1]	ND	5.35(0.07) c	ND	ND	2.8(0.05) b	ND	ND	14.51(0.16) d	5.64 (0.76) c	2.08 (0.36) b	5.39(0.05) c
Tyrosol acetate	ND	5.82(0.81) c	ND	8.59(0.86) d	ND	ND	5.16 (0.79) c	8.19(0.67) d	4.88 (0.51) c	ND	2.67(0.52) b
Decarboxymethyl ligstroside aglycone [2]	0.863(0.53) a	ND	ND	2.99(0.29) b	39.44(2.48) b	32.38(0.63) b	ND	95.26(1.65) c	ND	1.10(0.49) a	0.86(0.08) d
Decarboxymethyl ligstroside aglycone [1]	0.86(0.41) a	ND	ND	2.99(0.29) b	39.44(0.36) d	32.38(1.01) c	ND	95.26(1.1) e	ND	1.10(0.48) a	0.86(0.26) a
Pinoresinol [1] acetoxy pinoresinol	7.68(0.21) c	ND	23.36(1.05) d	33.39 (1.19) e	ND	ND	24.30(1.15) d	ND	ND	62.09(1.04) f	4.25(0.43) b
Cinnamic acid	3.26(0.43) b	17.27(0.05) f	9.14 (0.79) c	ND	ND	26.44(0.9) g	ND	ND	12.26(0.30) e	2.40(0.61) b	11.09(0.92) d
Ligsteroid aglycone [1]	ND	78.43(3.76) e	ND	ND	8.64(0.59) b	1.05(0.04) a	19.82(1.12) c	ND	61.54(0.58) d	ND	9.06 (0.16) b
Luteolin	4.8 (0.95) b	13.00(0.40) c	1.35(0.24) a	4.7(0.11) b	15.14(0.88) d	4.7 (0.81) b	4.64 (0.08) b	27.98(1.86) f	24.92(1.71) e	13.07(0.48) c	11.61(1.59) c
Oleuropein aglycone [3]	4.64(0.08) cd	2.56(0.66) b	2.18(0.92) b	ND	22.57(1.15) g	4.21(0.37) c	29.82(0.54) h	6.50(0.54) e	4.87 (0.67) cd	17.20 (0.10) f	5.55 (0.49) de
Apigenin	2.42(0.14) ab	6.28 (1.01) cd	5.96 (0.07) cd	24.60(4.50) f	2.16 (0.47) ab	3.95 (0.56) bc	28.83 (1.70) g	4.89(0.52) bcd	7.50 (1.03) d	0.1(0.02) a	11.13(0.81) e
Ligstroside aglycone [3]	4.41(0.63) d	20.35(1.09) f	2.87(0.19) bc	2.24(0.79) ab	7.08 (0.10) e	3.91 (0.22) cd	1.27(0.20) a	27.82(1.47) g	3.89(0.38) cd	3.77(0.51) cd	4.4(0.64) d
Total phenol	135.03(5.49) a	276.04(14.70) e	140.25(3.52) a	186.35(8.25) b	207.29(10.40) c	139.71(8.50) a	248.87(10.9) d	354.53(11.12) f	221.85(9.94) c	239.94(8.49) d	144.98(6.04) a

1=(Dialdehyde form), 2=(Oxidized dialdehyde form), 3=(Aldehyde and hydroxylic form).

