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Evaluation of Chemical Characteristics of Extra Virgin Olive Oils Extracted from Three Monovarieties of Mari, Arbequina and Koroneiki in Fadak and Gilvan Regions

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ABSTRACT: Olive oil in general due to it monounsaturation, stability, nutritive value, consistency, taste and aroma might be considered as a valuable and luxury oil. Annual importation of olive oil to Iran from Mediterranean countries is on a large scale although some olive oils are produced in Iran particularly in the north and some plantation has been applied in different provinces particularly in Fars. The value of this luxury oil either imported or produced internally provides a ground for adulteration. The aim of this work is to evaluate the chemical characteristics of three varieties (Mari, Arbequina, Koroneiki) of olive oils from two regions (Fadak and Gilvan).Three varieties of olive fruit in two regions were selected and the extracted oils were subjected to a series of chemical tests consisting of acid value, peroxide value, Iodine value, fatty acid profile as well as phenolic content and sterol composition. The results indicated that all of the samples in terms of acid and peroxide values were consistent with the values defined for extra virgin olive oil. The fatty acid composition indicated that oleic acid was the predominant fatty acid ranging from 56 to 77% of the total fatty acids indicating the Italian and Spanish origins. The analysis of sterol fraction showed that β-sitosterol was the major and the predominant sterol (88%). The phenolic concentration in this study was 100-250 mg/kg as gallic acid. The study illustrated that the investigated samples were in agreement with the standard of CODEX and the Iranian national standard in spite of differences obtained.

Keywords: *Fatty Acid Profile, Olive Oil, Phenolic Compounds, Sterol Composition.*

Introduction

Virgin olive oil is the oil from the fruit of olive tree (*olea Europa L.)* and is extracted solely by mechanical or physical methods. The oil is divided into several groups on the basis of acidity and sensory properties. The oil is classified as extra virgin, virgin, ordinary virgin, lampante virgin and refined olive oils (Anonymous, 2013).

Extra virgin and virgin olive oils are two popular classes of commercial varieties of olive oil and their free acidity in terms of oleic acid should not exceed 0.8 and 2 grams per 100 grams of oil respectively (Anonymous, 2013).

According to the International Olive Council the world production of virgin olive oil in the commercial year of 2013 – 2014 was about three million tons (commercial year for olive oil is between 1st of October to

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the 30th of September of the next year), and about 97% of production and over 80 percent of global consumption of virgin olive oil is related to the Mediterranean countries. Spain, Italy and Greece are the most important producers of this oil. Olive oil production in Iran is about four thousand tons per year (Anonymous, 2013).

The aim of this study is to investigate the chemical characteristics concerned with fatty acid profile, sterol composition and phenolic content in extra virgin olive oils that resulted from three monovarieties of olives, Mari, Arbequina and Koroneiki and compare the results with the national and international standards.

Materials and Methods

Manual samplings of healthy fruits of three varieties of Mari, Arbequina and Koroneiki from Fadak garden in Qom and Tarom Olive Research Station of the Ministry of Agriculture in Zanjan province were conducted. The samples of each variety in the range of three to four kilograms were collected. Samples were collected in the form of purple to black stage maturation index. The expression of maturity stage of olive fruit according to the international maturity index was used. In this method, olive is divided into 8 stages of maturity that are numbered from zero to eight. A sample of hundred olives was randomly selected. The number of fruits in each color group is counted and their maturity is determined according to the following formula:

$$MI = (a \times 0) + (b \times 1) + (c \times 2) + (d \times 3) + (e \times 4) + (f \times 5) + (g \times 6) + (h \times 7) /100$$

Where a, b, c, d, e, f, g, h denote the number of olives belonging to each of the 8 color categories.

Samples were transferred to Roodbar Olive Research Station with the maximum interval of two days for oil extraction. The samples were crushed and kneaded for 20 minutes at 25˚C. The virgin olive oil was extracted employing centrifugal force. The samples were kept in the dark glasses at 2˚C until required for analysis.

