Estimation of Avocado Oil (*Persea americana* Mill., Greek “Zutano” Variety) Volatile Fraction over Ripening by Classical and Ultrasound Extraction Using HS-SPME–GC–MS

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Abstract: The study of flavors and fragrances is a topic of rising interest from both marketing and scientific perspectives. Over the last few years, the cultivation of avocados has accelerated in Greece, with production levels elevated by 300%. There has been increasing attention from a number of growers and consumers on avocado oil, the volatiles of which form a key part of consumers’ purchase decisions. A previously unevaluated Zutano cultivar was chosen for this study. Extraction of the pulp oil was performed during three phases of ripening using Soxhlet and ultrasound techniques. Headspace-solid-phase microextraction (HS-SPME) and gas chromatography–mass spectrometry (GC–MS) were utilized in order to analyze the isolated volatile fraction. At least 44 compounds, including mainly terpenoids (61.7%) and non-terpenoid hydrocarbons (35.9%), presented in the Zutano variety, while (1S,6S,7S,8S)-1,3-dimethyl-8-propan-2-yltricyclo[4.4.0.02,7]dec-3-ene (a-copaene) and (1R,9S,Z)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene (β-caryophyllene) were in higher abundance. The composition of the volatiles was unaffected by the extraction techniques but was influenced by the ripening stage. Thus, during maturation, the volatile fraction fluctuates, with a significantly higher abundance of terpenoids during the fourth day of the ripe stage, whilst it decreases during over-ripening. These findings demonstrate that the Zutano variety can be used to produce an aromatic oil and hence could be used, among others, as an ingredient in cosmetic products.

Keywords: Zutano variety; avocado oil; Soxhlet extraction; ultrasound-assisted extraction; volatiles; ripening; over-ripe; HS-SPME–GC–MS

1. Introduction

The avocado (*Persea americana* Mill.) is a subtropical/tropical tree which is traditionally cultivated in Central America [1]. The growth of the avocado fruit depends on the cultivar and/or environmental conditions, and it is harvested when the fruit is horticulturally mature [1]. The production of avocados has become more widespread worldwide in the last few decades, with seven million tons produced in 2019 [2]. In Greece, avocados are mainly produced in Crete, Peloponnesse, and Rhodes, with an average of 9.3 tons produced in 2019 [2]. It is an emblematic crop for Crete, with 90% of the total Greek production concentrated in the prefecture of Chania. Furthermore, it is notable that production increased by about 300% between 2014 and 2019 [3].

Avocado oil is gaining considerable attention from business and research sectors, demonstrated by the expanding literature. It is renowned for its uses in cosmetics, the food processing industries, and edible oil [4,5]. Avocado oil is obtained from the fleshy...
The mesocarp (pulp) of the fruit and has a high nutrition value, biological effects, and healing properties [4,6,7].

The process of avocado oil extraction is quite similar to olive oil extraction. Generally, the extra virgin oil is extracted from high-quality fruit with minimal levels of rot and physiological disorders, while some rots or physiological disorders are permitted in virgin oil. In each case, extraction is carried out using only mechanical methods at low temperatures (<50 °C). For pure avocado oil, quality is not important, with low acidity, color, and a bland flavor, and for mixed avocado oil, blends with other oils are allowed. According to Woolf, [8] avocado oil might be classified as “extra virgin”, “virgin”, “pure”, or “mixed”. However, no global standardized physicochemical measurements have been implemented for such a categorization. The composition, quality, and yield of avocado oil are dependent on several factors, including fruit variety [9–11], harvesting time, and ripening stage [10,12,13]. The majority of scientists have focused their endeavors on assessing the “Hass” and “Fuerte” varieties or others with commercial value, while the “Zutano” variety has been studied by few researchers [14], i.e., there is a dearth of data regarding the cultivation of this variety within the Mediterranean. A further feature of the avocado is that if the fruit were to remain on the tree, ripening would not occur. Ripening takes place over a period of time, i.e., between 3–4 and 18–21 days following harvest [8]. The time duration is impacted by storage parameters, amongst additional extrinsic factors. Over-ripening may also occur. Different extraction techniques, conditions, and solvents are factors determining the avocado oil quality and yield [4,5]. An overview of extraction methods used in the last 20 years includes mainly liquid extraction using Soxhlet apparatus [15–27], homogenization [26,28], and microwave-assisted extraction (MAE) [19,25,27]. Several studies have focused on supercritical fluids [15,16,19–22,29] and mechanical extraction by cold pressure [17,18,30,31]. Lesser-used methods include extraction by enzymes [32] and ultrasound-assisted extraction (UAE) [15,16,19,30].