Table 4- Pearson coefficients of different phenolic compounds of olive oil

	Hydroxytyrosol	Tyrosol	Vanilic_acid	Vanillin	p_cumaric_acid	Hydroxytyrosol_acetate	Ferrulic_acid	o_cumaric_acid	d_m_oleuropein_a	Oleuropein	OAD_form	Tyrosol_acetate	D_ligstAo	DLAD_form	Ligsteroid_AgDform	Luteolin	OAAHform	Ligstroside_AAHform	Total_phenol
Hydroxytyrosol	-																		
Tyrosol	.647**	-																	
Vanilic_acid	-0/259	0/311	-																
Vanillin	0/279	0/129	0/034	-															
p_cumaric_acid	-0/026	0/264	0/264	.492**	-														
Hydroxytyrosol_acetate	0/212	0/125	0/086	0/063	.347*	-													
Ferrulic_acid	0/124	0/103	0/08	.497**	0/258	.676**	-												
o_cumaric_acid	-0/199	0/295	.433*	.476**	.727**	0/005	0/046	-											
d_m_oleuropein_a	.691**	0/128	.419*	0/141	.558*	.571*	.543*	0/216	-										
Oleuropein	.451**	0/135	0/074	-.392*	-0/159	-.414*	-.389*	0/155	.493**	-									
OAD_form	-0/211	0/297	0/196	-.412*	.808**	.413*	.449**	.698**	.399*	0/204	-								
Tyrosol_acetate	0/234	0/045	.423*	-.380*	.542**	0/025	0/092	.471**	.464**	0/296	.499**	-							
D_ligstAo	-0/007	0/302	0/056	-0/224	.639**	.834**	.661**	.407*	.549**	-0/308	.712**	0/242	-						
DLAD_form	-0/007	0/303	0/055	-0/224	.640**	.833**	.662**	.407*	.549**	-0/307	.713**	0/243	1.000**	-					
Ligsteroid_AgDform	-0/327	0/071	0/005	-0/252	-0/236	-.347*	-0/246	0/08	.461**	.931**	0/183	0/323	-0/293	-0/293	-				
Luteolin	-.387*	.440*	0/077	-0/329	.552**	.361*	.392*	.488**	0/206	.454**	.860**	.392*	.564**	.564**	.349*	-			
OAAHform	0/128	0/075	0/136	.779**	-0/286	.380*	.620**	.579**	0/209	-0/235	-0/146	0/035	0/035	0/035	0/107	0/006	-		
Ligstroside_AAHform	-0/025	0/097	0/014	-.388*	.577**	.420*	.468**	.518**	0/34	0.151	.837**	.471**	.697**	.698**	0/27	.628**	0/199	-	
Total_phenol	0/191	0/065	0/182	0/02	.406*	.439*	.655**	0.29	.568**	0.22	.723**	.618**	.549**	.549**	0/322	.697**	0/26	.758**	-

** Correlation is significant at the 0.01, * Correlation is significant at the 0.05 level.

Table 3 shows the phenolic pattern of the analyzed Iranian olive oils including 22 phenolic compounds. Secoiridoids were major phenolic compounds in olive oils which include mainly oleuropein, aglycon derivatives of oleuropein, demethyloleuropein, ligstroside. Hydroxytyrosol, tyrosol, oleuropein, luteolin, apigenin, and ligstroside aglycone (aldehyde and hydroxylic form) were detected in all samples, whereas caffeic acid was not found in any brands. Similar phenolic pattern was observed in Spanish olive oils (Franco et al., 2014). Consequently, caffeic acid in Turkish olive oil was not found or indicated in trace amount (Ocakoglu et al., 2009). According to ANOVA results, different content of oleuropein aglycone (aldehyde and hydroxylic form) were the main difference in phenolic fraction among selected brands (Table 3).

From the Table 3, it was found that high concentration of decarboxymethyl ligstroside aglycone (oxidized dialdehyde form and dialdehyde form), decarboxymethyl oleuropein aglycone (Dialdehyde form), *ortho*-Cumaric acid, ferrulic acid and *para*-cumaric acid were notable in the Z_2 sample. As pungent olive oils consist of higher amounts of deacetoxy ligsteroside aglycone, it could be concluded that this sample has strong pungency and throat irritation comparing to the other samples and results of sensory evaluation (Table 2) confirmed this finding. These results are similar to previous studies (Andrewes et al., 2003, Beauchamp et al., 2005, Boskou et al., 2005)

Hydroxytyrosol of Z_1 sample, was significantly ($p<0.05$) higher than the other brands (Table 3). Hydroxytyrosol is a simple phenol with significant antioxidant activity (Boskou et al., 2005). Holding of olives before oil extraction and consequent degradation of cell structure and mold growth may result in considerable loss of antioxidant. In order to have extra virgin olive oil with median defects of zero, it is necessary to extract the oil with no delay after harvesting. Table 2 shows that Z_1 sample had no defects and classified as EVOO.

