International Journal of Horticultural Science and Technology
Journal homepage: https://ijhst.ut.ac.ir

Analysis of Olive Oil Yield and Quality in Iranian Olive Varieties: A Study of the Minodasht Collection

Sayed Ali Hoseini[1], Azim Ghasemnezhad[1]*, Smail Seifi[1], Isa Karamatlou[2]

1 Department of Horticulture, Faculty of Agriculture, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
2 Minodasht Agricultural Jihad Center, Ministry of Agricultural Jihad, Minodasht, Golestan, Iran

*Corresponding author's email: ghasemnezhad@gau.ac.ir

ARTICLE INFO

Article history:

Received: 14 October 2024,
Received in revised form: 6 August 2025,
Accepted: 13 August 2025,

Article type:

Research paper

Keywords:

Alpha-tocopherol
Genotype,
HPLC,
NMR

ABSTRACT

This study aimed to identify and characterize olive cultivars with high nutritional value to enhance profitability in olive cultivation, olive oil production, and export. We focused on genotypes from the Minodasht olive collection, which represents a substantial portion of Iranian olive diversity. A two-year study (2020–2022) was conducted to evaluate 23 and later 30 genotypes for tocopherol content as well as qualitative and quantitative olive oil indices. After olive fruit collection, we analyzed olive oil quality by measuring acid value (free fatty acid percentage), peroxide value, and alpha-tocopherol content. The maturity index and oil content were assessed using nuclear magnetic resonance (NMR) spectroscopy based on dry weight, while alpha-tocopherol was quantified via high-performance liquid chromatography (HPLC) according to ISO 9936 standards. Our results showed significant variability among olive genotypes in tocopherol content and oil quality. In 2020, Azadshahr 2 and T12 had the highest alpha-tocopherol levels (273.89 and 222.67 mg L^{-1}, respectively), while T23 and Benvareh 7 had the lowest (168.34 and 169.95 mg L^{-1}). In 2021, T14 and Chamjeh 1 recorded the highest levels (192.87 and 192.85 mg L^{-1}), whereas Savari and Malek Shahi had the lowest (72.43 and 16.73 mg L^{-1}). Notably, significant differences in acidity were observed, with T23 and T24 showing the lowest values in 2020, and Shiraz and Torshk in 2021. Overall, genotypes Azadshahr 2, T12, T14, and Chamjeh 1 displayed superior olive oil yield and quality, highlighting their potential for improved cultivation and commercial use.

Introduction

The olive tree (*Olea europaea* L.) is a perennial plant with a diploid chromosome number of 46 (2n = 2x = 46) and a highly outcrossing reproductive system. It belongs to the olive family (Oleaceae), which includes about 25 genera and 600 species found in temperate and tropical regions worldwide. Since a significant portion of olive cultivation is aimed at olive oil production, the quality of olive oil is of primary importance in olive genotype selection (Carlos et al., 2019).

The chemical composition of olive oil can be categorized into two primary groups. Triacylglycerols, which account for 97–99% of olive oil, are the predominant components. These triacylglycerols are largely composed of monounsaturated fatty acids, specifically oleic acid. In addition, olive oil contains moderate levels of polyunsaturated fatty acids, such as linoleic and linolenic acids, and smaller quantities of saturated fatty acids, including palmitic and stearic acids (Jimenez-Lopez et al., 2020). The minor components of olive oil constitute a heterogeneous mixture of polar, non-polar, and amphiphilic compounds. This fraction encompasses a wide range of substances, such as hydrocarbons, tocopherols, phenolic compounds, sterols, chlorophylls, carotenoids,

mono- and diacylglycerols, fatty acid esters of fatty alcohols (waxes and sterol esters), triterpene alcohols, free fatty acids, and volatile compounds (Jimenez-Lopez et al., 2020; Gharby et al., 2021).

Virgin olive oil, being a direct extract of olives, is rich in secondary metabolites that accumulate in the olive fruit. This makes it a nutritionally dense food. The high content of phenolic compounds in virgin olive oil is indicative of its potent antioxidant properties. These phenolic compounds primarily include tocopherols. Tocopherols, in particular, are responsible for the vitamin E content of olive oil (Carlos et al., 2019). Antioxidants function by neutralizing reactive oxygen species, thereby safeguarding cells from oxidative stress. Reactive oxygen species are generated as a byproduct of cellular metabolism, and their production is elevated under various stress conditions. When these reactive species accumulate within cells, they can cause damage to DNA, proteins, and lipids (Basuny, 2019). As a result, antioxidants like tocopherols play a crucial role in maintaining equilibrium by regulating the generation and removal of reactive oxygen species.

Vitamin E, a group of compounds encompassing tocopherols, exhibits strong antioxidant properties (Cuomo et al., 2020). Alpha-tocopherol is the predominant form of vitamin E and demonstrates superior biological activity compared to other forms. This is due to its unique ability to be exclusively incorporated into very low-density lipoproteins (VLDL) via a specific liver protein (Carlos et al., 2019). The primary function of vitamin E is to act as an antioxidant, preventing lipid peroxidation in cellular membranes and neutralizing reactive oxygen species. Consequently, vitamin E plays a crucial role in protecting tissues from oxidative damage.

Tocopherols are primarily produced by olive oilseed plants and have been extensively studied for their potential to prevent various human diseases, including coronary heart disease and cancer (Foscolou et al., 2018; Delgado et al., 2020). They also play a multifaceted role in plants, participating in responses to environmental stressors, maintaining cell membrane integrity, facilitating signal transduction, and regulating light stress within chloroplasts. Beyond their well-known antioxidant properties, tocopherols are involved in diverse biological processes, including preventing lipid oxidation, mediating cell signaling, and modulating gene expression (Di Vincenzo et al., 2019).

The authentication of olive oil, traditionally reliant on chemical analyses and the assessment of metabolites like fatty acids, volatile compounds, and tocopherols, has undergone a paradigm shift with the advent of DNA-based methods. Molecular markers have become invaluable tools for tracing the origin and quality of olive oil products (Fanelli et al., 2021). The main purpose of this study is to determine the differences among the studied olive genotypes in terms of tocopherol content and the quantitative and qualitative indicators of Iranian olive genotypes.

Materials and Methods

This study was carried out over two consecutive growing seasons (2020 and 2022) within the Iranian olive germplasm collection located in Minodasht, Golestan Province, Iran. Given the biennial bearing habit of olive trees and the prevailing climatic conditions in 2021, the second experimental year was conducted in 2022. The collection, encompassing approximately four hectares, was divided into four distinct sections: the first dedicated to preserving Iranian olive cultivars and genotypes, the second to Mediterranean olive cultivars, and the third and fourth to propagation, breeding, and identification programs for stress-tolerant olive trees. A total of 93 Iranian native olive genotypes, 10 Iranian commercial cultivars, 20 Mediterranean cultivars, and 30 cold-tolerant olive genotypes were cultivated in this collection. Samples were collected according to criteria such as olive oil yield and cold tolerance.