Volatile compounds of avocado oil have a well-established role in aroma profiles and are one of the most important characteristics of the quality of the product. The combination of different volatile compounds forms the aroma character of avocado oil [33,34]. Some studies demonstrate that volatile compounds may differ depending on the variety [35,36], oil extraction solvents, or treatments [25,37]. One possible influence on the aromatic profile of avocado oil is the ripening phase, which, to date, has been poorly investigated.

In the current work, the volatiles from Greek Zutano avocado oil were acquired during three ripening phases, i.e., breaking, ripe, and overripe. Two diverse extraction methods were employed, i.e., Soxhlet extraction (SE) and ultrasound-assisted extraction (UAE). Headspace-solid-phase microextraction (HS-SPME) and gas chromatography–mass spectrometry (GC–MS) techniques were utilized to characterize the respective volatile fractions obtained.

2. Materials and Methods

2.1. Avocado Fruit Samples

Avocado fruits (Persea americana Mill., “Zutano” variety) were provided directly from producers at commercial maturity (firm) during the 2020 harvest year. The fruits were located in the Greek island of Crete (35°28′28.1″ N, 23°56′53.3″ E). The samples were stored in the dark at ambient temperature (24 ± 1 °C) for one day (breaking), four days (ripe), and eight days (overripe) (Figure 1). Then, samples were cut and lyophilized by freeze drying on a VirTis Freezemobile 25EL (SP Industries, 935 Mearns Rd, Warminster, PA, USA) to remove the water, and the solid residue was stored for 24 h at –20 ± 1 °C until oil extraction. Moreover, the percentage (% w/w) of dry matter (>19% w/w) was calculated according to Greek legislation for avocado commercial standards [38].
Avocado oils were extracted by classical SE and UAE techniques. The AOAC Official Method 948.22 was applied for the SE with some modifications. Approximately 10 g of avocado pulp powder was mixed with 625 mL petroleum ether (purity 99.0%) in a Soxhlet apparatus for 6 h at 50 °C. UAE was performed in a Grant ultrasonic water bath (Grant Instruments Ltd., Cambridge, UK) (300 × 140 × 150 mm³ internal dimensions) at the fixed frequency of 35 kHz. Approximately 10 g of avocado pulp powder was mixed with 80 mL of petroleum ether in an Erlenmeyer flask for 30 min at 25 °C. The organic solvent of each extract was totally evaporated under reduced pressure at 35 °C using a Laborota 4000 efficient rotary evaporator (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). The previous procedure was performed in triplicate and the received oily extracts were refrigerated at −20 ± 1 °C in a totally filled storing flask until GC analysis.

2.3. Isolation and Analysis of Avocado Oil Volatile Fraction

The isolation and analysis of the volatile compounds were performed using HS-SPME–GC–MS according to Xagoraris with few modifications [39]. An amount of 4 g of avocado oil alongside 1 μL of β-ionone (Alfa Aesar, Ward Hill, MA, USA) were placed into a 15 mL screw-top glass vial with PFTE/silicone septa. The vials were equilibrated for 30 min in a water bath at 60 °C under stirring at 700 rpm. Subsequently, the HS-SPME procedure was carried out using a triple-phase divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber 50/30 μm needle (Supelco, Bellefonte, PA, USA) with a length of 1 cm. The needle was inserted into the vial and exposed to the headspace for 30 min.

