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Evaluation of changes in olive oil quality indicators and fatty acid profile in response to harvest delay

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ABSTRACT

The quality of olive oil is influenced by its fatty acid composition and the concentration of phenolic compounds. This study was conducted to investigate the effect of harvesting time on the quality characteristics and fatty acid profile of olive oil from the Arbequina cultivar, which is one of the predominant varieties in the Rudbar region of Iran. The experiment was arranged in a completely randomized design with three different harvesting times: mid-November, late November, and mid-December. In this research, acidity, peroxide value, anisidine value, TOTOX index, phenolic compound concentration, and type and content of fatty acids were determined. The results showed that acidity, peroxide value, anisidine value, and TOTOX index increased significantly with delayed harvesting, while the phenolic compound concentration decreased from 2.20 to 1.40 mg GAE/kg oil. In contrast, by the end of the harvesting season, the levels of palmitic acid and oleic acid increased significantly. Moreover, the ratio of oleic acid to linoleic acid showed a significant increase from 3.89 to 5.70. Therefore, harvesting in late November is recommended to obtain olive oil with desirable quality characteristics, an acceptable level of phenolic compounds, and an optimal balance between chemical properties and oxidative stability.

1- Introduction

The olive fruit, scientifically known as Olea europaea, is an oval-shaped drupe composed of two main parts: the pericarp and the endocarp. The pericarp consists of the epicarp (skin) and the mesocarp (pulp or flesh), accounting for approximately 65% to 83% of the total fruit weight. The endocarp, which includes the stone or pit, comprises about 13% to 30% of the total fruit weight. The chemical composition of olive fruit includes approximately 50% water, 1.6% protein, 22% oil, 19.1% carbohydrates, 5.8% cellulose, and 1.5% ash. Other important constituents include pectins, organic acids, pigments, and phenolic glycosides. During the growth stages, the fruit weight increases until mid-November. Thereafter, the weight begins to decrease due to moisture loss. Consequently, the oil content of the fruit generally increases from October to December. Oil accumulation starts between late July and early August. In autumn and winter, the fruit turns black and its oil content reaches the maximum level [1]. Olive ripening is accompanied by fruit enlargement and characteristic changes in skin color, which in many cases follow a four-stage sequence: green, light green, purple, and reddish-purple pigmentation. As ripening progresses across the entire fruit surface, the degree of darkening gradually increases until the fruit eventually becomes completely black. Olive oil is considered one of the most valuable vegetable oils due to its nutritional composition and health benefits, as it contains approximately 76% to 85% unsaturated fatty acids. The chemical composition and fatty acid profile of olive oil are influenced by several factors, including harvesting time and the geographical region of cultivation. Recent studies indicate that these two factors significantly affect oil quality, oxidative stability, and the content of phenolic compounds and unsaturated fatty acids [2]. Natural olive oil is produced using extraction systems such as pressing, centrifugation, and percolation systems [3]. The major components of olive oil include triglycerides, diglycerides, and free fatty acids, while the minor components consist of pigments, tocopherols, and phenolic compounds. Increased acidity is associated with the activity of endogenous lipase enzymes, which hydrolyze triglycerides and release free fatty acids [4]. In general, harvesting time (early or late harvest), olive cultivar and plant genetics, climatic and environmental conditions including temperature, light, rainfall, and soil, as well as the ripening index and oil content of the fruit, are considered key factors affecting the fatty acid profile of olive oil. In addition, harvesting time and postharvest storage conditions of olives are important factors that significantly influence not only the oil yield but also its quality. Compared with other vegetable oils, the quality of olive oil depends on the balanced composition of fatty acids (especially unsaturated fatty acids) and the presence of phenolic and aromatic compounds (such as aldehydes and six-carbon alcohols), and is directly related to the harvesting stage of olive fruits. Climatic and regional conditions are among the factors responsible for differences in harvesting time across various regions. Furthermore, harvesting time is one of the key determinants of olive oil quality. As the fruit matures, the oil content increases; however, the optimum stage in terms of fatty acid composition is usually achieved before the maximum oil content is reached. This is because lipid oxidation can be affected by numerous internal and external factors, including fatty acid type and concentration, antioxidant activity, exposure to light, air temperature, and contact with oxygen. Lipid oxidation during storage not only reduces the nutritional quality of food but also generates oxidation products such as free radicals, which may lead to undesirable chemical reactions [5]. Studies have shown that early harvesting (green-purple stage) is associated with higher retention of phenolic and antioxidant compounds, whereas late harvesting (black stage) results in increased oil percentage but lower quality due to oxidation and reduced polyphenol content [6]. The most important phenolic compounds in olives include phenolic acids, phenolic alcohols, flavonoids, secoiridoids, and lignans, whose antioxidant activity increases with increasing concentration [7]. A study on superior olive cultivars from the Gorgan region (Mission, Belidi, and Roghani), harvested from mid-September to early January, showed that the oil content exhibited an increasing trend until December. Acidity also increased gradually at different harvesting times and rose more sharply from early December onward. Therefore, considering the changes in oil percentage and acidity, the optimum harvesting time for these cultivars was determined to be early December [8]. An investigation of the fatty acid profile in two types of olive oil, namely virgin and refined olive oil, demonstrated a close relationship between air temperature and the composition of certain fatty acids. Higher temperatures resulted in an increase in linoleic acid content and a decrease in oleic acid levels [9]. This study was conducted to evaluate the changes in fatty acid profiles and quality-related compounds of olive oil at different harvesting times.

