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Special Graduation Project

Characterization of olive oil from the variety of *Lefkoelia Serron* of four different times of harvest

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Abstract

The quality of olive oil is not just a matter of geography or processing technique but is also closely related to the specific variety of olive, cultivation practices, and the timing of the harvest. This study evaluated the quality of *Lefkoelia Serron* olive oil harvested at four different times (pre-mature, mature and post-mature) to see how it aligns with the standards set by the International Olive Council (IOC). Although *Lefkoelia Serron* has not been studied thoroughly yet, the focus was on understanding how the timing of the harvest influences key quality factors like acid value, oxidative stability, peroxide value, K-values, chlorophyll, carotenoid and phenolic content. The research was designed using a Completely Randomized Design with three replicates, and data was analyzed using ANOVA to identify any significant differences between the samples. The findings revealed that all olive oil samples met the criteria for Extra Virgin Olive Oil (EVOO). However, oils harvested earlier, especially in October, stood out with superior quality traits—lower acidity, greater resistance to oxidation, and higher phenolic content—compared to those harvested later. This suggests that harvesting olives earlier could be the key to producing high-quality olive oil. To preserve quality and strengthen market position, producers are encouraged to prioritize early harvesting and proper storage methods. Future studies might explore additional factors, such as different environmental conditions and more detailed chemical analyses, to deepen the understanding of what influences olive oil quality.

Keywords: Acid value, harvest timing, oxidative stability, phenolic content, quality indicators.

Resumen

Este estudio evaluó la calidad del aceite de oliva *Lefkoelia Serron* cosechado en cuatro momentos diferentes (pre-maduración, maduración y pos-maduración) para evaluar cómo se alinea con los estándares establecidos por el Consejo Oleícola Internacional (COI). El enfoque se centró en comprender cómo el momento de la cosecha influye en factores clave de calidad como el valor de acidez, la estabilidad oxidativa y el contenido fenólico. La investigación se diseñó utilizando un Diseño Completamente Aleatorizado con tres repeticiones, y los datos se analizaron mediante ANOVA para identificar diferencias significativas entre las muestras. Los resultados revelaron que todas las muestras de aceite de oliva cumplieron con los criterios para ser clasificadas como Aceite de Oliva Virgen Extra (AOVE). Sin embargo, los aceites cosechados más temprano, especialmente en octubre, destacaron por sus características de calidad superiores: menor acidez, mayor resistencia a la oxidación y mayor contenido fenólico, en comparación con los cosechados más tarde. Esto sugiere que la cosecha temprana de aceitunas podría ser clave para producir aceite de oliva de alta calidad. Para preservar la calidad y fortalecer la posición en el mercado, se recomienda a los productores priorizar la cosecha temprana y métodos de almacenamiento adecuados. Futuros estudios podrían explorar factores adicionales, como diferentes condiciones ambientales y análisis químicos más detallados, para profundizar en la comprensión de lo que influye en la calidad del aceite de oliva.

Palabras clave: Contenido fenólico, estabilidad oxidativa, indicadores de calidad, momento de la cosecha, valor de acidez.

Introduction

The olive tree (*Olea europaea*) has been a symbol of life and prosperity in the Mediterranean for thousands of years, deeply rooted in the region's culture and history. From prehistoric times, olives and their oil have played a crucial role in human diets, with evidence of their cultivation dating back nearly 6,000 years to the early Bronze Age (3150 to 1200 BCE) (Zohary et al., 2013). Artifacts like inscribed tablets, olive pits, and pieces of olive wood found in ancient burial sites suggest that olive cultivation began along the eastern Mediterranean coast, in what is now southern Turkey, Syria, Lebanon, Palestine, and Israel (Vossen, 2007). Olive oil was more than just a food source in ancient societies; records from around 2000 BCE in Syria show that it was a highly prized commodity, valued much higher than wine or seed oils (Vossen, 2007). The cultivation of olives spread alongside other important agricultural crops like grapes, dates, and figs, reflecting the interconnected nature of agriculture and trade in ancient times.

Growing olive trees from seeds is notoriously difficult because it takes about 10 to 15 years for the trees to bear fruit, and the resulting plants often do not have the same characteristics as the parent tree (Fabbri, 2004). To preserve desirable traits and ensure consistent oil quality, growers have relied on vegetative propagation methods, such as cuttings. These methods, along with innovations in cultivation and processing techniques, have been pivotal in shaping the modern olive oil industry.

Currently, olive oil remains a cornerstone of the global food industry. The International Olive Council (IOC) reported that in 2020–21, IOC member countries produced 2.8 million tons of olive oil, accounting for 93.3% of the world's total output. The European Union (EU) dominates this market, with Spain producing the largest share, followed by Italy, Greece, and Portugal (International Olive Council, 2022). These figures underline the economic importance of olive oil for these countries and its enduring relevance as a dietary staple.

The ways in which olive oil is extracted have changed dramatically over time, moving from traditional methods to modern technologies. The classic discontinuous (pressing) method, still used in

some traditional mills, involves crushing olives with millstones and extracting the oil using mechanical presses (Kalia & Kumar, 2017). This method is still found in small, non-motorized setups, but more efficient techniques like the continuous (centrifuging) method have largely replaced it. This modern method allows for continuous oil extraction and more efficient separation of the oil from byproducts such as pomace, thereby maximizing resource use and minimizing waste (Ranalli & Angerosa, 1996). This process also includes the sustainable use of byproducts, such as using the solid residue (pulp and olive stones) for animal feed or further processing it into olive pomace oil (Berbel & Posadillo, 2018).

In recent years, there has been increasing concern over the authenticity and quality of virgin olive oil, driven by both consumer demand and medical research highlighting its health benefits (García-González et al., 2008). This has led to the adoption of quality marks such as Protected Geographical Indication (PGI) and Protected Designation of Origin (PDO). These labels ensure that the oil comes from a specific geographical area, with qualities unique to that region (Janin et al., 2014). To meet these standards, producers must undergo rigorous testing for various chemical markers, including fatty acids, triacylglycerols, sterols, volatile components, and tocopherol levels (Gargouri et al., 2023). Harvest timing plays a critical role here; the ripeness of the olives affects these chemical parameters, influencing acidity, peroxide value, and phenolic content, which in turn shape the oil's taste and stability (Paul Vossen, 2016) (Jiménez Herrera et al., 2012).

The quality of olive oil is not just a matter of geography or processing technique but is also closely related to the specific variety of olive, cultivation practices, and the timing of the harvest. These factors affect the oil's chemical composition, sensory attributes, and shelf life. Several key indicators help assess olive oil quality: acidity, peroxide value (PV), K-values, chlorophyll and carotenoid content, induction time, bitterness intensity, and total phenolic content. For example, acidity levels, measured as the percentage of free fatty acids (FFA), signal the extent of hydrolysis or degradation in the oil, which can result from poor handling or delayed processing and infestation off the fruit specially with olive fruit fly. Lower acidity

levels are preferred as they suggest better taste and longer shelf life. The IOC has established that extra virgin olive oils must have an acidity level below 0.8%, virgin olive oils must be below 2% to ensure high quality, olive oil composed of refined and virgin olive oils and olive-pomace oil below 1% (Al Henefat, 2022).

Similarly, the peroxide value (PV) is an important measure of the oil's freshness and oxidative state, with higher PVs indicating more oxidation and potential rancidity due to exposure to oxygen and light during production or storage (Ross & Sheng, 2014). The IOC recommends that extra virgin olive oils should have a PV lower than 20 milliequivalents (meq) of active oxygen per kilogram, with values above 10 meq/kg indicating less stability and shorter shelf life. The K-values, measured with ultraviolet spectrophotometry, provide further insights into the oil’s stability or adulteration with seed oils, with higher values suggesting more significant deterioration (International Olive Council, 2019).