The analysis of volatile compounds was performed using a Thermo GC-Trace ultra, coupled with a Thermo mass spectrometer DSQ II (Thermo Fisher Scientific Inc., Waltham, MA, USA). The GC inlet temperature was 260 °C in splitless mode for 3 min with a 0.8 mm injector liner (SGE International Pty Ltd., Ringwood, Australia). The column used was a Restek Rtx-5MS (30 m × 2.25 mm i.d., 0.25 μm film thickness) (Restek, Bellefonte, PA, USA). The carrier gas was helium at a 1 mL·min⁻¹ flow rate. The column was maintained at 40 °C, held for 6 min, then heated to 120 °C at a rate of 5 °C·min⁻¹, then heated to 160 °C at a rate of 3 °C·min⁻¹, then heated to 250 °C at a rate of 15 °C·min⁻¹ and held at 260 °C, held for 6 min, then heated to 280 °C at a rate of 5 °C·min⁻¹, and held at 280 °C for 10 min.

The analysis was performed using a Thermo TRACE Ultra gas chromatograph.
250 °C for 1 min [39]. The temperature conditions of the mass spectrometer were: transfer line (290 °C), source (240 °C), and quadrupole (150 °C). Electron impact was 70 eV, and mass spectra were recorded at the 35–650 mass range. Retention index (RI) values were calculated using n-alkane (C8-C20) standards (Supelco, Bellefonte, PA, USA). The peak identification was carried out with the Wiley 275 mass spectra library and masses spectral data and arithmetic index provided by Adams [40]. Quantification of volatile compounds was accomplished by dividing the peak areas of the compounds by the peak area of the internal standard (β-ionone) and multiplying this ratio by the initial concentration of the internal standard.

2.4. Statistical Analysis

All chromatographic data were acquired by analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) using the SPSS v.25 (IBM, SPSS, Statistics) software. The mean values were calculated in Microsoft Excel 2013.

3. Results and Discussion

3.1. Estimation of Avocado Oil Yield

SE and UAE are classical methods that are commonly used for avocado oil extraction [7]. These processes have total solvent penetration into the oil membranes of avocado fruit [7]. Furthermore, these have been widely used to determine the theoretical maximum oil yield of avocado [41]. However, some factors, including variety, drying method, organic solvent, and temperature, should be taken into account in oil recovery. For each instance, the current data concur with previous publications [7]. Avocado oils were weighed to measure the oily mass, and all yields were calculated from 46.76 to 66.22% (w/w) for SE and 31.97 to 54.54% (w/w) for the UAE method. Similarly to our results, in a previous study, SE produced a 64.76% (w/w) oil yield and UAE produced a 54.63% (w/w) oil yield [19].

3.2. Volatile Compounds Analysis

The volatile compounds and their semi-quantification in avocado oil are expressed as average values and are summarized in Table 1. The identified fraction was characterized by at least 44 components, including terpenoids, hydrocarbons, aldehydes, and ketones. Terpenoids were the dominant fraction of volatiles, with an average relative abundance of 61.7%, whilst hydrocarbons (non-terpenoids) were 35.9%. Thus, the avocado oil fragrance from the Zutano cultivar was characterized by many terpenoids with high abundance. The results show that the volatile fraction of this variety of oil is rich in terpenoids.

As reported previously by Tan [5], the quality and quantity of volatile compounds detected in avocado oil are affected by several factors such as variety, extraction conditions (e.g., organic solvent, temperature, time), and analytical technique (isolation or analysis parameters). Avocado oil obtained from P. americana Mill. sourced from a Mexico City regional market was studied by Moreno [25], who identified 36 volatile substances using four diverse extraction methods. It should be noted that the largest number of compounds (15 volatiles) were identified using microwaves and Soxhlet and hexane as extractors. Furthermore, in a recent study by Liu [37], 40 volatile compounds were detected using different extraction methods (squeezing, supercritical carbon dioxide, and aqueous). Nineteen volatile materials were identified by Bukykurt [33] in cold-pressed avocado oil grown in regions of Turkey.