2-Materials and Methods

To investigate the effect of olive harvesting time on phenolic content, oxidative stability, and the fatty acid profile of olive oil, an experiment was conducted based on a completely randomized design with three replications. For this purpose, olives of the Arbequina cultivar (a widely cultivated cultivar in the Rudbar region) were used in one of the

The oil extraction process was carried out in one of the modern factories of the county, and the samples were collected at three different harvesting times and transferred to the oil laboratory. The treatments included harvesting the first sample at the beginning of the oil extraction season (mid-November), the second sample in the middle of the oil extraction season (late November), and the third sample in mid-December. All chemicals used in the experiments were supplied by Merck.

2.1. Sample Preparation and Oil Extraction

Olives were harvested manually by workers and transported without any damage in perforated plastic baskets to the processing factory. The oil extraction process began within a maximum of 7 hours after harvesting. At the beginning of the process, cleaning and removal of twigs, leaves, and other debris accompanying the fruits were carried out mechanically. The olives were then transferred to a washing tank and cleaned thoroughly with fresh water to remove dust and soil particles.

After washing, the olives were drained using perforated vibrating conveyors, then dried and crushed. Subsequently, the olive paste was heated at 32°C for 2.5 hours to facilitate oil extraction and then transferred to a decanter to remove solid materials and a large proportion of the water content from the paste. Pure oil was finally obtained using a centrifuge. After this stage, oil samples were collected and transferred to the laboratory in dark glass containers [1].

2.2. Chemical Analyses and Oxidative Stability of Olive Oil

Peroxide value was determined according to the Iranian National Standard No. 4179; acidity was measured based on Iranian National Standard No. 4178; anisidine value was determined according to Iranian National Standard No. 4093; and the Totox index was calculated as the sum of twice the peroxide value and the anisidine value for the oil samples extracted from olive fruits [10,11].

2.3. Identification and Determination of Olive Oil Fatty Acids

Fatty acid profile analysis was carried out according to Iranian National Standards No. 4090 and 4091. For the identification and quantitative determination of fatty acids, fatty acid methyl esters (The fatty acids were then analyzed using a Varian CP-3800 gas chromatograph equipped with a flame ionization detector (FID) and a 50 m BPX-70 capillary column packed with diethylene glycol succinate. The following fatty acids were identified and quantified: palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3). Their contents were expressed as percentages of the peak area. Helium was used as the carrier gas. The column temperature was programmed from 160°C to 210°C. The detector temperature was set at 230°C, the injector temperature at 250°C, and the injection volume was 1 µL [12].

