

CHARACTERIZATION OF VIRGIN OLIVE OILS (*OLEA EUROPAEA* L.) FROM THREE MAIN IRANIAN CULTIVARS, 'ZARD', 'ROGHANI' AND 'MARI' IN KAZEROON REGION

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ABSTRACT

The quality of virgin olive oil of three major Iranian cultivars including 'Zard', 'Roghani', and 'Mari', grown in Kazeroon region located in southern part of Iran, was examined in 2008. There were significant differences among fatty acids content of all three cultivars. 'Mari' had the highest oleic acid, and the lowest palmitic acid. Oleic acid to linoleic acid ratio in all studied cultivars was higher than the reported levels in the olive oils from other countries. Results from the quantification of phenolic compounds indicated that 'Zard' cultivar had the highest tyrosol and 'Mari' had the highest cinamic acid and vanilic acid. The oil of 'Zard' had the highest chlorophyll and 'Roghani' showed the highest carotenoid content. Results here confirm that there were significant differences ($p < 0.05$) in the features, biochemical characteristics and nutritional value among these cultivars.

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Introduction

Olive (*Olea europaea*) is one of the few trees that can still produce fruits even on rock and unproductive land (18). Virgin olive oil has a delicate and unique flavour that distinguishes it from other edible vegetable oils (3). Quantity and quality of substances existing in the virgin olive oil such as fatty acids, phenolics, chlorophyll and carotenoids are affected by various factors including the type of the olive cultivar (2, 4, 10, 13), climatic conditions (1, 22, 24), ripening stage (21), irrigation management (23) and the extraction methods (20). Among these factors, cultivar is undoubtedly one of the most important. However, it is often ignored, either through lack of varietal information, or because the saled oil is a mixture of various varieties or even because emphasis has been laid only on its place of origin (12).

There are various local olive cultivars in Iran taking up the most olive-growing area. Among these cultivars, 'Zard' is ranked as the first regarding to tree population (around 64 percent) and oil production. 'Roghani' and 'Mari' cultivars are positioned below 'Zard' with around 20 and 2 percent of the total olive trees orchards, respectively. Among various areas of Iran, Kazeroon region in the south of the country has been allocated to olive growing because of appropriate climatic conditions. In addition, the area of plantation is still expanding. There have been several reports in other countries regarding to the effects of the cultivar type and climate on the quality of the oils obtained from various cultivars (2, 4, 10, 13, 22). However, few studies have been carried out in Iran on the quality of

olive oils obtained from various cultivars, including 'Zard', 'Roghani', and 'Mari'. Since the main part the manufactured olive oil in Iran is from the above mentioned cultivars but, the quality of oil that is obtained from them have not been studied to date, this experiment was conducted to evaluate the three cultivars grown in Kazeroon region climate.

Materials and Methods

Fruit samples

Healthy olive fruit samples (*Olea europaea* L.) of 'Mari', 'Roghani' and 'Zard' cultivars were picked at industrial optimum ripening stage. It was determined, according to the skin color which changed to greenish-yellow as a maturity index. This experiment was conducted during the crop season of 2006-2007 in the olive orchard of Kazeroon Olive Research Station located in south of Iran. The average annual precipitation was 500 mm with the majority in October, December, and January. Average annual temperature of the experimental orchard site is 24°C; the altitude is 860 m, 29°37' N of latitude and 51°39' E of longitude.

Oil extraction

For oil extraction, the washed fruits were ground by the hammer mill. The paste was kept in the room temperature for 30 minutes. To accelerate oil extraction, 100 ml of lukewarm water was added and mixed using a mixer and then was centrifuged in 5000 rpm for 20 minutes. The obtained oil samples were kept in dark at 4°C until use.

Solvents and standards

The solvents used in this study were of analytical or HPLC grade. The standard of hydroxytyrosol was purchased from

Cayman (USA). All of the other standards and solvents were purchased from Merck (Germany).

