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Fatty acids, volatile and phenolic composition, quality and sensory profile of two Albanian Kalinjot extra virgin olive oils

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ORIGINAL ARTICLE

Abstract

Olive oil production plays a key role in the Albanian economy, with considerable potential for expansion in global markets. This study characterizes two monovarietal Kalinjot extra virgin olive oils, the country's most common cultivar. Both samples conform to EU quality standards for free acidity (0.2 ± 0.0 - 0.3 ± 0.0 %), peroxide values (6.0 ± 0.3 - 6.7 ± 0.1 mEq O_2/kg), and extinction coefficients (K_{232}: 1.80 ± 0.08 – 1.84 ± 0.01; K_{268}: 0.14 ± 0.02 – 0.15 ± 0.02), as well as fatty acid composition and sensory analysis, which also highlighted distinctive positive attributes. Moreover, oxidation stability index, total phenols, and both phenolic and volatile profiles. These results, combined with existing scientific literature, underscore the potential of Kalinjot monovarietal extra virgin olive oil for obtaining a possible geographical indication.

Keywords: Kalinjot, monovarietal, extra virgin olive oil, sensory analysis, composition, Albania

Introduction

Albania is a country endowed with diverse ecological niches and rich biological and landscape diversity of olive trees. Located within the Mediterranean Basin, it lies in the first and second favorable climatic zones for olive cultivation, making it suitable for growing olive trees at altitudes of up to 560 meters (Fraga *et al*., 2020; Topi *et al*., 2021). The country's geographic position, along with its geological, hydrological, climatic, and soil characteristics contribute to this diversity (Gixhari *et al*., 2014). Olive tree cultivation has been steadily expanding worldwide due to its significant economic value, especially in Mediterranean countries (Fraga *et al*., 2020), with wild olives having been present in Albania for around 12,000 years (Gixhari *et al*., 2014). Olives and olive oil production are a key and promising sector of Albania's economy (Kycyk, 2020). In 2021, Albania ranked 14th in olive fruit production and 20th in olive oil production, with a yield of approximately 11,500 tons (FAOSTAT, 2021). Since 2009, Albania has seen a significant increase in olive oil production, exceeding 10,000 tonnes annually since 2012

(FAOSTAT), supported by government subsidies for new plantations (Pugliese *et al*., 2018; MARD, 2021). By 2012, olive oil processing in the country was primarily carried out using three-phase decanters (Kapaj and Kapaj, 2012).

Nevertheless, the Albanian olive oil market still faces challenges in competing internationally, primarily due to limited production, export barriers, and the absence of certifications (Boja, 2020). However, the country is making progress toward developing certification schemes to improve its competitiveness, in line with ISO standards and organic certification (Boja, 2020). A promising example of this effort is the first organic certification granted to a local company, which has successfully begun exporting to Switzerland (Kapaj and Kapaj, 2012; Boja, 2020). Furthermore, with the approval of Law No. 8/2019 on quality schemes, which aligns with EU regulation 1151/2012, a new framework has been established for certifying products under quality schemes such as TSG, PGI and PDO.

For countries aiming to join the EU, geographical indications (GIs) are pivotal in trade negotiations and in safeguarding the protection of unique regional products (Kokthi *et al*., 2021; Kokthi and Kruja, 2017). The EU acquis requires potential candidate countries to adopt and implement EU legislation, including laws related to intellectual property rights, which encompass the protection and recognition of GIs. However, the success of GIs is closely tied to consumer preferences, awareness, and demand for high-quality, authentic, and region-specific products (Bytyçi *et al*., 2024; Kokthi *et al*., 2021).

Olive oil quality parameters are influenced by various factors, including cultivar, region of cultivation, and the associated pedoclimatic conditions, as well as the timing of the harvest and the technologies employed for harvesting and oil extraction (Deiana *et al*., 2019). Some studies have demonstrated how the quality of Albanian olive oil varies with olive tree varieties and cultivation regions (Morina and Kongoli, 2022), as well as how cultivars and local geographical origin impact phenolic and volatile profiles (Topi *et al*., 2019; 2020).

In Albania, there are at least 22 native olive cultivars, recognized through genetic studies and distributed across six regions: Tirana, Vlora, Kruje, Elbasan, Berat, and Lezhe (Morina and Kongoli, 2022). The main cultivars in these regions include Kalinjot, Kokërrmadh i Beratit, Mixani, Ulliri i Bardhë i Tiranës, Krypsi i Krujës, and Kallmet (Belaj *et al*., 2003). The cultivar Kalinjot, also known as Kaninjot, is the most important autochthonous cultivar in Albania and is believed to originate from the village of Kanina in the Vlora region (Ismaili, 2017). This cultivar is widely found in the districts of Vlora, Saranda, Delvina, Mallakaster, Fier, and Lushnje. The olive tree is

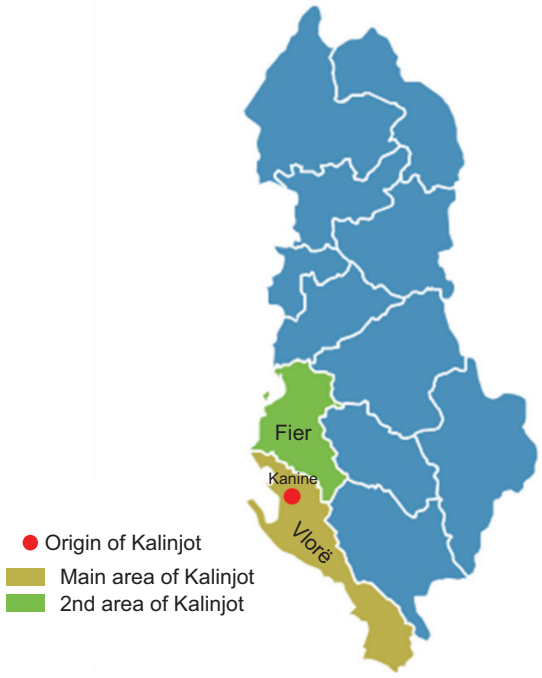

Figure 1. The distribution area of Kalinjot in Albania (based on data from Institute of Plant Genetic Resources, part of Agricultural University of Tirana).

robust and voluminous, with an average open branching structure, and the fruit has a slightly oval-spherical shape. It is highly adaptable to rooting but is sensitive to pests such as *Bactrocera oleae*, *Cycloconium oleaginum*, and *Pseudomonas savastanoi*. The cultivar is also resistant to cold and drought, although it enters production relatively late after planting.

Kalinjot is also found alongside other local cultivars, such as "Pulazeqini" and "Olivaster," with many of these cultivars coexisting harmoniously. It is known for its good and alternating production, characterized by medium-late and gradual fruit ripening. Kalinjot is valued both for its high yield of good-quality, aromatic olive oil and for its use as table olives (Thomaj & Panajoti, 2003).