2.4. Determination of Oil-Soluble Polyphenols

The concentration of polyphenols in the oil was estimated using an alcoholic extract and the Folin–Ciocalteu reagent. For each extracted alcoholic sample, 0.1 to 0.4 mL was transferred into a 10 mL volumetric flask. Then, 5 mL of distilled water and 0.5 mL of Folin–Ciocalteu reagent were added. After 3 minutes, 1 mL of saturated sodium carbonate solution was added to each flask. The mixtures were thoroughly homogenized and then diluted to volume with distilled water. The absorbance of the samples was measured after 1 hour at a wavelength of 555 nm against a blank sample [13].

2.5. Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) with SPSS 16. When significant differences were observed, means were compared using Duncan's multiple range test (Duncan test).

3-Results and Discussion

The quality and characteristics of olive oil are influenced by several factors such as cultivar type, growing region, and harvesting time [1].

3.1. Evaluation of Chemical Properties and Oxidative Stability of Oil

Acidity, expressed as free fatty acid content based on the predominant fatty acid in the sample (oleic acid), showed significant differences among treatments at the 1% probability level. According to the mean comparison table, acidity values (meq KOH/g oil) were 0.17 (mid-November), 0.51 (late November), and 2.11 (mid-December). The observed changes in acidity indicate a significant increasing trend ($p < 0.01$), suggesting greater susceptibility to enzymatic hydrolysis. Increased activity of hydrolytic enzymes such as lipase at the later stages of fruit ripening can accelerate hydrolytic reactions, leading to higher acidity. Structural changes in the fruit, such as softening, membrane damage, increased cell permeability, and the release of degradative enzymes, may also contribute to increased oil acidity. Furthermore, previous studies have confirmed a relationship between moisture loss and increased acidity at the final harvesting stages of fruits [14]. On the other hand, the increase in acidity may also be attributed to enhanced activity of molds and bacteria producing lipase, which increases the population of hydrolytic microorganisms. In some cases, the accumulation of free fatty acids may also result from partial degradation processes, which further contributes to increased acidity [15]. The peroxide value represents the concentration of peroxides in an oil or fat sample and indicates the intensity of oxidative deterioration. It is commonly used as an index of oil stability. Changes in peroxide value were significant at the 1% probability level. The peroxide values in the treatments were 4.46 (mid-November), 4.87 (late November), and 5.53 (mid-December), expressed as meq O_2/kg oil (Table 1). The results show an increasing trend in peroxide value with delayed harvesting time. However, compared with the maximum acceptable peroxide value defined for olive oil quality classification (20 meq O_2/kg), all samples were still classified as high-quality olive oil [16]. Delaying harvest time and increasing olive fruit ripening may lead to a higher risk of physical damage, softening, and thinning of cell walls. In addition, a decrease in temperature can reduce antioxidant concentration. Therefore, the increased activity of lipolytic enzymes appears to be a likely factor contributing to these changes [17].

The results of anisidine value (AV) changes in Table 1 show that delaying the harvesting time led to a significant increase in anisidine value. The lowest value was observed in the first harvesting stage (0.45 mg/kg oil), while the highest value was recorded in the final stage (0.54 mg/kg oil).

The increase in anisidine value (AV) can be attributed to several key factors. First, as olive fruits reach full ripening in late autumn, enzymatic and oxidative activities within the fruit increase. This leads to the formation and accumulation of secondary oxidation products, particularly aldehydic compounds, which directly contribute to higher AV levels. In addition, prolonged fruit exposure on the tree may enhance lipid oxidation due to increased susceptibility of cell structures to oxygen, light, and temperature fluctuations. The reduction in natural antioxidant compounds during late ripening stages further accelerates oxidative processes, resulting in greater formation of secondary oxidation products such as aldehydes, which are measured by the anisidine index. Oxidative enzymes directly affect the anisidine value [18]. Secondly, changes in the fatty acid composition of the fruit during autumn—particularly the relative increase in oleic acid—may increase the oil's susceptibility to oxidation, even in the presence of high levels of polyphenols [19]. On the other hand, physical factors also play an important role in this phenomenon. More mature fruits in late autumn are softer and therefore more prone to mechanical damage during harvesting and transport. Such damage leads to cell wall disruption and the release of enzymes and lipid substrates, which accelerates oxidative reactions. Furthermore, any delay in processing harvested fruits during this period—even for a few hours—can promote microbial activity and increase the temperature of the fruit mass due to cellular respiration. All of these factors contribute to the observed increase in anisidine value [20].