A typical chromatogram of avocado oil’s ultrasound technique is presented in Figure 2. However, no qualitative changes were observed among the chromatograms that emerged from different extraction techniques or ripening stages. The total ion chromatograms reveal the capture of seven peaks that characterize the dominant volatile profile of avocado oil from Zutano cultivar. These peaks include 2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (α-pinene); 1-methyl-4-prop-1-en-2-ylcyclohex-1-ene (D-limonene); 4,10-dimethyl-7-propan-2-yltricyclo[4.4.0.01,5]dec-3-ene (α-cubebene); (1R,9S,Z)-1,3-dimethyl-8-propan-2-yltricyclo[4.4.0.02,7]dec-3-ene (a-copaene); (1R,9S,Z)-4,11,11-trimethyl-8-methylenebicy-
Compounds 2022, 2, FOR PEER REVIEW 6 of 12

Others

\( \text{trans-decahydronaphthalene} \quad 493-02-7 \) 16.1 1057 0.34 0.10 0.25

\( \text{dimethyl-6-(4-methylpent-3-enyl)bicyclo[3.1.1]hept-2-ene} \quad (\alpha-\text{bergamotene}) \); and \( (1\text{S},2\text{S},4\text{R})-1,3\text{-dimethyl-8-propan-2-yltricyclo[4.4.0.02,7]dec-3-ene} \) (\( \beta \)-caryophyllene); \( 2,6\text{-dimethyl-6-(4-methylpent-3-enyl)bicyclo[3.1.1]hept-2-ene} \) (\( \beta \)-farnesene), and \( (1\text{S},2\text{S},4\text{R})-1\text{-methyl-4-(prop-1-en-2-yl)cyclohex-1-ene} \) (D-limonene); \( (1\text{R},9\text{S},Z)-4,11,11\text{-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene} \) (\( \beta \)-curcumene) as the most abundant compounds in avocado oil, while Moreno [25] confirmed the above results.

**Figure 2.** A characteristic gas chromatogram of avocado oil (Persea americana Mill., Greek “Zutano” variety) from UAE. (P1) 2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (\( \alpha \)-pinene); (P2) 1-methyl-4-(prop-1-en-2-yl)cyclohex-1-ene (D-limonene); (P3) 4,10-dimethyl-7-propan-2-yltricyclo[4.4.0.0^1,5]dec-3-ene (\( \alpha \)-cubebene); (P4) (15,6S,7S,8S)-1,3-dimethyl-8-propan-2-yltricyclo[4.4.0.0^2,7]dec-3-ene (\( \alpha \)-copaene); (P5) (1R,9S,Z)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene (\( \beta \)-caryophyllene); (P6) 2,6-dimethyl-6-(4-methylpent-3-enyl)bicyclo[3.1.1]hept-2-ene (\( \alpha \)-bergamotene); (P7) (1E,4E,8E)-2,6,6,9-tetramethylcycloundeca-1,4,8-triene (humulene); (I.S) Internal Standard.

3.3. Estimation of Volatiles over Extraction Method

Although the SE and UAE techniques provided different oil yields, the analysis of the volatile compounds by HS-SPME–GC–MS showed similar results. In both techniques, a solvent is used for extraction, which permeates the oily cells to engage with the lipid compounds [41]. The main difference between these techniques was the time and temperature of extraction. In SE, the avocado fruit is repeatedly brought into contact with an organic solvent in a relatively mid–high temperature of 50 °C for 6 h, whereas in UAE, the avocado fruit has been used for accelerated extraction [30] in a relatively controlled mid–low temperature of 25 °C for 15 min.

The extraction techniques did not have statistically significant differences between their qualification and quantification results. Only three components including octan-2-one, (6Z)-7,11-dimethyl-3-methylidenedodeca-1,6,10-triene (\( \beta \)-farnesene), and (15,2S,4R)-1-ethyl-1-methyl-2,4-bis(prop-1-en-2-yl)cyclohexane (\( \beta \)-elemene) could potentially vary between the SE and UAE methods. However, an analysis of the variance indicated that the p-values were higher than 0.05 for all the volatile compounds.

