Physicochemical changes in olive oil (cv. Koroneiki) due to fruit ripening and extraction method

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Abstract

This study was conducted to find the effects of fruit ripening and extraction method on the quantity and quality of the Koroneiki olive oil. The oil samples extracted in six ripening stages were used for further quality analysis. Quality assessment of the oil was conducted according to the standard methods and the composition of fatty acids was measured by gas chromatography. The results showed that there were some significant differences in the percentage of oil among different fruit tissues and harvesting times. In all tissues, the percentage of oil in dry matter increased significantly from 12 Oct to 4 Jan. The mesocarp and seed had significantly higher percentages of oil compared to endocarp. The extraction method and harvesting time had some significant influences on peroxide value. Furthermore, the extraction method showed a significant effect on the refractive index, but not on the other quality traits studied. The oil extracted using soxhlet had higher peroxide value, but lower refractive index compared to the oil extracted by centrifugation. The harvesting time showed some significant effects on the percentage of free fatty acids, K232 value, K270 value and the amounts of total chlorophylls and total carotenoids. There were a higher percentage of free fatty acids in the oil samples produced in later harvesting times. The oil extracted on 1 Mar showed the highest percentage of free fatty acids. On the other hand, the oil samples obtained from earlier harvesting times had higher values of K232 and K270 and higher amounts of total chlorophylls and carotenoids. The results showed an increase in the percentage of oleic acid and linoleic acid during the harvesting times; while, there was a slight decrease in the percentages of other fatty acids studied.

Key words: fatty acids; fruit tissues; *Olea europaea*; physicochemical traits; chlorophylls; carotenoids

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Introduction

Olive (*Olea europaea* L.) is one of the most ancient fruit trees and has been cultivated for its oil in the countries surrounding the Mediterranean Sea for thousands of years (Bertrand, 2002). Olive oil is a natural fruit juice, obtained from the fruit, with a unique composition and quality. Moreover, olive oil is one of the very few oils that can be consumed in its natural form, thus preserving all its natural elements. Olive oil is a key component of the

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traditional Mediterranean diet, which is believed to be associated with a relatively long life and good health. Consumption of olive oil has also increased outside the Mediterranean basin due to the tendency of consumers to buy least-processed foods.

The fatty acid composition of olive oil has attracted much attention due to its valuable influence on human health. The cancer rates among the Mediterranean regions are significantly lower than in the rest of the world. This has been attributed to the high dietary intake of olive oil (Trichopoulou, et al. 2000). Several case studies among Mediterranean populations concluded that the risk of breast cancer was lower for women who frequently consumed olive oil (Landa, et al. 1994; La Vecchia, et al. 1995).

Olive oil quality is influenced by a number of factors such as harvest period, extraction procedure, production area and cultivar (Ranalli, et al., 2001; Ben Temime, et al., 2006; Baccouri, et al., 2007; Salvador, et al., 2007). Several metabolic processes take place in olive fruits during ripening, which are reflected in the quality traits and nutritional value of the extracted oil. The majority of olive oil produced is not of the best quality, as the fruit has not been picked at the optimal harvesting time (Beltrán, et al., 2005).

The aim of this study was to investigate the effect of ripening stage on oil accumulation in different fruit tissues and the influence of fruit ripening and extraction method on oil quality in olive cultivar Koroneiki.

Materials and Methods

This study was conducted in season 2013-2014 in a completely randomized design with three replications. The fruit samples were collected from an olive collection located in Golestan province, north of Iran. The fruits were harvested in six ripening stages every three weeks from 12 Oct to 4 Jan and then 8 weeks later on 1 Mar. All the trees were in good physiological condition and grown with supplementary irrigation and fertilization.

The oil was extracted using the soxhlet method and the percentage of oil was recorded in both dry and fresh matters in all harvesting times and in three different fruit tissues including mesocarp, endocarp, and seed, separately. Since the oil extracted using this method was under the influence of heating during the procedure, the oil was also extracted using centrifugation method but only from the whole fruit. For this, the fruit samples were processed by a standard discontinuous procedure in which the fruits were crushed with a hammer crusher, malaxed for 30 min at 24 °C and the oil was extracted by centrifugation at 5000 rpm. The oil from both extraction methods was used for further quality analysis.