Pigments like chlorophylls and carotenoids significantly impact the quality and appeal of olive oil. Chlorophyll contributes to the green color of fresh olive oil and has antioxidant properties that help gauge the oil's freshness (Bajoub et al., 2018). The balance between chlorophyll and carotenoids, which are more yellow orange in hue, depends on the olive variety and ripeness. Higher chlorophyll content is typical of early-harvest oils, while carotenoids become more prominent as olives ripen, affecting both the color and stability of the oil (Minguez-Mosquera et al., 1991). The degradation of these pigments can influence the perceived freshness and sensory appeal of the oil (Lino et al., 2024). The oxidation induction time, a critical factor in determining the quality and shelf life of olive oil, is measured by the Rancimat method to assess oxidative stability. The resistance of the oil to oxidative degradation can be predicted with the aid of an accurate assessment of induction time (Asbbane et al., 2024). Bitterness intensity is strongly correlated with phenolic content, which enhances both the oil's sensory qualities and health benefits. Bitterness intensity is an important quality criterion in olive oil characterization. Assessing bitterness is crucial in figuring out when to harvest the oil at its best and in assessing its sensory profile and stability, all of which

have a big impact on marketability and consumer preference. Moreover, the bitterness-causing phenolic chemicals are recognized for their antioxidant qualities, which enhance the nutritional value of olive oil and its shelf life. Total phenolic content (TPC) measurement is important because phenolic chemicals affect the oil's sensory qualities, oxidative stability, and nutritional value. These substances are well-known for having potent antioxidant properties, which extend the shelf life of olive oil and offer health advantages like lowering the risk of heart disease and some types of cancer (Bendini et al., 2007).

This study aims to dive deep into the characterization of the *Lefkoelia Serron* olive oil variety, examining how different harvest times affect quality indicators like acidity, peroxide value, K-values, chlorophyll and carotenoid levels, induction time, bitterness intensity, and phenolic content. These parameters are crucial for determining the quality, authenticity, and nutritional value of olive oil. By understanding how these factors vary with harvest timing, producers can refine their harvesting strategies to ensure better quality, higher antioxidant levels, and improved oxidative stability. This information is not only vital for maintaining the premium status of olive oil but also for meeting the evolving demands of the global market, where authenticity and quality are increasingly prized.

Materials and Methods

Study Location

This study was conducted at the American Farm School Research Campus, specifically in the Life Sciences Laboratory of Perrotis College, located in Thessaloniki, Greece.

Materials

The study utilized olive oil samples from the *Lefkoelia Serron* variety. The samples were harvested during different periods in 2022 and 2023 by students from Perrotis College, which allowed for an in-depth examination of how the timing of harvest affects the chemical properties and overall quality of the olive oil.

Sample Collection and Description

Sample 1**:** Harvested on October 17, 2023

Sample 2: Harvested on November 14, 2023

Sample 3: Harvested on December 4, 2023

Sample 4: Harvested in 2022 (sample aged one year)

These samples were selected to represent various stages of olive maturity, thereby providing insights into the impact of harvest timing on the olive oil's quality. Each harvest date was carefully recorded to correlate the level of ripeness with changes in the oil's chemical and sensory characteristics, a critical factor for determining the optimal harvest period for quality olive oil production (Vossen, 2013).

Olive Oil Extraction Process

The extraction process was standardized to ensure consistency and reliability across all samples. Three distinct batches of olives, representing different harvest times (pre-mature, mature, and post-mature), this process used the ABENCOR® system which underwent controlled processing steps as follows:

Pre-Mature Harvest Batch

Sample Preparation

The first batch comprised olives harvested before full maturation. For each extraction, 700 grams of olives were weighed precisely.

Grinding and Malaxation: The olives were grounded using a mechanical grinder, with the addition of 100 mL of distilled water during grinding to facilitate oil release. The ground olive paste then underwent a malaxation process, a crucial step in olive oil production where the paste is slowly mixed to enhance oil yield and flavor development. Malaxation was performed at 25 °C for 30 minutes, following recommended practices for optimal aroma development (Tsimidou, 2006).

Centrifugation: The olive paste was then transferred to a centrifuge, where an additional 100 mL of distilled water was added to aid in separating the oil from the solid components. Approximately 12 kg of olives were processed in this manner, and the extracted oil was stored in dark glass bottles under nitrogen to minimize oxidation and preserve quality.

Mature Harvest Batch

Process Modifications: The second batch consisted of olives harvested at a typical stage of maturity. The same extraction steps were followed, except for reducing the amount of water used. Only 100 mL of distilled water was added during grinding, and no additional water was added during centrifugation. This modification was based on the observation that the higher moisture content in these olives could reduce oil yield if excess water was added (El-Emam, 2023). A total of 7 kg of olives were processed for this batch.

Post-Mature Harvest Batch

Further Adjustments: The third batch consisted of olives harvested after maturation. Initially, the same process was applied as in the first batch. However, due to the lower oil yield observed, adjustments

were made by reducing the amount of water added during malaxation to 20 mL. This adjustment helped counter the high moisture content in the overripe olives, which can affect the efficiency of oil extraction (M. I. Minguez-Mosquera et al., 1990). For this batch, 7.3 kg of olives were used.

Analytical Methods

Determination of Acid Value

This method is commonly used for assessing free fatty acid (FFA) levels in oils by titrating the sample with a standardized sodium hydroxide (NaOH) solution until a color change is observed with phenolphthalein as the indicator. The acid value is reported as the amount of NaOH (in milligrams) needed to neutralize the free acids in 1 gram of oil.

Procedure: Five grams of each olive oil sample were accurately weighed into a conical flask. A solution of 100 mL of an ethanol/ether mixture (1:1) was added, and the mixture was thoroughly stirred. A few drops of phenolphthalein were added as an indicator, and titration was performed with 0.1 N NaOH until a stable pink color appeared, indicating the endpoint. The volume of NaOH used was recorded, and the acid value was calculated using the formula:

$$\%FFA(asoleic) = \frac{V \times N \times 282}{1000 \times W} \times 100 \quad [1]$$

Where:

%FFA = Percent free fatty acid (g/100 g), expressed as oleic acid

V = Volume of NaOH titrant (mL)

N = Normality of NaOH titrant

W = Sample mass (g)

Determination of K Values

K values were determined using the official method of the International Olive Council (IOC), which involves spectrophotometric analysis in the ultraviolet region to assess the quality of the oil and detect

any degradation products.

Procedure: For each sample, 0.05 grams of oil were weighed and transferred to a 10 mL graduated flask for 232 and 0.5 for 270. The samples were diluted with cyclohexane and homogenized. A spectrophotometer with quartz cells was used to measure the absorbance at 232 and 270 nm. The extinction coefficients (K values) were calculated to determine the degree of oxidation:

$$K\lambda = \frac{E\lambda}{c*s} \quad [2]$$

Where:

K = Specific extinction at wavelength λ

E = Extinction measured at wavelength λ

c = Concentration of the solution (g/100 mL)

s = Path length of the quartz cell (cm)

ΔK Value Calculation:

$$\Delta K = \left| Km - \left(\frac{K\lambda m-4 + K\lambda m+4}{2} \right) \right| \quad [3]$$

Where Km is the extinction at the maximum absorption wavelength.

Induction Time (Rancimat Method)

The oxidative stability of the olive oils was assessed using the Rancimat method, which determines the induction time—a measure of the oil's resistance to oxidation under accelerated conditions (Velasco & Dobarganes, 2002).

Procedure: Each oil sample (3 grams) was placed in a Rancimat apparatus at a temperature of 110°C with an airflow rate of 20 L/h. The induction time, which indicates when the oil begins to oxidize rapidly, was recorded for each sample.

Determination of Chlorophyll Pigments (IUPAC method)

The determination of chlorophyll pigments in olive oils was carried out using a modified method based on spectrophotometric analysis. The absorbance of the samples was measured at 630 nm, 670 nm,

and 710 nm using a spectrophotometer equipped with a 10 mm quartz cell. Prior to analysis, the oil samples were homogenized to ensure uniformity. Spectrophotometric measurements were conducted against air instead of using a reference cell to correct background absorption.

Chlorophyll pigments were determined by measuring the absorbance at 670 nm and correcting the results for background absorption. The content of chlorophyll pigments was calculated using the absorptivity of pheophytin a, the primary chlorophyll pigment in olive oils. The chlorophyll content was expressed in milligrams of pheophytin a per kilogram of oil. All measurements were performed in triplicate, and the results were reported as the mean ± standard deviation. (AOCS Official Method Cc 13d-55).