Table 1- Acidity values, peroxide values, and oxidative stability measured over three harvesting period

Third harvesting period	Second harvesting period	First harvesting period	Permissible limit according to the standard	Test parameters
$2.11^{a} \pm 0.060$	$0.51^{b} \pm 0.036$	$0.17^{c} \pm 0.003$	≤ 0.8	Acidity value mg KOH/g (oil)
$5.53^{a} \pm 0.251$	$4.87^{b} \pm 0.205$	$4.46^{b} \pm 0.055$	≤ 20	Peroxide value (meqO2/kg oil)
$0.54^{a} \pm 0.004$	$0.49^{b} \pm 0.003$	$0.41^{c} \pm 0.00$	-	Anisidine value (mg/kg oil)
11.600	10.230	9.330	< 10	Totox

The results are presented as mean ± standard deviation.

2-3- Evaluation of Oil-Soluble Polyphenols

Phenolic compounds play a major role in the oxidative stability and sensory properties of olive oil. The table presents the mean polyphenol contents of the samples at 20/2 (mid-November), 11/2 (late November), and 04/1 (mid-December), expressed as (mg GAE/kg oil). The results showed a decreasing trend in polyphenol content with delayed harvesting time, and this reduction became statistically significant from late November onward (Figure 1).

In general, genetics, harvesting time, and fruit ripening stage have been reported as the most important factors affecting the extent of these changes [21]. The reduction in phenolic compounds due to delayed harvesting may be related to the occurrence of enzymatic activities responsible for the degradation of phenolic compounds. In addition, lower temperatures may disrupt the biosynthesis mechanisms of phenolic compounds, ultimately leading to a decrease in their concentration. Since phenolic compounds are able to inhibit lipid oxidation chain reactions by donating a hydrogen atom to free radicals, the increase in acidity and peroxide indices during the harvesting period may be attributed to the decline in polyphenol concentration over the same period [5].