It is evident that the mass transfer and molecular affinity amongst petroleum ether and the targeted compounds exerted a higher influence on acquiring and retaining the volatiles. In this context, comparing the advantages and drawbacks between SE and UAE, the latter appeared more cost-effective and environmentally advantageous.
Table 1. Volatile compounds isolated from headspace of avocado oil (mg kg\(^{-1}\)).

| Volatile Compounds | CAS Number | RT \(^{a}\) | RI \(^{b}\) | Soxhlet Breaking | Soxhlet Ripe | Soxhlet Overripe | UAE Breaking | UAE Ripe | UAE Overripe |
|--------------------|------------|-------------|-------------|-----------------|-------------|----------------|----------------|-------------|----------------|
| Hydrocarbons (Non Terpenoids) | | | | | | | | | |
| 2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (x-pinene) | 7785-70-8 | 11.5 | 929 | 1.79 | 2.57 | 0.74 | 1.80 | 1.95 | 0.36 |
| 2,3,5,6-tetramethylbicyclo[2.2.2]octane (β-myrcene) | 133-33-3 | 13.7 | 988 | 0.96 | 0.94 | 0.33 | 0.96 | 0.64 | 0.39 |
| 1-methyl-4,4-dimethyl-2,6,7-tricyclo[3.3.1.1\(7,11\)]hept-3-ene (D-limonene) | 99-85-4 | 16.1 | 1057 | 0.00 | 0.10 | 0.00 | 0.00 | 0.11 | 0.00 |
| 1-isopropyl-4-methylcyclohexa-1,4-diene (y-terpinene) | 138-86-3 | 15.1 | 1028 | 0.99 | 1.50 | 0.27 | 0.99 | 1.20 | 0.31 |
| 1-methyl-4-methyl-2,6,7-tricyclo[3.3.1.1\(7,11\)]hept-3-ene (β-bergamotene) | 586-62-9 | 17.0 | 1085 | 0.15 | 0.07 | 0.04 | 0.15 | 0.07 | 0.03 |
| 1-methyl-4-prop-1-en-2-ylidenecyclohexa-1,4-diene (x-cubebene) | 1195-32-0 | 17.2 | 1090 | 0.09 | 0.14 | 0.01 | 0.09 | 0.14 | 0.02 |
| 4,10-dimethyl-7-prop-2-yltricyclo[4.4.0.0\(3,8\)]dec-3-ene (cumbene) | 17699-14-8 | 25.0 | 1346 | 1.48 | 1.70 | 1.19 | 1.70 | 2.28 | 1.42 |
| (1S,5S,7S,9S,11R,13R)-1,3-dimethyl-8-prop-2-yltricyclo[4.4.0.0\(3,8\)]dec-3-ene (x-copaene) | 3856-25-5 | 26.0 | 1377 | 3.80 | 5.27 | 3.66 | 3.81 | 7.15 | 4.24 |
| (1R,9S)-4,6,9-trimethyl-8-methylenecyclo[7.2.0]undec-4-ene (β-caryophyllene) | 87-44-5 | 27.5 | 1422 | 5.78 | 8.07 | 4.32 | 5.78 | 9.84 | 4.73 |
| 2,6-dimethyl-1-[4-methylpent-3-enyl]bicyclo[3.1.1]hept-2-ene (x-bisabolene) | 13474-59-4 | 27.8 | 1432 | 1.05 | 1.55 | 1.09 | 1.05 | 2.24 | 1.21 |
| (6Z)-7,11-dimethyl-3-methylenedodeca-1,6,10-triene (β-farnesene) | 28973-97-9 | 28.4 | 1450 | 0.05 | 0.07 | 0.03 | 0.05 | 0.13 | 0.08 |
| (1E,4E,8E)-2,6,6,9-tetramethylcycloocta-1,8-triene (humulene) | 6753-98-6 | 28.6 | 1454 | 0.54 | 0.76 | 0.45 | 0.54 | 0.93 | 0.52 |
| (1αR,4αS,7αS,8αS)-7-methyl-3-methylene-1-propan-2-yl-2,3,4a,5,6,8a-hexahydronaphthalene (α-murolone) | 25246-27-9 | 28.7 | 1448 | 0.13 | 0.16 | 0.11 | 0.13 | 0.20 | 0.14 |
| (15α,5α,8βR)-4,7-dimethyl-1-propan-2-yl-1,2,4a,5,6,8a-hexahydronaphthalene (y-murolone) | 30021-74-0 | 29.2 | 1473 | 0.08 | 0.15 | 0.08 | 0.08 | 0.21 | 0.12 |
| (15α,5α,8βR)-4,7-dimethyl-1-propan-2-yl-1,2,4a,5,6,8a-hexahydronaphthalene (α-murolone) | 31983-22-9 | 30.0 | 1497 | 0.08 | 0.10 | 0.05 | 0.05 | 0.14 | 0.09 |
| (4S)-1-methyl-4-[6-methylhept-1-en-2-yl]cyclohexene (β-sisabolene) | 495-61-4 | 30.3 | 1506 | 0.14 | 0.23 | 0.24 | 0.14 | 0.36 | 0.30 |
| (1R,4a,8aS)-7-methyl-4-methylene-1-propan-2-yl-2,3,4a,5,6,8a-hexahydronaphthalene (β-murolone) | 39029-41-9 | 30.5 | 1511 | 0.04 | 0.08 | 0.04 | 0.04 | 0.09 | 0.06 |
| (15α,8αR)-4,7-dimethyl-1-propan-2-yl-1,2,3,5,6,8a-hexahydronaphthalene (β-cadinene) | 483-76-1 | 30.7 | 1517 | 0.40 | 0.65 | 0.51 | 0.40 | 0.66 | 0.57 |
| 4-isopropyl-1,6-dimethyl-1,2,3,4a,7,8a-hexahydronaphthalene (β-cadinene) | 16728-99-7 | 31.1 | 1503 | 0.04 | 0.07 | 0.05 | 0.04 | 0.08 | 0.06 |
| (15α,7αR)-4,7-dimethyl-1-propan-2-yl-1,2-dihydrornaphthalene (α-calacorene) | 21391-99-1 | 31.4 | 1538 | 0.04 | 0.08 | 0.09 | 0.05 | 0.08 | 0.09 |