All the chemicals used for qualitative and quantitative analysis were of analytical grads purchased from Merck Chemical Company of Germany. The acidity was determined according to the Iranian national standard number 4178 by the titration of samples with potassium hydroxide using phenolphthalein as indicator. Peroxide value was determined according to the Iranian national standard number 4179 by the titration of the oil samples with sodium thiosulphate using starch indicator. Iodine values of the oil samples representing the unsaturation were calculated based on the AOCS standard method number 85- cd 1c (Firestone *et al*., 1985). The Cox value showing the level of oxidation of oils based on the unsaturated fatty acid is calculated by the following equation (Fatemi & Hammond, 1980).

$$\text{Cox index} = [(1 \times \% \text{ oleic acid}) + (10.3 \times \text{percent linoleic acid}) + (21.6 \times \text{percent linolenic acid})] / 100$$

The fatty acid compositions and profiles were determined by the formation of fatty acid methyl ester according to the national standard method number 4090. The derivatives were applied to a Young lin model YL 6500 gas chromatograph equipped with cpsil 88 capillary column and flame ionization detector according to the national standard method number 4091 using hydrogen as the carrier gas. The sterols were fractionated and isolated from the nonsaponifiable matter by TLC using silica gel G type 60 as described by Ghavami *et al*. (2008). The total phenolic compounds in oil was quantitatively determined using Folin - Ciocalteu procedure using spectrophotometry method at 765 nm and reported in terms of gallic acid (Montodero *et al*., 1992). The statistical

analysis was performed by One-way ANOVA (ANOVA, $P<0.05$) using SPSS 16 statistical software and in case of significant differences in the data and comparison of the treatments means were determined using Duncan method. All the analyses were carried out in triplicate order.

Results and Discussion

Figure 1 shows the peroxide values of the samples in term of milliequivalents of peroxide per kilogram of the sample and Figure 2 shows the free acidity of the samples in term of grams of oleic acid per 100 grams of oil. All the samples were within the permissible limit in terms of IOC and Codex. The highest peroxide index related to Mari of Fadak (7.7 milliequivalents of peroxide per kg of oil) and the lowest value related to Koroneiki of Gilvan (2.5 milliequivalents of oxygen per kg of oil). The highest free fatty acid concentration was related to the Arbequina of Gilvan sample (0.3) and the lowest value related to Koroneiki of Fadak sample (0.2). This might give suggestions concerned with the quality of the olives and the virgin olive oils extracted with respect to both oxidative and hydrolytic rancidity.