Quality assessment of the oil was conducted only in four last harvesting times. Refractive index was recorded at 20 °C according to AOCS (1989) method. Peroxide value was measured using the method suggested by Mania-Djebali et al. (2012). Free fatty acids were estimated according to the procedure described by Boskou (1996). The value of K232 and K272 were estimated through the procedure of Boskou (1996). Total chlorophylls and carotenoids were recorded by the method of Minguez-Mosquera et al. (1998). The composition of fatty acids was measured by gas chromatography (YL6000) using the method suggested by Commission Regulation 702/07 (2007).

All the mentioned traits were measured in three replicates. Two-way analysis of variance was performed and the means compared by least significant difference (LSD). Data were suitably transformed to satisfy the assumptions of normality and constant variance prior to analysis. Data processing was performed using SAS software (Version 9.1).

Results

The analysis of variance showed that there were some significant differences ($P< 0.001$) in the percentage of oil in dry matter among different fruit tissues and harvesting times (Fig. I). The results showed that in all tissues the percentage of oil increased significantly from Oct 12 to Jan 4. Two last harvesting times, including Jan 4 and Mar 1 were not different significantly in accumulating oil in all tissues. In the mesocarp, for example, the percentage of the oil had an increase from 35.52% on Oct 12 to 61.16% on Mar 1. Similarly, in endocarps and seeds, the lowest

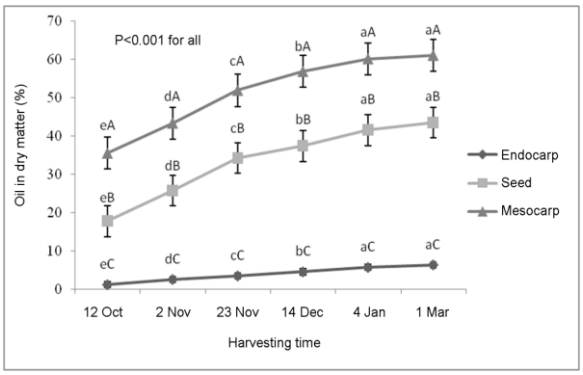

Fig. I. The percentage of oil in dry matter of different fruit tissues and harvesting times in olive cultivar Koroneiki.; Different lower-case and capital letters represent significant differences at P≤0.01 among the harvesting times and fruit tissues, respectively.

percentage of oil was seen in the first step and the highest percentage of oil was recorded on Jan 4 and Mar 1, respectively when the oil accumulation in the whole fruit were stable. The results of this study also indicated that there were significant differences in the percentages of oil among the different tissues of the fruit in all harvesting times (Fig. I). In all harvesting times from Oct 12 to Mar 1, the mesocarp had the highest percentage of the oil and the endocarp had the lowest one.

The analysis of variance also showed that there were some significant differences (P< 0.001) in the percentage of oil in fresh matter among different fruit tissues and harvesting times (Fig. II). The results indicated that the percentage of oil increased in the endocarp, seed, and mesocarp during the harvesting times from Oct 12 to Mar 1

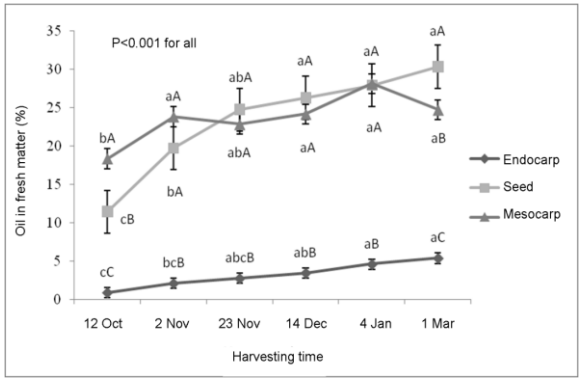

Fig. II. The percentage of oil in fresh matter of different fruit tissues and harvesting times in olive cultivar Koroneiki; Different lower-case and capital letters represent significant differences at P≤0.01 among the harvesting times and fruit tissues, respectively.

though the differences among some harvesting times were not statistically significant. The percentage of oil increased in the mesocarp from Oct 12 to Nov 2, after which that it did not change until the last harvesting time. According to the results (Fig. II) there were some significant differences in the percentage of oil among the mesocarp, seed, and endocarp of the fruits. The percentage of oil in the mesocarp was higher than seeds at the first harvesting time (Oct 12) while it was lower in the last harvesting time (Mar 1). Both tissues had the same percentage of oil from Nov 2 to Jan 4. The endocarp had the lowest percentage of oil in all harvesting times comparing to the mesocarp and seed.

