

Fatty acids composition and pigments changing of virgin olive oil (*Olea europea* L.) in five cultivars grown in Iran

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Abstract

This study was conducted to evaluate some quality indices, fatty acids and pigments of five olive oil cultivars (*Olea europea* L.), namely 'Zard', 'Arbequina', 'Coratina', 'Frangivento' and 'Beledy'. The results showed high levels of oleic acid in the olive cultivars, ranging from 76.08% in 'Beledy' cultivar up to 80.72% in 'Coratina', whereas low levels of linoleic acid (2.30% to 3.41%) were obtained in 'Beledy' and 'Coratina' cultivars, respectively. Gas-chromatographic analyses highlighted that 'Coratina' cultivar appears to have the highest content of monounsaturated fatty acids (81.35 %) and polyunsaturated fatty acids (3.86 %). Moreover the oil of 'Coratina' had the highest amount of chlorophyll (9.51 mg kg⁻¹) and carotenoid (4.97 mg kg⁻¹). A clear and statistically significant difference ($p < 0.05$) was observed for the total phenol content within the cultivars. Total phenol of 'Zard' cultivar was the highest (181.63 mg kg⁻¹), while the lowest (148.42 mg kg⁻¹) was in the 'Frangivento' olive oil. Also intermediate content of total phenols was measured in 'Arbequina', 'Coratina' and 'Beledy' cultivars (152.29, 168.72 and 170.52 mg kg⁻¹, respectively). The radical scavenging activity of virgin olive oil from 'Zard' cultivar was higher (69.44% reduction) than that of other cultivars, while 'Arbequina' cultivar had the lowest (51.08% reduction). As all of the studied cultivars were grown in the same orchard with the same pedo-climatic condition, the results show that the studied analytical parameters were greatly influenced by the cultivar.

Keywords: Virgin olive oil, Olive cultivars, Pigments, Fatty acids

Introduction

The importance of virgin olive oil is related to its high levels of monounsaturated fatty acids (mainly oleic acid), and several antioxidants (Ocañoğlu et al., 2009). The oxidative stability, sensory quality and health properties of virgin olive oil stem from a prominent and well-balanced chemical composition (Bendini et al., 2007). The high content of oleic acid in olive oil serves to slow down penetration of fatty acids into arterial walls (Charbonnier, 1982). Oil with higher monounsaturated fatty acids (MUFAs) and lower saturated fatty acids (SFAs) are preferred because of the proven beneficial effect of MUFAs on serum cholesterol levels (Baccouri et al., 2008). Olive oil quality is related to the chemical composition of the oil, and its oxidative stability and sensory characteristics. These parameters are affected by olive cultivar (Vinha et al., 2005; Cerretani et al., 2006; Baccouri et al., 2007; Tura et al., 2007; Manai et al., 2008), climatic conditions (Vinha et al., 2005; Tura et al., 2007), ripening stage (Salvador et al., 2001; Beltran et al., 2005), irrigation management (Tovar et al., 2001) and the extraction system (Ranalli et al., 2001). Among these factors, the variety factor is undoubtedly one of the most important, but despite this, it is often ignored because the oil has been mixed with oils of different varieties or even because emphasis has been laid only on its place of origin (Lanteri et al., 2002). There are various local cultivars of olive in Iran which take up the most olive-growing area. 'Zard' alone, covering 64% of the olive-growing surface and oil production, is spread from the north to the south. Also many cultivars, such as 'Arbequina' (from Spain), 'Coratina' and 'Frangivento' (from Italy) and 'Beledy'