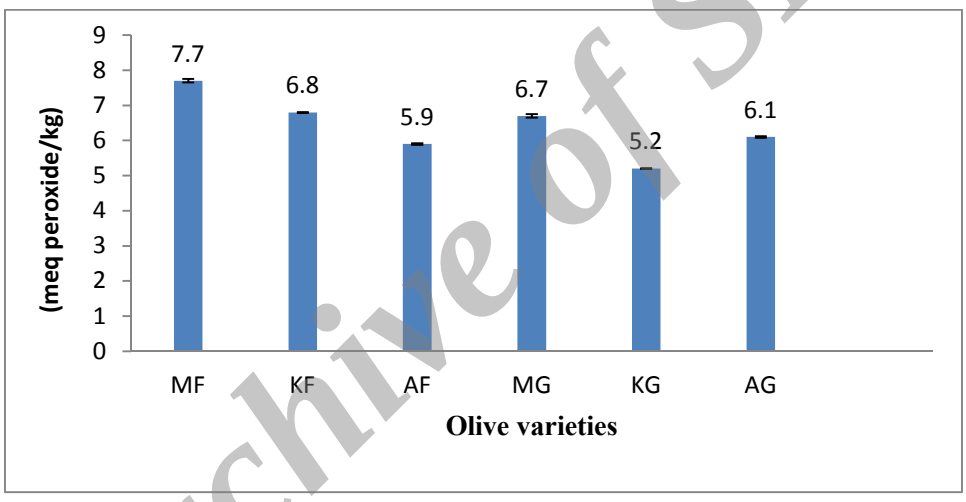

Fig. 1. Comparison of peroxide value of olive oil samples

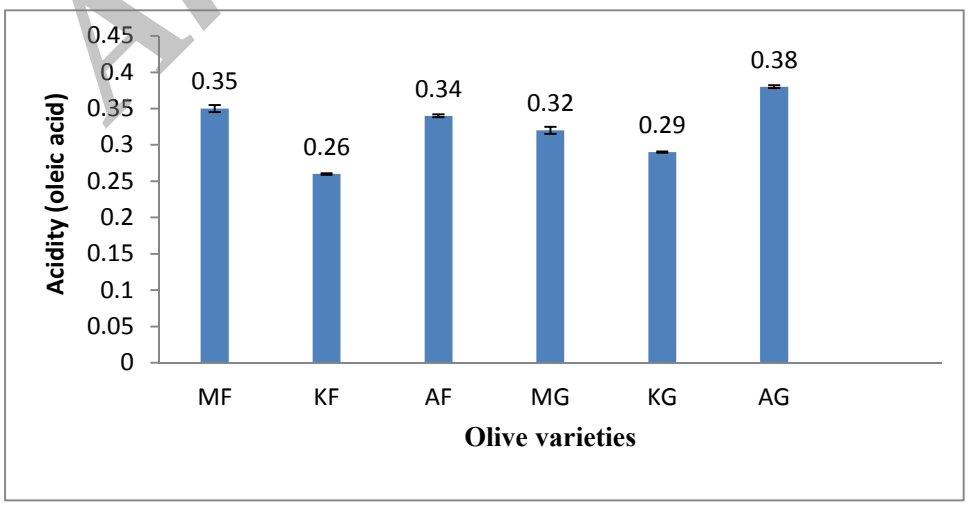

Fig. 2. Comparison of free acidity of olive oil samples

Table 1 indicates the fatty acid composition of the examined extra virgin olive oils. Among the saturated fatty acids as described by the Iranian national standards as well as the International Olive Council, palmitic acid represents 7.5 to 20 percent of the constituent fatty acids. Based on the statistical analysis, there was a significant difference between palmitic acid of the samples ($p<0.05$). Arbequina variety of Gilvan had the highest (19.75%) and the Mari variety of Gilvan had the lowest concentration (11.4%). According to Codex and the IOC standards, stearic acid content of olive oils represent 0.5-5% of the total fatty acids. In this respect, there was a significant difference between the samples ($p<0.05$), but stearic acid contents of all the samples were within the allowable range. Koroneinki variety of Fadak and Marie of Gilvan had the highest (2.55%) and the