The study showed that harvesting time did not have any significant effect on the refractive index. The results of the present study also revealed that the extraction method had a significant influence on the refractive index of the extracted olive oil (Table 1) and the centrifugation method had a higher refractive index as compared to soxhlet method. In both methods and at all harvesting times, the refractive index was within the limits established by International Olive Oil Council (1.4677-1.4705) (IOOC, 2012). There were some statistically significant differences in peroxide value of the oils between the extraction methods (P=0.04) and also among the harvesting times (P=0.03). The peroxide value of the oil extracted by soxhlet was more than the oil extracted by centrifugation method. According to the results, the highest peroxide values were observed at two first harvesting time.

The extraction method did not have any significant influence on the other physiochemical traits including the percentage of free fatty acids, K232 and K270 values, and the amounts of total chlorophylls and carotenoids while the harvesting time showed significant effects on all these traits. Also the results indicated that there were higher percentages of free fatty acids in the oil samples produced at later harvesting times. The oil extracted from the fruits harvested on Mar 1 showed the highest percentage of free fatty acids (0.69%). According to the results of this paper, the percentage of free fatty acids did not exceed the upper limit established by IOOC (0.8%). The oil samples obtained from earlier harvesting times had a higher value of K232 and K270.

Table 1
The influence of harvesting time and extraction method on some physiochemical traits of olive oil (cv. Koroneiki)

Treatment	Refractive index (at 20 °C)	Peroxide value (meq/kg)	Free fatty acids (%)	K232 value	K270 value	Total chlorophylls (mg/kg)	Total carotenoids (mg/kg)
Harvesting time	P = 0.3	P = 0.03	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P = 0.004
23 Nov	1.469	8.35 ab	0.43 c	0.0252 a	0.0359 a	1.641 a	0.980 a
14 Dec	1.469	8.96 a	0.57 b	0.0251 ab	0.0081 b	1.080 b	0.796 b
4 Jan	1.469	7.18 b	0.58 b	0.0249 ab	0.0112 b	0.857 c	0.680 bc
1 Mar	1.470	7.50 b	0.69 a	0.0247 b	0.0046 b	0.843 c	0.568 c
Extraction Method	P = 0.03	P = 0.04	P = 0.2	P = 0.09	P = 0.8	P = 0.3	P = 0.6
Centrifugation	1.470	7.54	0.56	0.0248	0.0156	1.078	0.737
Soxhlet	1.469	8.46	0.58	0.0251	0.0143	1.135	0.771

Different letters within each column represent statistical differences at $P \leq 0.01$, LSD test.

The oil samples extracted from earlier harvesting times had also higher amounts of total chlorophylls and carotenoids. Total chlorophylls, for instance, was higher on Nov 23 (1.641 mg/kg) and lower on Jan 4 and Mar 1 (0.857 and 0.843 mg/kg, respectively). The interaction between the harvesting time and the extraction method was not significant in all traits studied.

Oleic acid was the major fatty acid found in all olive oils studied (Fig. III. A). The results showed an increase in the percentage of oleic acid during the fruit ripening. Similarly, the percentage of linoleic acid increased from Nov 23 to Mar 1 (Fig. III. B) while there was a slight decrease in the percentages of linolenic acid during this time (Fig. III. C). Changes observed from Dec 14 to Mar 1 in the percentages of oleic and linoleic acids during ripening led to a gradual decrease in oleic/linoleic acid ratio. According to the results presented here, in both saturated fatty acids, i.e., palmitic acid and stearic acid, there was a slight reduction during the harvesting times (Figs. III. D and E).

The study showed that there was no significant difference in the percentages of oleic, linolenic, palmitic, and stearic acid between the two extraction methods (Table 2). In contrast, the extraction method revealed a significant influence (P=0.029) on the percentage of linoleic acid and this fatty acid was higher in the soxhlet method.