(from Syria) have been imported from the other countries. The oldest olive orchards and the first olive research station is located in north of Iran in Roudbar region. These orchards were used as mother stocks for producing olive plantlet for establishment of new production orchards. Few cultivars are grown commercially in Iran, while most of them have a local diffusion. Iranian government is planning to increase olive cultivation area from 80,000 to 600,000 ha (Omran-Sabbaghi et al., 2007). Establishment of new olive industry requires well-characterized cultivars with well-balanced oil chemical composition or cultivars with elite agronomic characteristics. Several authors have already studied the influence of genetic and agronomic factors on olive oil quality in some Spanish (Salvador et al., 2001) and Italian (Cerretani et al., 2006; Tura et al., 2007) cultivars. Nevertheless, there is scarce information available on the influence of cultivar on olive oil quality in the north of Iran (Hashempour et al., 2009). Almost all studies on Iranian virgin olive oil have focused on characterization of local varieties (Ramezani-Kharazi, 2008; Hashempour et al., 2009; Torkzaban et al., 2009). As a result, there is a lack of information on the chemical characteristics of several varieties that are imported from the other countries. As far as we know, little is known about the nature and/or the chemical composition of the oil of the Spanish, Italian and Syrian cultivars introduced in Iran and employed in olive research stations. Therefore, the aim of this experiment is to gain knowledge about some quality characteristics of the most important Iranian cultivar ('Zard') and foreign cultivars

Table 1. Description of olive oil studied samples (Mean values*).

Quality parameters	Cultivar				
	Zard	Coratina	Frangivento	Beledy	Arbequina
Free acidity (% oleic acid)	0.34 c	0.51 a	0.52 a	0.42 a	0.38 bc
Peroxide value (meq O ₂ kg ⁻¹ oil)	6.52 c	5.57 c	9.72 a	10.08 a	8.12 b
K ₂₃₂	0.60 d	0.90 c	0.99 b	1.22 a	0.83 c
K ₂₇₀	0.082 ab	0.079 b	0.093 a	0.084 ab	0.067 c
Chlorophyll (mg kg ⁻¹)	6.99 b	9.51 a	5.77 b	5.91 b	5.69 b
carotenoid (mg kg ⁻¹)	3.02 c	4.97 a	3.31 bc	3.37 cb	3.75 b
Ripening index**	3	3.2	3.1	2.8	2.9

*Means within a row followed by the same letter are not significantly different at $P < 0.05$.

**Ripening index was calculated according to method proposed by the International Olive Oil Council (IOOC, 1984).

(‘Arbequina’, ‘Coratina’, ‘Frangivento’ and ‘Beledy’) in the north of Iran during 2007-2008 crop years.

Materials and methods

Olive fruits samples

Olive fruits from five varieties grown in the olive orchard of Roudbar Olive Research Station located in north of Iran: ‘Zard’ from Iran, ‘Coratina’, ‘Frangivento’ from Italy and ‘Beledy’ from Syria were used according to their skin and pulp color (Ripening index) during 2006-2007. The olive ripeness index was calculated according to the method proposed by the International Olive Oil Council (IOOC, 1984). The climate of this region is of Mediterranean type with hot summers and mild winters having an annual average rainfall, which varies from 250 to 350 mm; the average annual temperature: 17°C (Iran Meteorological Organization 2009) and; coordinates of the experimental site is 48° 36' N 24° 29' E and asl 300 m.

Oil extraction

Olive fruits were crushed with a hammer mill and were slowly mixed for 30 min at 25°C. Then oil was extracted by centrifugation at 5000 rpm over 15 min without addition of water or chemicals. The obtained oil was transferred into a dark glass bottle and stored in dark at 4°C.

Determination of quality parameters

Free acidity (% oleic acid per 100 g olive oil), peroxide level (meq O₂ kg⁻¹ oil) and UV absorption characteristics (K₂₃₂ and K₂₇₀) were determined according to the European Community EEC Reg. 2568/91 (1991).

Determination of fatty acids composition

The fatty acids composition was determined as methyl esters by gas chromatography (GC) according to methods described in regulation of EEC 2568/91. Fatty acid methyl esters were prepared by vigorous shaking of a solution of each olive oil sample in n-hexane (0.2 g in 3 mL) with 0.4 mL 2 N methanolic potassium hydroxide solution. Chromatographic analysis was performed on a Hewlett Packard 5890N gas chromatograph equipped with a FID detector (Hewlett Packard, Palo Alto, CA, USA), using a fused-silica capillary column (30 m × 0.25 μm i.d. × 0.25 μm film thickness, HP Supelco, Inc., Bellefonte, PA, USA). The injector and detector temperatures were maintained at 220 °C and 260 °C, respectively; the oven temperature was set at 210 °C.