Phenolic alcohols of olive oils which consist of tyrosol and hydroxytyrosol varied between 4-31 mg/kg (Table 3). The lowest amounts of the phenolic alcohol were observed in Qa_2, G, and F samples (3.77, 5.59, and 7.5 mg/kg respectively) with no significant differences (Table 3). Phenol alcohols of Turkish olive oil were reported between 4.86-14.56 mg/kg (Alkan et al., 2012). These compounds are related to the freshness of the olive oils which was mentioned before (Reboredo-Rodríguez et al., 2018). Concentration of hydroxytyrosol was often higher than tyrosol (Boskou et al., 2005), which was in accordance with the results obtained in this study. Although there were some exceptions in our study (Go_1, Go_2, and Qa_2) and also in the study conducted before, which reported that amounts of tyrosol was higher than hydroxytyrosol (Ocakoglu et al., 2009).

The main phenolic acids observed in this study were vanillic acid, *o*-cumaric acid, *p*-cumaric acid, and ferrulic acid (Table 3). In Turkish olive oils, vanillic acid, syringic acid and *p*-cumaric acid were identified (Ocakoglu et al., 2009). The oleuropein content of the samples varies between 1.71 and 33.08 mg/kg for Go_3 and G samples, respectively. During maturation of olive, the oleorupein continuously was reduced and replaced by its derivatives such as de methyl oleuropein. Therefore, the maximum content of oleuropein in G sample may indicated that other sample of olive oils with lower oleuropein contents have been made from more ripe olives. The main compounds which contributed to the bitterness of olive oil are oleuropein aglycone and other secoiridoid derivatives of hydroxytyrosol (García et al., 2001, Vitaglione et al., 2015). It was, therefore; expected that Go_2 and Z_2 samples have minimum and maximum bitterness respectively, which was confirmed by the results of sensory evaluation (Table 2).

Flavonoids levels represented by luteolin and apigenin varied between 1.35-27.98 mg/kg and 0.1-28.83 mg/kg, respectively. This finding consistent with the work done before, which reported flavonoids range of 0.05-30 mg/kg (Franco et al., 2014). These flavonoid compounds were determined in most of the Spanish, Italian, Portuguese and Turkish olive oils (Vinha et al., 2005, Bendini et al., 2007, Ocakoglu et al., 2009, Franco et al., 2014).

As can be seen in Table 3, the concentration of total phenolic compounds of the samples varies from 135.03 mg tyrosol/ kg for Go_1 to 354.53 mg tyrosol/ kg for Z_2 sample. These were within the range for extra virgin olive oil (100-800 mg kg^{-1}), which was indicated before (Tresserra-Rimbau and Lamuela-Raventos, 2017). The wide range of total phenol content was reported between 50 and 1000 mg/kg and usually described in the range of 100-300 mg/kg (Boskou et al., 2006). In general terms, TPC amounts of various samples showed significant differences ($p<0.05$). There are a good and significant correlations between TPC and ligstroside aglycone-aldehyde and hydroxylic form ($r=0.758$), luteolin ($r=0.697$), oleuropein aglycone-dialdehyde form ($r=0.723$) (Table 4). Also, there is a good correlation

between TPC and stability of the olive oil, as total phenol content increased, the shelf life of the oil was also increased (Boskou et al., 2005). Although, when TPC exceeds 300 mg/kg, the oil may have a bitter taste, which Iranian consumers would not prefer despite its high nutritional values. Therefore, samples with TPC less than 300 mg/kg could be recommended for olive oil consumers in Iran.

According to the European Food Safety Authority (EFSA), not all the phenolic compounds are beneficial for health, and olive oils containing at least 5 mg of hydroxytyrosol and its derivatives such as oleuropein complex and tyrosol, per 20 g of olive oil are allowed to be labeled for protection of blood lipids from oxidative stress (EFSA Panel on Dietetic Products and Allergies, 2012). All olive oil samples in this study met this criterion, due to the amount of hydroxytyrosol and its derivatives (Table 3).

Due to the fact that the phenolic compounds could be deemed as fingerprint, they could be used to describe and categorize the product (Alkan et al., 2012). In order to obtain the overall schematic pattern of sample classification regarding phenolic compounds, the information carried by the original variables was projected onto a smaller number of underlying variables, called principal components and their values as scores outlined in Table 5. The first principal component (PC1) covers as much of the variation in the data as possible (63.2%). The second principal component (PC2) is orthogonal to the first and covers as much of the remaining variation as possible (22.8%). The eigenvalues of correlation matrix which are equal to the variances of the principal component, when they are greater than one, were used. Therefore, the first two principal components which explained 86% of the data variability or total variance was an adequate amount of variation explained the data. Consequently, the other proportions which explain smaller amount of the variability in data, were not important to include (Table 5).

