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Understanding the Effect of the Position of Fruit in the Canopy of Olive Trees on Oil Characteristics

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The characteristics of olive fruits, which strongly affect both the quality and quantity of olive oil, are affected by the position of the fruit in the canopy of olive trees. The present study aimed to understand the net effect of the position of the fruit in the canopy on the oil characteristics by comparing oils extracted from ripened olive fruits harvested at different heights in the tree canopy. Chemical analyses, measuring oil quality parameters, fatty acid composition and volatile compounds, showed higher values of acidic parameters, an increase in oxidative off-flavors, a decrease in olive oil aroma and a reduction in the concentration of oleic acid in oils extracted from fruits harvested from the higher canopy layers compared with those from the lower canopy layers. This suggested that lipid oxidation was greater in these oils compared with oils from fruit harvested from the lower layers. However, oils obtained from fruits from the higher layers exhibited higher antioxidant activities and contents of photoprotectors such as phenolics and carotenoids, which act as defense compounds under strong lighting conditions. These results suggest that environmental lighting conditions strongly affect oil quality and provide relevant information for methods of harvesting fruits destined for the production of high-quality olive oil.

1. Introduction

The olive (*Olea europaea* L.) is an evergreen tree, native to the Mediterranean basin and parts of Asia, and is now widely cultivated in many other areas of the world for the production of olive oil and table olives. Olive oil, one of the world's major edible oils, is a component of the Mediterranean diet, and is appreciated for its aroma, taste, color and nutritive features that distinguish it from other vegetable oils. Extra virgin olive oil (EVOO), prized for its sensory and nutritional properties, has the highest quality category of olive oil because it is extracted from fresh olive fruits using only physical processing with no added chemicals. Olive oil is a main source of oleic acid (> 55% of total fatty acids) and has the lowest content of linoleic acid compared with other vegetable oils (IOC, 2019). Although linoleic acid is an essential fatty acid, it is not suitable for cooking purposes because of the trans fatty acids which are linked to increased risk of cardiovascular disease. While vegetable oils such as olive oil with higher levels of oleic acid are preferred for food and industrial purposes, an increase of oleic acid levels leads to oxidative stability and reduces the risk of cardiovascular disease. Therefore, olive oil is often preferred as a conventional cooking oil and as a high-quality premium edible oil.
When olive oil is produced, its characteristics are influenced by several factors during the harvesting and extraction processes. In particular, the decisions made when harvesting olive fruits, such as the timing of the harvest, the maturity of the olive fruit and the cultivar, have a central role in determining the oil yield, and its quality and chemical composition. The timing of the harvest has the most influence on the yield, quality, sensory attributes and chemical composition of the olive oil, which are all of particular interest to the oil producers (Salvador et al., 2001). Currently, the proportion of intensively-run olive orchards has been increasing to help improve olive productivity because of the rapid increase in the size of the olive oil market. Therefore, the environmental conditions (e.g., light, temperature and soil composition) for olive cultivation and production have also become important factors affecting the oil characteristics.
For plants, light is an important environmental factor influencing photosynthesis and the production of secondary metabolites such as phenolic compounds. It has been estimated that approximately 2% of all carbons

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photosynthesized in plants are transformed into phenolic compounds (Robards and Antolovich, 1997). Phenolic compounds are produced by plants mainly to counteract the effects of adverse conditions, such as UV radiation. Plant phenolic compounds are thought to play an important role as defense compounds under environmental lighting conditions. A deficiency in their content increases the production of free radicals and other oxidative species because the phenolics in the oil provide antioxidant activity. As well as phenolic compounds, studies on phytochemicals in olive fruit have revealed the presence of a variety of bioactive secondary metabolites such as triterpenic compounds, tocopherols, sterols and pigments (Ghanbari et al., 2012). These bioactive compounds in the fruit are transferred to the extracted oil so are responsible for many health benefits from ingesting the oil.