In this experiment, sampling was conducted over two experimental years from olive fruiting cultivars. After fruit collection, olive oil was extracted from all genotypes to assess oil yield and quality parameters, including acid value (free fatty acid content), peroxide value, and tocopherol content. Olive oil was extracted using a conventional cold extraction method. Freshly harvested, ripe olives were cleaned to remove leaves, stems, and debris, and then washed with potable water. The cleaned olives were crushed using a stainless-steel hammer mill to produce a uniform paste. The paste was malaxated at 25 ± 1 °C for 30 minutes to promote oil droplet coalescence. Oil was then separated from the paste using a two-phase centrifugation system. The extracted oil was decanted and filtered to remove residual water and solids. Finally, the oil was stored in dark glass bottles at 15 °C in the absence of light and air until further analysis (Fanelli et al., 2021).

A two-year study (2020–2022) was thus undertaken on the olive germplasm collection to evaluate variation in olive oil quality characteristics among different Iranian olive genotypes. Adverse weather conditions in 2020 limited fruiting to only 23 genotypes. In 2021, olive fruit set was completely absent due to snowfall during the flowering period. However, in 2022, favorable weather conditions enabled 30 genotypes to reach the fruiting stage.

Maturity index and olive oil content

Maturity index (MI) was assessed visually according to a standardized scale based on olive fruit skin and flesh color. A random sample of 50 olive fruits was categorized into MI codes and counted. Olive oil

content was determined quantitatively using nuclear magnetic resonance (NMR) spectroscopy, a non-destructive technique that measures the hydrogen content of the sample.

Olive oil acidity

Free fatty acid (FFA) content is a key indicator of olive oil quality and oxidative degradation, commonly used to assess the degree of hydrolytic rancidity. It is expressed as the percentage of oleic acid by weight. FFA content was determined following the standard titrimetric method described by the International Olive Council (IOC). Briefly, 10 g of olive oil was accurately weighed into an Erlenmeyer flask and dissolved in a neutralized mixture of ethanol and diethyl ether (1:1, v/v). A few drops of phenolphthalein indicator were added to the solution, which was then titrated with standardized 0.1 N sodium hydroxide (NaOH) until a persistent pink endpoint was observed (Delgado et al., 2020).

Peroxide value

Peroxide value (PV) is a measure of the primary oxidation products (hydroperoxides) formed during the initial stages of lipid oxidation and is expressed in milliequivalents of active oxygen per kg of oil (meq O_2 kg^{-1}). The PV of the olive oil samples was determined according to the official method of the International Organization for Standardization (ISO 3960:2017), with slight modifications. Approximately, 5.0 g of oil was accurately weighed into a glass-stoppered Erlenmeyer flask, followed by 30 mL of a solvent mixture of glacial acetic acid and chloroform (3:2, v/v), which was swirled until the oil was completely dissolved. Subsequently, 0.5 mL of saturated potassium iodide (KI) solution was added, and the flask was immediately stoppered and kept in the dark for 1 min with occasional shaking to allow liberation of iodine from the reaction of KI with hydroperoxides. After incubation, 30 mL of distilled water was added, and the liberated iodine was titrated with standardized 0.01 N sodium thiosulfate ($Na_2S_2O_3$) until the yellow color faded. At this point, a few drops of freshly prepared 1% starch solution were added as an indicator, turning the solution blue. Titration was continued until the blue color just disappeared, indicating the endpoint. A blank determination (without oil) was also performed under identical conditions (Gharby et al., 2021).

Alpha-tocopherol content

Alpha-tocopherol, a vitamin E compound, acts as an antioxidant in olive oils, protecting them from oxidation and rancidity. Its content was determined quantitatively using high-performance liquid chromatography (HPLC), following the method of Gimeno et al. (2000) with minor modifications. Approximately, 1.0 g of olive oil was accurately weighed and dissolved in 5 mL of n-hexane. The solution was filtered through a 0.45 µm PTFE membrane filter and transferred into an HPLC vial. HPLC analysis was performed using a system equipped with a UV–Vis detector set at 292 nm, corresponding to the maximum absorbance wavelength of alpha-tocopherol. Separation was achieved with a C18 reversed-phase column (250 mm × 4.6 mm i.d., 5 µm particle size) maintained at room temperature. The mobile phase consisted of methanol and water (97:3, v/v), delivered at a flow rate of 1.0 mL min^{-1} under isocratic conditions. The injection volume was 20 µL. Quantification was carried out by external standard calibration. A series of alpha-tocopherol standard solutions (1–100 µg mL^{-1}) were prepared in n-hexane to construct the calibration curve. Sample concentrations were determined by comparing peak areas with those of the standards. All measurements were performed in triplicate, and results were expressed as milligrams of alpha-tocopherol per kilogram of oil (mg kg^{-1}) (Cuomo et al., 2020).

Statistical analysis

The study was conducted using a completely randomized design with three replications per genotype. Data were analyzed with SAS software using t-tests and ANOVA, with significance determined at $p < 0.01$. Each replication consisted of 30 fruits per tree, with three trees sampled per genotype, for a total of about 100 fruits per genotype. Since this was a genotype characterization study, no treatments were applied. Harvest data were not available for 2021 due to severe snowfall that destroyed the olive fruits.

Results

The maturity index

The maturity index indicates the level of olive fruit ripeness at harvest time. In 2020, Mondan 3 and T23 genotypes had the highest maturity indices, measuring 4.96 and 9.04, respectively. Conversely, the Benvare 7 and T24 genotypes had the lowest maturity indices (1.05) (Fig. 1).

In 2022, genotypes T4 and Gilan-Gharb exhibited the highest maturity index values (4.64 and 4.49, respectively), while Katefe Goshe and Minoodasht 3 showed the lowest values (1.25 and 1.46, respectively) (Fig. 2). Rastmi Ouzmechlouei et al. (2015) reported that delaying olive harvest significantly decreased antioxidant compounds in olive oil, including total chlorophyll, total carotenoids, total phenols, and total flavonoids, in four olive cultivars (Zard, Roghani, Arbequina, and Koratina) from the Rudbar region. They also observed that, although antioxidant activity decreased with delayed harvest, the olive fruit's oil content increased significantly. This finding is

consistent with the current study, where variation in oil content and maturity index demonstrated significant differences and highlighted the measurable qualities of olive fruits. Dag et al. (2011) also investigated the effect of harvest time and maturity index on olive oil yield and quality. Their results showed that olive oil accumulation increased throughout the ripening season, leading to higher oil yield, while oil quality decreased, as measured by free fatty acids, peroxide value, and phenolic compounds. The ripening stage was also found to influence fatty acid and pigment composition: linoleic and palmitic acids increased, while oleic acid decreased. Additionally, the levels of chlorophyll and carotenoid pigments in olive oil declined as ripening progressed.