Table 5- Eigen value and cumulative percent of variances for the two main principal components

No	Phenolic compound	Principle 1	Principle 2
1	Hydroxytyrosol	0.023	0.722
2	Decarboxymethyl Oleuropein-aglycone-dialdehyde form	0.272	0.541
3	Oleuropein-aglycone-dialdehyde form	0.403	-0.180
4	Decarboxymethyl ligstroside aglycone-oxidised dialdehyde form	0.401	0.059
5	Decarboxymethyl ligstroside aglycone- dialdehyde form	0.396	0.053
6	Luteolin	0.338	-0.380
7	Total phenol	0.379	0.024
8	Tyrosols	0.433	-0.038
	Cumulative Percent	63.2	86
	Eigen value	0.632	0.228

In the first component, “Oleuropein-aglycone-dialdehyde form”, “Decarboxymethyl ligstroside aglycone-oxidised dialdehyde form”, and Tyrosols with coefficients of 0.403, 0.401, and 0.433 are the more important corresponding variable in calculation the component and showed the most important and positive effect on the group separation. By increasing these compounds, the first component also increased. Consequently, Hydroxytyrosol and “Decarboxymethyl Oleuropein-aglycone-dialdehyde form” showed the highest positive effect on the second component with coefficients of 0.722, 0.541 respectively, which played a significant role in discrimination of the olive oils. The reason of high discriminating power of these compounds is the lack or trace amounts of these phenols in olive oil samples. This finding was in accordance to the results obtained for vanillin and syringic acid due to the different harvest years of Turkish olive oils (Ocakoglu et al., 2009).

Score plot which shows the scores of the second principal component versus the scores of the first principal components (Figure. 1) clearly displays the difference between samples. As shown in the Figure 1, Z_2 sample with TPC>300 mg tyrosol/kg, placed in one group, alone (Group1), which has the highest value of the first component. The other samples with TPC<300 mg tyrosol/kg, also placed in the other group (Group 2). Z_2 sample belongs to the Mediterranean climate with average temperature and elevation of 11ºC and 1638 meter, which is coldest and highest one in the studied climate regions (Table 1).

As shown in Figure 2, the loading plot associated with each component allows the most important variable to be selected. The loading plot of eight variables of olive oil samples shows that the most variables were placed in the positive and direct of the first component. The remaining eight variables were resulted after removing variables based on ANOVA, Pearson’s coefficients and small

coefficients of principal components. These variables which strongly influenced the components, were important in the group differentiation of the olive oil samples. Consequently, this variable showed the most significant correlation coefficients with the other variables (Table 4).

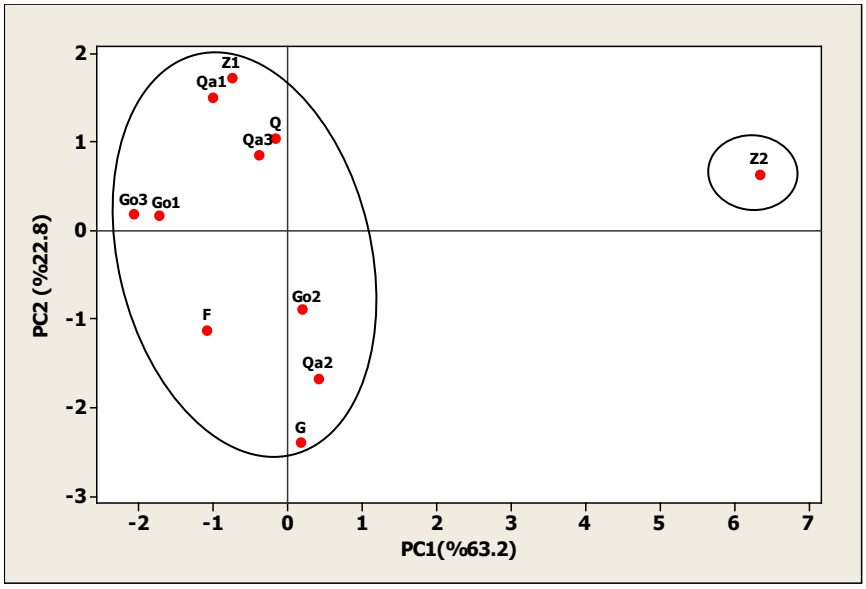

Figure 1. Score plot of Iranian olive oil samples scattered in principle components area based on the phenolic compounds.