Environmental lighting conditions can affect the oil characteristics. Information on bioactive compounds such as phenolics and the antioxidant activity of olive oil would help consumers become more aware of the level of beneficial phytochemicals present in this nutritious vegetable product. Investigating the effect of light levels on the content of phenolics and other antioxidants in olive oil would help identify new cultivation methods for enhancing the nutritional value of olive oil. The characteristics of olive fruit, such as grade of maturity, size, and oil content, have been reported to vary according to their position in the canopy of the tree, and are strongly related to the illumination level (Acebedo et al., 2000; Gómez-del-Campo et al., 2009; Connor et al., 2009). The oils extracted from olive fruits at different maturation grades have been analyzed to better understand the effects of fruit position at different heights in the canopy on the fruit characteristics (Gómez-del-Campo et al., 2009, 2012; Castillo-Ruiz et al., 2015). Fruit growth and maturity are more rapid in the higher canopy layers where a higher quantity of solar energy is available for photosynthesis. The quantity and quality of bioactive compounds in olive fruits vary according to their stage of development. For example, an increase in the content of phenolic compounds has been reported between the green and spotted stages of maturation then a decrease up to the mature (black) stage (Franco et al., 2014). Changes in the content of pigments, such as chlorophylls and carotenoids, also depend on the level of the light available to the plant. Overall, these studies have pointed out the effect of the higher and better illuminated canopy layers in facilitating olive fruit growth and development but more information is needed on how the position of the fruit in the canopy directly affects the olive oil characteristics to ensure the production of high-quality oil. Therefore, this study aims to quantify the net effect of the position of the fruit in the canopy on oil by comparing the characteristics of oils extracted from fruits with almost the same maturity index harvested at different heights in the canopy of olive trees.

2. Experimental

2.1 The orchards

Three self-rooted olive trees (*Olea europaea* L. cv. Mission) planted in east-west oriented rows in Shodoshima (Kagawa, Japan; 34.4° N, 134.1° E) were used. Each tree was 15 years old, vase-shaped, with two or three main branches and a trunk 0.5 m in diameter, and planted at a 5 × 5 m spacing. The mean tree height and width were relatively uniform at 4.0 ± 0.1 and 4.0 ± 0.3 m, respectively. The treatments investigated comprised three layers defined by the height from the ground to: the lower canopy, < 1.5 m; the middle canopy, 1.5–3.0 m; and the higher canopy, 3.0–4.0 m.

2.2 Harvesting and assessing maturity index of olive fruits

The olive maturity index was determined by assessing the skin color of each sample (García et al., 1996). By classifying the fruit from 0 to 7 based on their skin color, the fruits were harvested separately from each of the three heights in the canopy in mid-November 2020. The average fruit weight was determined from a randomly-taken sample of 100 healthy fruits.

2.3 Oil extraction

The oil was extracted as described previously (Kashiwagi et al., 2019). The oil phase was placed in a new tube and weighed. The oil yield was calculated as the oil mass as a percentage of the mass of fresh olives from which it was obtained. After weighing, the oils were filtered through filter paper then stored in dark glass bottles until analysis.

2.4 Analytical procedures

The free fatty acids, peroxide value, coefficient of specific extraction at 270 nm (K270) and total phenolic contents of the oil samples were measured using an OxiTester (CDR; Ginestra Fiorentina, Italy) (Kishimoto, 2021a). The α-tocopherol contents in the oil samples were measured by Japan Food Research Laboratories (Tokyo, Japan). The contents of chlorophylls and carotenoids were determined as described previously (Kishimoto, 2021a). The concentrations of hexanal and E2-hexenal in oil samples were determined through flash gas chromatography electronic nose analysis using a HERACLES II electronic nose (Alpha MOS, Toulouse, France) as described previously (Kishimoto, 2021b). The absorption spectra of the samples were recorded to determine the UV and visible light absorbance using a spectrophotometer UV-1800 (Shimadzu Co., Kyoto, Japan).