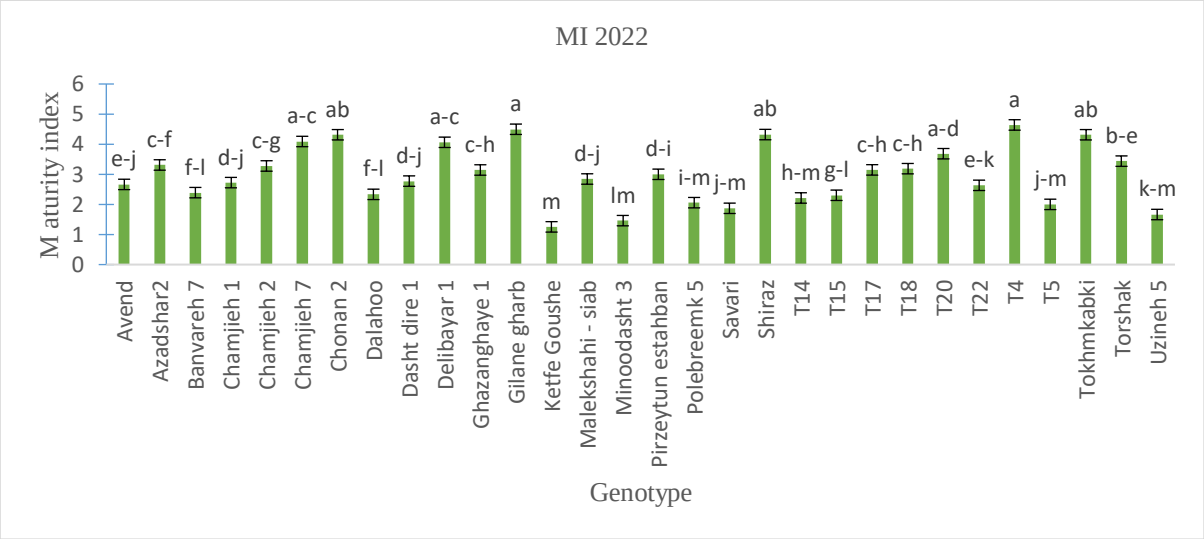

Fig. 1. Changes in the ripening index of olive fruit samples of the studied genotypes in 2022. Data are presented as mean values ± standard deviation (SD) based on three independent replicates per genotype. Statistical differences in ripening index among genotypes at each sampling point were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's least significant difference (LSD) test for mean comparisons at the 5% significance level ($P < 0.05$).

Olive oil percentage

The percentage of olive oil in olives serves as an indicator of extraction efficiency. Higher oil content reflects greater efficiency and generally better oil quality. In this study, during 2020, the Gilan-Gharb and Azadshahr 2 genotypes exhibited the highest oil content (44.14% and 43.47%, respectively), while Benvare 7 and Minoodasht 1 showed the lowest (26.68% and 26.92%, respectively) (Fig. 3).

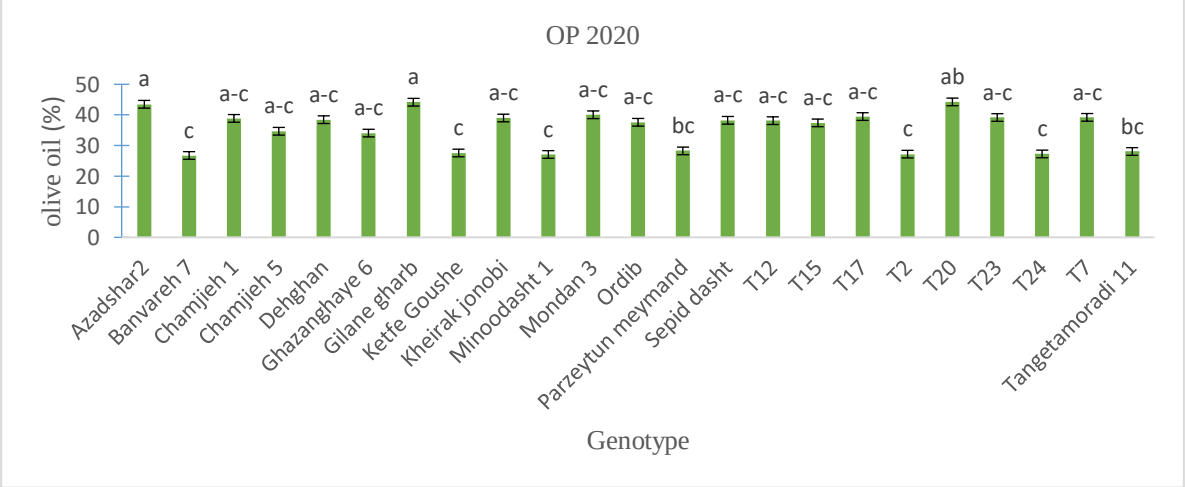

Fig. 2. Changes in the olive oil percentage of the studied genotypes in 2020. Values are expressed as mean ± standard deviation (SD) based on three independent replicates per genotype. Statistical analysis was conducted using one-way analysis of variance (ANOVA) to evaluate differences in oil content among genotypes at each sampling time. Means were compared using Tukey's least significant difference (LSD) test at the 5% significance level ($P < 0.05$).

In 2022, the Azadshahr 2 and T22 genotypes demonstrated the highest olive oil content, reaching 49.64% and 48.81%, respectively. In contrast, the Delibyar 1 and Malekshahi genotypes exhibited the lowest values, at 20.07% and 22.39%, respectively (Fig. 4). Previous research by Soltani et al. (2014) on both native and foreign olive cultivars and genotypes also reported significant differences ($P < 0.001$) in olive oil percentage, whether measured on a dry weight or fresh weight basis. In that study, all cultivars examined reached a suitable maturity level for oil extraction. Among them, the Blidi and Leccino cultivars showed the highest oil percentages in dry matter, at 44.52% and 42.32%, respectively. The Blidi cultivar also recorded the highest oil content on a fresh weight basis (27.19%), although this was not significantly different from the Zard and Arbequina cultivars.

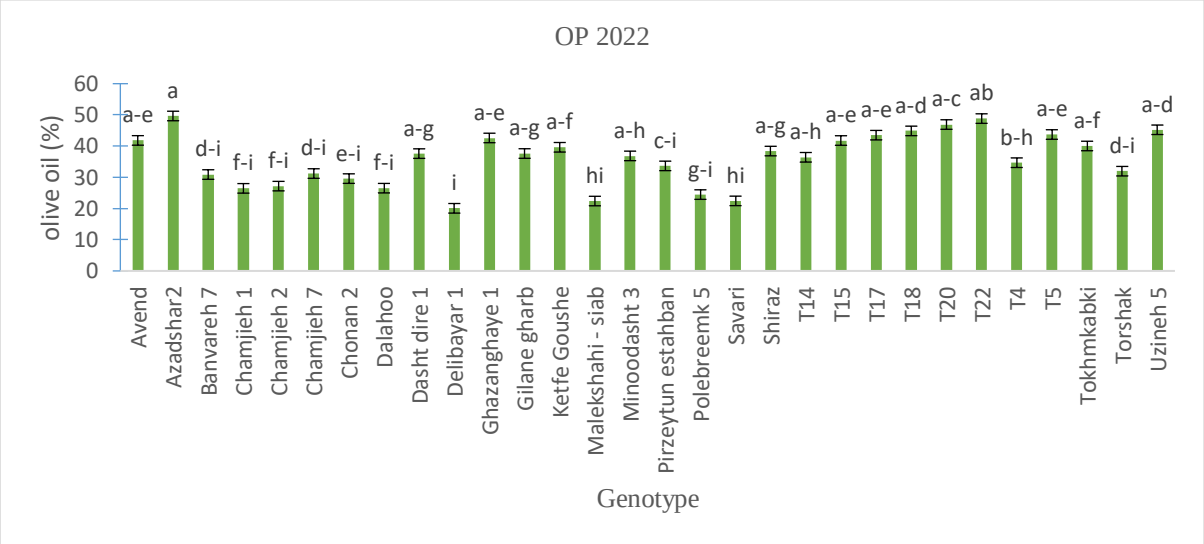

Fig. 3. Changes in the olive oil percentage of genotype samples in 2022. Data are presented as mean values ± standard deviation (SD) based on three independent replicates per genotype. Differences in oil content among genotypes at each sampling time were assessed using one-way analysis of variance (ANOVA). Where significant differences were detected, mean comparisons were performed using Tukey's least significant difference (LSD) test at a 5% significance level ($P < 0.05$).