Determination of Chlorophyll and Carotenoid Pigments

To determine the carotenoid content in the pigments, 1.5 g of the oil sample was dissolved in cyclohexane within a 5 mL volumetric flask. Spectrophotometric analysis was then performed at wavelengths of 670 nm and 470 nm, corresponding to the absorption peaks of the pigments under investigation. The absorption spectra were recorded using a spectrophotometer, and the results were expressed in terms of milligrams of pigment per kilogram of oil (mg pigment/kg oil) following the AOCS Official Method Cc 13i-96.

To calculate the concentrations of pheophytin a and lutein for Total Chlorophyll (TChl) and Total Carotenoids (TCar) respectively, specific equations were used. The experimental procedure was conducted in triplicate to ensure the precision of the results. The results were expressed as mg of pigment/kg oil; (pheophytin a and lutein 4 TChl and TCar respectively) by using these equations:

$$TChl = \frac{(A670 \times 1000000)}{(613 \times 100 \times d)} \quad [4]$$

$$TCar = \frac{(A470 \times 1000000)}{(2000 \times 100 \times d)} \quad [5]$$

Determination of Intensity of Bitterness

Bitterness intensity, closely related to the phenolic content, was determined using

spectrophotometric analysis to quantify polar phenolic compounds.

Procedure: One gram of oil was extracted with a methanol-water mixture (60:40 v/v), centrifuged, and the aqueous phase was measured at 225 nm. Oleuropein standards were used to construct a calibration curve for quantification.

IB= 13.33 K225 – 0.837 (Gutiérrez Rosales et al., 1992). [6]

Determination of Peroxide Value

The peroxide value, which indicates the extent of primary oxidation, was determined using a titration method based on AOCS Official Method Cd 8-53 (Kiritsakis & Markakis, 1991).

Procedure: Two grams of oil were dissolved in an acetic acid/chloroform mixture, and potassium iodide was added. The liberated iodine was titrated with sodium thiosulfate, and the peroxide value was calculated using:

$$Peroxide\ value = \frac{(S-B)\times N}{W} \times 1000 \quad [7]$$

Where:

Peroxide value = mEq peroxide per kg of sample

S = volume of titrant (ml) for sample

B = volume of titrant (ml) for blank

N = normality of $Na_2S_2O_3$ solution

1000 = conversion of units (g/kg)

W = sample mass (g)

Determination of Total Phenols

Total phenolic content was measured using the Folin-Ciocalteu method, which quantifies the reducing capacity of phenolic compounds in olive oil (Bendini et al., 2007).

Procedure**:** Suitable aliquots of the polar extract (1 mL) were transferred in a 10 mL volumetric flask and, subsequently, water (5 mL) and the Folin-Ciocalteu reagent (0.5 mL) were added. After 3 min, 1 mL

of saturated (35%, w/v) sodium carbonate solution was added to the reaction mixture. The solution was diluted with water to 10 mL and after one hour in dark surrounding, the absorbance at 725 nm was measured against a blank solution with a spectrophotometer. Calibration curve was constructed using standard solutions of gallic acid (10-100 μg/mL). TP contents were expressed as gallic acid equivalents (GAE).

Sample Selection and Treatments

Olive oil samples were collected from four distinct harvest periods: early harvest (October 17, 2023), mid-harvest (November 14, 2023), late harvest (December 4, 2023), and a set of one-year-old samples from the 2022 harvest. These samples were carefully chosen to represent a range of harvest timings, which are known to influence the chemical composition and sensory qualities of olive oil (Vossen, 2007). These parameters are widely recognized in both the industry and academic research as essential markers of olive oil quality.

Experimental Design and Statistical Analysis

The study used a Completely Randomized Design (CRD) with three replications for each treatment to evaluate the impact of different harvest periods on the quality of olive oil from the *Lefkoelia Serron* variety. This design ensured that each treatment had an equal chance of being allocated to any experimental unit, reducing potential biases and enhancing the credibility of the findings. Replicating each treatment three times further improved the accuracy of the results by allowing for better estimation of variability and treatment effects.

Statistical Methods

To analyze the data, Analysis of Variance (ANOVA) was employed to determine whether there were significant differences among the treatment means. ANOVA is well-suited for comparing the means of multiple groups—in this case, different harvest times—to ascertain if any statistically significant differences exist. Prior to conducting ANOVA, tests for normality and homogeneity of variances were

performed to confirm that the data met the necessary assumptions for the analysis. If the data did not meet these assumptions, appropriate transformations, such as logarithmic or square root transformations, were applied to achieve normality and homogeneity.

When significant differences were identified through ANOVA, Duncan's Multiple Range Test (DMRT) was used for follow-up analysis to identify which specific treatments differed from one another. DMRT is particularly effective in controlling the Type I error rate when comparing multiple treatment means, making it suitable for studies like this with a moderate number of treatments.

Model Adequacy and Diagnostic Checks

The combination of a CRD, rigorous use of ANOVA, post-hoc testing with DMRT, and thorough diagnostic checks provided a reliable and comprehensive approach to understanding how harvest timing affects the quality attributes of *Lefkoelia Serron* olive oil.

Results and Discussion

Acid Value

The acid value (AV) of olive oil is a critical indicator of quality that reflects the extent of free fatty acid (FFA) content due to triglyceride hydrolysis. This parameter is essential because it influences the classification of olive oil according to the International Olive Council (IOC) standards. A higher AV generally suggests that there has been more extensive breakdown of triglycerides, often due to enzymatic activity or oxidation, which is undesirable as it reduces the oil's quality and shelf life. In contrast, a lower AV indicates fresher oil, which is considered a marker of superior quality (International Olive Council, 2022).

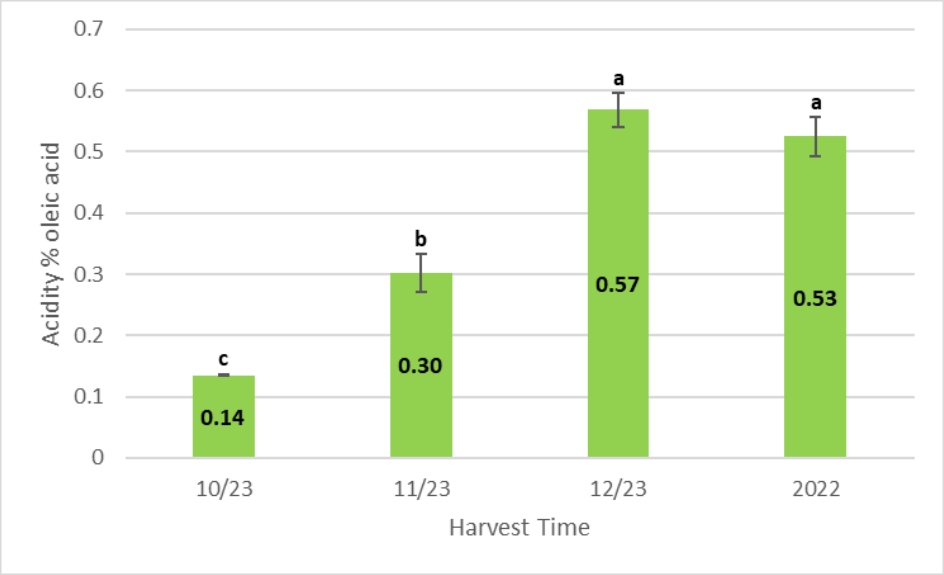
Results showed that oils from the 1223 and 2022 harvest periods had higher AVs (0.5689% and 0.5254%, respectively), whereas those from earlier harvests (1123 and 1023) exhibited notably lower AVs (0.3025% and 0.1353%). This pattern suggests that later harvests might lead to increased oxidation or prolonged enzyme activity, potentially degrading the oil's quality. This observation aligns with previous studies which emphasize that the timing of harvest significantly impacts the chemical composition of olive oil. Delaying the harvest often results in increased FFA levels due to prolonged exposure to environmental conditions, such as light and oxygen, which promote oxidative processes.