2.5 Total antioxidant capacity assay

The antioxidant power of the oil samples was evaluated using the PAO-SO Test kit (Japan Institute for the Control of Aging, Nikken Seil Co., Ltd., Shizuoka, Japan), according to the manufacturer's instructions.

2.6 Analysis of fatty acid composition

The composition of fatty acids was determined by near infrared spectrophotometry using a SpectraStar 2600 XT-R analyzer (Unity Scientific, Milford, MA, USA). The analytical conditions were as follows: the oil sample (1.5 mL) was put in the ring cup then placed on the sample stage of the analyzer. The system was controlled by UScan chemometric software (Unity Scientific), incorporating calibrations for common constituents such as the fatty acid composition of olive oil.

2.7 Statistical analysis

Data are presented as means ± standard deviation from three replicates. The data were analyzed using one-way analysis of variance followed by the Student's *t*-test in Microsoft Excel. Differences between mean values were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1 Effect of fruit position in the canopy of olive trees on the maturity index

The relationship between the properties of olive fruits from the three layers was investigated by using separate samples harvested from each canopy layer so that their maturity indices could be compared. Table 1 shows that the position of the fruit in the canopy significantly affected its maturity index with the highest value obtained in fruits harvested from the higher canopy layers ($p < 0.05$). This trend has also been reported for another olive cultivar, 'Arbequina' (Ghanbari et al., 2012; Castillo-Ruiz et al., 2015). The mean fresh weight of the olive fruit was 4.5 ± 0.1 g, with no significant difference between those harvested from the higher and lower layers, although fruit size has been reported to vary in canopy layers of different height. This difference may have been caused by the comparison in the present study between ripe olives (black skin) with almost the same maturity index. Previous studies have reported that the percentage of immature olives (green skin) is greater in the lower than in the higher canopy layers (Ghanbari et al., 2012; Gómez-del-Campo et al., 2012; Castillo-Ruiz et al., 2015). Therefore, the weight of olive fruits harvested from the lower canopy layers should be less than those from the higher layers. This therefore confirmed that the development of olive fruits was significantly affected by their position in the vase-shaped olive canopies with the more illuminated canopy layers yielding fruit with a higher maturity index.

Table 1: Effect of the position of fruit in the canopy on its mean maturity index.

Fruit canopy location	Mean maturity index
Higher	5.8 ± 0.4
Middle	5.0 ± 0.3
Lower	4.4 ± 0.3

3.2 Analysis of conventional quality parameters

The net effect of the position of the fruit in the canopy of olive trees on the quality and quantity of the oil was determined by comparing olive oils extracted from ripened black olives with almost the same maturity index of six harvested from canopy layers at two different heights (higher and lower). The olive fruits harvested from the middle canopy layers were not included in this analysis, because there were no significant differences in the maturity index between fruits harvested from the middle layers and those from the higher or lower layers (Table 1) (Gómez-del-Campo et al., 2012; Castillo-Ruiz et al., 2015). Table 2 shows that fruits harvested from the higher canopy layers yielded more oil as a percentage of total wet matter than those from the lower layers ($p < 0.05$). This could be related to the higher oil content of fruits harvested from the higher canopy layers (Ghanbari et al., 2012; Castillo-Ruiz et al., 2015). The values of the three quality parameters, free fatty acids, peroxide value and K270, of the extracted oils, which have been legally established for evaluating the quality level, were within the internationally-recognized limits for the commercial quality "extra", the highest quality level for EVOO (Table 2). The values of these parameters were very low and affected by the position of the fruit in the canopy, increasing with the height of the fruit growing layer. This indicated that lipid oxidation was significantly increased in oils extracted from fruits harvested from the higher canopy layers compared with those from the lower layers ($p < 0.05$). These results suggest that differences in light distribution in the olive canopy zones may be partially responsible for distinct patterns of oil accumulation and oil quality.

Table 2: Comparison of the yield and quality parameters of oil samples.