Fatty acids, peroxide value, and UV Spectrophotometric indices (K_{232} , K_{270})

The quality indices of fatty acids, peroxide value, and specific extinction coefficient of K_{232} and K_{270} and ΔK were calculated from absorption at 232 and 270 nm, respectively, by a UV spectrophotometer (JENWAY - 6405 UV Visible spectrophotometer, England) according to the European Commission Regulation EEC/2565/91 (6).

Determination of chlorophyll and carotenoid compounds

Pigments of chlorophyll and carotenoid were determined by a spectrophotometer according to Minguez-Mosquera et al. (1991): 1 g of olive oil was dissolved in 10 ml of iso-octane. The absorbance of the solution was measured at 670 and 470 nm for chlorophyll and carotenoid, respectively.

Fatty acids analysis

The fatty acid composition of the olive oil was determined as acid methyl esters (FAMES). FAMES were prepared by vigorous shaking of a solution of each olive oil sample in hexane (0.2 g in 3 ml) with 0.4 ml of 2 N methanolic potassium hydroxide solution, then 2 μ l were injected into the GC (HP5890N, Hewlett Packard, USA) with a FID detector. The carrier gas was helium at a flow through the column (50 m length \times 0.25 μ m i.d. \times 0.25 mm film thickness, HP 5) of 1 ml/min, according to the method of European Regulation Commission 2568/91 (6 and 5). The temperature of the injector and detector were set at 250°C and the oven temperature at 210°C. The results were expressed as relative area percent of the total FAMES. Iodine (IV) were calculated from the percentage of fatty acids (14) using the formula: $IV = (\text{palmitoleic \%} \times 1.001) + (\text{oleic \%} \times 0.899) + (\text{linoleic \%} \times 1.814) + (\text{linolenic \%} \times 2.737)$.

Phenolic compounds analysis

The amounts of phenolic compounds of virgin olive oil were determined using method by Montedoro et al. (1993): 14 g of olive was shaken well in 14 ml of methanol/water solution (80:20, v:v). Then the solution was centrifuged for 10 minutes at 5000 rpm and the phase of methanol and water was separated; whereas the oil phase was extracted for another three times. The hydroalcoholic extracts were compounded and then evaporated in vacuum by Rotary Evaporator at the temperature of 35°C until a syrupy consistency was achieved. Then it was resuspended to 15 ml using acetonitrile and was washed three times with 20 ml of hexane to remove the fats. After hexane was removed, 10 ml of acetonitrile was evaporated in vacuum at the temperature of 35°C and the remaining was solved in 1 ml of methanol and 50 μ l of it was injected into the HPLC. The HPLC system was composed of a 4.6 mm \times 155 mm Symmetray® C18 5 μ m column, coupled with UV detector (Waters 486). The flow rate was 0.8 ml/min; the mobile phase used was 0.1% acetic acid in water (A) and acetonitrile (B) for a total running time of 30 min, and the gradient changed as follows: solvent B started at 5% increased to 10% in 6 min, to BIOTECHNOL. & BIOTECHNOL. EQ. 24/2010/4

20% in 17 min, to 70% in 20 min and to 100% in 1 min until the end running. Identification of compounds was achieved by comparing their retention time values with those of standards. Concentration of phenolic compound in the extracts was calculated comparing HPLC peak areas with those of standards. The amount of total phenol was also measured according to Gutfinger et al. (1981) using spectrophotometer at 760 nm and the results were expressed in mg caffeic acid.

Statistical analysis

The complete data were evaluated by randomized block design, with three blocks (replications) each containing six trees, using SAS software (version 9.1). Samples were a mixture of six tree fruits. The results were displayed in mean values and standard deviation (n=3). Significance of the differences among the numbers was determined by the variance analysis using Duncan's multiple range test. The level of significance was determined as $P < 0.05$.