Despite the Kalinjot cultivar being reported in various studies as the most widely cultivated, covering approximately 50% of the plantation area, and being used both for table olives and olive oil extraction (Topi *et al*., 2021), the structure of olive cultivation in Albania has shifted since 2009 with the introduction of new cultivars from abroad. Although the Ministry of Agriculture and Rural Development in Albania is working on creating an olive cadastre, this project is still ongoing (MARD, personal communication, March 18, 2024). The average fruit and stone weight of Kalinjot is 3.6 g and 0.5 g, respectively, with an extractability rate that can reach up to 28% w/w (Topi *et al*., 2021). Kalinjot is a high-oleic-acid cultivar, with a polyunsaturated fatty acid n-6/n-3 ratio of

approximately 10 (Topi *et al*., 2021). In a comparative study of volatile profiles between Kalinjot from the Vlora region, Kalinjot from the Himara region, and the Bardhi Tirana cultivar, Kalinjot from Himara exhibited the highest aroma concentration, followed by Kalinjot from Vlora and Bardhi Tirana (Topi *et al*., 2019). In general, the Kalinjot variety contained 29 volatile compounds, with aldehydes—particularly (*E*)-2-hexenal—being the most prominent (Topi *et al*., 2019). Velo and Topi (2017) found similarities between the main Albanian cultivars and those from Italy and Greece (Velo and Topi, 2017). Current scientific literature on Albanian autochthonous olive oils is somewhat limited, with studies typically focusing on specific compounds (such as phenolic compounds, aroma profiles, fatty acids, etc.) individually. However, a comprehensive compositional and sensory characterization of these varieties is lacking. This study aims to address this gap by analyzing two monovarietal Kalinjot extra virgin olive oils produced at two different mills in the South-West of Albania—Musai and Ulliri i Artë—located in the counties of Vlora and Fier. By integrating various analytical techniques and sensory evaluations, this research seeks to provide a more holistic and multi-dimensional perspective on Kalinjot olive oil quality. Quality parameters and sensory profiles were determined in accordance with EU Regulations 2022/2105 and 2022/2104. Additionally, volatile compounds were analyzed using solid-phase microextraction gas chromatography-mass spectrometry (SPME-GC-MS) and high-speed gas chromatography-ion mobility spectrometry (HS-GC-IMS) for rapid screening. Total phenols were measured using the Folin–Ciocâlteu method, and phenolic compounds were separated and quantified by high-performance liquid chromatography with diode-array detection (HPLC-DAD).

Virgin olive oils from the Kalinjot variety, Albania's most widely cultivated native cultivar, warrant detailed study to define their unique characteristics and establish a specific production standard. This could pave the way for a designation of origin, which would not only enhance the product's visibility in international markets but also help boost Albanian olive oil exports.

Materials and Methods

Samples

The two samples analyzed, Kalinjot1 and Kanlijot2, were produced from 100% monovarietal Kalinjot olives, harvested in November 2022 from two different oil mills located in the southwest of Albania: Musai and Ulliri i Artë, in the counties of Vlore and Fier, respectively. Prior to compositional and sensory analyses, the olive oil was stored in dark glass bottles, protected from air and light, and only opened when the analysis was conducted. Since the volatile analysis was performed later, the samples were kept at -18°C in dark glass bottles. Before use, the samples were allowed to reach room temperature and were gently shaken.

Peroxide value

To determine the peroxide value (PV) of the samples, an iodometric titration was performed according to the EU official method specified in regulation (EU Reg. 2022/2105). The amount of substance to be analyzed was based on the expected peroxide content; in this case, 3 g of olive oil were weighed. The olive oil samples were dissolved in 15 mL of acetic acid and 10 mL of chloroform. Next, 1 mL of a saturated potassium iodide solution was added and titrated with a standardized sodium thiosulfate solution, using iodine-starch as an indicator. The analysis was performed in triplicate for each sample. Finally, the peroxide value, expressed as milliequivalents of active oxygen per kilogram of oil (mEq O_2/kg), was calculated using the following formula: P.V. = ((V × T) / m) × 1000, where V is the volume of titrant used, T is the normality of the solution, and m is the mass of the sample, expressed in grams.

Ultraviolet spectrophotometric indices

Following the methods described in the EU official regulations (EU Reg. 2022/2105), absorbance at 268, 270, 272, 276, and 232 nm was measured. These specific wavelengths enable the detection of conjugated dienoic and trienoic fatty acids. For the first four absorbance values (268, 270, 272, and 276 nm), a 1% solution of the olive oil samples dissolved in isooctane was used. However, this concentration was too high to accurately measure absorbance at 232 nm, so a 0.2% solution was prepared for that wavelength. The measurements were repeated three times. Finally, the extinction coefficients were calculated from the absorbance values obtained using the Lambert–Beer equation (Kλ = Aλ / c*s).

Oxidation stability

Oxidation stability was measured using the 892 Rancimat instrument (Metrohm, Filderstadt, Germany). For this analysis, 5 g of the samples were exposed to accelerated oxidation by heating to 110°C with a constant air flow of 9 L/h. The air stream carries volatile oxidation products to a vessel containing 50 mL of distilled water. The instrument measures the conductivity of the water and records the time when a sudden exponential increase is detected. This time, known as the induction time, reflects the sample's

resistance to oxidation. Three replicates of each sample were analyzed. Finally, the instrumental software calculates the induction period, which is expressed in hours.

Free acidity

The acidity of the olive oil samples was determined by titration with an ethanolic solution of potassium hydroxide, as described in the EU official methods (EU Reg. 2022/2105). The olive oil samples were weighed according to the expected acidity percentage, as specified in the official method. In this case, an aliquot of 20 g per sample was diluted in 100 mL of a neutralized solution of ethyl ether and ethanol (1:1). The samples were then titrated with a 0.1 mol/L potassium hydroxide solution, using phenolphthalein as an indicator. The titrations were performed in triplicate for each sample. Finally, the acidity was expressed as the percentage of oleic acid, calculated by multiplying the volume of titrant used by its concentration in mol/L, then by the molar mass of oleic acid (282 g/mol), and dividing the result by 10 and by the weight of the sample (in grams).