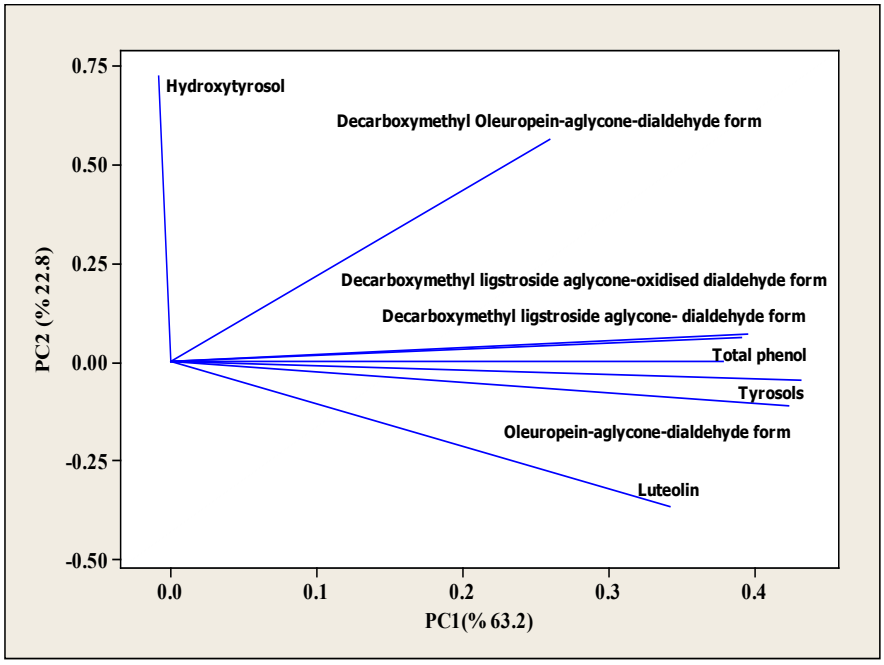

Figure 2. Loading Plot of the first two principal components PC1 and PC2.

According to the classification established by TPC, it can be concluded that Z_2 sample were within the category of high content phenolic compound (350<TPC<550 mg tyrosol/kg), samples with 200<TPC<300 mg tyrosol/kg including Q, G, Go_2, Qa_2, and Z_1 were within the intermediate content,

and samples with TPC<200 mg tyrosol/kg including Go_1, Go_3, F, Qa_1, and Qa_3 were within the low content phenolic compounds (Franco et al., 2014). VOO rich in phenolic content has more stability during cooking (Olivero-David et al., 2014). According to this concept, it could be considered that group with TPC less than 200mg/kg as the less stable olive oils and Z_2 sample with the highest TPC, as the most stable one PCA was not able to completely separate these samples based on TPC, but in some cases such as Go_1 and Go_3 or Qa_1 and Qa_3 , they were placed near as can be seen in score plot which belongs to the same climate conditions. In the other study conducted before (Shavakhi et al., 2020), fatty acid profiles of the Iranian olive oils using the same samples revealed that samples of Go_1, Z_1, and Qa_1 were placed in high quality group of olive oil. Since the quality of olive oil was affected by various variables such as cultivar, geographical origin, irrigation, climatic conditions, processes condition, degree of maturity, harvesting methods, freshness of fruit before oil extraction, extraction method and storage conditions (Boskou et al., 2005, Franco et al., 2014, Servili et al., 2014, Reboredo-Rodríguez et al., 2018) therefore, overall quality assessment of the olive oils requires a holistic view of different experiments approaches.