Table 1. Fatty acid compositions of some Iranian olive oils

	AF	KF	MF	AG	KG	MG
C16:0	18.45±0.05^{b}	12.75±0.35^{d}	13.00±0.60^{d}	19.75±0.15^{a}	15.75±0.05^{c}	11.40±0.10^{e}
C16:1t	0.10±0.00^{a}	0.10±0.00^{a}	0.10±0.00^{a}	0.15±0.05^{a}	0.05±0.05^{a}	0.01±0.00^{a}
C16:1c	3.10±0.00^{a}	1.40±0.10^{c}	1.15±0.050^{d}	2.85±0.05^{b}	1.00±0.00^{d}	0.95±0.05^{d}
C17:0	0.05±0.00	-	-	-	-	-
C17:1	0.30±0.00^{a}	0.10±0.00^{b}	0.10±0.00^{b}	0.15±0.05^{b}	0.10±0.00^{b}	0.01±0.00^{b}
C18:0	1.65±0.05^{e}	2.55±0.05^{c}	2.30±0.00^{d}	1.55±0.05^{e}	2.20±0.00^{d}	2.55±0.05^{c}
C18:1	62.00±0.1^{f}	74.05±0.35^{b}	74.75±0.45^{b}	56.30±0.1^{h}	72.95±0.15^{c}	76.90±0.1^{a}
C18:2	12.90±0.00^{e}	7.40±0.10^{g}	7.10±0.00^{h}	18.00±0.1^{c}	6.15±0.05^{j}	6.70±0.00^{i}
C18:3	0.35±0.05^{ab}	0.40±0.00^{a}	0.35±0.00^{ab}	0.30±0.00^{b}	0.40±0.00^{a}	0.40±0.00^{a}
C20:0	0.20±0.00^{a}	0.20±0.00^{a}	0.20±0.00^{a}	0.20±0.00^{a}	0.25±0.05^{a}	0.25±0.05^{a}
C20:1	0.75±0.05^{b}	1.00±0.00^{a}	0.65±0.05^{bcd}	0.70±0.00^{bc}	0.95±0.05^{a}	0.50±0.00^{e}
C22:0	0.10±0.00^{a}	0.05±0.05^{a}	0.10±0.00^{a}	0.05±0.05^{a}	0.10±0.05^{a}	0.15±0.05^{a}
C22:1	0.05±0.00^{b}	-	-	-	-	-
S	20.40±0.05^{ab}	15.50±0.35^{de}	15.60±0.60^{de}	21.55±0.15^{a}	18.30±0.10^{c}	14.35±0.05^{e}
US	79.50±0.05^{de}	84.40±0.35^{ab}	84.20±0.40^{b}	78.45±0.25^{e}	81.6±0.2^{c}	85.6±0.05^{a}
MUS	66.3±0.00^{f}	76.65±0.25^{b}	76.75±0.35^{b}	60.15±0.15^{g}	75.05±0.15^{c}	78.55±0.05^{a}
PU	13.25±0.05^{e}	7.80±0.10^{g}	7.45±0.05^{h}	18.3±0.10^{c}	6.55±0.05^{j}	7.10±0.00^{i}
S/U	0.257±0.00^{ab}	0.18±0.004^{de}	0.19±0.008^{de}	0.27±0.002^{a}	0.22±0.001^{c}	0.167±0.00^{e}
M/P	5.00±0.01^{f}	9.82±0.09^{d}	10.3±0.02^{c}	3.28±0.009^{h}	11.45±0.06^{a}	11.06±0.007^{b}
Oleic/linoleic	4.80±0.007^{f}	10.00±0.087^{d}	10.52±0.06^{c}	3.12±0.011^{h}	11.86±0.07^{a}	11.47±0.014^{b}
Cox value	2.02±0.009^{f}	1.58±0.013^{g}	1.55±0.015^{gh}	2.48±0.011^{c}	1.44±0.006^{i}	1.54±0.001^{h}
IV	83.76±0.13^{c}	83.24±0.39^{c}	82.65±0.45^{c}	87.40±0.32^{b}	79.55±0.26^{d}	83.67±0.04^{c}

Arbequina of Gilvan had the lowest contents (1.65%) of this saturated fatty acid. Oleic acid is the most abundant fatty acid in olive oil and the limits assigned in accordance with the national standard of Iran and the IOC is between 55 to 83%. All the samples were within this limit, but there were statistically significant differences amongthe samples ($p<0.05$). The Mari of Gilvan had the highest value (76.9%) while Arbequina of Gilvan had the lowest value (56.3%) regarding oleic acid. According to Codex standards and the IOC standard, linoleic acid content is between 3.5-21% of the total fatty acid. There were statistically significant differences among the samples examined in this respect. Arbequina ofGilvan had the highest (19.05%) and Koroneiki of Gilvan had the lowest (6.15%) concentrations of this polyunsaturated fatty acid.