Results and Discussion

Free acidity, peroxide value and UV spectrophotometric indices

All the analyzed oils showed very low values for the regulated physicochemical parameters (acidity $\leq 0.8\%$; peroxide value ≤ 20 m equiv. O_2 kg^{-1} ; $K_{270} \leq 0.22$; $K_{232} \leq 2.5$), with all of them falling within the ranges established for "extra virgin olive oil" category, as required by Regulation EC/1989/2003 (EEC, 2003) and Codex Alimentarius, (2003) (Table 1).

Fatty acids compound

Fatty acid compositions of the oils were different depending on the variety (Table 2). Oleic acid (C18:1) was the main monounsaturated fatty acid, representing high concentrations (72.60%-77.92%) (Table 2). Another monounsaturated fatty acid was the palmitoleic acid (C16:1) which had the highest mean values (1.77%) in the oil of 'Roghani' cultivar and the least mean values (1.04%) in the oil of 'Zard' cultivar. Palmitic acid (C16:0), the major saturated fatty acid in olive oil, had the highest mean values (19.10%) in the oil of 'Roghani' cultivar in comparison with oils of 'Zard' and 'Mari' cultivars in which the mean values were 17.26 and 14.05%, respectively (Table 2). Stearic acid (C18:0) which is an important saturated fatty acid in olive oil, did not show any significant difference in the three cultivars. The levels of linoleic acid (C18:2) and linolenic acid (C18:3) which are considered as polyunsaturated fatty acids had not any significance in the cultivars. The ratio of oleic acid to linoleic acid did not show any significant differences at $P < 0.05$.

Pigment contents

The levels of chlorophyll and carotenoids of virgin olive oils from the three cultivars showed significant differences ($P < 0.05$). The oil of 'Zard' cultivar had the highest level of chlorophyll with the mean values of 8.79 mg/kg in comparison with the oils of 'Mari' and 'Roghani' cultivars which had the averages of 5.14 and 8.25 mg/kg, respectively. While, the oil of

TABLE 1

Quality indices of virgin oils from cultivars Zard, Roghani and Mari from the Kazeroon region

Quality indicatess	Cultivar			EVOO (EEC, 2003)
	Zard	Roghani	Mari	
Free acidity (oleic acid, %)	0.32±0.02a	0.28±0.02a	0.32±0.03a	≤0.8
Proxide value (meq O2.kg oil)	5.62±0.35b	7.75±0.39a	5.56±0.27	≤20
K232	0.74±0.02a	0.72±0.03a	0.68±0.07a	≤2.5
K270	0.078±0.01b	0.093±0.02a	0.076±0.01a	≤0.22
MI*	3	3.4	3.2	

Mean ± SD (n=3): Means in each column with the same letters are not significantly different at 5% of probability by Duncan's multiple range test.

*Maturity index fruits olive

TABLE 2

Fatty acids composition of virgin olive oils from cultivars Zard, Roghani and Mari of Kazeroon region (results are expressed as percents)

Fatty acid composition (%)	Cultivar		
	Zard	Roghani	Mari
Palmitic acid (C 16:0)	17.26±0.68a	19.10±0.52a	14.05±0.24b
Palmitoleic acid (C 16:1)	1.04±0.05b	1.77±0.19a	1.24±0.09ab
Stearic acid (C 18:0)	1.91±0.06a	2.32±0.14a	2.49±0.2b
Oleic acid (C 18:1)	74.98±1.25b	72.60±1.18c	77.92±0.88a
Linoleic acid (C 18:2)	3.74±0.21a	3.51±0.22a	3.84±0.14a
Linolenic acid (C 18:3)	0.45±0.03a	0.46±0.02a	0.40±0.01a
ΣSaturated fatty acids* (SFAs)	19.17±0.62a	21.42±0.46a	16.50±0.51b
ΣMonounsaturated fatty acids (MSFAs)	76.02±1.24b	74.34±0.98c	78.69±1.01a
ΣPolyunsaturated fatty acids (PUSFAs)	4.19±0.22a	3.97±0.23a	4.25±0.15a
ΣMonounsaturated fatty	18.21±0.65a	18.91±1.4a	18.59±0.92a
Oleic acid. Linoleic acid	20.12±0.78a	20.87±1.6a	20.23±1.04a
Iodine value (IV)	76.44±0.69ab	74.65±0.38b	79.36±0.77b

Mean ± SD (n=3): Means in each column with the same letters are not significantly different at 5% of probability by Duncan's multiple range test.