4. Conclusion

This study showed that virgin olive oils could be discriminated by differences in their phenolic compounds. PCA was able to discriminate olive oil samples based on their phenolic profiles. Since the first two components account for the most of the variation in the data (86%), they could be used to assess the data structure, detect clusters, outlier and trends. In general terms, two distinct groups were observed based on separate distribution in the data, including TPC<300 mg tyrosol/kg olive oil and TPC>300 mg tyrosol/kg of olive oil based on their richness in total phenolic compounds**,** which could be related to the organoleptic characteristics, and consequently possible health claims of the olive oil. The results of this work will help define qualitative analysis strategies for olive oils. Moreover, based on the polyphenolic components, it can be considered a valid approach to enhance the quality and consider the needs of the producer and the consumer. In addition to other qualitative parameters, phenolic compounds assessment may have potential for quality ranking of the olive oils. It also can be applied to confirm the results of sensorial assessment of the oils.

References

Alkan, D., Tokatli, F., & Ozen, B. (2012). Phenolic characterization and geographical classification of commercial extra virgin olive oils produced in Turkey. *Journal of the American Oil Chemists' Society,* 89(2), 261-268.

Amanpour, A., Kelebek, H., & Selli, S. (2019). LC-DAD-ESI-MS/MS–based phenolic profiling and antioxidant activity in Turkish cv. Nizip Yaglik olive oils from different maturity olives. *Journal of Mass Spectrometry,* 54(3), 227-238.

Andrewes, P., Busch, J. L. H. C., de Joode,T., Groenewegen, A., & Alexandre, H. (2003). Sensory Properties of Virgin Olive Oil Polyphenols: Identification of Deacetoxy-ligstroside Aglycon as a Key Contributor to Pungency. *Journal of Agricultural and Food Chemistry,* 51(5), 1415-1420.

Beauchamp, G. K., Keast, R. S., Morel, D., Lin, J., Pika, J., Han, Q., Lee, C. H., Smith, A. B., & Breslin, P. A. (2005). Ibuprofen-like activity in extra-virgin olive oil. *Nature,* 437(7055), 45-46.

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

Boskou, D., Blekas, G., & Tsimidou, M. (2005). Phenolic compounds in olive oil and olives. *Current Topics in Nutraceutical Research,* 3(2), 125-136.

Boskou, D., Blekas, G., & Tsimidou, M. (2006). Olive oil composition. *Olive Oil*, Elsevier**,** 41-72.

EFSA Panel on Dietetic Products, Nutrition and Allergies (2012). Scientific Opinion on the substantiation of a health claim related to polyphenols in olive and maintenance of normal blood HDL cholesterol concentrations (ID 1639, further assessment) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA Journal,* 10(8), 2848.

Franco, M. N., Galeano-Díaz, T., Sánchez, J., De Miguel, C., & Martín-Vertedor, D. (2014). Total phenolic compounds and tocopherols profiles of seven olive oil varieties grown in the south-west of Spain. *Journal of Oleo Science,* 63(2), 115-125.

Garcia-Gonzalez, D. L., Tena, N., & Aparicio, R. (2010). "Quality Characterization of the New Virgin Olive Oil Var. Sikitita by Phenols and Volatile Compounds. *Journal of Agricultural and Food Chemistry,* 58(14), 8357-8364.

García, J. M., Yousfi, K., Mateos, R., Olmo, M., & Cert, A. (2001). "Reduction of oil bitterness by heating of olive (Olea europaea) fruits. *Journal of Agricultural and Food Chemistry,* 49(9), 4231-4235.

IOC. (2017). Determination of biophenols in olive oils by HPLC, (COI)T.20/Doc No 29/Rev.1.

IOC. (2018a). Sensory analysis of olive oil method for the organoleptic assessment of virgin olive oil, (COI)T.20/Doc No 15/Rev.10

IOC. (2018b). Trade standard applying to olive oils and olive-pomace oils. COI/T.15/NC No 3/Rev. 12.

Lerma-García, M. J., Lantano, C., Chiavaro, E., Cerretani, L., Herrero-Martínez, J. M., & Simó-Alfonso, E. F. (2009). Classification of extra virgin olive oils according to their geographical origin using phenolic compound profiles obtained by capillary electrochromatography. *Food Research International,* 42(10), 1446-1452.

Ocakoglu, D., Tokatli, F., Ozen, B. and Korel, F. (2009). Distribution of simple phenols, phenolic acids and flavonoids in Turkish monovarietal extra virgin olive oils for two harvest years. *Food Chemistry*,113(2), 401-410.