Free fatty acids (FFA)

One of the most important quality factors of vegetable olive oils, especially olive oil, is the percentage of free fatty acids. The level of free fatty acids is directly related to the degree of olive oil degradation and oxidation. The content of free fatty acids in olive oil is influenced by various factors, including olive fruit quality and maturity, extraction method, and storage conditions. Saturated fatty acids such as palmitic and stearic acids, and unsaturated fatty acids such as oleic and linoleic acids, are among the important fatty acids found in olive oil. In 2020, genotypes T23 and T24 had the lowest percentage of free fatty acids (0.3 and 0.4%, respectively) among the studied cultivars. In contrast, Gilan-Gharb and Benvare 7 genotypes showed the highest acidity (1.2 and 1.1%, respectively) (Fig. 5). However, the level of this trait in all studied genotypes was below the threshold for the accumulation of free fatty acids based on the olive oil quality index. An increase in free fatty acids is greatly influenced by olive fruit damage, olive fruit quality, and the duration and temperature of olive oil extraction from the olive fruit (Mailer, 2006).

In 2022, the Shiraz and Tershk genotypes exhibited the lowest acidity values, measuring 0.20% and 0.23%, respectively. In contrast, genotypes T5 and Chonan 2 showed the highest acidity, at 1.20% and 0.93%, respectively. Importantly, the acid values for all genotypes remained well below the permissible limit, confirming the high quality of the olive oil in this collection (Fig. 6).

These findings are consistent with previous studies highlighting the influence of genetic diversity on free fatty acid levels in olive oil. For instance, Hashempour et al. (2021) reported significant differences in free fatty acid content among five olive cultivars. Similarly, Arafat et al. (2016) found that olive oil quality traits, such as oxidative stability, fatty acid composition, and sterol content, are strongly affected by the cultivar's genetic characteristics. Tuberoso et al. (2016), while working on four Italian cultivars, demonstrated that total chlorophyll, carotenoid pigments, phenolic compounds, and antioxidant capacity varied significantly depending on cultivar type. They also noted that the hydrophilic fraction and specific components, such as oleuropein and decarboxymethyl oleuropein, were influenced by genetic background.

The observed variation in free fatty acid levels among olive cultivars can be attributed to multiple

factors, including genotype, environmental conditions, cultivation and harvesting practices, and processing methods. Genetic differences, in particular, play a crucial role, as different olive genotypes possess distinct genes involved in fatty acid synthesis and metabolism. This genetic variation underlies the differences in free fatty acid content observed among cultivars.

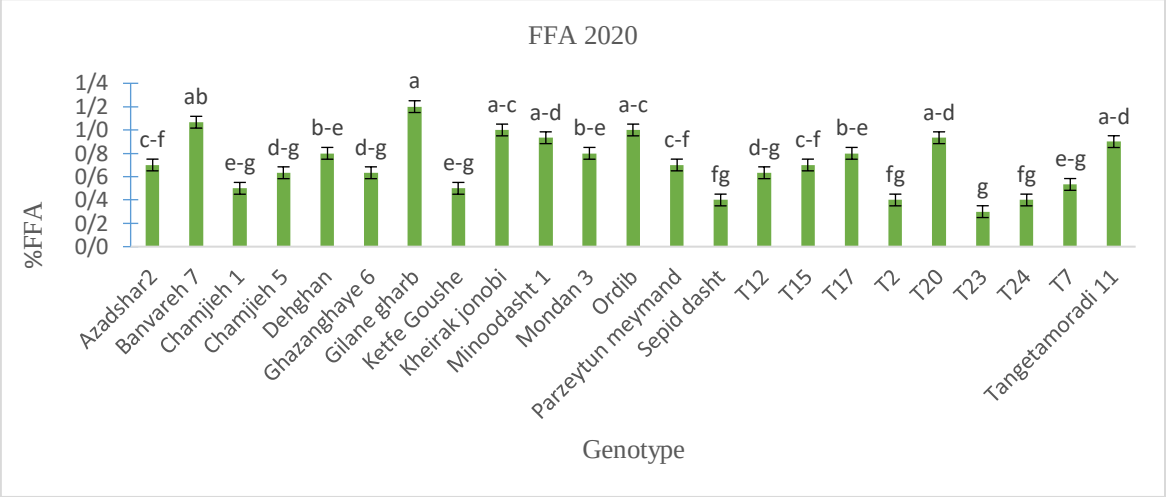

Fig. 4. Changes in acid value of olive oil samples of the studied genotypes in 2020. Results are expressed as mean ± standard deviation (SD) based on three independent replicates per genotype. Statistical differences in acid value among genotypes at each sampling time were evaluated using one-way analysis of variance (ANOVA). Mean separation was performed using Tukey's least significant difference (LSD) test at the 5% significance level ($P < 0.05$).

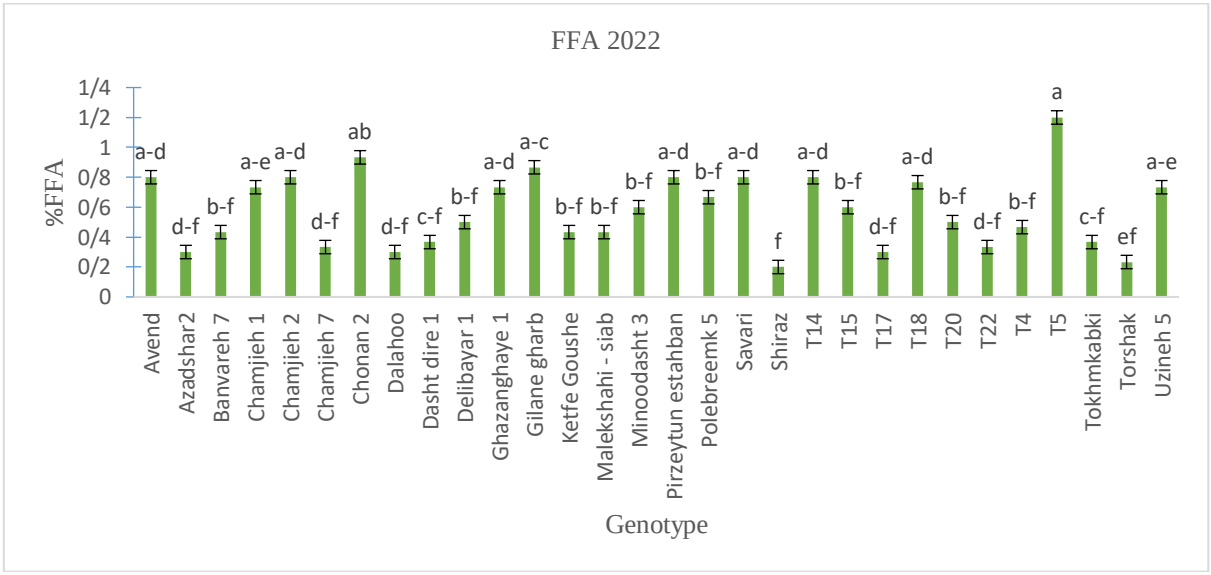

Fig. 5. Changes in acid value of olive oil samples of the studied genotypes in 2022. Values are presented as mean ± standard deviation (SD) from three independent replicates per genotype. One-way analysis of variance (ANOVA) was used to determine significant differences in acid value among genotypes at each sampling time. Tukey's least significant difference (LSD) test was applied for post hoc mean comparisons at a significance level of $P < 0.05$.