Phenolic compound analysis

Phenolic extraction

Polar phenolic compounds were extracted from Albanian olive oil samples and then analyzed and quantified by HPLC, following the method proposed by the International Olive Council (2017), as described below. Exactly 2 g of olive oil were weighed into a 10-mL PTFE screw-cap glass tube, to which 1 mL of a syringic acid solution (c = 0.015 mg/mL), used as an internal standard, was added. The solution was prepared in methanol/water (4:1, v/v) from a stock solution of the same compound (c = 1.51 mg/mL). After vortexing for 30 s, 5 mL of a methanol/water (4:1, v/v) extraction solution was added to the sample, which was then shaken for 1 min, placed in an ultrasonic bath for 15 min at room temperature, and finally centrifuged at 3075 x g for 25 min. Approximately 1.5 mL of the supernatant was transferred to a PP centrifuge microtube and stored at -18°C before HPLC analysis. Extracts for total phenolic content analysis by UV/Vis spectrophotometry were prepared using the same procedure, except that 1 mL of methanol/water (4:1, v/v) was used instead of the internal standard solution.

Colorimetric determination of the total phenolic content (TPC) by UV/Vis spectrophotometry

TPC was measured following the Folin-Ciocalteau procedure as described by Singleton and Rossi (1965), briefly outlined as follows. For this determination, 7.3 mL of water, 0.2 mL of hydroalcoholic extract (without syringic acid), 0.5 mL of Folin-Ciocalteau reagent, and 2.0 mL of 15% (w/v) sodium carbonate were transferred to a 10-mL PTFE screw-cap glass tube and shaken by hand for 5 s. The mixture was then kept in the dark at room temperature. After 2 h (and no longer than 8 h), the absorbance of the solution was measured at 750 nm using a single-beam spectrophotometer (mod. UV-5600) from Hinotek (Ningbo, China). Water was used as the reference solution to adjust the absorbance to zero. TPC was calculated using a gallic acid calibration curve. From a stock solution (c = 2.01 mg/mL in methanol/water 4:1, v/v), diluted solutions were prepared in the same solvent mixture, with concentrations ranging from 0.0025 to 0.25 mg/mL (seven calibration points, $r^2 > 0.99$). The observed absorbance values were corrected by subtracting the absorbance of a blank sample, prepared by replacing the hydroalcoholic extract with 0.2 mL of methanol/water (4:1, v/v). Extracts, gallic acid standard solutions, and blanks were analyzed in duplicate.

Determination of individual phenolic compound by high performance liquid chromatography (HPLC)

Before HPLC analysis, 1 mL of each extract, with the internal standard added, was filtered into an HPLC glass vial through a 3-mL plastic syringe using a PVDF filter (diameter: 13 mm, pore size: 0.45 µm). Phenolic extracts were analyzed in linear gradient elution mode on a Nexera Series ultra-high-performance liquid chromatograph (Shimadzu, Kyoto, Japan), equipped with a solvent delivery module including two binary pumps and a degassing unit (mod. LC-40Bx3), a system controller (mod. CBM-40), a UV-VIS photodiode array detector (mod. SPD-M40), an autosampler for liquid samples (mod. SIL-40Cx3), and a column oven (mod. CTO-40S). The mobile phases were: A) 0.2% (v/v) orthophosphoric acid in water, and B) acetonitrile/methanol (1:1, v/v). Methanol/water (4:1, v/v) was used as a cleaning solution for the autosampler syringe before and after each sample injection. The solvents for both the mobile phase and cleaning solution were of chromatographic grade and were degassed in an ultrasonic bath for 15 min at room temperature. Mobile phase A and the cleaning solution were also filtered through a nylon membrane filter (diameter: 47 mm, pore size: 0.45 µm) before sonication to remove any possible contaminants present in the water. The chromatographic separation was carried out at a controlled temperature of 35°C using a SphereClone™ 5 µm ODS(2) 80 Å LC column (250 × 4.6 mm i.d.) from Phenomenex (Torrance, CA, USA); no pre-column protection system was used. The gradient elution program was as follows: 0-40 min, 96% to 50% A; 40-45 min, 50% to 40% A; 45-60 min, 40% to 0% A; 60-70 min, 0% A; 70-72 min, 0% to 96% A; 72-82 min, 96% A. The flow rate was 1.0 mL/min, and the injection volume was 20 µL. HPLC traces were acquired at 280 nm, and absorption spectra were recorded from 190 to 400 nm. Data were processed and filed using the LabSolutions software (version 5.97) from Shimadzu.

An external calibration standard solution, containing syringic acid (c = 0.015 mg/mL) and tyrosol (c = 0.030 mg/mL), was prepared in methanol/water (4:1, v/v) from stock solutions of the same compounds (c = 1.52 mg/mL for syringic acid and c = 3.04 mg/mL for tyrosol). This solution was used to calculate the response factors (RFs) of the external calibration standards and the ratio of syringic acid-to-tyrosol response factors (RRF). The RRF was 4.9, within the range suggested by the International Olive Council (2017). The relative retention time (RRT) of each peak was calculated with respect to the retention time of syringic acid, and compound identification was performed by comparing RRTs and UV spectra with data reported by the International Olive Council (2017). The content of each identified phenolic compound was calculated based on the amount of syringic acid, the peak area, and the area of the syringic acid peak corrected by the RRF (International Olive Council, 2017). The purity of syringic acid and tyrosol was assessed in solutions prepared at concentrations of 1.51 mg/mL and 1.49 mg/mL, respectively, in methanol/water (4:1, v/v). Each extract and standard solution was injected in duplicate. Standard solutions were filtered through PVDF filters prior to HPLC analysis, as were the olive oil extracts.

Fatty acid profile

The analysis of fatty acids in the virgin olive oil sample was performed following the standardized procedures outlined by the EU official methods (EU Reg. 2022/2105). Approximately 0.1 g of the sample was weighed into a test tube, dissolved in 2 mL of hexane, and transmethylated with 0.2 mL of a 2N KOH methanolic solution. The mixture was then centrifuged at 1800 rpm for 5 min. The supernatant was carefully collected, and 1 µL was injected into a GC-FID 8000 (Fisons Instruments, Glasgow, UK), equipped with a split-splitless injector system. A Restek RXT 2330 capillary column (30 m × 0.25 mm i.d., 0.2 µm film thickness, 90% biscyanopropyl, 10% phenylcyanopropyl polysiloxane) was used (Restek, Centre County, PA, U.S.). Helium was employed as the carrier gas at a constant pressure of 70 kPa, and the split ratio was set to 1:30. The oven temperature was programmed from 100°C to 240°C at a rate of 4°C/min, and the final temperature was held for 20 min. Data acquisition was carried out using Chrom Card Data System software (version 2.3.1). Peaks were identified using a reference compound mix, FAME Supelco 37 (Darmstadt, Germany). The data are expressed as the percentage of total fatty acids (area%). Three replicates were performed for each sample.