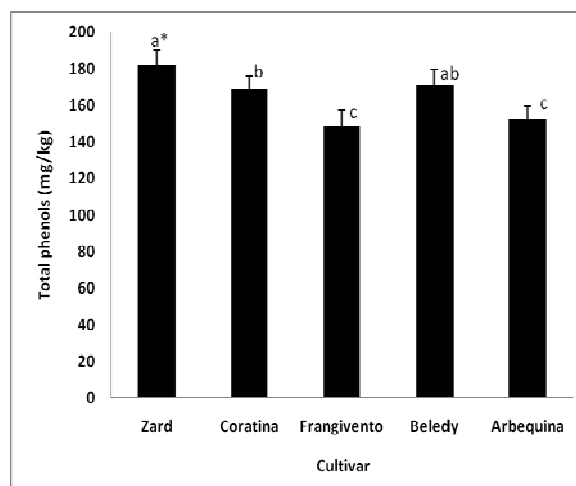


Fig 1. Total phenols of the virgin olive oils from five cultivars grown in Iran. * Different lettering show results significantly different at $P < 0.05$. Vertical bars show standard error

Helium was employed as the carrier gas with a flow rate of 1 mL/min according to the method of European Regulation 2568/91 (EEC, 1991). Fatty acids were identified by comparing retention times with those of standard compounds. Iodin value (IV) was calculated from the percentage of fatty acids (Maestri et al., 1998).

Determination of chlorophyll and carotenoid compounds

Pigments of chlorophyll and carotenoid were determined by the spectrophotometer (Jenway /6405 UV-England, 1997) using the method described by Minguez-Mosquera et al (1991): One g of the sample olive oil was dissolved in 10 ml of iso-octane solution. The solution absorption was read in the wavelength of 670 nm for chlorophyll and the absorption was read 470 nm for carotenoid. The results are expressed as milligram of carotenoid or chlorophyll per kilogram oil.

Total phenol content

The total phenol content of the olive oil extracts were determined according to Montedoro et al (1992) by the Folin–Ciocalteu spectrophotometric method (Jenway /6405 UV-England, 1997) at 765 nm, and results were expressed as milligram gallic acid per kilogram oil.

Table 2. Fatty acid composition of studied olive oils samples (Mean values*)

Fatty acid composition (%)	Cultivar				
	Zard	Coratina	Frangivento	Beledy	Arbequina
Palmitic acid	13.55 b	11.41 c	11.37 c	16.03 a	14.79 ba
Palmitoleic acid	1.14 b	0.53 c	0.83 c	1.94 a	1.7 1a
Stearic acid	2.62 ba	1.98 c	3.09 a	2.27 bc	1.99 c
Oleic acid	79.24 ab	80.72 a	80.42 ab	76.08 b	76.81ab
Linoleic acid	2.64 bc	3.41 a	3.05ba	2.30 c	2.48 c
Linolenic acid	0.36 b	0.45 a	0.42 ba	0.41 ba	0.40 ba
∑Saturated fatty acids	16.17 b	13.69 c	14.46 c	18.30 a	16.7 8b
∑Monounsaturated fatty acids	80.37 a	81.35 a	81.25 a	78.21 a	78.52 a
∑Polyunsaturated fatty acids	3 bc	3.86 a	3.48 ba	2.71 c	2.89 c
Iodine value (IV)	78.15 abc	80.62 a	79.83 ab	75.61 c	76.38 bc

*Means within a row followed by the same letter are not significantly different at $P < 0.05$

Radical scavenging capacity

The olive oil samples were examined for their capacity to scavenge the stable 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) (Kalantzakis et al., 2006). Of the oil solution in ethyl acetate (10%, wt/vol), 1 mL was added to 4 mL of a freshly prepared DPPH (10^{-4} M in ethyl acetate) solution in a 10 mL test tube. The reaction mixture was then shaken vigorously for 10 s in a Vortex apparatus, and the tube was maintained in the dark for 30 min, after which a steady state was reached. The absorbance of the mixture was measured by the spectrophotometer (Jenway /6405 UV-England, 1997) at 515 nm against a blank solution. A control sample (no oil) was prepared and measured daily. A refined olive oil (Minerva S.A. edible Oils, Shimatari, Viotia, Greece) devoid of pro-oxidants/antioxidants was used for comparison.