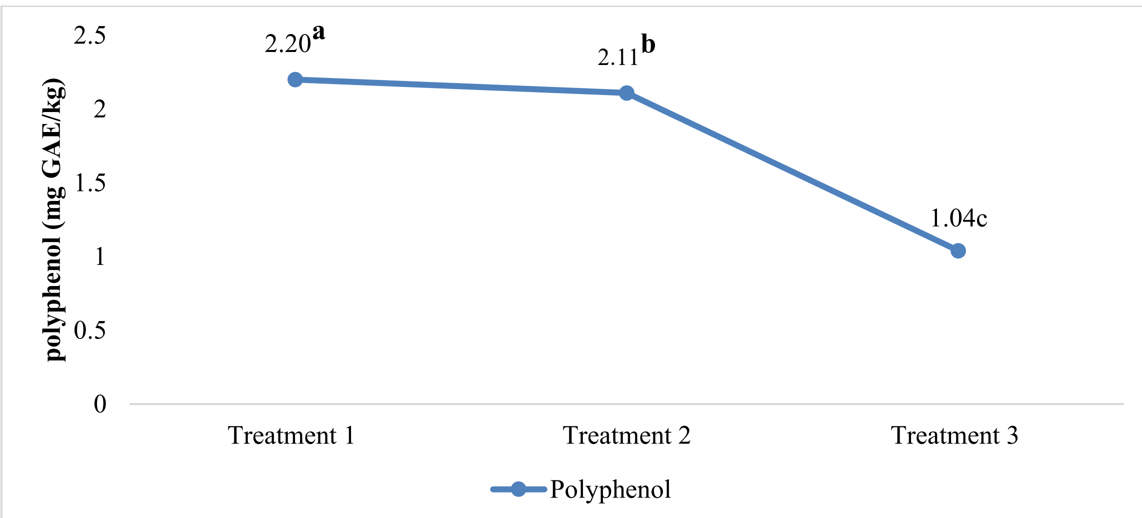

Figure 1- Trend of changes in polyphenolic compounds soluble in oil

t1: oil obtained from olives harvested at the beginning of the harvest periodt2: oil obtained from olives harvested in the middle of the harvest periodt3: oil obtained from olives harvested at the end of the harvest period

3-3- Evaluation of Fatty Acid Composition

According to Table 2, significant differences were observed among the measured fatty acid contents of the samples harvested at different times ($p \leq 0.05$). The results indicated that unsaturated fatty acids were present at higher levels than saturated fatty acids. Among the saturated fatty acids, palmitic and stearic acids were the predominant components.

Palmitic acid showed a significant increasing trend during the three harvesting periods, with values of 8.71%, 15.16%, and 15.84%, respectively. In contrast, stearic acid decreased significantly with fruit ripening, and the measured values were reported as 3.19%, 2.19%, and 2.09%, respectively. Among the unsaturated fatty acids, data analysis revealed a significant increase in oleic acid from 66.91% to 68.2%, and subsequently to 69.8% with delayed harvesting time. In contrast, linoleic acid showed a significant decreasing trend, declining from 17.2% at the beginning of the harvesting period to 12.01%, and finally to 12.19% at the end of the harvesting season. Oleic acid represented the major unsaturated fatty acid in olive oil.

Previous studies have confirmed a negative correlation between palmitic and oleic acids. Based on these findings, increased ripening in olive fruit is associated with an increase in palmitic acid accompanied by a corresponding increase in oleic acid content [1]. The increase in oleic acid and the reduction in linoleic acid with delayed harvesting resulted in a significant rise in the oleic acid to linoleic acid ratio from 3.89% to 5.7%. This ratio has been introduced by researchers as an indicator of oxidative stability and is strongly influenced by temperature changes. Studies have shown that a decrease of 10°C in temperature may increase oleic acid content by approximately 20%. In addition, lower temperatures appear to reduce linoleic acid levels, thereby increasing the oleic acid/linoleic acid ratio and consequently improving oxidative stability. Researchers have also reported a positive correlation between the ratio of oleic acid to polyunsaturated fatty acids and the concentration of phenolic compounds as an indicator for evaluating olive oil oxidative resistance [1].

In the present study, this ratio was measured as 3.78, 5.82, and 5.41 for the three harvesting times, respectively (Figure 2). Increased activity of enzymes involved in the formation of double bonds in fatty acid chains during ripening may enhance the conversion of stearic acid to oleic acid by up to 15% [6]. Furthermore, reduced activity of enzymes responsible for converting oleic acid to linoleic acid in mid-December may explain the observed reduction in linoleic acid content.

Moreover, when harvesting occurs at advanced stages of ripening, stearic acid may undergo catabolism as an energy source, resulting in reductions of up to 40% in its concentration [22]. Similar studies have reported that the highest oleic acid content in olive fruit is observed during mid-December harvests [23].

Table 2- The fatty acid composition of olive oil samples evaluated across three different harvest periods