Volatile compounds analysis

To investigate the volatile profile of the two olive oil samples, solid-phase microextraction followed by gas chromatography-mass spectrometry (SPME-GC-MS) was performed. Olive oil samples and the internal standard (IS) mixture were prepared as described in Casadei et al. (2024). The IS mixture, containing 4-methyl-2-pentanol at an approximate concentration of 50 mg/kg, was formulated in refined olive oil and added to the oil sample for the qualitative and quantitative analysis of the headspace volatile fraction. Specifically, 1.9 g of the sample was placed in a 20 mL glass vial, along with 0.1 g of the IS, and the vial was hermetically sealed with a polytetrafluorethylene (PTFE) septum. To equilibrate the molecules in the headspace, the vial was agitated at 40°C for 10 min. The septum was pierced with the SPME needle, and the fiber was exposed to the headspace at 40°C for 40 min. Afterward, the fiber was inserted into the injector port of the GC and held for 5 min at 250°C, with the purge valve set to a 1:10 split ratio. The sample was then injected into a polar-phase capillary column (TG-WAXMS: 60 m length, 0.25 mm internal diameter, and 0.50 µm coating; Thermo Fisher Scientific, Waltham, MA, USA) of a GC equipped with a mass spectrometry (MS) detector (QP2010 Ultra, Shimadzu, Kyoto, Japan). The ion source was set to 200°C, and the transfer line temperature was set to 260°C. The MS analyzer was operated in full-scan mode with a m/z range from 30 to 250, a scan speed of 454 (m/z)/s, and an electron energy of 70 eV. Helium was used as the carrier gas. The oven temperature was initially held at 40°C for 10 min, then programmed to increase by 3°C/min to a final temperature of 200°C. A cleaning step was included after the temperature program, where the temperature was increased to 250°C at 20°C/min and held for 5 min to prepare the column for the next analysis.

In addition, a screening analysis of the volatile fraction was performed using a gas chromatography coupled with ion-mobility spectrometry (GC-IMS) Flavourspec® instrument (G.A.S. Dortmund, Dortmund, Germany), with a nitrogen generator (Microprogel, Pordenone, Italy) and an autosampler unit (HT2000H, HTA s.r.l., Brescia, Italy). For each sample, a Hamilton syringe with a 51 mm needle was used to withdraw 100 µL from the headspace, which was then introduced into a splitless heated injector (2 mm I.D., 6.5 mm O.D., 78.5 mm fused quartz glass). A low-polar column (FS-SE-54-CB-0.5, 30 m in length, 0.32 mm internal diameter, and 0.5 µm film thickness, composed of 94% methyl, 5% phenyl, and 1% vinylsilicone) was used for the first separation. The eluate was then subjected to a second separation using the IMS, which was equipped with a tritium ionizing radioactive source at 5000 V and a 9.8 cm long drift tube, supplied by Gesellschaft für Analytische Sensorsysteme mbH (G.A.S.) in Dortmund, Germany.

Sensory analysis

Sensory analysis was conducted by the Professional Committee of DISTAL (Department of Agricultural and

Food Sciences, University of Bologna, recognized by the Italian Ministry of Agriculture, Food Sovereignty, and Forests) following the official procedure outlined in EU Reg. 2022/2104 and EU Reg. 2022/2105. The olive oil samples were sensory profiled based on the intensity of defects and three main positive attributes (fruity, bitter, and pungent), as described in the aforementioned regulations (EU Reg. 2022/2104 and EU Reg. 2022/2105).

Statistical analysis

The data analysis was conducted using Microsoft® Excel 2016 (Microsoft Corp., Redmond, WA).

Results and Discussion

The present discussion focuses on the compositional and sensory profiles of two Kalinjot virgin olive oils, aiming to complement the information gathered by previous research on specific quality aspects such as phenols (Topi *et al*., 2020; Velo and Topi, 2017; Kongoli *et al*., 2011), fatty acids (Kongoli *et al*., 2011; Hysi and Kongoli, 2015; Velo and Topi, 2017), and volatile compounds (Topi *et al*., 2019). The goal is to contribute to a holistic understanding of the oil obtained from this unique variety. This is particularly relevant for potential future applications for quality labels, such as PDO (Protected Designation of Origin) and PGI (Protected Geographical Indication), which could be linked to the geographical origin of the oil. Such labels would help standardize and improve both the quality and market value of the oil, thereby benefiting local farmers and producers (Lopes *et al*., 2022). In the following discussion, EU Reg. 2022/2104 is used as a reference for the quality and purity parameters of virgin olive oils, despite Albania's current status as a non-EU member state.

Basic quality parameters

Both virgin olive oils presented basic quality values below the limits imposed by EU Reg. 2022/2104 for extra virgin olive oils.

Free acidity measurements quantify the amount of free fatty acids, which are products of the hydrolysis of triglycerides, also releasing diglycerides and monoglycerides. This parameter is an important indicator of oil quality, reflecting the quality of the starting olives, the timing and storage conditions of the olives, and the effectiveness of the separation process between the oil and the wastewater. In both samples, the acidity was below the maximum value specified by EU Reg. 2022/2104 for extra virgin olive oils (see Table 1). Free acidity in Kalinjot virgin olive oils analyzed in previous studies has consistently been below 0.8%, ranging from 0.2% to 0.6% in Kongoli *et al*. (2015), with an average value of 0.3% in Veizi *et al*. (2020), and ranging from 0.3% to 0.8% in Morina and Kongoli (2022). These values reflect the consistent attention to the basic quality of olive oil produced in this geographical area, as early as 2015 (Muço *et al*., 2015).

Peroxide value (PV) is an important parameter for quantifying primary oxidation products and is expressed in mEq O_2/kg. Both samples reported values well below the limit of 20 mEq O_2/kg set by EU Reg. 2022/2104. Additionally, other studies on the Kalinjot variety have shown low PV levels, such as 4.9 mEq O_2/kg (Kongoli *et al*., 2011), 6.32 mEq O_2/kg (Veizi *et al*., 2020), and 7.71 mEq O_2/kg (Morina and Kongoli, 2022).

Spectrophotometric investigation in the ultraviolet (233 nm, 268 nm, and ΔK) is related to the amount of dienoic and trienoic conjugated fatty acids, which are secondary oxidation products. Both samples showed values below the limit set by EU Reg. 2022/2104 for extra virgin olive oils. Similar findings have been reported in the literature (Kongoli *et al*., 2011; Hysi and Kongoli, 2015; Veizi *et al*., 2020; Morina and Kongoli, 2022).

Moreover, as an additional analysis not included among those officially outlined in EU Reg. 2022/2104, the Rancimat test measures the induction period—i.e., the time, in hours, that an oil subjected to forced oxidation (at 110°C with an air stream of 9 L/h) takes to transition from the slower phase of oxidation to the faster propagation phase. This is an important parameter for assessing the potential shelf life of the oil (Farhoosh, 2007). Both Kalinjot olive oil samples had an induction time exceeding 20 h. These results suggest that the oils exhibit good stability and are within the average range for extra virgin olive oils when compared with some Italian varieties (Di Lecce *et al*., 2020). In particular, Kalinjot1 showed a relatively long induction period of 29.8 h.