Discussion

The results of this study showed that the percentage of oil in dry matter of all tissues increased significantly during the harvesting times though the two last harvesting times were not different significantly. Similarly, the results indicated that the percentage of oil in fresh matter increased in the endocarp, seeds, and mesocarp during the harvesting times. The percentage of oil increased in the mesocarp until Nov 2, but after that it did not change. These changes may be due to losing water from mature fruits at that time of

Table 2
The influence of extraction method on the percentage of major fatty acids in olive oil (cv. Koroneiki)

Fatty acid	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Palmitic acid (%)	Stearic acid (%)
Soxhlet	77.67	5.99	1.09	11	2.49
Centrifugation	78.83	5.18	1.1	10.6	2.45
P-value	0.201	0.029	0.886	0.686	0.343
Limits accepted [a]	55-83	3.5-21	< 1	7.5-20	0.5-5

[a]: IOOC

the growing season (Inglese et al., 1994). Previous studies showed that the percentage of oil increases during early fruit ripening and decreases slightly as fruit becomes overripe (Salvador et al., 2001). The results of this study confirm the findings of Lavee and Wonder (2004). They showed that the percentage of oil has been increased from September to December in cultivars Barnea and Manzanillo. Connor and Fereres (2005) reported that at normal harvesting time for oil production, the percentage of oil in the seed (27%) was a little lower than in the mesocarp (30%). These results agree with the findings of the present study.

The study showed that in both methods and at all harvesting times, the refractive index was within the limits established by International Olive Oil Council (IOOC) (1.4677-1.4705) (IOOC, 2012). The peroxide value of the oil extracted by soxhlet was higher than the centrifugation method. These results are in agreement with those of Matos et al. (2007) and Baccoury et al. (2007). In all oil samples, the peroxide value was less than 20; therefore, they could be classified as virgin olive oil according to the limits established by IOOC (IOOC, 2012).

The results indicated that there were a higher percentage of free fatty acids in the oil samples produced at later harvesting times. This is in agreement with previous studies which reported that as ripening progressed, an increase was observed in free acidity in different cultivars, such as Arbequina (Garcia et al., 1996), Picual (Gutierrez et al., 1999), and Correggiolo (Rotondi and Magli, 2004). The relatively low free acidity observed was due to the healthy fruits sampled and to their rapid processing. On the other hand, the oil samples obtained from earlier harvesting times had a higher value of K232 and K270. These results are in accordance with the results reported by Matos et al. (2007) for other cultivars such as Madural and Verdeal. Compounds of secondary oxidation (aldehydes, ketones etc.) contribute to K270, but compounds of oxidation of the conjugated dienes contribute to K232 (Kiritsakis et al. 2002). The K232 and K270 values were within the limits accepted by IOOC (K232 less than 2.5 and K270 less than 0.22) (IOOC, 2012).

The results of present study also showed that the oil samples extracted from earlier