The radical scavenging activity toward DPPH was expressed as the % reduction in DPPH concentration by the constituents of the oils: % [DPPH] red = $100 \times (1 - [DPPH]_{30} / [DPPH]_0)$, where $[DPPH]_0$ and $[DPPH]_{30}$ were the concentrations of DPPH in the control sample ($t = 0$) and in the test mixture after the 30 min reaction, respectively.

Statistical analysis

The data were evaluated in the form of complete by randomized block design, with five blocks (replications) each containing nine trees, using SAS software (version 9.1). Samples were a mixture of six tree fruits. The results were displayed in mean values with standard deviation ($n=3$). Significance of the differences among the numbers was determined by the variance analysis using Duncan's multiple range tests (Steel and Torrie., 1982). The level of significance was determined as $p < 0.05$.

Results and discussion

Free acidity, peroxide value and UV spectrophotometric indices

Quality parameters of olive oils from the studied cultivars are shown in Table 1. Statistically significant differences were obtained in free acidity, peroxide value, K_{232} and K_{270} between oils from the studied cultivars ($p < 0.05$). The maximum level of free acidity was in 'Frangivento' (0.52%) while 'Zard' had the lowest free acidity (0.10%).

The relatively lower free acidity observed in tested samples, is due to the use of only healthy fruits and typically small scale of system used for the processing. The content of peroxide value from 'Beledy' and 'Frangivento' cultivars were significantly higher (10.08 and 9.72 meq O_2 kg^{-1} , respectively) than other cultivars and 'Coratina' olive oil showed significantly the lowest (5.57 meq O_2 kg^{-1}). This behavior can be explained by a difference in the activity of the enzyme lipoxygenase in these cultivars. These results are in agreement with the results of Ramezani-Kharazi (2008) on 'Zard', 'Roghani', and 'Shengeh' grown in Iran, that reporting the content of free acidity and peroxide value in oils were affected by the olive cultivars. Concerning K_{232} , relatively low levels were observed in the analyzed samples with the highest values observed in 'Beledy' (1.22), whereas the lowest found in 'Zard' (0.60 %). 'Frangivento' cultivar had the highest level of K_{270} (0.093), while was measured the lowest (0.069) in 'Arbequina'. These results are in agreement with those of other authors (Salvador et al., 2001; Baccouri et al., 2008). Based on the above results, analyzed oil of all samples showed very low values of the evaluated physicochemical parameters (acidity $\leq 0.8\%$; peroxide value ≤ 20 meq O_2 kg^{-1} ; $K_{270} \leq 0.22$; $K_{232} \leq 2.5$), i.e. all of them falling within the ranges established for "extra virgin olive oil" category, as required by Regulation of EC/1989/2003 (EEC, 2003). These high quality indices are translated into a high quality of oil which could be due to the use of healthy fruits and typically small scale of system used for the processing procedures.

Fatty acid composition

As shown in Table 2, many fatty acids were detected in the virgin olive oils of the cultivars. Palmitic and oleic acids were considered as major fatty acids while palmitoleic, stearic, linolenic and linoleic acids were low. Results illustrate a significant difference in the content of these acids in different cultivars ($p < 0.05$). The five analyzed virgin olive oils showed fatty acid composition (Table 2) in compliance with established limits (EEC, 2003) and with ranges depending on the cultivars except for linoleic acid. The level of linoleic acid was lower than established limits (EEC, 2003). The level of palmitic acid was observed at the lowest value in 'Frangivento' olive oil (11.37%) and the highest value of palmitic acid was observed in 'Beledy' (16.03 %). Oleic acid, the major