Total phenols and phenolic profile

Total phenols were determined using the Folin-Ciocalteu method and HPLC (Figure 2). Both samples contained phenolic compounds, with slightly higher amounts found in Kalinjot1 (Table 2). These values align with the induction period measurement, which was also higher for Kalinjot1. Phenolic compounds are the primary antioxidants in olive oil (Aparicio & Harwood, 2013). Furthermore, the HPLC results are consistent with the study by Topi *et al*. (2020), which reported a total phenol content of 248.34 ± 1.96 mg/kg for the Kalinjot variety in the same geographical area as Kalinjot1 (Vlora).

Table 1. Results (mean ± SD, n = 3) for the basic quality parameters (free acidity, peroxide value, induction period, K268, K232, ΔK) of the analyzed extra virgin olive oils, presented as the average of three replicates. The related EU limits, when applicable, are also included (EU Reg. 2022/2104).

Sample	Free acidity (%)*	POV (mEq O_2/kg)*	K_{268}*	K_{232}*	ΔK*	Induction period (h)
Kalinjot1	0.2 ± 0.0	6.0 ± 0.3	0.15 ± 0.02	1.80 ± 0.08	-0.003 ± 0.00	29.8 ± 1.7
Kalinjot2	0.3 ± 0.0	6.7 ± 0.1	0.14 ± 0.01	1.84 ± 0.01	-0.002 ± 0.01	23.9 ± 1.1

* In Reg. UE 2022/2104, extra virgin olive oil limits are: free acidity < 0.8 %; POV < 20 mEq O_2/kg; K_{268} < 0.22; K_{232} < 0.25; ΔK < 0.01.

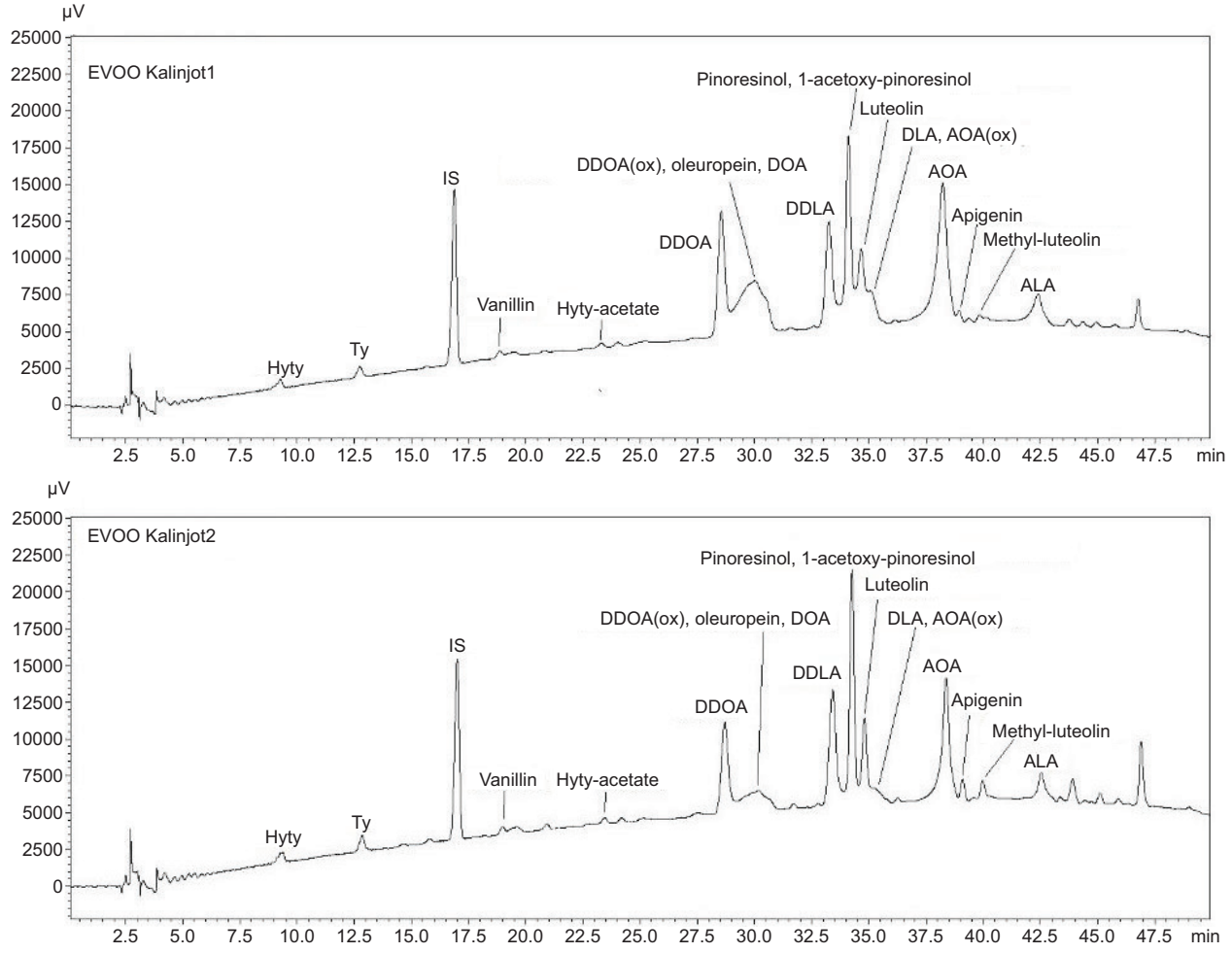

Figure 2. HPLC chromatograms of the phenolic compound analysis for the two Kalinjot virgin olive oil samples, labeled as Kalinjot1 and Kalinjot2.

The Folin-Ciocalteu method, however, reported higher values for total phenols compared to the sum of the concentrations determined by HPLC. This discrepancy may be due to the overestimating effect of the Folin-Ciocalteu procedure, which is known to be influenced by non-phenolic compounds such as ascorbic acid, amines, and, in particular, sugars. These substances can lead to an overestimation of total phenolic content in spectrophotometric assays (Escarpa and González, 2001; Tsao and Yang, 2003).

On the other hand, the HPLC method proposed and adopted by the IOC quantifies phenols using syringic acid and tyrosol as reference compounds, due to the lack of commercial standards for bound phenolics. This approach may lead to an underestimation of the actual amount of more complex secoiridoid forms (Mastralexi *et al*., 2014). Additionally, the IOC method does not apply a response factor to account for the molecular mass of the identified bound phenolic compounds.