*ΣSum of major saturated fatty acids

ΣSum of major monounsaturated fatty acids

ΣSum of major polyunsaturated fatty acids

ΣSum of major MSFAs . ΣSum of major (PUSFAs)

TABLE 3

Pigments and phenolic compounds of virgin oils from cultivars Zard, Roghani and Mari of Kazeroon region (results are expressed as mg/kg)

Pigments and phenolic compounds	Cultivar		
	Zard	Roghani	Mari
Total phenol	172±5b	179±7a	169±8b
Total chlorophylls (mg/kg)	8.79±0.09a	8.35±0.63a	5.14±0.29b
Total carotenoid (mg/kg)	3.49±0.39b	4.37±0.18a	2.30±0.20c
Hydroxytyrosol (mg/kg)	0.51±0.08a	0.49±0.07a	0.46±0.09a
Tysol (mg/kg)	1.18±0.14a	0.75±0.09b	1.03±0.13a
Cinamic acid (mg/kg)	0.63±0.09b	0.74±0.08b	1.40±0.15a
Vanilic acid (mg/kg)	0.81±0.11b	1.06±0.10a	1.18±0.18a

Mean ± SD (n=3): Means in each column with the same letters are not significantly different at 5% of probability by Duncan's multiple range test

'Roghani' cultivar had the highest concentration of carotenoid with the mean values of 4.37 mg/kg in comparison with oils of 'Zard' and 'Mari' cultivars which had the mean values of 3.49 and 2.30 mg/kg, respectively (**Table 3**).

Phenolic compounds

In this research, four major phenolic compounds of virgin olive oil (tyrosol, hydroxytyrosol, cinamic acid, vanilic acid) have been studied by HPLC. **Table 3** shows the concentrations of the phenolic compounds that were identified and the data were expressed as mg/kg for samples of virgin olive oils obtained from 'Zard', 'Roghani' and 'Mari', cultivars. Significant differences between cultivars ($p < 0.05$) were observed in the amounts of phenolic compounds. In comparison with 'Mari' and 'Roghani' cultivars which had 1.03 and 0.75 mg/kg of the phenolic compound of tyrosol, in their oils respectively, 'Zard' cultivar oil had higher levels of tyrosol- 1.47 mg/kg. The oil of 'Mari' cultivar had the highest levels of the phenolic compounds of cinamic acid and vanilic acid (1.4 and 1.18 mg/kg, respectively). Moreover, the total level of phenol in the oils of the cultivars showed significant differences and the oil of 'Roghani' cultivar had higher mean amounts of total phenol- 179.1 mg/kg in comparison with the oils of 'Zard' and 'Mari' cultivars which had the mean values of 172 and 169.9 mg/kg, respectively (**Table 3**).

The results here, confirmed that there were significant differences in the features and characteristics of virgin olive oils of 'Zard', 'Roghani' and 'Mari' cultivars in Kazeroon region. This study showed that the oil of 'Mari' had the highest content of oleic acid. High content of this unsaturated fatty acid is positively correlated with the stability of olive oil (**Table 2**). In addition, the oil of 'Mari' had lower content of the palmitic acid in comparison with the two other cultivars; high content of this saturated fatty acid is unfavorable in olive oil. These results are consistent with the results of Ramezani-Kharazi (2008) on 'Zard', 'Roghani', and 'Shengeh' in Roudbar region located in the north of Iran. In contrast, linoleic acid content of olive oil in the same cultivars in the north of Iran (19) and in selected cultivars from some other countries (1, 2, 13) has been higher than that for Kazeroon.