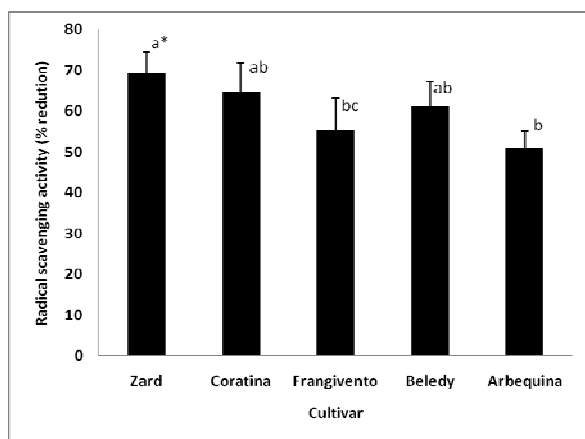


Fig 2. Radical scavenging activity of the virgin olive oils from five cultivars grown in Iran. *Different lettering show results significantly different at $P < 0.05$. Vertical bars show standard error.

monounsaturated fatty acid, showed also a wide variability depending on the cultivars. The highest content of oleic acid was observed in 'Coratina', (80.72%), while it was the lowest in 'Beledy', (76.08%). 'Arbequina', 'Frangivento' and 'Zard' showed relatively high levels (76.81, 80.42 and 79.24, respectively). The lowest percentage of Linoleic acid was observed in 'Beledy' (2.3%) whereas the highest value was observed in 'Coratina' (3.41%). The increase in oleic acid content is due to the triacylglycerols active biosynthesis which takes place throughout fruit ripening, involving a fall in the relative percentage of palmitic acid content. On the other hand, the increase in linoleic acid content is due to the transformation of oleic acid into linoleic acid by the oleate desaturase activity which is active during triacylglycerol biosynthesis (Gutiérrez et al., 1999; Sanchez and Harwood., 2002). These differences in level of palmitic, oleic and linoleic acids could be explained by the differing activities of the enzymes during triacylglycerol biosynthesis among studied cultivars. In contrast with this study, the level of linoleic acid in the other cultivars grown in the north of Iran (Ramezani-Kharazi., 2008) and reports of other countries (Baccouri et al., 2008; Manai et al., 2008) were higher than these values. These differences might be due to the genetical and environmental factors or ripening indices. The minor fatty acids components of the virgin olive oils, i.e. palmitoleic, stearic, and linolenic acids, also showed significant differences among five olive oils studied ($p < 0.05$). The oil of 'Beledy' cultivar had the highest level of palmitoleic acid (1.94%) and the oil of 'Coratina' cultivar had the lowest level of palmitoleic acid (0.53%). The highest content of stearic acid as important saturated fatty acid were found in 'Frangivento' oil (3.09%) and the lowest were found in 'Coratina' (1.98%) compared with 'Arbequina', 'Beledy' and 'Zard' cultivars (1.99%, 2.27%, 2.62%, respectively). The oil of 'Coratina' cultivar had the highest level of linolenic acid (0.45%), and 'Zard' had the lowest level of linolenic acid (0.36%). The percentage of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) in the studied cultivars were also evaluated. 'Beledy' oil was rich in total SFAs (18.30 %) due to its higher content of palmitic acid which represents the major SFAs fraction. The total MUFAs showed no significant difference among cultivars. 'Coratina' cultivar

had the highest level of MUFAs (81.35%) and the oil of 'Beledy' cultivar had the lowest level of MUFAs (78.21%). 'Coratina' oil was rich in total PUFAs (3.86%), due to its higher content of linoleic acid whereas 'Beledy' oil had the lowest PUFAs (2.71%), due to its lower content of linoleic acid. Variations in fatty acid composition observed in olive oil samples (Table 2) might be related to both genetic factors and environmental conditions during the development and maturity of the fruit. These results are in accordance with the results of other researches (Baccouri et al., 2007; Manai et al., 2008; Ramezani-Kharazi, 2008). They noted that several agronomic parameters could change the fatty acid composition of olive oil. The most studied aspects include cultivar and origin, fruit ripening, and pedoclimatic conditions. The oil of 'Coratina' cultivar had the highest Iodine value (IV) (80.62%) because of its high contents of oleic acid representing the major fatty acid component of that fraction. The lowest Iodine value (75.61%) was observed in 'Beledy' cultivar, due to its lower content of oleic acid.