The findings are visually represented in Figure 1, which illustrates the fluctuations in AV across the different harvest periods. The oil from the earliest harvest (1023) had the lowest AV (0.135%), reflecting its freshness and high quality, while the oil from the 1223 harvest had the highest AV (0.569%), indicating greater exposure to conditions that promote lipid degradation. Even though all samples remained within the IOC's limit for extra virgin olive oil (0.8%), the variation in AV underscores the importance of harvest timing in maintaining optimal oil quality. Oils from earlier harvests (1023 and 1123) showed significantly lower AVs, suggesting they are less hydrolyzed and therefore of higher quality, while oils from later harvests (2022 and 1223) exhibited levels that, if not carefully managed (Tsimidou, 2013). The acidity does not change after the olive oil has been produced.

These results emphasize the importance of proper harvest timing and handling practices to minimize damage and ensure that olive oil maintains its desirable qualities.

Figure 1

Acid values (AV) for olive oils from different harvest times.



Note. Acidity percentage expressed as oleic acid for different harvest times (10/23, 11/23, 12/23, 2022). Values represent the mean ± standard error of three replicates. Different letters (a-c) above the bars indicate significant differences ($P < 0.05$) between harvest times as determined by statistical analysis.

K Values (K232, K270 & ΔK)

To further understand the oxidative state of the olive oils, specific extinction coefficients, known as K values, were measured using UV spectrophotometry. The K232 and K270 values are particularly valuable for assessing the extent of oxidation in olive oil. The K232 value indicates the presence of primary oxidation products, such as conjugated dienes and hydroperoxides, while the K270 value reflects secondary oxidation products, such as aldehydes and ketones, which are formed from the breakdown of hydroperoxides (Frankel, 2010).

The findings are provided in table 1, the data revealed that the oil from the 1123 harvest had the highest K232 value (1.0537), indicating a greater presence of primary oxidation products, likely due to

prolonged exposure to oxygen or light. In contrast, the 2022 harvest oil had the lowest K232 value (0.623), suggesting that it had undergone less oxidation. This pattern of higher K232 values in oils from mid-season harvests suggests they may have been exposed to more oxidative conditions, a finding supported by earlier research. The K270 values mirrored this trend, with the 1123 harvest oil showing a higher value (0.146) compared to the 2022 oil (0.125), further indicating more advanced oxidation that could negatively impact the oil's flavor and stability (Boskou, 2006).

In terms of ΔK values, which help distinguish high-quality virgin olive oils from those mixed with refined oils, all samples showed low values ranging from 0.001 to 0.004. This range aligns with IOC standards for extra virgin olive oil, confirming that minimal refining and oxidation occurred across all samples. The lowest ΔK value in the 2022 sample reflects a relatively stable oil with minimal degradation, reinforcing the idea that oils harvested earlier generally have higher antioxidant levels and are more suitable for longer storage (Servili et al., 2004).

Table 1

Absorbance coefficients (K266, K270, K274) and ΔK for samples from different harvest times.

	Mean ± SD		
Sample	K232	K270	ΔK
10/23	0.660 ± 0.021^{c}	0.140 ± 0.006^{a}	0.001 ± 0.001^{b}
11/23	1.054 ± 0.098^{a}	0.120 ± 0.004^{c}	0.001 ± 0.000^{b}
12/23	0.843 ± 0.075^{b}	0.143 ± 0.002^{a}	0.001 ± 0.000^{b}
2022	0.664 ± 0.066^{c}	0.130 ± 0.004^{b}	0.004 ± 0.000^{a}
Prob	0.0020	0.0028	0.0036
C.V. (%)	8.14	2.93	33.61

Note. Values of K232, K270 and ΔK for different samples (1023, 1123, 1223, 2022) are presented as mean ± standard deviation. Each value represents the mean of three replicates. Different superscript letters (a-c) indicate significant differences ($P < 0.05$) between samples for each parameter, as determined by statistical analysis. Prob: Probability. C.V.: Coefficient of variation.

Oxidative Stability (Rancimat*)*

Oxidative stability is another essential factor in determining the quality and shelf life of olive oil.

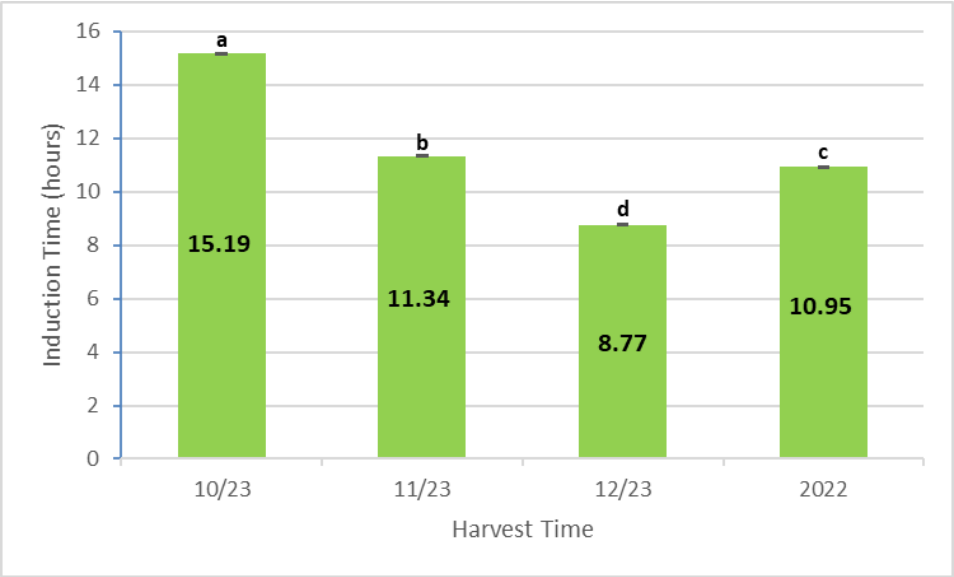
The Rancimat method, which measures induction time (IT), was employed in this study to evaluate how long an oil can resist oxidation under controlled conditions. Longer induction times generally indicate greater oxidative stability, which is highly desirable for maintaining oil quality during storage and distribution (Velasco & Dobarganes, 2002).

The results of the Rancimat, shown in Figure 1, showed that oils from different harvest periods varied significantly in their oxidative stability. The oil from the 1023 harvest exhibited the highest stability, with an induction time of 15.19 hours, while the oil from the 1223 harvest showed the lowest stability, with an induction time of 8.77 hours. Oils from the 1123 and 2022 harvests had intermediate stability, with induction times of 11.34 and 10.95 hours, respectively. These findings support the notion that oils from earlier harvests, which typically have higher levels of phenolic compounds, are more resistant to oxidation (Tsimidou, 2006). Phenolic compounds are known for their antioxidant properties, which can help protect the oil from oxidative damage and prolong its shelf life (Bendini et al., 2007).

The consistency of these results with other measurements, such as lower K values and higher phenolic content, underscores the significant impact of harvest timing on the oxidative stability of olive oil. This suggests that careful planning of the harvest period can help in producing olive oils with superior quality and longer shelf life.

Figure 2

Rancimat induction times for olive oils from different harvest times.



Note. Induction time (hours) measured by Rancimat for different harvest times (10/23, 11/23, 12/23, 2022). Values represent the mean ± standard error of three replicates. Different letters (a-d) above the bars indicate significant differences ($P < 0.05$) between harvest times as determined by statistical analysis.

Chlorophyll and Carotenoids

Chlorophyll and carotenoids are important pigments in olive oil that affect its color, quality, and stability. Chlorophylls contribute to the green color of olive oils and possess antioxidant properties, while carotenoids provide a yellowish hue and also offer antioxidant benefits (Minguez-Mosquera et al., 1991). In this study, pigment levels across different harvest periods were measured using the IUPAC spectrophotometric method and specific absorbance readings at 470 nm (for carotenoids) and 670 nm (for chlorophylls).