Parameters	Canopy height	
	Higher	Lower
Oil yield (%)	6.7 ± 0.8	5.1 ± 0.4
Free fatty acids (%)	0.15 ± 0.03	0.06 ± 0.02
Peroxide value (meqO_2/kg)	4.9 ± 0.5	1.7 ± 0.3
K270	0.079 ± 0.009	0.024 ± 0.007

3.3 Analysis of volatile compounds

As well as the conventional oxidative markers discussed above, the contents of two particular volatile compounds in the extracted oils, hexanal and E2-hexenal, were also investigated. E2-hexenal contributes significantly to the aroma of olive oil and is related to the positive sensory characteristics of almond and green olive fruits but hexanal is directly related to oxidative off-flavors (Kalua et al., 2007; Kishimoto, 2021a, 2021b). A higher hexanal/E2-hexenal ratio indicates a higher degree of oxidation in the oil, because hexanal can also be formed by the auto-oxidation of unsaturated fatty acids (Jiménez et al., 2007). Therefore, these compounds can be recommended as reliable markers of olive oil oxidation. Table 3 shows that the contents of hexanal and E2-hexenal in oils extracted from olive fruits harvested from the higher canopy layers were significantly higher and lower, respectively, than in oil from fruits harvested from the lower layers ($p < 0.05$). This indicated that oxidative alterations had occurred in the oils extracted from fruits harvested from the higher canopy layers. This observation was confirmed by the higher values of the conventional oxidative parameters measured in the oils extracted from fruit harvested from the higher layers (Table 2).

Table 3: Comparison of hexanal and E2-hexenal contents in oil samples.

Volatile compounds	Canopy height	
	Higher	Lower
Hexanal (ppm)	6.5 ± 0.1	5.5 ± 0.1
E2-hexenal (ppm)	17.6 ± 0.8	19.3 ± 0.4

3.4 Analysis of antioxidants and antioxidant power

EVOOs are rich in antioxidants such as phenolic compounds, α-tocopherol and carotenoids (Acebedo et al., 2000). Table 4 shows the content of these three antioxidants and the values of antioxidant power in the oils extracted from the olive fruits harvested at different canopy heights. The oils extracted from the fruits harvested from the higher canopy height had greater contents of phenolics and carotenoids than from those harvested from the lower height but there was no difference in the α-tocopherol content. Table 5 shows that the UV and visible short-wave light absorption abilities of oil extracted from olive fruits harvested from the higher canopy height were significantly higher than those of oils harvested from the lower height ($p < 0.05$). The common response of plants to ambient sunlight is to produce compounds which absorb UV-visible light, so-called photoprotectors such as phenolics and carotenoids. These compounds, which play a key role in counteracting oxidative stress induced by light and preventing the penetration of sunlight into the fruits, are transferred into the oil (Gómez-del-Campo, 2009). The contents of chlorophyll pigments have been reported to decrease throughout the process of ripening, while the contents of carotenoids decrease more gradually and discontinuously (Gandul-Rojas et al., 1999). In the present study, no chlorophylls were detected in the oil of fruit from either canopy height (data not shown) but the carotenoids content was higher in the oils from fruit harvested from the higher canopy height ($p < 0.05$). Oils extracted from fruits harvested from the higher canopy height also exhibited a higher level of antioxidant power and phenolic and carotenoid contents ($p < 0.05$). The high coefficients of determination between the measured antioxidant capacity and the contents of phenolics and carotenoids (R^2 = 0.9869 and 0.9370, respectively) suggest that phenolics and carotenoids functions as the major antioxidant components and photoprotectors in the oils, which may quench the oxidative stress induced by sunlight.

Table 4: Comparison of antioxidant contents and antioxidant power of oil samples.

Chemical parameters	Canopy height	
	Higher	Lower
Total phenolic content (mg/kg)	294 ± 32	194 ± 21
α-Tocopherol content (mg/kg)	160 ± 7	168 ± 4
Total carotenoids content (mg/kg)	0.8 ± 0.1	0.5 ± 0.1
Antioxidant power (μM)	13,211 ± 459	9,842 ± 805

Table 5: Comparison of light absorption abilities of oil samples.