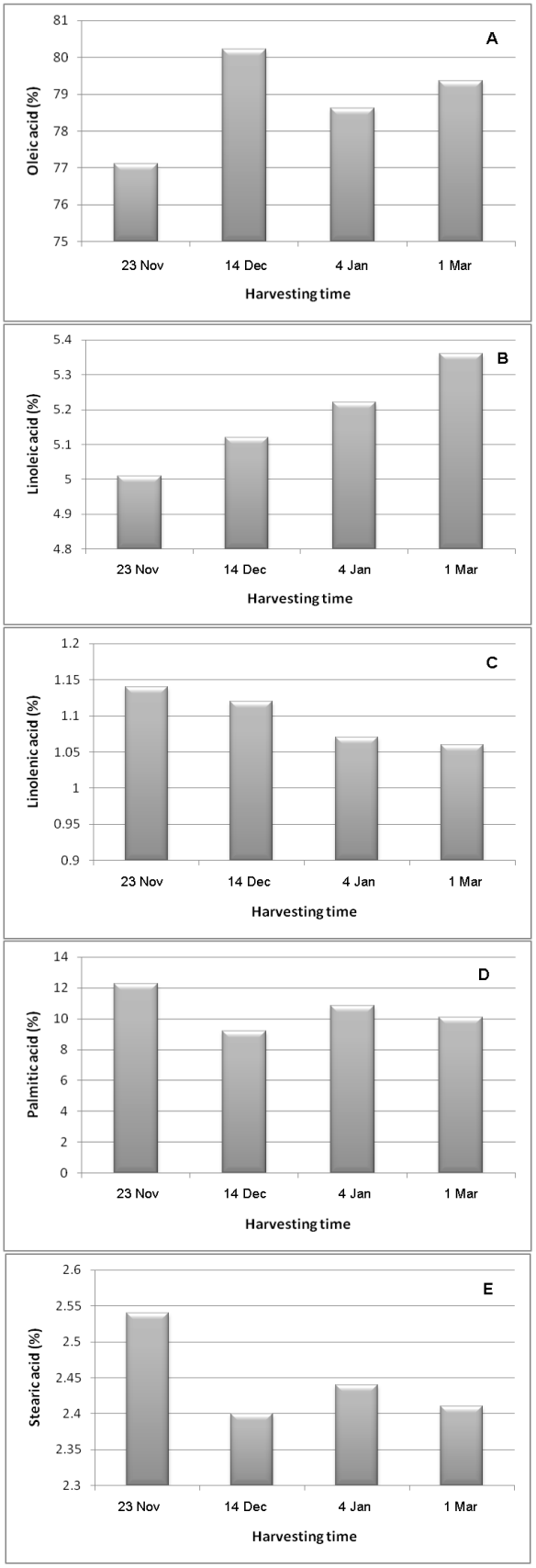

Fig. III. The influence of harvesting time on the percentage of major fatty acids in olive oil (cv. Koroneiki)

harvesting times had also higher amounts of total chlorophylls and carotenoids. These results agree with other reported data (Minguez-Mosquera et al., 1990; Beltran et al., 2005; Baccoury et al., 2007). Both total chlorophylls and carotenoids are considered to have an important role in the quality of oils, largely because of their action as photo-sensitizers (Cert, et al. 2000). In addition to their antioxidant activities, these pigments are responsible for the color of the oils, which influences their selection by consumers.

Fatty acids are the most important components in olive oil. There are only a few types of fatty acids in olive oil, but the proportions of each influence the the oil characteristics. Unsaturated fatty acids are considered to be the healthiest fatty acids and have great values in a healthy diet (Unal and Nergiz, 2003). These fats have various health benefits when they replace saturated fats in the diet. The fatty acid profile is an important parameter in the shelf life of olive oil and is affected by two major factors: the cultivar and the ripening stage (Aparicio and Luna, 2002). The most important unsaturated fatty acids in olive oil are the monounsaturated oleic acid, the polyunsaturated linoleic acid, and the polyunsaturated linolenic acid. On the other hand, the major saturated fatty acids in olive oil are palmitic acid and stearic acid.

High intake of oleic acid was reported to be the reason for the decrease in the rates of coronary artery disease in the Mediterranean countries (Martinez-Gonzalez and Sanchez-Villegas, 2004). The results showed an increase in the percentage of oleic acid and linoleic acid but a slight decrease in the percentages of linolenic acid. Changes observed during ripening led to a gradual decrease in oleic/linoleic acid ratio, which confirms the findings of Rotondi et al. (2004). All fatty acids found in the oil samples were within the limits established by IOOC for virgin olive oil except for linolenic acid which exceeded the upper limit slightly (IOOC, 2012).

In conclusion, the results showed that there were some significant differences in the percentage of oil in dry and fresh matter among different fruit tissues and harvesting times. The harvesting time and extraction method had a significant influence on peroxide value. The extraction method also showed a significant effect on the refractive index but not on the other quality traits studied. In fatty acid profile, the extraction method had a significant effect on linoleic acid but not on the other fatty acids. In contrast, the harvesting time had some significant effects on all other traits except the refractive index. The best harvesting time is considered when the fruit mesocarp has a perfect fatty acid balance. As olives overripe, the ratio of oleic acid to linoleic acid decreases considerably, due to an increase of linoleic acid (Rotondi et al., 2004). Consequently, according to the findings of this study, the best time to harvest Koroneiki olive in Northern Iran is mid-Dec. More studies are needed to understand whether the oil extracted from various fruit tissues have different physiochemical traits or not.

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