The highest concentration of chlorophyll was found in oils from the 2022 harvest (30.73 mg of pheophytin per kilogram), followed by the 1023 harvest (9.98 mg/kg), the 1123 harvest (5.52 mg/kg), and the 1223 harvest (2.24 mg/kg) (Figure 3). These results suggest that early harvest oils tend to retain more

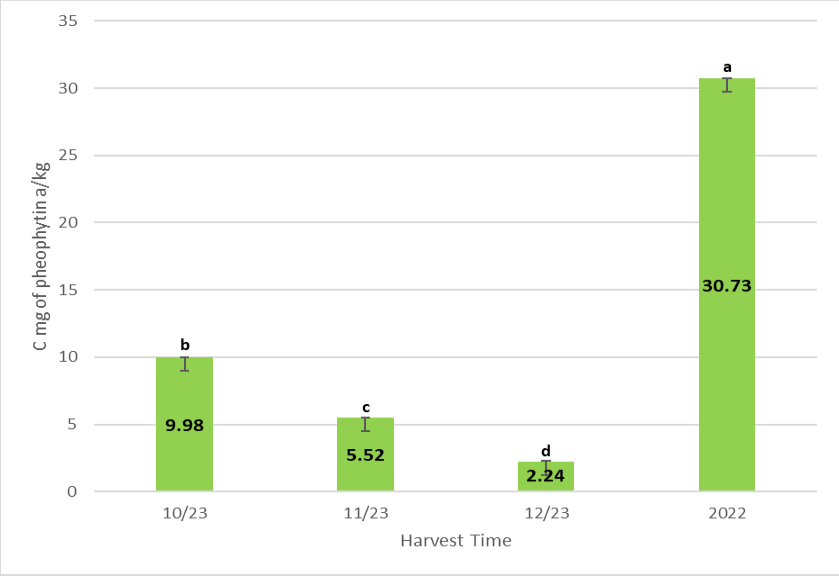
chlorophyll, which contributes to their greener color and better stability. As olives mature, chlorophyll is broken down into pheophytins, leading to a change in oil color from green to more olive-brown shades, which may affect perceived freshness and quality (Minguez-Mosquera et al., 1990).

Similarly, carotenoid content was highest in early harvest oils (9.98 mg/kg in the 1023 harvest) and lowest in later harvest oils (2.24 mg/kg in the 1223 harvest) (Figure 4). The decline in carotenoid levels as the harvest season progresses may be due to enzymatic activity and oxidation, leading to color changes and potentially reduced antioxidant capacity (Psomiadou & Tsimidou, 2001).

The balance between chlorophyll and carotenoid levels plays a significant role in determining the color and stability of olive oil. Oils from early harvests, such as the 2022 sample, showed higher chlorophyll-to-carotenoid ratios, resulting in a more vibrant green color and enhanced stability. In contrast, oils from later harvests exhibited lower ratios, indicating a shift towards yellow or golden hues, which could be perceived as reduced quality.

Figure 3

Total chlorophyll measurements (IUPAC method)

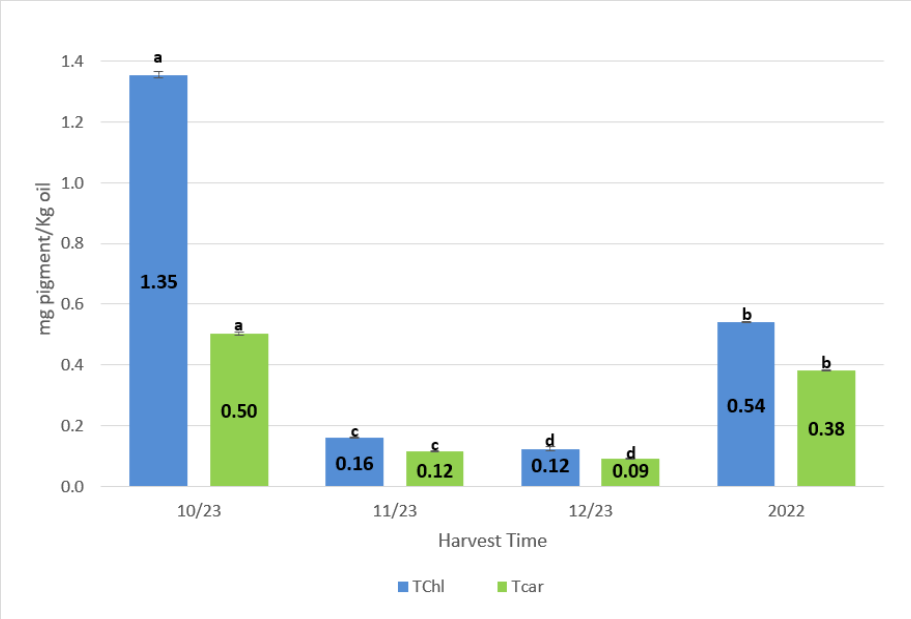


Note. Total chlorophyll measurements expressed as mg of pheophytin/kg, determined using the IUPAC method for different harvest times (10/23, 11/23, 12/23, 2022). Values represent the mean ± standard error of three replicates. Different letters (a-d) above the bars indicate significant

differences ($P < 0.05$) between harvest times as determined by statistical analysis.

Figure 4

Chlorophyll and carotenoids absorbance measurements at 470 nm and 670 nm for olive oils.



Note. Chlorophyll and carotenoids measurements are expressed as pigment content (mg pigment/kg oil) for different harvest times (10/23, 11/23, 12/23, 2022). Values represent the mean ± standard error of three replicates. Different letters (a-d) above the bars indicate significant differences ($P < 0.05$) between harvest times for each pigment, as determined by statistical analysis. TChl: Total clorophyll. Tcar: Total carotenoid.

Intensity of Bitterness

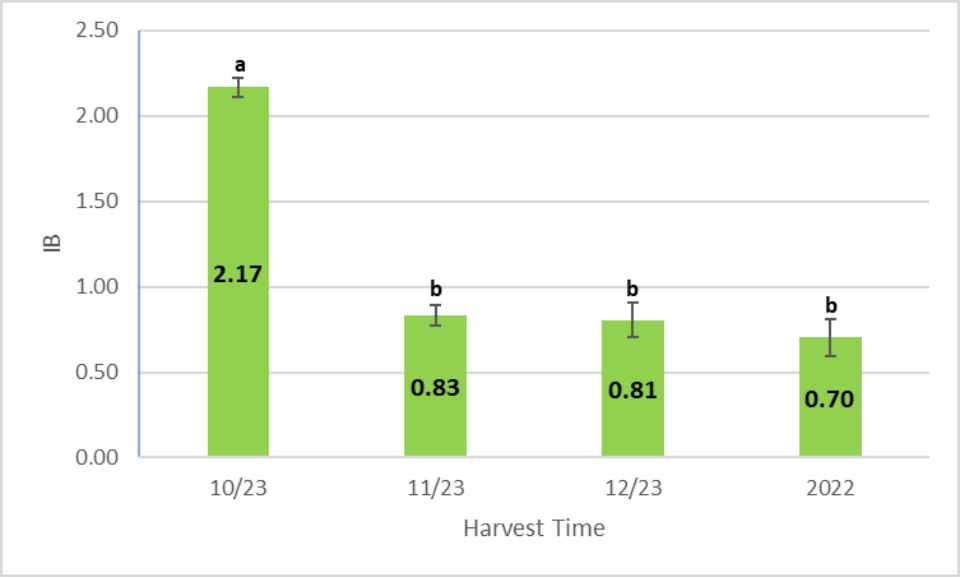
The intensity of bitterness (IB) in olive oil is closely related to the concentration of polar phenolic compounds, particularly secoiridoid derivatives like oleuropein and ligstroside aglycones, which are measured at 225 nm. Bitterness is generally considered a desirable trait in extra virgin olive oil because it reflects the presence of healthy phenolic compounds (Inarejos-Garcia et al., 2009). The bitterness levels in olive oils varied significantly by harvest time as shown in Figure 5. The oil from the 1023 harvest showed the highest bitterness (IB = 2.17), while the oil from the 2022 harvest had the lowest bitterness (IB = 0.70). Oils from the 1123 and 1223 harvests exhibited intermediate bitterness levels (IB = 0.83 and 0.81, respectively). This variation is largely due to differences in phenolic compound concentrations, which tend to decrease as olives ripen, reducing bitterness (Servili et al., 2004).