Peroxide (peroxide value) of olive oil

Peroxide in olive oil acts as a natural oxidant and is formed due to the presence of saturated and unsaturated fatty acids. Its levels may increase significantly when the oil is exposed to light, heat, or air, leading to reduced quality and the development of unpleasant odors. The peroxide value is therefore an important indicator of the degree of oxidation in olive oil, with lower values reflecting greater stability and higher quality. In 2020, genotypes T20 and Banvareh 7 recorded the lowest peroxide values (5.50 and 5.56 meq kg^{-1}, respectively), whereas Ordib and T23 showed the highest values (11.86 and 11.83 meq kg^{-1}, respectively) (Fig. 7). Nevertheless, the peroxide levels of all studied genotypes were below the permissible limit of 20 meq kg^{-1}, confirming the overall high quality of the oils from this collection.

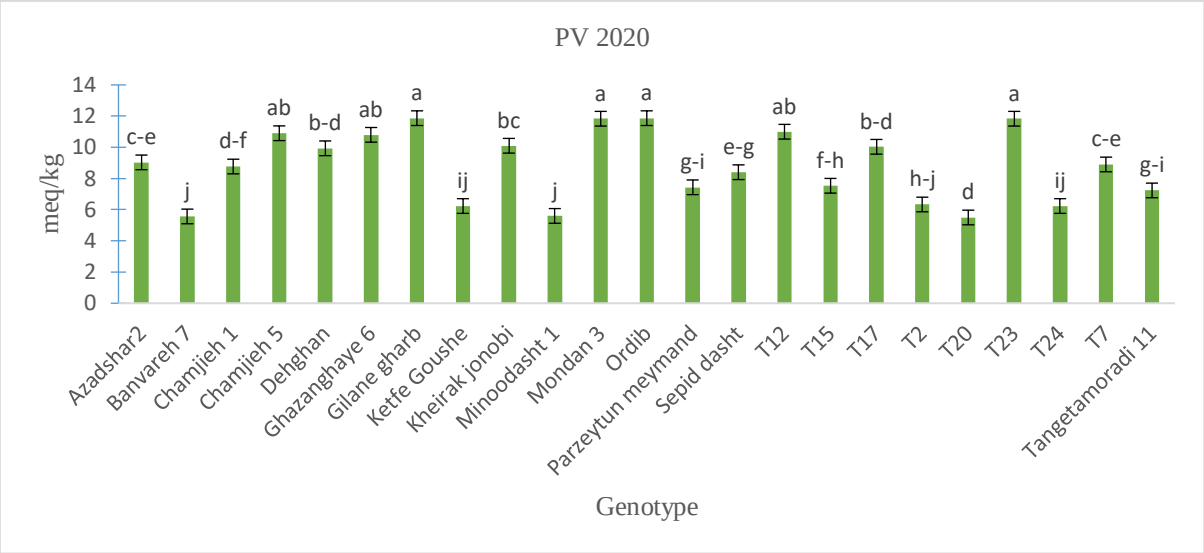

Fig. 6. Changes in peroxide value of olive oil samples of the studied genotypes in 2020. Data are expressed as mean ± standard deviation (SD) from three independent replicates per genotype. One-way analysis of variance (ANOVA) was used to assess differences in peroxide value among genotypes at each sampling time. Tukey's least significant difference (LSD) test was applied for multiple comparisons at a significance level of $P < 0.05$.

In 2022, the Tershk and Dalahu genotypes recorded the lowest peroxide values, at 7.2 and 7.3 meq kg^{-1}, respectively, whereas the Avand and T14 genotypes showed the highest values, at 10.56 and 10.46 meq kg^{-1}, respectively (Fig. 8). Soltani et al. (2014) also reported significant differences in olive oil percentage, both on a dry and fresh weight basis, among various native and foreign olive cultivars. In their study, the highest peroxide value was observed in the Leccino cultivar (11.33 meq O_2 kg^{-1}), while the lowest was in Zard (7.80 meq O_2 kg^{-1}).

Peroxide value is a key indicator of olive oil degradation, as peroxide is the primary product of lipid oxidation. Importantly, it should not exceed the permissible limit. In general, the higher the degree of unsaturation in olive oil, the greater its susceptibility to oxidation (Mailer, 2006).

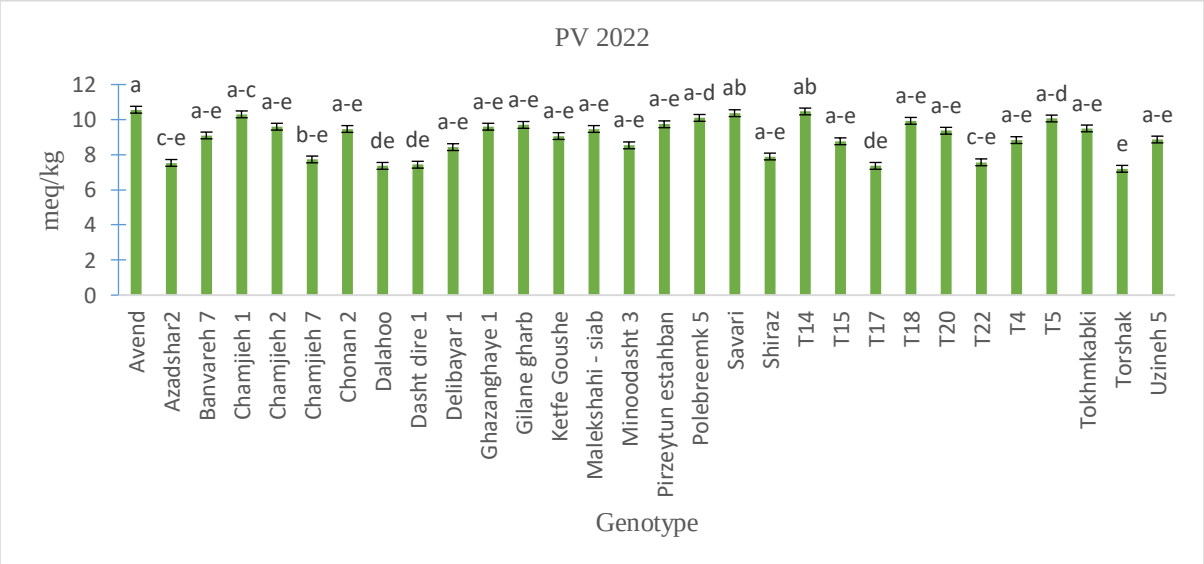

Fig. 7. Changes in peroxide value of olive oil samples of the studied genotypes in 2022. Values are presented as mean ± standard deviation (SD) from three independent replicates per genotype. Statistical analysis was performed using one-way analysis of variance (ANOVA) to identify significant differences in peroxide value among genotypes at each sampling time. Tukey's honest significant difference (HSD) test was used for mean comparison at the 5% significance level ($P < 0.05$).