Light components	Canopy height	
	Higher	Lower
Total UV (290–400 nm)	112.8 ± 5.8	92.2 ± 4.9
Visible short-wave (400–500 nm)	17.4 ± 2.5	11.5 ± 0.2

3.5 Analysis of fatty acid composition

Table 6 shows the effect of the canopy height on the fatty acid composition of the oils extracted from the olive fruits. Fatty acids such as heptadecanoic (C17:0) and heptadecenoic acids (C17:1) were present at very low concentrations (< 0.1%) in all the oils so were not further considered in the present study (data not shown). The concentrations of linolenic (C18:3), arachidic (C20:0) and eicosenoic acids (C20:1) in the extracted oils did not vary with the position of the fruit in the canopy but the contents of other fatty acids were significantly affected by this factor. The concentration of oleic acid was significantly lower in the oil of fruit from the higher canopy height ($p < 0.05$), whereas the concentrations of the other fatty acids (palmitic, palmitoleic, stearic and linoleic) were significantly higher ($p < 0.05$). These results were consistent with reports on another olive cultivar, 'Arbequina' (Ghanbari et al., 2012; Acebedo et al., 2000; Connor et al., 2009; Gómez-del-Campo et al., 2012). In general, light is known to affect the synthesis of fatty acids in plants. A high level of illumination has been reported to allow olive calli to activate oleate desaturation leading to the formation of linoleic acid (Hernández et al., 2008). In the present study, the fruits would have received more solar energy at the higher canopy height, which would have enabled fatty acid synthesis and oleic acid desaturation in the olive cells to increase. Therefore, the fruits harvested from the more illuminated canopy layers may have a higher oil content (Table 2) and a lower concentration of oleic acid. The slightly elevated linoleic acid content may have contributed to the oxidation of the oils extracted from the fruits harvested from the higher canopy heights (Table 2), leading to a negative effect on oil quality (Mao et al., 2020).

Table 6: Fatty acid composition of olive oil according to the position of the olive fruits in the tree canopy.

Fatty acids	Canopy height	
	Higher	Lower
Palmitic (C16:0)	10.0 ± 0.6	9.8 ± 0.6
Palmitoleic (C16:1)	0.74 ± 0.03	0.71 ± 0.07
Stearic (C18:0)	1.89 ± 0.01	1.88 ± 0.01
Oleic (C18:1)	67.2 ± 1.7	68.4 ± 1.3
Linoleic (C18:2)	8.6 ± 0.2	8.5 ± 0.2
Linolenic (C18:3)	0.93 ± 0.01	0.93 ± 0.01
Arachidic (C20:0)	0.32 ± 0.01	0.33 ± 0.01
Eicosenoic (C20:1)	0.27 ± 0.01	0.27 ± 0.01

4. Conclusions

The present study characterized oils extracted from fruits harvested at different heights in the canopy of olive trees and found that the oil quality varied according to the position of the fruit in the canopy. Olive fruits from the higher canopy layers received more illumination thus producing oil that was richer in phytochemicals such as phenolic compounds and carotenoids. This could improve their antioxidative activity to quench any oxidative stress induced by sunlight and provide a level of photoprotective activity able to reduce the penetration of UV- and visible-light radiation. However, the higher level of illumination may also lead to greater lipid oxidation in the olive fruits, to the formation of hexanal causing oxidative off-flavors, to a decrease in olive oil aroma compounds such as E-hexenal, and a lower concentration of oleic acid which has health benefits. Overall, the oils obtained from olive fruit harvested at the different canopy positions complied with the requirements for EVOO based on chemical parameters set out by the International Olive Council (2019). These results suggest that environmental lighting conditions play an important role in the production of EVOO by controlling olive oil quality regarding, for example, the amounts of phytochemicals present and the extent of oil oxidation.

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