The ratio between oleic acid and linoleic acid in all of the three cultivars is higher than what has been reported for olive oils from other areas (1, 2, 13) (**Table 2**) showing lower linoleic acid and/or higher oleic acid content. Total saturated fatty acids in the oil of 'Roghani' cultivar were higher than those of 'Mari' and 'Zard' cultivars due to the higher level of palmitic acid. Whereas, total unsaturated fatty acids in the oil of 'Mari' cultivar was higher than 'Zard' and 'Roghani' due to high level of oleic acid. However, the levels of fatty acids in the oils of the studied cultivars for all the samples were in the admitted range of European Commission Regulation (EEC, 2003). Variations in fatty acid composition were observed in the olive oil samples (**Table 2**) that might probably be related to both genetic factors and environmental conditions. In addition, Iodon Index in the oil of 'Mari' cultivar was higher in

comparison with the oils of 'Zard' and 'Roghani' cultivars due to high level of oleic acid. (**Table 2**).

The color of olive oils is an important characteristic of quality, and plays a key role as a factor of acceptability among consumers (7, 8). Chlorophylls and carotenoids also have biological and health properties (3). The oil of 'Zard' cultivar had the highest level of chlorophyll and the oil of 'Roghani' cultivar had the highest concentration of carotenoid. The oil of 'Mari' cultivar was poor in levels of chlorophyll and carotenoid (**Table 3**). This may be attributed to varietals differences with characteristic biosynthetic or catabolic pathways. These results are consistent with the findings of Giuffrida et al (2007) who showed that the contents of chlorophyll and carotenoid in the sample oils obtained from various cultivars are distinct.

The amount of phenolic compounds is an important factor when calculating the quality of virgin olive oil, because of their involvement in its resistance to oxidation and its sharp bitter taste. As shown in **Table 3**, significant differences between cultivars ($p < 0.05$) were observed in the phenolic contents. The most representative phenolic compound in all studied olive oils was the tyrosol. The concentration of this compound was different and distinct in the oils of 'Mari', 'Zard' and 'Roghani' cultivars (**Table 3**). The oil of 'Zard' cultivar had higher levels of tyrosol in comparison with 'Mari' and 'Roghani' cultivars. The amount of hydroxytyrosol in the studied virgin olive oils from the three cultivars did not show any significant difference and concentrations were identified in lower contents in comparison with other phenolic compounds. While, analysis of phenolic compounds of olive oils in the north of Iran (19) did not show hydroxytyrosol in the olive oils of 'Roghani' and 'Shengeh' cultivars or it was seen in very low contents. Other identified and quantified phenols in the studied virgin olive oils were vanilic acid, and cinamic acid. The oil of 'Mari' cultivar had higher levels of cinnamic acid and vanilic acid compared with that of 'Roghani' and 'Zard' cultivars (**Table 3**). These results are in accordance with the findings of Ramezani-Kharrazi (2008) and Ocakoglu et al. (2009) reporting that oils' phenolic content is a varietal characterisation index.

The total level of phenolic compounds in the oil of the studied cultivars showed significant differences and the oil of 'Roghani' cultivar had higher amounts of total phenolics in comparison with the oils of 'Zard' and 'Mari' cultivars. These amounts were in lower contents than what was reported for the oils of studied cultivars from Roudbar region in the north of Iran (19). The difference in the results may be due to the different climatic conditions of the regions, different systems of extracting oil or even the year of conducting the experiment. Therefore, all of the results put in evidence that oil quality of 'Mari' cultivar could be the best in the Kazeroon region.

Conclusions

The results here showed significant differences in the features of olive oils in studied cultivars. Variations in fatty acid composition and phenolic compounds were observed in the olive oil samples probably due to both genetic factors and

environmental conditions. Therefore, the levels of fatty acids in the oils of the studied cultivars were in the ranges established for “extra virgin olive oil” category. According to the analytic parameters that were examined here we can conclude that the oil quality of ‘Mari’ cultivar could be the best in the Kazeroon region and other regions with similar climatic conditions in order to produce high quality olive oil.

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