Table 2. MAmounts of total and individual phenolic compounds in the Kalinjot virgin olive oil samples, Kalinjot1 and Kalinjot2, determined by HPLC, along with total phenols measured using the Folin-Ciocâlteu method (mean ± SD, n = 3).

Phenolic compounds (mg/kg)[1]	Kalinjot1	Kalinjot2
Hyty	TR[2]	TR
Ty	TR	3.78 ± 0.03
Vanilin	TR	TR
Hydroxytyrosyl acetate	ND[2]	TR
DDOA	39.48 ± 0.58	28.78 ± 0.18
DDOA (ox), oleuropein, DOA	57.67 ± 1.08	20.93 ± 0.35
DDLA	34.67 ± 0.55	36.92 ± 0.28
Pinoresinol, 1-acetoxy-pinoresinol	46.97 ± 0.77	48.68 ± 0.16
Luteolin	25.62 ± 0.26	21.32 ± 0.12
DLA, AOA (ox)	10.88 ± 0.28	5.85 ± 0.18
AOA	65.67 ± 1.01	41.85 ± 0.35
Apigenin	TR	3.92 ± 0.10
Methyl-luteolin	TR	3.35 ± 0.39
ALA	11.70 ± 0.13	8.90 ± 0.33
Total secoiridoids (HPLC)	220.05 ± 3.07	147.02 ± 0.14
Total phenols (HPLC)	292.63 ± 4.04	224.22 ± 1.06
mg secoiridoid/20 g EVOO (health claim) (HPLC)	4.40 ± 0.06	2.90 ± 0.0
mg GA/20 g EVOO (health claim) (Folin-Ciocâlteu)	7.00 ± 0.12	5.36 ± 0.10
Total phenols (mg GA/kg) (Folin-Ciocâlteu)	350.23 ± 6.02	268.17 ± 4.88

Hyty: hydroxytyrosol; Ty: tyrosol (ty); DDOA: decarboxymethyl oleuropein aglycone, dialdehyde form (DDOA); DDOA (ox): decarboxymethyl oleuropein aglycone, oxidized dialdehyde form; DOA: oleuropein aglycone, dialdehyde form; DDLA: decarboxymethyl ligstroside aglycone, dialdehyde form; DLA: ligstroside aglycone, dialdehyde form; AOA (ox): oleuropein aglycone, oxidized aldehyde and hydroxylic form; AOA: oleuropein aglycone, aldehyde and hydroxylic form; ALA: ligstroside aglycone, aldehyde and hydroxylic form; TR: traces; ND: not detected.

Kalinjot1 and Kalinjot2 meet the required concentration of secoiridoids (5 mg/kg) for the health claim according to EFSA (EFSA, 2011) when the results from the Folin-Ciocâlteu method are considered [Table 2]. However, both virgin olive oil samples fall short of this threshold when calculated using the HPLC data [Table 2], unless correction factors are applied. Nevertheless, it is important to note that the concentrations are quite close to the health claim limit, even though the olive harvest occurred in late November. Therefore, it would be valuable to analyze the total phenol content of olive oils produced at the beginning of the season, to monitor their evolution throughout the harvest period and provide this quality information to producers. It is well established that as olives ripen, the concentration of phenols decreases (Morelló *et al*., 2004). The two olive oil samples exhibited similar phenolic profiles [Table 2]; however, methyl-luteolin and apigenin were quantifiable only in Kalinjot2.

Fatty acids profile

Fatty acid composition was determined, identifying 13 compounds [Table 3]. Importantly, each of these identified compounds falls below the limits established by EU Reg. 2022/2104 for extra virgin olive oils. Furthermore, it is noteworthy that these results are consistent with previous research on the fatty acid composition of Kalinjot virgin olive oils, as documented by Muço *et al*. (2015), Velo and Topi (2017), Veizi *et al*. (2019), and Morina and Kongoli (2022). The samples contain a significant amount of oleic acid, the primary monounsaturated fatty acid in olive oil, which is one of the main contributors to the health benefits associated with olive oil, particularly in reducing the risk of cardiovascular diseases (Lu *et al*., 2024)

Volatile compounds

The volatile profile of the two Kalinjot olive oil samples was investigated using SPME-GC-MS. A total of 31 volatile compounds were identified and quantified, as shown in Table 4.

The olive oils analyzed exhibited a volatile profile primarily composed of C5 and C6 compounds derived from the LOX pathway.

The results for the Kalinjot1 sample show that the volatile compounds in the highest concentrations belong to the chemical class of hydrocarbons (3.77 mg/kg), followed by products from sugar fermentation (3.40 mg/kg), penten dimers (2.61 mg/kg), alcohols (1.72 mg/kg), ketones (1.64 mg/kg), products of autoxidation (0.96 mg/kg), aldehydes (0.64 mg/kg), esters (0.62 mg/kg), and products of amino acid metabolism (0.18 mg/kg). Both olive oil samples shared prominent aldehyde compounds, such as (*Z*)-2-Hexenal, Hexanal, and (*E*)-2-pentenal, which are known for their green notes (Genovese *et al*., 2021). In particular, hexanal, the major aldehyde compound, is associated with green and fruity flavors (Morales *et al*., 2013) and cut grass (Di Vaio *et al*., 2021). Likewise, key alcohols, such as (*Z*)-3-Hexen-1-ol and 1-Hexanol, along with the primary ketone, 1-penten-3-one, contribute to the characteristic positive sensory attribute of "green" (Genovese *et al*., 2021). Overall, a predominance of volatiles linked to positive attributes was observed in high concentrations. Conversely, those associated with defects, such as

Table 3. Fatty acid composition of the Kalinjot virgin olive oil samples, Kalinjot1 and Kalinjot2 (mean ± SD, n = 3).

Fatty acids (%)		Kalinjot1	Kalinjot2	EU Reg. 2022/2104.
Myristic acid	C14:0	0.01 ± 0.00	0.01 ± 0.00	≤0.03
Palmitic acid	C16:0	11.02 ± 0.28	9.78 ± 0.01	7.00–20.00
Palmitoleic acid	C16:1 (*n*-9)	0.49 ± 0.01	0.39 ± 0.00	0.30–3.50
Heptadecanoic acid	C17:0	0.12 ± 0.00	0.14 ± 0.01	≤0.4
Heptadecanoic acid	C17:1 (*n*-9)	0.18 ± 0.01	0.21 ± 0.00	≤0.4
Stearic acid	C18:0	2.65 ± 0.06	2.83 ± 0.03	0.50–5.00
Oleic acid	C18:1 (*n*-9)	75.39 ± 0.09	76.82 ± 0.05	55.00–85.00
Linoleic acid	C18:2 (*n*-6)	8.74 ± 0.09	8.27 ± 0.02	2.50–21.00
Arachidic acid	C20:0	0.39 ± 0.04	0.44 ± 0.02	≤0.6
Linolenic acid	C18:3	0.60 ± 0.01	0.61 ± 0.01	≤1
Eicosenoic acid	C20:1 (*n*-9)	0.29 ± 0.04	0.33 ± 0.01	≤0.5
Behenic acid	C22:0	0.10 ± 0.00	0.11 ± 0.00	≤0.2
Lignoceric acid	C24:0	0.05 ± 0.01	0.06 ± 0.00	≤0.2

Table 4. Volatile compounds identified and quantified by SPME-GC-MS (mean ± SD, n = 3).