Regarding the oil resistance to oxidation, Koroneiki of Gilvan that had the least Cox value (1.44) therefore had the highest resistance to oxidation while Arbequina of Gilvan with the highest Cox value (2.48) had the lowest resistance to oxidation. Cox value of each sample is presented in Figure 3. Iodine number is the degree of unsaturation of the oil. In respect of Iodine value all the samples were within the limit in respect of this value (75-94) as described by codex (2003) but significant differences existed between the samples ($p<0.05$).

Table 2 presents the sterol composition of the extracted oils. In all the cases, β-sitosterol was the dominant sterol followed by Δ^5-avenosterol and campesterol. According to the Iranian national standard and the IOC standard, the total nominal amount of apparent β-sitosterol must be equal or greater than 93% of the total sterols. In this respect, there were significant differences between the samples ($p<0.05$), but all the samples were within the allowable range (Iranian national standard, 1446).

Table 2. Sterol compositions of some Iranian olive oils [1]

	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	Clerosterol	β-sitosterol	Δ^5-avenasterol	Stigmastadienol	Stigmastenol	Δ^7-avenasterol	Apparent β- sitosterol	Campestrol/ Stigmasterol
AF	0.01±0.01^{c}	0.00±0.00^{c}	4.51±0.00^{bc}	0.57±0.01^{f}	0.79±0.01^{d}	87.68±0.16^{efg}	4.88±0.02^{b}	0.45±0.00^{c}	0.06±0.01^{e}	1.01±0.10^{ab}	94.10±0.04^{b}	7.83±0.19^{c}
KF	0.09±0.01^{b}	0.01±0.00^{c}	4.38±0.17^{bc}	0.44±0.03^{fg}	0.68±0.02^{e}	89.62±0.28^{c}	2.87±0.13^{d}	0.64±0.01^{a}	0.42±0.01^{c}	0.84±0.04^{bc}	94.40±0.18^{b}	9.82±0.37^{b}
MF	0.00±0.00^{c}	0.00±0.00^{c}	3.81±0.15^{d}	0.76±0.05^{e}	0.96±0.04^{ab}	92.08±0.28^{a}	1.78±0.09^{f}	0.34±0.04^{d}	0.02±0.02^{e}	0.24±0.02^{d}	94.47±0.16^{b}	5.01±0.55^{de}
AG	0.12±0.00^{b}	0.01±0.00^{c}	4.77±0.11^{b}	1.32±0.02^{c}	0.56±0.04^{f}	89.74±0.17^{c}	2.20±0.09^{e}	0.49±0.01^{bc}	0.03±0.00^{e}	0.74±0.05^{c}	93.21±0.03^{cd}	3.61±0.15^{e}
KG	0.13±0.01^{b}	0.21±0.03^{a}	4.34±0.03^{c}	0.95±0.05^{d}	0.89±0.00^{bc}	87.30±0.38^{fg}	4.81±0.07^{b}	0.53±0.01^{b}	0.04±0.01^{b}	0.64±0.01^{c}	93.94±0.45^{b}	4.56±0.29^{de}
MG	0.01±0.01^{c}	0.12±0.03^{b}	3.26±0.07^{e}	2.19±0.02^{b}	0.61±0.02^{ef}	91.05±0.07^{b}	1.43±0.02^{g}	0.01±0.00^{f}	0.66±0.02^{b}	0.65±0.03^{c}	93.81±0.09^{bc}	1.48±0.05^{f}

[1] Lowercase letters denote comparison of mean (column comparison) of sterolic compounds in olive oil varieties (P <0/05).

Virgin olive oil is a good source of phenolic compounds. These compounds have important roles in oxidative stability of olive oil. Figure 4 presents the concentrations of the total phenolic compounds in the examined oil samples where there were significant differences between the samples ($p<0.05$).