Fatty acid composition (%)	Treatment 1	Treatment 2	Treatment 3
Palmitic acid (C16:0)	8.710^{c}± 0.037	15.160^{b}± 0.015	15.840^{a}± 0.040
Stearic acid (C18:0)	3.190^{a}± 0.055	2.190^{b}± 0.045	2.090^{b}± 0.011
Oleic acid (C18:1)	66.910^{c}± 0.047	68.200^{b}± 0.066	69.800$^{a\pm}$ 0.035
Linoleic (C18:2)	17.200^{a}± 0.040	12.010^{b}± 0.023	12.190^{b}± 0.050
Linolenic acid (C18:3)	0.490^{b}± 0.040	0.710^{a}± 0.035	0.700^{a}± 0.020
Arachidic acid (C20:0)	0.290^{b}±0.030	0.350^{ab}± 0.025	0.370^{a}± 0.017
Total monounsaturated fatty acids: ΣMUFA	66.910^{c}± 0.047	68.200^{b}± 0.066	69.800^{a}± 0.035
Total Polyunsaturated fatty acid:ΣPUFA	17.690^{a}± 0.080	11.720^{c}± 0.058	12.890^{a}± 0.070
Total saturated fatty acid :ΣSFA	12.190^{c}± 0.122	17.700^{b}± 0.085	18.300^{a}± 0.068
ΣMUFA/ ΣPUFA Ratio	3.780^{b}± 0.020	5.82^{a}± 0.030	5.415^{a}± 0.030

The results are presented as mean ± standard deviation.

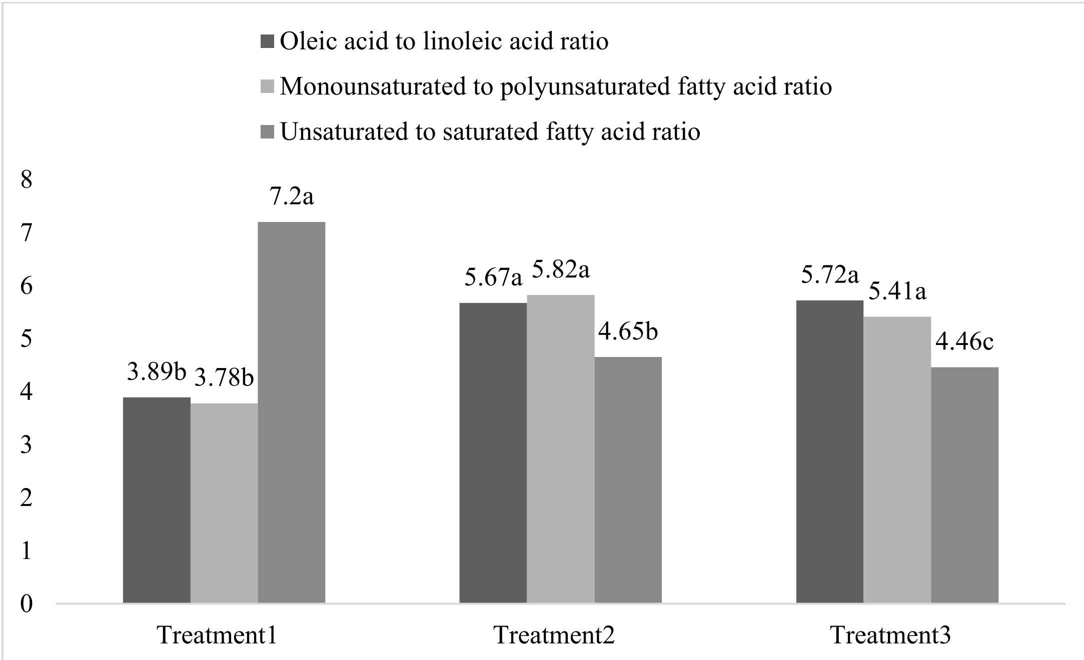

Figure 2- Graph of the oleic acid ratio, the monounsaturated to polyunsaturated fatty acids ratio (ΣMUFA/ ΣPUFA), and the unsaturated to saturated fatty acids ratio (ΣUFA/ ΣSFA)

Harvest period 1: Oleic acid to linoleic acid ration 3.89, Monounsaturated to Polyunsaturated fatty acids ratio ((ΣMUFA/ ΣPUFA) 3.78, Unsaturated to saturated fatty acids ratio ((ΣUFA/ ΣSFA) 7.2, Harvest period 2: Oleic acid to linoleic acid ration 5.67, Monounsaturated to Polyunsaturated fatty acids ratio ((ΣMUFA/ ΣPUFA) 5.82, Unsaturated to saturated fatty acids ratio ((ΣUFA/ ΣSFA) 4.65, Harvest period 3: Oleic acid to linoleic acid ration 5.72, Monounsaturated to Polyunsaturated fatty acids ratio ((ΣMUFA/ ΣPUFA) 5.41, Unsaturated to saturated fatty acids ratio ((ΣUFA/ ΣSFA) 4.46