Volatile compounds (mg/kg)	Kalinjot1	Kalinjot2	LRI*
C6 LnA - Aldehydes	0.23 ± 0.04	0.31 ± 0.03	0.03
2-Hexenal (*E*)	0.12 ± 0.02	0.16 ± 0.02	0.02
2-Hexenal (*Z*)	0.11 ± 0.02	0.15 ± 0.01	0.01
C6 LnA Alcohols	0.73 ± 0.05	1.11 ± 0.12	0.12
3-Hexen-1-ol (*E*)	0.01 ± 0.00	0.02 ± 0.00	0.00
3-Hexen-1-ol (*Z*)	0.72 ± 0.05	1.08 ± 0.12	0.12
C6 LnA Esters	0.67 ± 0.05	0.43 ± 0.02	0.02
3- hexenyl acetate (*Z*)	0.62 ± 0.05	0.36 ± 0.02	0.02
Hexyl acetate	0.05 ± 0.01	0.07 ± 0.01	0.01
C6 LA Aldehydes	0.35 ± 0.03	0.70 ± 0.10	0.10
Hexanal	0.35 ± 0.03	0.70 ± 0.10	0.10
C6 LA Alcohols	0.12 ± 0.01	0.24 ± 0.03	0.03
1-Hexanol	0.12 ± 0.01	0.24 ± 0.03	0.03
C5 LnA Aldehyeds	0.06 ± 0.01	0.06 ± 0.01	0.01
2-pentenal (*E*)	0.06 ± 0.01	0.06 ± 0.01	0.01
C5 LnA Alcohols	0.87 ± 0.05	0.82 ± 0.06	0.06
1-Penten-3-ol	0.48 ± 0.02	0.48 ± 0.03	0.03
3-pentanol	0.07 ±0.01	0.02 ± 0.00	0.00
2-Penten-1-olo (*E*)	0.04 ± 0.00	0.04 ± 0.01	0.01
2-penten-1-ol (*Z*)	0.28 ± 0.02	0.27 ± 0.02	0.02
C5 LnA Ketones	0.97 ± 0.15	0.47 ± 0.07	0.07
1-Penten-3-One	0.97 ± 0.15	0.47 ± 0.07	0.07
C5 LA Ketones	0.67 ± 0.05	0.82 ± 0.05	0.05
3-Pentanone	0.67 ± 0.05	0.82 ± 0.05	0.05
Penten dimers	2.61 ± 0.30	2.01 ± 0.25	0.25
3-Ethyl-1,5-Octadiene (1)	0.21 ± 0.03	0.19 ± 0.04	0.04
3-Ethyl-1,5-Octadiene (2)	0.17 ± 0.01	0.15 ± 0.01	0.01
3-Ethyl-1,5-Octadiene (3)	1.08 ± 0.13	0.74 ± 0.10	0.10
3-Ethyl-1,5-Octadiene (4)	0.84 ± 0.12	0.67 ± 0.10	0.10
3-Ethyl-1,5-Octadiene (5)	0.32 ± 0.04	0.26 ± 0.02	0.02
Hydrocarbons	3.77 ± 0.26	6.32 ± 0.27	0.27
1,7-Nonadiene,4,8-dymethyl	0.53 ± 0.02	0.42 ± 0.06	0.06
B cis ocimene	0.15 ± 0.01	0.43 ± 0.04	0.04
Geranil nitrile	0.39 ± 0.02	1.25 ± 0.07	0.07
a-farnesene	0.04 ± 0.01	0.11 ± 0.01	0.01
1-tetradecene	2.66 ± 0.24	4.11 ± 0.18	0.18
Auto oxidation	0.96 ± 0.11	1.32 ± 0.14	0.14
2,4-Hexadienal (*E,E*)	0.96 ± 0.11	1.32 ± 0.14	0.14
AA metabolism	0.18 ± 0.01	0.60 ± 0.03	0.03
Butanal-2-Methyl	0.12 ± 0.01	0.37 ± 0.02	0.02
Butanal-3-Methyl	0.06 ± 0.01	0.23 ± 0.01	0.01
Sugars fermentation	3.35 ± 0.18	2.09 ± 0.12	0.12
Ethyl Acetate	0.42 ± 0.07	0.31 ± 0.03	0.03
Methanol	0.57 ± 0.05	0.80 ± 0.03	0.03
Ethanol	1.93 ± 0.05	0.43 ± 0.01	0.01
Acetic acid, hexyl ester	0.31 ± 0.01	0.32 ± 0.06	0.06
Propanoic acid	0.01 ± 0.00	0.02 ± 0.00	0.00
Butanoic acid	0.02 ± 0.00	0.03 ± 0.00	0.00
1-butanol,3-methyl	0.09 ± 0.00	0.18 ± 0.01	0.01

*LRI: Linear Retention Indices.