The acid value, peroxide value and some other indices indicate the state of the oils or olive fruit in term of hydrolytic and oxidative rancidity but other factors namely fatty acid profile and sterol composition might indicate the variety of fruits or extracted oils (Yildirim, 2009). Fatty acid composition in different oils produced varies according to the region, altitude, climate, olive varieties and maturity index at the harvest (Boskou, 1996). Researchers have classified olive oils into two categories, the oils with low concentration of oleic acid and the oils with high concentration of oleic

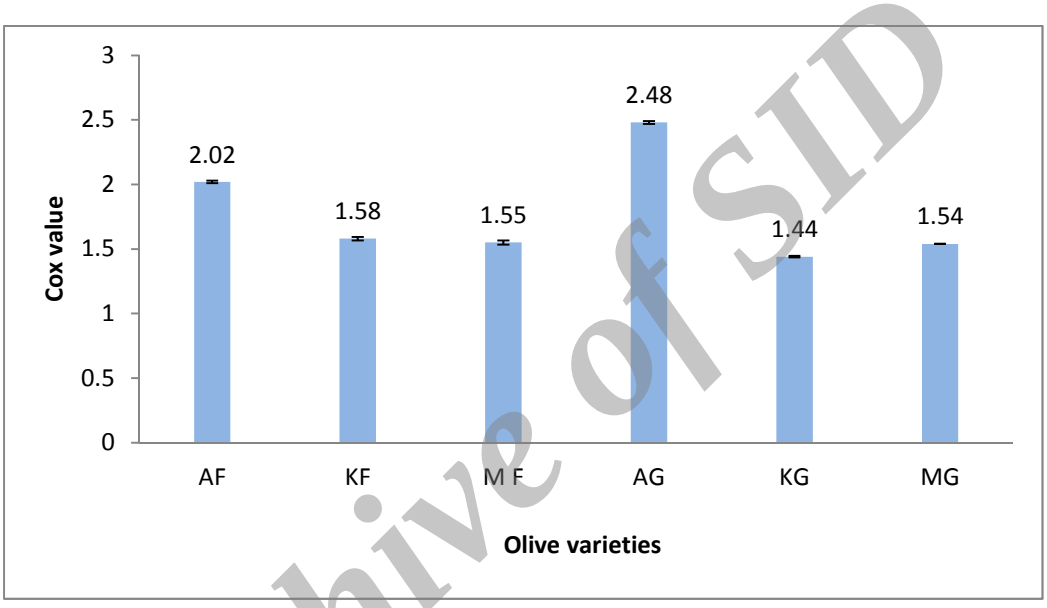

Fig. 3. Comparison of Cox value of olive oil samples

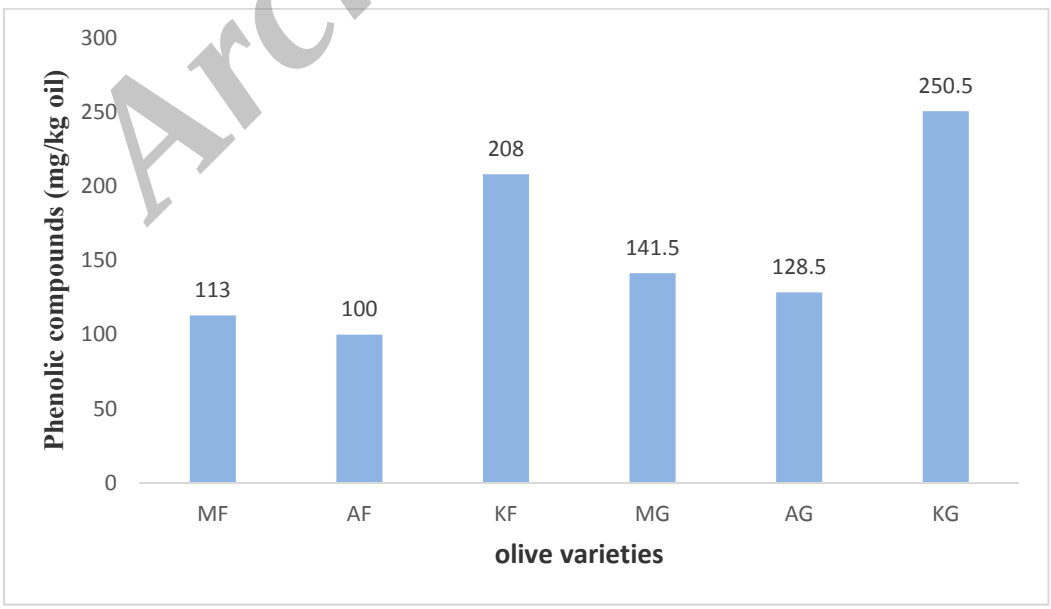

Fig. 4. Comparison of phenolic compounds in olive oil samples

acid. Spanish, Italian and Greek olive oils are among the first while the Tunisian olive oil is placed in the second category. The results presented in Table 1 indicate that the most abundant fatty acids in all the samples are oleic acid followed by palmitic, linoleic and stearic acids respectively. Palmitoleic and gadoleic and some other acids are present in small quantities. These results were consistent with other studies (Hashempour *et al*., 2010). The difference between fatty acid compositions in different samples might be due to differences in varieties used for oil extraction and difference in cultivation factors. Rondanini *et al* (2011) noted that there is an inverse correlation between oleic and linoleic acid contents. Oleic and linoleic acid ratio varies from 3.12 to 11.86 in the Koroneiki of Gilvan and Arbequina of Gilvan respectively. The higher this ratio, the greater is the oxidative resistance of the oil. Koroneiki of Gilvan had the highest ratio of oleic to linoleic acid