Oils from early harvests, such as those from 1023, have higher levels of phenolic compounds like oleuropein aglycone, which is known for its distinctive bitter taste. In contrast, oils from later harvests, such as the 2022 harvest, contain fewer bitter compounds, resulting in a milder flavor profile. These findings are consistent with previous research by Servili et al. (2004) and Bendini et al. (2007), which demonstrated a strong correlation between phenolic content and bitterness intensity.

Various factors, including olive ripeness, environmental conditions, and processing methods, significantly influence the phenolic content and bitterness of olive oils, thereby affecting both sensory qualities and consumer preferences. Understanding these factors is crucial for producers aiming to optimize the sensory qualities of their olive oils.

Figure 5

Bitterness intensity (IB) for olive oils from different harvest times.



Note. Intensity of bitterness (IB) for different harvest times (10/23, 11/23, 12/23, 2022). Values represent the mean ± standard error of three replicates. Different letters (a and b) above the bars indicate significant differences ($P < 0.05$) between harvest times as determined by statistical analysis.

Peroxide Value

The peroxide value (PV) is a crucial indicator of the primary oxidation state of olive oil and is

essential for evaluating its quality and shelf life. PV measures the amount of peroxides formed in the early stages of lipid oxidation and is widely used to assess the oxidative rancidity of oils (Kiritsakis & Markakis, 1987). In this study, PVs of oils from different harvest times were measured using the AOCS Cd 8-53 standard titration method.

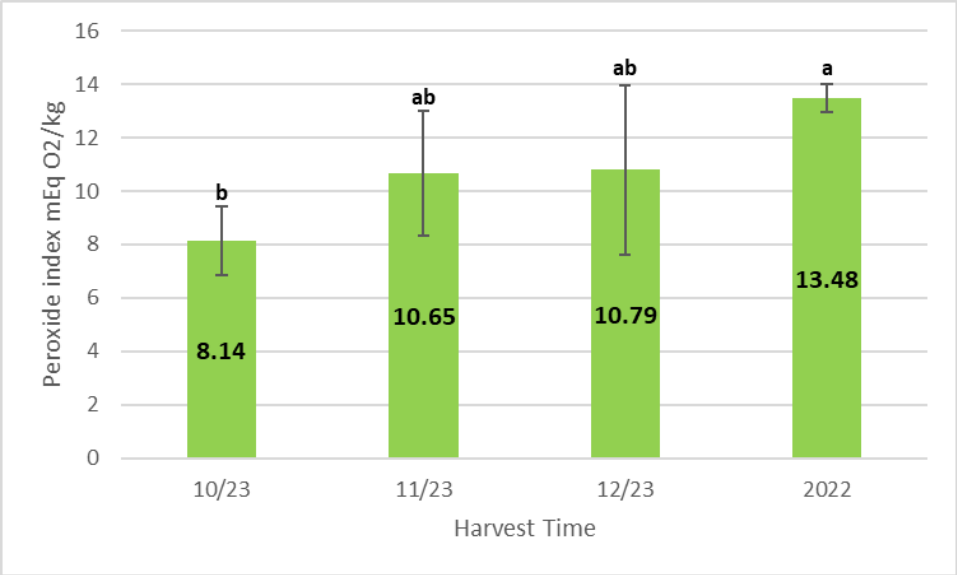
Figure 6 shows the range of PVs for the different harvests. The oil from the 1023 harvest had the lowest PV at 8.14 meq O_2/kg, which qualifies as "excellent conservation," while the oil from the 2022 harvest had the highest PV at 13.48 meq O_2/kg, categorized as "good conservation." Oils from the 1123 and 1223 harvests were also within the "good conservation" range, with PVs of 10.65 and 10.79 meq O_2/kg, respectively.

An "excellent conservation" classification (PV < 10 meq O_2/kg) suggests superior oxidative stability, leading to a longer shelf life and better preservation of sensory qualities. Conversely, a "good conservation" rating (PV between 10 and 15 meq O_2/kg) indicates moderate oxidative stability, still within the standards for extra virgin or virgin olive oil as defined by the IOC (2015). Higher PVs in oils from later harvests, such as the 2022 oil, suggest a greater tendency toward oxidation, potentially due to prolonged exposure to environmental factors (Kiritsakis & Markakis, 1987). The lower PV of 8.14 meq O_2/kg for the 1023 harvest indicates greater stability and a potentially longer shelf life.

These findings underscore the critical role of optimal harvest timing and proper handling practices in preserving the quality and oxidative stability of olive oil (International Olive Council, 2019). Moreover, they highlight the importance of maintaining low peroxide levels to ensure the longevity and flavor stability of the oil.

Figure 6

Peroxide values (PV) for olive oils from different harvest times.



Note. Peroxide index values (mEq O2_22/kg) for different harvest times (10/23, 11/23, 12/23, 2022). Values represent the mean ± standard error of three replicates. Different letters (a-b) above the bars indicate significant differences ($P < 0.05$) between harvest times as determined by statistical analysis.

Total Phenols

Olive oils are characterized by their total phenolic (TP) content, which varies significantly throughout the harvest season, indicating differences in oil quality and potential antioxidant capacity. Phenolic compounds are important for the health benefits and stability of olive oil, as they exhibit strong antioxidant properties that prevent oxidation (Tsimidou, 2013). In this study, after extracting the phenolic compounds using a methanol-water ($MeOH/H_2O$) solvent system, the total phenolic content was measured using the Folin-Ciocalteu Assay. The calibration curve, constructed with gallic acid due to its similarity to phenolic compounds found in olive oil, demonstrated a high degree of linearity ($R^2 = 0.9971$), confirming the assay's reliability for measuring phenolic content.

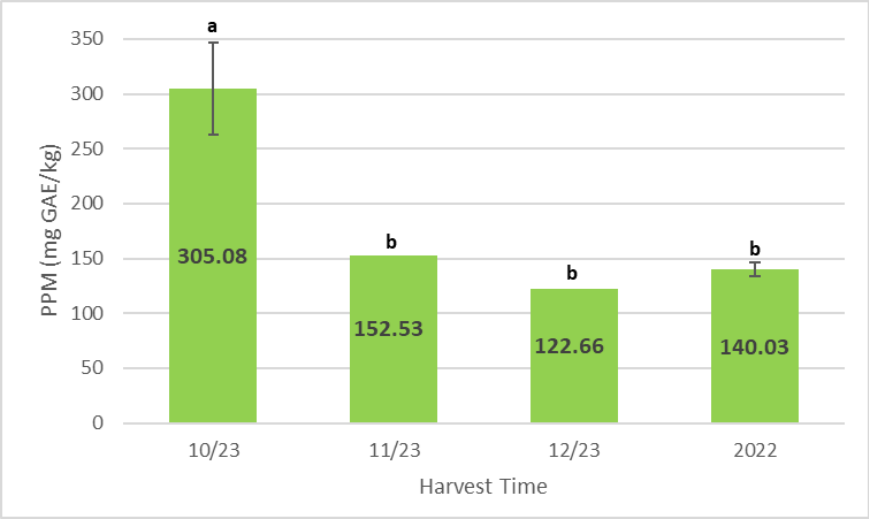
The study found significantly higher levels of phenolic compounds in the oil from the 1023 harvest (305.08 mg/kg), suggesting that oils produced from earlier harvests are richer in phenolics, contributing

to their antioxidant capacity. In contrast, the oil from the 1223 harvest had much lower phenolic content (122.66 mg/kg), likely due to oxidative degradation or increased exposure to environmental factors over time. The oils from the 1123 and 2022 harvests displayed intermediate phenolic levels, at 152.53 mg/kg and 140.03 mg/kg, respectively, showing a trend of decreasing phenolic content in oils from later harvests. The findings are shown in Figure 7.

These results suggest that optimizing the timing of olive harvesting could help preserve phenolic content, thereby maintaining the quality and health benefits associated with these compounds in olive oil. Furthermore, the significant differences in phenolic content between the harvest times underline the importance of considering both chemical and sensory qualities when determining the best time to harvest olives for oil production.