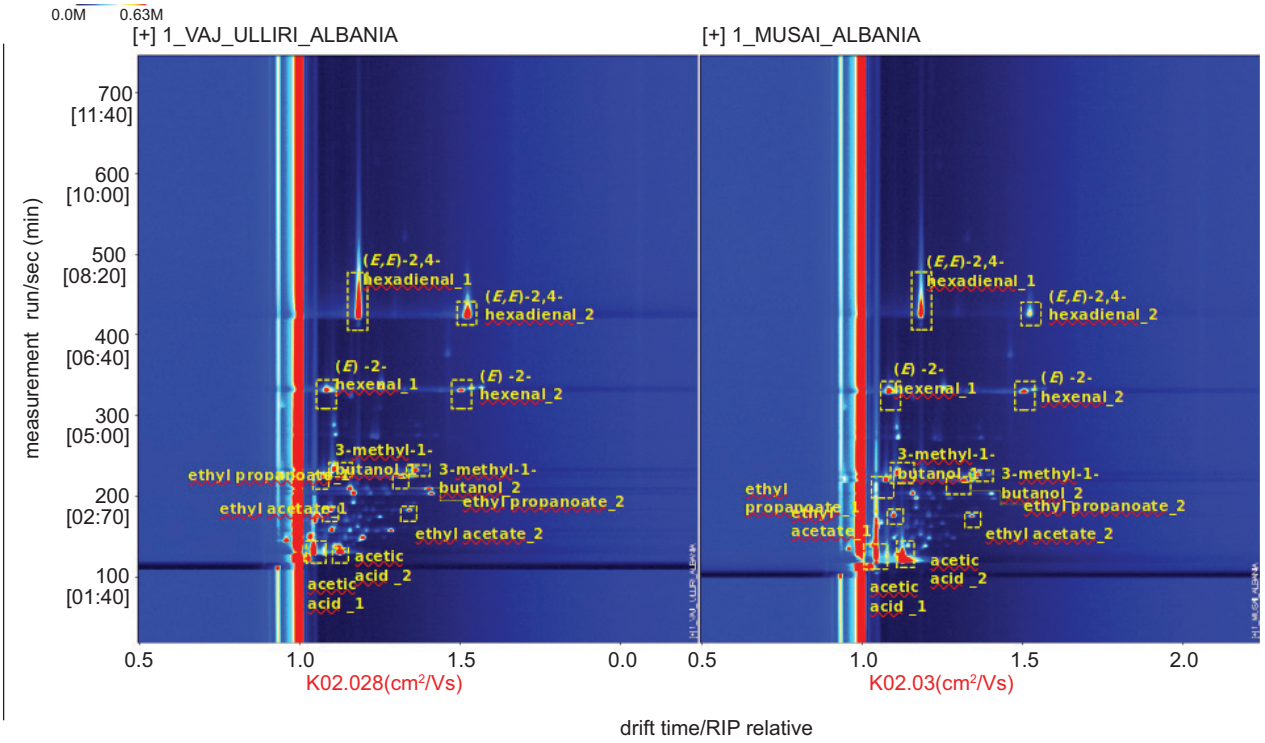

Figure 3. Heat maps showing twelve volatile compounds found, by HS-GC-IMS, in Kalinjot1 (left) and Kalinjot2 (right).

butanoic acid and acetic acid, were detected at notably lower levels. Furthermore, the results align with the volatile profile of Kalinjot olive oil investigated by Topi *et al.* (2019).

Volatile compounds were also analyzed using HS-GC-IMS, a cost-effective and rapid technique for identifying aroma digital fingerprints (Garrido-Delgado *et al.*, 2015). In the heat maps (Figure 3), 12 compounds were identified through comparison with the standards injected under the same analytical conditions. Specifically, (*E*)-2-Hexenal, which is crucial for fruity notes, is clearly evident, among others. Compounds associated with defects, such as ethyl acetate, acetic acid, ethyl propanoate, and 3-methyl-1-butanol, were also present but remained below the sensory perception threshold.

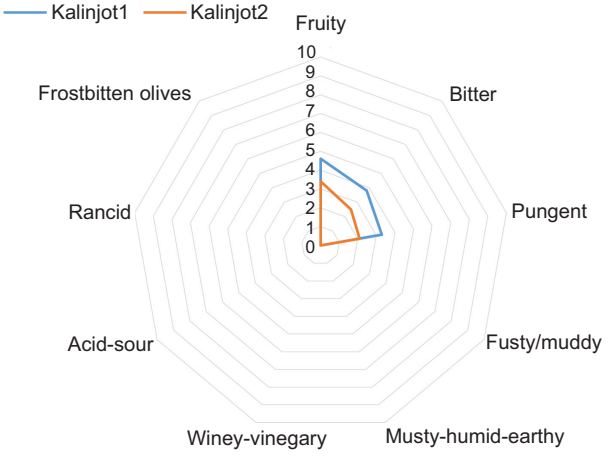

Figure 4. Spider graphs of sensory analysis of Kalinjot1 and Kalinjot2.

Sensory analysis

The sensory analysis was conducted according to the method known as the Panel test (EU Reg. 2022/2104) by the professional committee of virgin olive oil tasters at the University of Bologna. Both Kalinjot1 and Kalinjot2 fall into the commercial category of "extra virgin olive oil," showing no sensory defects and a median fruity score above 0 (EU Reg. 2022/2104) [Figure 2]. In Kalinjot1, all three main positive attributes—fruity, bitter, and pungent—were perceived with medium intensity. Additionally, Kalinjot1 was characterized by two secondary positive attributes, tomato and grass, with medians of 2.0 and 3.0, respectively. In contrast, Kalinjot2 exhibited medium intensity only for the fruity attribute, with lighter intensity for bitterness and pungency (Figure 4). Hysi and Kongoli (2015) reported median values of 3.0 for the positive attributes of fruity, bitter, and pungent, and also noted the presence of a secondary positive attribute, apple.

This outcome is consistent with the findings regarding VOCs and phenol content. Specifically, the higher bitterness and pungency of Kalinjot1 compared to Kalinjot2 aligns with the higher phenolic content and longer induction period observed in Kalinjot1 (Dierkes *et al*., 2012; Vulcano *et al*., 2015). Additionally, literature indicates that bitterness and pungency are also linked to the presence of 1-penten-3-one (Olmo-Cunillera *et al*., 2022). This is in agreement with the higher concentration of 1-penten-3-one and the more pronounced bitterness and pungency in Kalinjot1. Furthermore, this volatile compound is associated with the secondary attribute of tomato (Tura *et al*., 2008), which was perceived only in the Kalinjot1 extra virgin olive oil.

Conclusions

This study builds on existing knowledge in the scientific literature and provides valuable insights into the compositional and sensory characterization of monovarietal Kalinjot extra virgin olive oils. Both oils analyzed meet the quality criteria set by the European Union for extra virgin olive oils, as well as the minimum secoiridoids concentration required for the health claim, which was approximately evaluated using the Folin-Ciocâlteu method. Additionally, both oils exhibit induction periods greater than 20 h (29.8 h for Kalinjot1 and 23.9 h for Kalinjot2), a positive indicator for their shelf-life. A total of 33 volatile compounds were identified via GC-MS, with a predominant presence of aldehydes, key alcohols, and a primary ketone, all contributing to the distinctive green notes. Furthermore, GC-IMS analysis highlighted twelve volatile compounds, including those associated with fruity notes. Two secondary attributes, grass and tomato, were also perceived in the sensory analysis. Given the promising results, it would be valuable to further assess the consistency of these findings by analyzing additional monovarietal Kalinjot extra virgin olive oils. Such research could be instrumental in the valorization of Kalinjot oils and the potential application for a designation of origin.

Authors Contribution

Conceptualization T.G.T.; formal analysis S.Z., E.C., M.M., F.F., S.B.; writing-original draft preparation S.Z., F.F., S.B.; writing-review and editing all authors. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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