Alpha tocopherol

Tocopherols are antioxidant compounds found in olive oil. Higher levels of tocopherols indicate better health properties and higher quality olive oil. In 2020, Azadshahr 2 and T12 genotypes had the highest levels of alpha-tocopherol (273.89 and 267.22, respectively), while T23 and Benvare 7 genotypes showed the lowest levels of alpha-tocopherol (76.11 and 76.71, respectively) (Fig. 9). In 2022, genotypes T14 and Chamjieh 1 had the highest levels of alpha-tocopherol (192.87 and 192.85, respectively), while Savari and Malek Shahi genotypes showed the lowest levels of alpha-tocopherol (72.43 and 73.16, respectively).

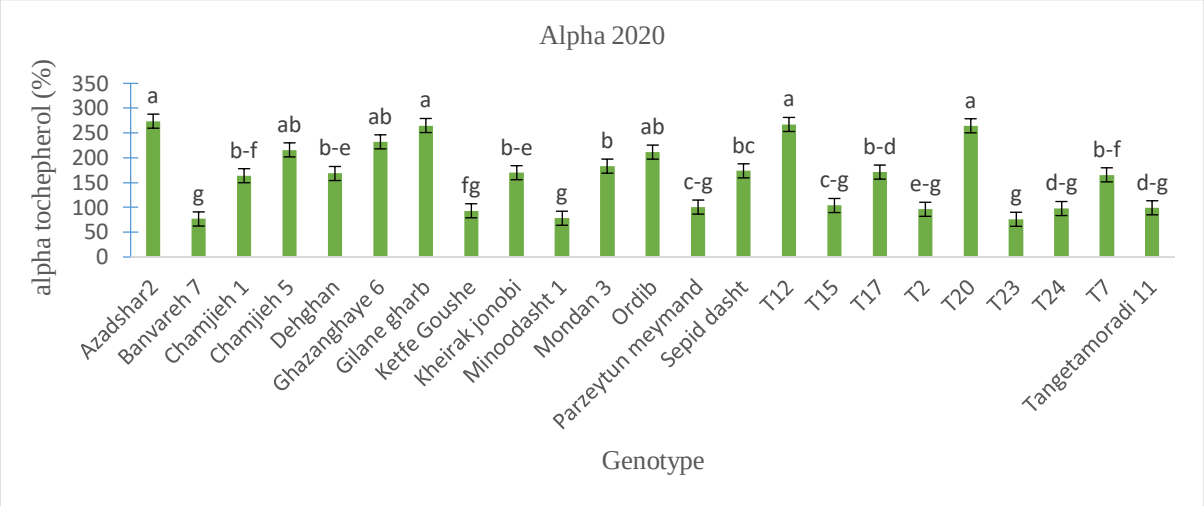

Fig. 8. Changes in alpha-tocopherol content of olive oil samples of the studied genotypes in 2020. Data are presented as mean ± standard deviation (SD) from three independent replicates per genotype. One-way analysis of variance (ANOVA) was used to evaluate significant differences in alpha-tocopherol content among genotypes at each sampling time. Tukey's least significant difference (LSD) test was applied to separate means at the 5% significance level ($P < 0.05$).

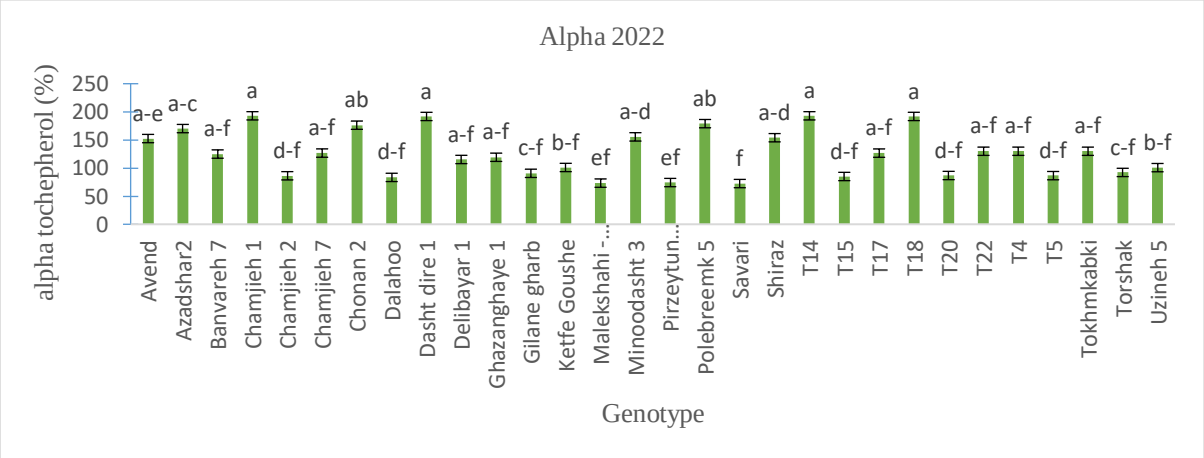

Fig. 9. Changes in alpha-tocopherol content of olive oil samples of the studied genotypes in 2022. Values are expressed as mean ± standard deviation (SD) based on three independent replicates per genotype. One-way analysis of variance (ANOVA) detected significant differences in alpha-tocopherol content among genotypes at each sampling time. Means were compared using Tukey's least significant difference (LSD) test at the 5% significance level ($P < 0.05$).

Discussion

Results from both years revealed significant differences in alpha-tocopherol content among the studied genotypes. These differences can be attributed to both genetic diversity and environmental factors. Genetic variability among olive cultivars influences alpha-tocopherol accumulation, with some cultivars naturally producing higher levels of this antioxidant. Environmental conditions such as climate and sunlight also play a role, although the samples in this study were grown under similar conditions. Even so, genetic differences can result in varying responses to environmental exposure. Previous studies support these findings. Perez-Lopez et al. (2008) reported that vitamin E content in virgin olive oil was strongly dependent on cultivar, ranging from 89 to 1410 mg tocopherol per kg of oil, whereas the influence of climatic conditions, season, and ripening stage was

comparatively lower. Similarly, Arjmand Fard et al. (2015), in a study on Amygdal, Manzanilla, and Arbequina cultivars grown in the Darab region of Fars province, found significant differences in chemical composition, including tocopherol content, fatty acid profile, acid value, iodine value, and saponification value. The acid value of most cultivars exceeded the limits set by the Codex Alimentarius Commission and the International Olive Council, indicating higher lipolytic activity in the studied region. Importantly, although tocopherol levels varied among cultivars, alpha-tocopherol was consistently the predominant compound in all olive oils.