Figure 7

Total phenolics (TP) for olive oils from different harvest times.



Note. Total phenols content (PPM, mg/kg) for different harvest times (10/23, 11/23, 12/23, 2022). Values represent the mean ± standard error of three replicates. Different letters (a and b) above the bars indicate significant differences ($P < 0.05$) between harvest times as determined by statistical analysis.

Correlation Study

At a significance level of less than 0.0001, the table displays the correlations between the

variables Time to oxidize and Total Phenols. With a correlation value of 0.92812, a very strong positive association between the two variables was seen in this correlation. This correlation coefficient, which is higher than 0.7, is regarded as high and indicates that the amount of time needed for oxidation to happen increases along with the total amount of phenols. It is crucial to emphasize that both values had significant statistical significance, supporting the theory that this association represents a regular trend in the data rather than being the result of chance. This finding implies that the product's stability against oxidation—a crucial component of its longevity and quality—is directly influenced by the total amount of phenols.

The strong correlation, as shown in Table 2, between Total Phenols and Time to Oxidize suggests that the phenols in the mixture significantly boost resistance to oxidation, delaying the process until later.

Table 2

Correlation analysis between total phenols and oxidation time at a significance level (< 0.005)

Pearson Correlation Coefficients		
	Time to Oxidize	Total Phenols
Time to Oxidize	1.00000	0.92812 <.0001
Total Phenols	0.92812 <.0001	1.00000

Conclusions

Using critical quality parameters like acid value, oxidative stability, specific extinction coefficients, chlorophyll, carotenoid concentrations, total phenolic content, peroxide value, and bitterness intensity, the study could assess how harvest timing affected the quality of *Lefkoelia Serron* olive oils. The results show that these parameters, which are crucial for the categorization and positioning of olive oils on the market, are significantly influenced by the timing of the olive harvest.

All olive oil samples, regardless of harvest time, met the criteria for classification as Extra Virgin Olive Oil (EVOO) according to the standards set by the International Olive Council (IOC). However, early harvests of olive oils typically showed superior quality indicators, such as reduced acid levels, increased oxidative stability, higher phenolic content, and favorable sensory qualities. These attributes imply that early-harvest oils are better suited for premium classification due to their more consistent profile and strong antioxidant capability.

A strong positive correlation (Pearson correlation coefficient of 0.928, $p < 0.0001$) was found between the total phenolic content and oxidative stability (time to oxidize). This significant correlation suggests that oils with higher phenolic content tend to exhibit prolonged oxidative stability, supporting the importance of phenolics in enhancing the antioxidant properties and shelf life of olive oils.

Recommendations

Conduct sensory evaluation panels to link the phenolic content and fatty acid profiles of Lefkoelia Serron olive oil with sensory attributes such as fruitiness, pungency, and bitterness.

Expand the chemical study of Lefkoelia Serron olive oil by fully describing the profile of volatile compounds, the detailed fatty acid composition, sterols, and levels of tocopherol by using modern separation techniques, such as GC-MS and HPLC, for a full basis on flavor, nutrition, and stability.

Expand the date range from very early to very late to investigate the optimal timing that will bring the best balance of flavor, antioxidants, and stability in Lefkoelia Serron olive oil. Take into consideration environmental factors like rainfall, temperature, and soil conditions to make more precise recommendations on harvesting.

Evaluate the long-term storage stability of Lefkoelia Serron olive oil under different conditions, such as varying temperatures, light exposure, and packaging materials, to identify the best methods for preserving quality, and compare different packaging options like dark glass, PET, and tin cans.

Conduct comparative studies with other varieties from this region and other international types, too, to identify the peculiarities of Lefkoelia Serron olive oil, which could justify specific targeted marketing and the development of a PGI for this variety.

Investigate clinical examination of specific health benefits of the high polyphenol content in early-harvest Lefkoelia Serron olive oil for anti-inflammatory and antioxidant effects will give this product great appeal among health-conscious consumers. (Kim et al., 2017)

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Appendices

Appendix A

Raw numbers table from Acidity

Sample	Mass (g)	C (N) KOH	ΔV	M	Acidity % oleic acid
1a	5.02	0.1	0.24	282	**13.48%**
1b	4.99	0.1	0.24	282	**13.56%**
1c	5.00	0.1	0.24	282	**13.54%**
2a	5	0.1	0.54	282	**30.46%**
2b	5.2	0.1	0.5	282	**27.12%**
2c	5.1	0.1	0.6	282	**33.18%**
3a	4.99	0.1	0.94	282	**53.12%**
3b	4.99	0.1	1.06	282	**59.90%**
3c	4.99	0.1	1.02	282	**57.64%**
4a	5.01	0.1	1	282	**56.29%**
4b	5.02	0.1	0.9	282	**50.56%**
4c	5	0.1	0.9	282	**50.76%**

Appendix B

Raw numbers table from Peroxide Values

Sample	Mass (g)	Titration Volume (ml)	C (N)	Peroxide Value
blank	2.04	0.8	0.01	0.00
1a	2.01	2.2	0.01	6.97
1b	2.01	2.4	0.01	7.96
1c	2.00	2.7	0.01	9.50
2a	2.01	2.4	0.01	7.96
2b	2.00	3.2	0.01	12.00
2c	2.00	3.2	0.01	12.00
3a	2.00	2.5	0.01	8.50
3b	2.01	2.7	0.01	9.45
3c	2.01	3.7	0.01	14.43
4a	2.00	3.6	0.01	14.00
4b	2.01	3.4	0.01	12.94
4c	2.00	3.5	0.01	13.50

Appendix C

Raw numbers from K-values

# Sample	Mass (g)	232nm	Kλ
1a	0.058	0.6764	0.68
1b	0.054	0.6367	0.64
1c	0.053	0.6666	0.67
2a	0.062	1.1299	1.13
2b	0.061	1.0875	1.09
2c	0.054	0.9436	0.94
3a	0.065	0.9291	0.93
3b	0.053	0.8043	0.80
3c	0.058	0.7952	0.80
4a	0.052	0.623	0.62
4b	0.065	0.7399	0.74
4c	0.049	0.6288	0.63

Appendix D

Raw numbers from Phenols

Sample	Absorbance at 725nm
1a	0.5114
1b	0.5661
1c	0.6959
2a	0.2473
2b	0.2583
2c	0.2318
3a	0.1943
3b	0.1541
3c	0.1861
4a	0.2283
4b	0.216
4c	0.2082

Appendix E

Raw numbers from Rancimat

Sample	Mass (g)	Induction Time (hrs)
1a	3.014	15.19
1b	3.008	15.17
1c	3.016	15.20
2a	3.006	11.34
2b	3.003	11.33
2c	3.011	11.36
3a	3.017	8.77
3b	3.015	8.78
3c	3.007	8.75
4a	3.014	10.95
4b	3.010	10.96
4c	3.009	10.93

Appendix F

Raw numbers of results of the Total Chlorophyll measurement (IUPAC METHOD)

#Sample	630nm	670nm	710nm	C mg of pheophytin/kg
1a	1.7751	1.9088	1.7526	10.01
1b	1.7724	1.9050	1.7491	9.96
1c	1.7701	1.9031	1.7478	9.95
2a	0.0486	0.1193	0.0300	5.52
2b	0.0365	0.1076	0.0189	5.52
2c	0.0349	0.1061	0.0176	5.51
3a	0.0334	0.0606	0.0218	2.28
3b	0.0210	0.0480	0.0106	2.22
3c	0.0185	0.0456	0.0081	2.23
4a	0.0660	0.4892	0.0216	30.76
4b	0.0628	0.4857	0.0189	30.72
4c	0.0608	0.4836	0.0171	30.71