Olivero-David, R., Mena, C., Pérez-Jimenez, M. A., Sastre, B., Bastida, S., Márquez-Ruiz, G., & Sánchez-Muniz, F. J. (2014). Influence of Picual Olive Ripening on Virgin Olive Oil Alteration and Stability during Potato Frying. *Journal of Agricultural and Food Chemistry,* 62(48): 11637-11646.

Pandey, K. B., & Rizvi, S. I. (2009). Plant Polyphenols as Dietary Antioxidants in Human Health and Disease. *Oxidative Medicine and Cellular Longevity,* 2(5), 270-278.

Piravi-Vanak, Z., Ghasemi, J. B., Ghavami, M., Ezzatpanah, H., & Zolfonoun, E. (2012). The Influence of Growing Region on Fatty Acids and Sterol Composition of Iranian Olive Oils by Unsupervised Clustering Methods. *Journal of the American Oil Chemists' Society,* 89(3): 371-378.

Ranalli, A., Modesti, G., Patumi, M., & Fontanazza, G. (2000). The compositional quality and sensory properties of virgin olive oil from a new olive cultivar I-77. *Food Chemistry,* 69(1), 37-46.

Reboredo-Rodríguez, P., González-Barreiro, C., Cancho-Grande, B., Forbes-Hernández, T. Y., Gasparrini, M., Afrin, S., Cianciosi, D., Carrasco-Pancorbo, A., Simal-Gándara, J., Giampieri, F., & Battino, M. (2018). Characterization of phenolic extracts from Brava extra virgin olive oils and their cytotoxic effects on MCF-7 breast cancer cells. *Food and Chemical Toxicology,* 119, 73-85.

Rodrigues, N., Casal, S., Pinho, T., Peres, A. M., Bento, A., Baptista, P., & Pereira, J. A. (2019). Ancient olive trees as a source of olive oils rich in phenolic compounds. *Food Chemistry,* 276, 231-239.

Rodríguez-López, P., Lozano-Sanchez, J., Borrás-Linares, I., Emanuelli, T., Menéndez, J. A., & Segura-Carretero, A. (2020). Structure–Biological Activity Relationships of Extra-Virgin Olive Oil Phenolic Compounds: Health Properties and Bioavailability. *Antioxidants*, 9(8), p.685.

Servili, M., Sordini, B., Esposto, S., Urbani, S., Veneziani, G., Di Maio, I., Selvaggini, R. and Taticchi, A. (2014). Biological Activities of Phenolic Compounds of Extra Virgin Olive Oil. *Antioxidants,* 3(1), 1-23.

Shavakhi, F., Boo, H. C., Osman, A., & Ghazali, H. M. (2011). Application of zNose™ for classification of enzymatically-macerated and steamed pumpkin using principal component analysis. *International Food Research Journal,* 18, 311-318.

Shavakhi, F., Moradi, P., & Rahmani, A. (2020). Quality classification of some of the Iranian olive oils using chemometric tools. *Iranian Journal of Biosystem Engineering,* 51(2), 419-431.

Squeo, G., Caponio, F., Paradiso, V. M., Summo, C., Pasqualone, A., Khmelinskii, I., & Sikorska, E. (2019). Evaluation of total phenolic content in virgin olive oil using fluorescence excitation–emission spectroscopy coupled with chemometrics. *Journal of the Science of Food and Agriculture,* 99(5), 2513-2520.

Tresserra-Rimbau, A., & Lamuela-Raventos, R. M. (2017). "22 Olives and olive oil: a Mediterranean source of polyphenols. *Olives and Olive Oil as Functional Foods: Bioactivity, Chemistry and Processing*, 417.

Vinha, A. F., Ferreres, F., Silva, B. M., Valentao, P., Gonçalves, A., Pereira, J. A., Oliveira, M.B., Seabra, R.M. & Andrade, P. B. (2005). Phenolic profiles of Portuguese olive fruits (Olea europaea L.): Influences of cultivar and geographical origin. *Food Chemistry,* 89(4), 561-568.

Vitaglione, P., Savarese, M., Paduano, A., Scalfi, L., Fogliano, V., & Sacchi, R. (2015). Healthy Virgin Olive Oil: A Matter of Bitterness. *Critical Reviews in Food Science and Nutrition,* 55(13): 1808-1818.