In another study, Cunha et al. (2006) examined 18 olive cultivars in Portugal and reported that tocopherol content in the resulting olive oils ranged from 93 to 260 mg kg^{-1}. Similarly, a study on five major Greek olive cultivars found alpha-tocopherol levels ranging from 74 to 242 mg kg^{-1} in olive fruits (Georgiadou et al., 2019). Baccouri et al. (2008) investigated the effect of genotype on tocopherol content in seven different olive cultivars grown under similar conditions and observed tocopherol levels ranging from 310 to 780 mg kg^{-1} of olive oil. In the tocopherol biosynthesis pathway, five proteins, including methyltransferase and cyclase enzymes, play key roles (Lushchak and Semchuk, 2012). Therefore, genetic differences in the expression of genes encoding these enzymes, along with environmental influences on their activity, are major determinants of tocopherol production and accumulation. Because of the biennial bearing habit of olive trees and snow-related damage, only eight genotypes were evaluated in both years, allowing for a two-year comparison. Analysis of variance revealed that the interaction between year and genotype was significant at the 1% probability level for all measured traits (Table 1).

Table 1. Analysis of variance of genotype and year data on quantitative and qualitative characteristics of olive oil.

Sources of change	degree of freedom	OP	FFA	Pv	MI	Alpha
Genotype	7	32.82^{ns}	0.99**	5.6^{ns}	0.13^{ns}	35420.4**
year	1	234.50**	0.19**	9.6**	6.42**	13486.98**
Genotype×year	7	88.50**	0.12**	8.96**	1.62**	11733.05**
Error	28	6.18	0.01	0.52	0.09	183.21

* and ** are significant at 5 and 1% level, respectively, and ^{ns} is not significant.

Genotype effect

The highly significant differences observed in alpha-tocopherol and fatty acid content reflect substantial genetic diversity for these traits. This indicates that different genotypes inherently possess varying capacities to produce olive oil with these characteristics. For instance, some genotypes may have a greater ability to produce olive oil rich in alpha-tocopherol, a potent antioxidant. Olive oil is a product of olive tree metabolism and is strongly influenced by genetics or cultivar. Olive cultivars differ widely in traits such as yield, growth habit, early or biennial bearing, cold tolerance, flowering and ripening time, disease susceptibility, fruit size, pulp-to-stone ratio, oil content, composition of major and minor components, and organoleptic properties (Sadeghi, 2016). The quality of olive oil is defined by a set of characteristics that enhance nutritional value and increase consumer appeal. Among the most important quality indicators are the ratio of monounsaturated to polyunsaturated fatty acids (MUFA/PUFA), oxidative stability (peroxide value), and fatty acid profile, all of which are strongly influenced by factors such as cultivar, fruit maturity, yield, and tree vigor. The MUFA/PUFA ratio, or oleic/linoleic ratio, is considered the best predictor of oxidative stability (Beltran and Rio, 2004).

Effect of the year

The highly significant differences ($P<0.01$) observed in all traits indicate that environmental conditions each year substantially impact the chemical composition of olive oil. Factors such as rainfall, temperature, sunlight, and pest pressure can directly influence olive tree growth and, consequently, the quality of the produced oil. Ben Rouina et al. (2002), studying various olive cultivars in the hot and dry regions of Tunisia, reported a significant reduction in photosynthesis and final tree growth under drought and heat stress, which in turn affected both olive fruit and oil quality. Similarly, Hosseini et al. (2010) investigated the effects of different levels of drought stress on two olive cultivars and found that net photosynthesis, stomatal conductance, and transpiration decreased in both. The Arbequina cultivar, however, performed better than the Zard cultivar, likely due to its superior ability to maintain leaf water status and photosynthetic activity under stress, indicating greater drought tolerance.

Interaction effect of genotype and year

The highly significant differences ($P<0.01$) observed in all traits indicate that genotypes respond differently to varying environmental conditions. In other words, a genotype that performs best in one year may not necessarily perform optimally in the following year. This phenomenon, known as "genotype by environment interaction," highlights the complex interplay between genetics and environment. Olive oil quality results from the interaction between the olive tree (genetics or cultivar) and its growing environment, including climate, temperature, rainfall, and water availability, as well as horticultural management practices such as yield, shading, leaf-to-fruit ratio, pruning, and fertilization. These factors collectively influence the chemical composition, color, aroma, and flavor of olive oil, contributing to the unique identity and characteristics of each cultivar, including fatty acids, sterols, and hydrocarbons (Sadeghi, 2016). Dida et al. (1994) investigated the relationship between environmental conditions and the quality of Arbequina olive oil, demonstrating that oils produced under different climatic conditions exhibited distinct qualities.

Conclusions

The findings of this experiment highlighted the significant roles of both genotype and year in shaping the quantitative and qualitative characteristics of olive oil. Moreover, the observed interaction between genotype and environmental conditions across years highlights the complexity of olive oil quality as a trait influenced by both genetic makeup and environmental variability. These results emphasize the importance of multi-faceted approaches in future research to optimize olive oil quality through both genetic selection and environmental management. To further understand the underlying mechanisms, future studies should incorporate integrative approaches such as genomic, metabolomic, and gene expression analyses. Genomic studies can identify loci associated with desirable traits, including oil composition, yield stability, and stress tolerance. Metabolomic profiling can help elucidate the biochemical pathways responsible for flavor, aroma, and nutritional quality, while gene expression analysis can provide insights into the dynamic responses of different genotypes under varying environmental conditions. Additionally, long-term, multi-location trials would be valuable for assessing genotype stability and adaptability across diverse climatic and agronomic conditions. Such trials can help determine whether the superior genotypes identified in the current study maintain their performance over time and across regions. From a practical standpoint, modern breeding techniques, including marker-assisted selection and genomic selection, should be employed to develop new cultivars that combine multiple desirable traits, such as high oil yield, superior sensory and nutritional profiles, and resilience to environmental stresses like drought and pests. The use of biotechnological tools could further accelerate the development of elite olive varieties.

Acknowledgments

We sincerely thank the caretakers of the Minodasht collection, which was established in collaboration with Dr. Beski's collection and the National Genetics Center of Iran, for providing the foundation and support necessary for this study.

Authors Contributions

Supervised the research and revised the manuscript critically for important intellectual content, AG; collected the data, performed data analysis, and wrote the first draft of the manuscript, SH; contributed to data interpretation and manuscript revision, ES; assisted with statistical analysis and graphical presentation, IK. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Conflict of Interest

The authors indicate no conflict of interest in this work.