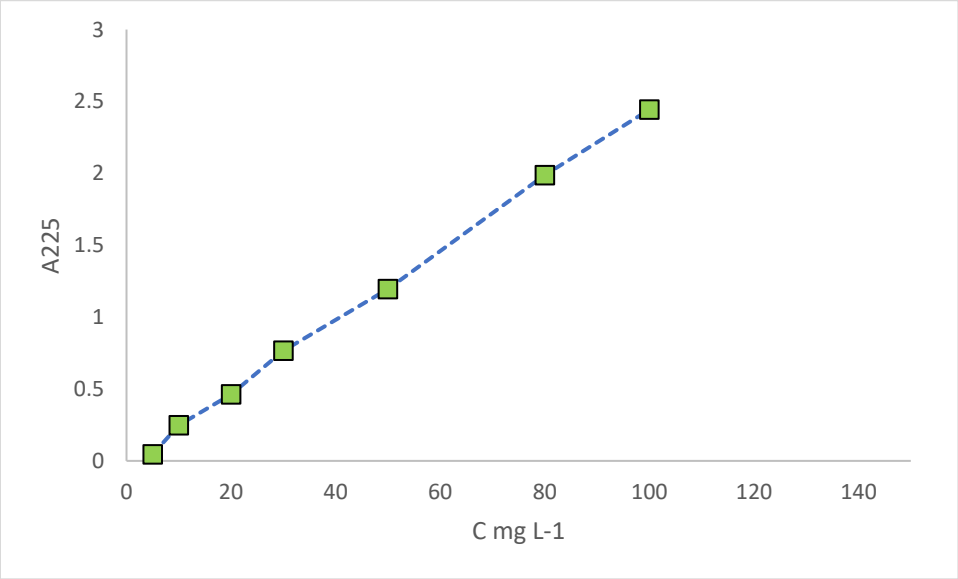
Appendix G

Raw numbers of results of the Bitterness

Sample	Mass (g)	225nm	K225	IB
1a	1.001	0.565	0.226	2.17
1b	1.000	0.553	0.221	2.11
1c	1.000	0.573	0.229	2.22
2a	1.001	0.326	0.130	0.90
2b	1.000	0.305	0.122	0.79
2c	0.999	0.309	0.124	0.81
3a	1.001	0.309	0.124	0.81
3b	0.999	0.326	0.130	0.90
3c	0.999	0.289	0.116	0.70
4a	0.999	0.310	0.124	0.82
4b	1.001	0.271	0.108	0.60
4c	1.001	0.287	0.115	0.69

Appendix H

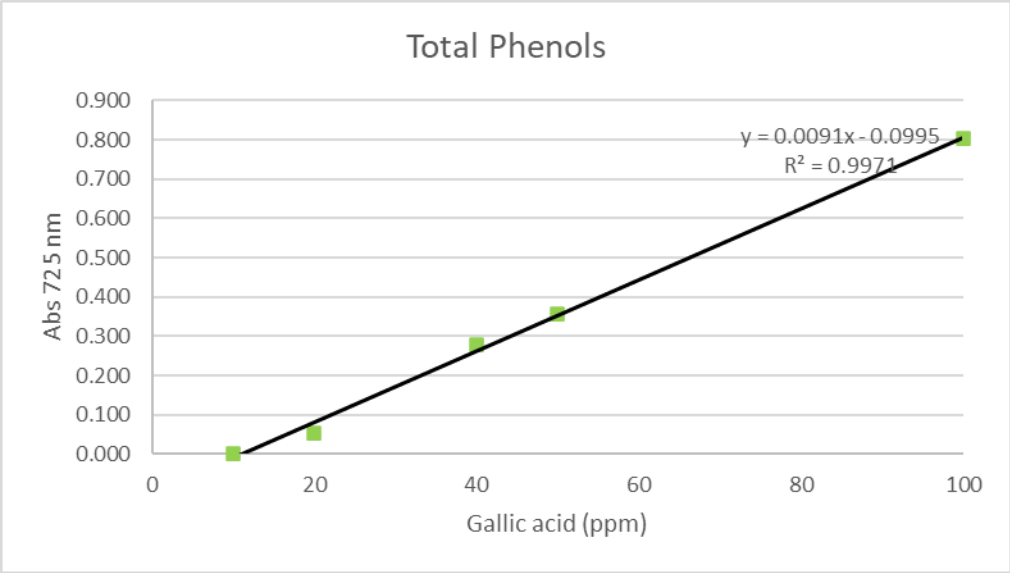
Reference Curve for Oleuropein (Spectrophotometry) Used to Measure the Bitterness Index in Olive Oil.



Note. Calibration curve showing the relationship between concentration (C, mg L−1^{-1}−1) and absorbance at 225 nm (A225). The data points represent mean values from three replicates, and the dashed line indicates the linear regression fitted to the data points.

Appendix I

Calibration Curve for Total Phenol Determination in Oils Using Gallic Acid as a Standard



Note: Absorbance at 725 nm (Abs 725 nm) is measured for varying concentrations of gallic acid (ppm) to determine total phenolic content in oil samples. Each data point on the calibration curve represents the mean of three replicates. Different letters (a-e) indicate significant differences ($P < 0.05$) between treatments and concentrations as determined by statistical analysis.

Appendix J

Quality criteria

	Extra virgin olive oil	Virgin olive oil	Ordinary virgin olive oil	Lampante virgin olive oil *	Refined olive oil	Olive Oil (ROO+VOOs)	Crude olive pomace oil	Refined olive pomace oil	Olive pomace oil (ROPO+VOOs)
4.1 Organoleptic characteristics									
- odour and taste					acceptable	good		acceptable	good
-									
. median of defect									
. median of the fruity attribute	Me = 0.0 Me > 0.0	0.0 < Me ≤ 3.5 Me > 0.0	3.5 <Me ≤ 6.0**	Me > 6.0					
- colour					light yellow	light, yellow to green		light, yellow to brownish yellow	light, yellow to green
- aspect at 20°C for 24 hours					limpid	limpid		limpid	limpid
4.2. Free acidity % m/m expressed in oleic acid	≤ 0.80	≤ 2.0	≤ 3.3	> 3.3	≤ 0.30	≤ 1.00	no limit	≤ 0.30	≤ 1.00
4.3. Peroxide value in milleq. peroxide oxygen per kg/oil	≤ 20.0	≤ 20.0	≤ 20.0	no limit	≤ 5.0	≤ 15.0	no limit	≤ 5.0	≤ 15.0

* It is not obligatory for the criteria in 4.1, 4.2 and 4.3 to be concurrent; one is sufficient.

** Or when the median of the defect is less than or equal to 3.5 and the median of the fruity attribute is equal to 0.0.

Appendix K

Quality criteria (contd.)

	Extra virgin olive oil	Virgin olive oil	Ordinary virgin olive oil	Lampante virgin olive oil	Refined olive oil	Olive Oil (ROO+VOOs)	Crude olive pomace oil	Refined olive pomace oil	Olive pomace oil (ROPO+VOOs)
4.4. Absorbency in ultra-violet ($K^{1\%}_{1cm}$)									
- 270 nm (cyclohexane) / 268 nm (iso-octane)	≤ 0.22	≤ 0.25	≤ 0.30		≤ 1.25	≤ 1.15		≤ 2.00	≤ 1.70
- ΔK	≤ 0.01	≤ 0.01	≤ 0.01		≤ 0.16	≤ 0.15		≤ 0.20	≤ 0.18
- 232 nm*	≤ 2.50**	≤ 2.60**							
4.5. Moisture and volatile matter (% m/m)	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.3	≤ 0.1	≤ 0.1	≤ 1.5	≤ 0.1	≤ 0.1
4.6. Insoluble impurities in light petroleum % m/m	≤ 0.10	≤ 0.10	≤ 0.10	≤ 0.20	≤ 0.05	≤ 0.05		≤ 0.05	≤ 0.05
4.7. Flash point	-	-	-	-	-	-	≥ 120 °C	-	-
4.8. Trace metals mg/kg									
Iron	≤ 3.0	≤ 3.0	≤ 3.0	≤ 3.0	≤ 3.0	≤ 3.0		≤ 3.0	≤ 3.0
Copper	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1		≤ 0.1	≤ 0.1
4.9. Fatty acid ethyl esters (FAEEs)	≤ 35 mg/kg								
4.10. Phenols content	See section 11.21								

* This determination is solely for application by commercial partners on an optional basis.

** Commercial partners in the country of retail sale may require compliance with these limits when the oil is made available to the end consumer.