Sterols are the most important non-glycerol components of olive oil that are employed for the authenticity of the oil (Boskou, 1996). Previous researchers have reported that β-sitosterol accounts for over 70% of the total sterols in the olive oil (Boskou, 1996). This is in agreement with the results obtained in this study. Itoh *et al.* (1981) and Rivera del Alamo *et al*. (2004) reported that the amount of Δ^5-avenosterol in VOO is between 5-20% of the total sterols. The amount of this compound in the present study was 1.43 (Mari of Fadak) to 4.88 (Arbequina of Fadak). Wang *et al.* in 2002 reported that this fraction has shown antioxidative properties at high temperatures.

According to Codex and IOC regulations, maximum campesterol concentration should be less than 4% of the total sterols, but Lanuzza *et al.* (1995) and Rivera del Alamo (2004) reported higher concentrations. Apparent β-sitosterol that is the sum of β-sitosterol and four other sterols formed following the decomposition of β-sitosterol. must be higher than 93% of the total sterol. This parameter is used to evaluate the authenticity of olive oil (Casas *et al*., 2004).

Phenolic compounds are natural hydrophilic antioxidants and are qualitative assessment parameters for olive oil. These compounds are evaluated by Folin-Ciocalteu reagent and spectrophotometry (Gallina-Toschi *et al*., 2005). Bolandnazar *et al*. (2011) reported the phenolic compounds in some Iranian olive oils during four months of fruit maturation and growth. In 2006, researchers reported that the Iranian olive's phenolic levels are lower than the European ones (Haghighat-Kharazi *et al*., 2012). The amounts of phenolic compounds measured in this study ranged from 100 (Arbequina of Fadak) to 250 (koroneiki of Gilvan) mg as gallic acid per kilogram of oil, which was consistent with the results of previous researchers (Hashempour *et al*., 2010).

Conclusion

The results of this study showed that all the samples had the necessary chemical characteristics as defined for extra virgin olive oil. In terms of fatty acids compositions all the samples had low palmitic and linoleic acids that are indices for Italian and Spanish olive oils. In terms of sterols composition and total sterol content, all the samples were within permissible limit as set by the Codex and the Iranian national standard.

Acknowledgement

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