References

Arafat SM, Basuny AMM, Elsayed ME, Soliman HM. 2016. Effect of pedological, cultivar and climatic condition on sterols and quality indices of olive oil. Scientia Agriculturae 13(1), 23–29. https://doi.org/10.1002/jsfa.1887

Arjmand Fard L, Shahriari S, Ghavami M. 2021. Qualitative evaluation of virgin olive oil produced from different olive cultivars in Darab-Shiraz region. Iranian Food Science and Technology Research Journal 17(5), 819–829. https://doi.org/10.22067/ifstrj.v18i1.86095

Baccouri B, Zarrouk W, Baccouri O, Guerfel M, Nouairi I, Krichene D, Zarrouk M. 2008. Composition, quality and oxidative stability of virgin olive oils from some selected wild olives (Olea europaea L. subsp. Oleaster). Grasas y Aceites 59(4), 346–351. https://doi.org/10.3989/gya.2008.v59.i4.528

Basuny AM. 2019. Antioxidants in Olive Oil. In: Antioxidants, 1–14. IntechOpen. https://doi.org/10.5772/intechopen.84540

Beltrán G, del Río C, Sánchez S, Martínez L. 2004. Seasonal changes in olive fruit characteristics and

olive oil accumulation during ripening process. Journal of the Science of Food and Agriculture 84(13), 1783–1790. https://doi.org/10.1002/jsfa.1887

Ben Rouina B, Trigui A, Boukhris M. 2002. Effect of the climate and the soil conditions on crops performance of the "Chemlali de Sfax" olive trees. Acta Horticulturae 586, 285–289. https://doi.org/10.17660/ActaHortic.2002.586.55

Carlos S, Pérez A, León L, Pascual M, de la Rosa R, Belaj A. 2019. Analysis of Olive (*Olea europaea* L.) Genetic Resources in Relation to the Content of Vitamin E in Virgin Olive Oil. Antioxidants 8(8), 242. https://doi.org/10.3390/antiox8080242

Cunha SC, Amaral JS, Fernandes JO, Oliveira MBPP. 2006. Quantification of tocopherols and tocotrienols in Portuguese olive oils using HPLC with three different detection systems. Journal of Agricultural and Food Chemistry 54(9), 3351–3356. https://doi.org/10.1021/jf053238g

Cuomo F, Cinelli G, Chirascu C, Marconi E, Lopez F. 2020. Antioxidant effect of vitamins in olive oil emulsion. Colloids and Interfaces 4(2), 23. https://doi.org/10.3390/colloids4020023

Dag A, Kerem Z, Yogev N, Zipori I, Lavee S, Ben-David E. 2011. Influence of time of harvest and maturity index on olive oil yield and quality. Scientia Horticulturae 127, 358–366. https://doi.org/10.1016/j.scienta.2010.11.008

Deidda P, Bandino G, Solinas M, Nieddu G, Orru V, Serraiocco A, Spano D. 1994. Olive oil quality in relation to environmental conditions. Acta Horticulturae 356, 354–357. https://doi.org/10.17660/ActaHortic.1994.356.71

Delgado A, Al-Hamimi S, Ramadan MF, Wit MD, Durazzo A, Nyam KL, Issaoui M. 2020. Contribution of tocols to food sensorial properties, stability, and overall quality. Journal of Food Quality 2020, Article ID 8885865. https://doi.org/10.1155/2020/8885865

Di Vincenzo A, Tana C, El Hadi H, Pagano C, Vettor R, Rossato M. 2019. Antioxidant, anti-inflammatory, and metabolic properties of tocopherols and tocotrienols: Clinical implications for vitamin E supplementation in diabetic kidney disease. International Journal of Molecular Sciences 20(20), 5101. https://doi.org/10.3390/ijms20205101

Fanelli V, Mascio I, Miazzi MM, Savoia MA, De Giovanni C, Montemurro C. 2021. Molecular approaches to agri-food traceability and authentication: An updated review. Foods 10(7), 1644. https://doi.org/10.3390/foods10071644

Foscolou A, Critselis E, Panagiotakos D. 2018. Olive oil consumption and human health: A narrative review. Maturitas 118, 60–66. https://doi.org/10.1016/j.maturitas.2018.10.013

Georgiadou EC, Koubouris G, Goulas V, Sergentani C, Nikoloudakis N, Manganaris GA, Fotopoulos V. 2019. Genotype-dependent regulation of vitamin E biosynthesis in olive fruits as revealed through metabolic and transcriptional profiles. Plant Biology 21(4), 604–614. https://doi.org/10.1111/plb.12950

Gharby S, Hajib A, Ibourki M, Sakar EH, Nounah I, Moudden HE, Elibrahimi M, Harhar H. 2021. Induced changes in olive oil subjected to various chemical refining steps: A comparative study of quality indices, fatty acids, bioactive minor components, and oxidation stability kinetic parameters. Chemical Data Collections 33, 100702. https://doi.org/10.1016/j.cdc.2021.100702

Hashempour A, Azimi M, Abdollahi A, Fatahi Moghadam J. 2021. Evaluation of qualitative traits and bioactive compounds of some cultivars and promising genotypes in Tarom region in Zanjan province. Food Science and Technology 18(11), 99–110. Available at: https://agris.fao.org/search/en/providers/124062/records/65255a210ca71c9f6d70776c

Jiménez-López C, Carpena M, Lourenço-Lopes C, Gallardo-Gómez M, Lorenzo JM, Barba FJ, Prieto MA, Simal-Gándara J. 2020. Bioactive compounds and quality of extra virgin olive oil. Foods 9(8), 1014. https://doi.org/10.3390/foods9081014

Kassa A, Konrad H, Geburek T. 2019. Molecular diversity and gene flow within and among different subspecies of the wild olive (*Olea europaea* L.): A review. Flora 250, 18–26. https://doi.org/10.1016/j.flora.2018.11.014

Lushchak VI, Semchuk NM. 2012. Tocopherol biosynthesis: Chemistry, regulation and effects of environmental factors. Acta Physiologiae Plantarum 34(5), 1607–1628. https://doi.org/10.1007/s11738-012-0988-9

Mailer R. 2006. Chemistry and quality of olive oil. NSW Department of Primary Industries, Primefact 227. Available at: https://www.dpi.nsw.gov.au/content/agriculture/horticulture/olives/olives-general/chemistry-and-quality-olive-oil

Pérez-López D, Ribas F, Moriana A, Rapoport HF, De Juan A. 2008. Influence of temperature on the growth and development of olive (*Olea europaea* L.) trees. Journal of Horticultural Science & Biotechnology 83(2), 171–176. https://doi.org/10.1080/14620316.2008.11512366

Rabiei M. 2021. Commercial olive genotypes. Sivan Sayan. (Note: DOI not available)

Rostami Ozumchuluei S, Ghasemnezhad M, Ramzani Malekroudi M. 2016. Effect of olive fruit harvest time on olive oil yield and quality of some olive (*Olea europaea* L.) cultivars in Roudbar region. Journal of Crop Production and Processing 5(4), 115–124. Available at: https://fsct.modares.ac.ir/article-7-6605-en.html

Soltani S, Seifi S, Ghasemnejad A, Fereidooni H. 2014. The study of some native and exotic olive cultivars and genotypes in terms of morphological diversity, olive oil quality and fatty acid composition. Journal of Plant Production 23(2), 1–22. (Note: DOI not available)

Tuberoso CIG, Jerković I, Maldini M, Serreli G. 2016. Phenolic compounds, antioxidant activity, and other characteristics of extra virgin olive oils from Italian autochthonous varieties Tonda di Villacidro, Tonda di Cagliari, Semidana, and Bosana. Journal of Chemistry 2016, Article ID 8462741. https://doi.org/10.1155/2016/8462741