4- Conclusion

The evaluation of chemical characteristics and oxidative stability of olive oil at different harvesting stages (mid-November, late November, and mid-December) demonstrated that harvesting time significantly affects oil quality. The results indicated that delayed harvesting led to an increase in acidity due to enzymatic hydrolysis and microbial activity, as well as increases in peroxide and anisidine values as a consequence of progressive oxidation. In contrast, phenolic compounds decreased because of enzymatic degradation and reduced biosynthesis.

On the other hand, changes in fatty acid composition, including increased oleic acid and decreased linoleic acid, contributed to a relative improvement in oxidative stability during the later stages of ripening. However, this advantage may be offset by the simultaneous increase in deterioration indices such as acidity and peroxide value.

Therefore, late November appears to be the optimum harvesting time for achieving a balance between oxidative stability and chemical quality. At this stage, acidity and peroxide values remain within desirable limits, phenolic compounds are still present at acceptable levels, and the oleic acid/linoleic acid ratio is improved. Although harvesting in mid-December may further increase oleic acid content and improve the oleic/linoleic ratio, the accompanying rise in hydrolytic and oxidative deterioration ultimately reduces the overall oil quality. Environmental factors such as temperature and humidity, along with physiological factors including fruit softening, enzymatic activity, and cellular respiration, appear to play key roles in these observed changes. Consequently, for the production of olive oil with desirable quality characteristics, harvesting in late November, combined with rapid processing and proper storage conditions to minimize enzymatic and oxidative spoilage, is recommended.

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Competing Interests
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مقاله علمی_پژوهشی

ارزیابی تغییرات شاخص‌های کیفی و پروفیل اسیدهای چرب روغن‌زیتون در پاسخ به تأخیر در برداشت

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چکیده

کیفیت روغن‌زیتون به ترکیب اسیدهای چرب و غلظت ترکیبات فنلی بستگی دارد. این مطالعه به‌منظور بررسی تأثیر زمان برداشت زیتون بر ویژگی‌های کیفی و پروفیل اسیدهای چرب روغن‌زیتون بر اساس طرح کاملاً تصادفی، بر زیتون رقم آربکین (رقم پرتکرار در منطقه رودبار در سه زمان برداشت مختلف (اواسط آبان، اواخر آبان و اواسط آذر) انجام شد. مقدار اسیدیته، اندیس پراکسید، عدد آنیزیدین، اندیس توتوکس، غلظت ترکیبات فنلی محلول در روغن اندازه‌گیری و نوع و میزان اسیدهای چرب بررسی گردید. نتایج نشان داد که مقادیر اسیدیته، پراکسید، عدد آنیزیدین، عدد توتوکس با به تعویق انداختن زمان برداشت، به‌طور معنی‌داری روند صعودی داشتند، درصورتی‌که میزان ترکیبات فنلی از (۲/۲۰mg GAE/kg oil) به (۱/٤۰ mg GAE/kg oil) کاهش یافت. از طرف دیگر با نزدیک شدن به اواخر فصل روغن‌کشی میزان پالمیتیک‌اسید و اولئیک اسید رشدی صعودی معنی‌دار نشان دادند. بعلاوه میزان نسبت اولئیک اسید به لینولئیک اسید نیز با افزایش معنی‌دار از ۳/۸۹٪ به ۵/۷٪ همراه بود. بنابراین برای استحصال روغن‌زیتون با ویژگی‌های کیفی مطلوب، میزان قابل‌قبول ترکیبات فنولی و برقراری تعادل بین ویژگی‌های شیمیایی و پایداری اکسیداتیو روغن، برداشت در اواخر آبان ماه پیشنهاد می‌گردد.