Pigment contents ($mg\ kg^{-1}$)

The natural pigment contents of the oils are important as the quality parameters because they correlate with colour and play a key role as a factor of sensorial acceptability among consumers. Virgin olive oil presents a colour range from green–yellow to golden, depending on the variety and the stage of maturity (Salvador et al., 2000). As shown in Table 1, significant differences among cultivars ($p < 0.05$) were also observed in pigment contents. Chlorophylls and carotenoids ranged, respectively from 5.69 to 9.51 $mg\ kg^{-1}$ and from 3.02 to 4.97 $mg\ kg^{-1}$ for all of the studied cultivars. These results showed that significant differences among cultivars ($p < 0.05$) were also observed in pigment contents. These findings are in agreement with the results of Psomiadou and Tsimidou (2001). Moreover Giufrida et al., (2007) reported that the presence of the pigment in the oil depends on several factors, such as the cultivar, soil and climatic conditions, fruit ripeness and the processing procedures.

Total phenol content ($mg\ kg^{-1}$)

Virgin olive oil is well known for its high content of phenolic substances, amount of oleic acid and tocopherols that are thought to have health-promoting properties (Owen et al., 2000). As seen in Fig. 1, significant differences among olive oil samples of different varieties were observed ($p < 0.05$). Among them, 'Zard' had the highest total phenol content (181.63 $mg\ kg^{-1}$), while 'Frangivento' olive oil had the lowest (148.42 $mg\ kg^{-1}$). Also 'Arbequina', 'Coratina', and 'Beledy' cultivars had intermediate content of total phenols (152.29, 168.72, 170.52 $mg\ kg^{-1}$, respectively). It is generally accepted that the level of phenolic compounds is varied in oils obtained from different cultivars and sites. Vinha et al. (2005) showed that discrimination between olive oil samples with the same geographical origin and different cultivars was possible. In another study by Cerretani et al., 2006, the phenolic composition was found to be not useful in discriminating the olive oil samples due to the fact that the phenolic content of oils was affected not only by the olive cultivars, but also by the climatic and environmental conditions, agronomic practice and the technological

process. In this study, olive fruits were harvested about at the same time from the same olive orchard, where the trees were subjected to similar agronomic procedures. Furthermore, olive oils were extracted by the similar process. For these reasons, it seems that difference in the content of total phenol among studied cultivars in this study is due to different genetically responses of these cultivars to same environment conditions or the effects of harvest period. Total phenol content of the samples in this study could be considered as medium-high levels in accordance with previous reports (Aparicio et al., 1999; Psomiadou and Tsimidou., 2001; Cerretani et al., 2006; Ocakoglu et al., 2009).

Radical scavenging activity (%reduction)

Results of this study showed significant ($p < 0.05$) differences in radical scavenging activity in the oils obtained from the studied cultivars. It can be seen from Fig. 2 that the radical scavenging activity of virgin olive oil in 'Zard' cultivar was higher (69.44%reduction) than that of other cultivars, while 'Arbequina' cultivar had the lowest (51.08% reduction) radical scavenging activity. This activity is necessarily due to higher phenol level in 'Zard' cultivar and lower phenol level in 'Arbequina' cultivar (Fig. 1). This result is agreement with other researchers (Kalantzakis et al., 2006).

Conclusion

Based on the results of this study, analyzed oil of all samples fall within the ranges established for "extra virgin olive oil" category. The results here showed high levels of oleic acid and low levels of linoleic acid in studied cultivars. The content of total phenol and radical scavenging activity were the highest in 'Zard' cultivar. The results also highlighted that the analytical parameters were influenced by the genetic characteristic, namely cultivar. Interestingly, 'Coratina' and 'Zard' are good cultivars for plantation in Roudbar region and other regions with similar climatic conditions in order to produce of olive oils with high quality.

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