**Notes:**
- RT: Retention time (min)
- RI: Retention index
- CAS: Chemical abstracts service number
- Soxhlet: Soxhlet extraction
- UAE: Ultra-performance liquid chromatography

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- RI: Retention index
- RT: Retention time (min)
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3.4. Estimation of Volatiles over Ripening

The volatile fraction resulting from avocado oil extraction with petroleum ether and isolated by the HS-SPME technique gave mainly terpenoids and hydrocarbons (non-terpenoids). In contrast to previous studies, trace amounts of aldehydes, ketones, and other compounds were detected [25,33,34,36,37], which may be attributed to the sampling method or to the differences among avocado varieties.

The ripening stages (breaking, ripe, and overripe) indicated major differences in the semi-quantification of volatile compounds. The hydrocarbon (non-terpenoids) contents were found to be 14.41, 6.57, and 17.62 mg·kg\(^{-1}\) for breaking, ripe, and overripe samples over SE and 14.80, 2.89, and 14.14 mg·kg\(^{-1}\) over UAE, respectively. The corresponding terpenoids contents were found to be 18.44, 24.68, and 14.27 mg·kg\(^{-1}\) for breaking, ripe, and overripe samples over SE and 18.67, 28.74, and 15.50 mg·kg\(^{-1}\) over UAE. The above results show that, during maturation, the volatile fraction fluctuates. On the fourth day of maturation, the abundance of hydrocarbons was significantly lower (\(p < 0.05\)) compared with the first day, while it increased on the eighth day. In contrast, the abundance of terpenoids was significantly higher (\(p < 0.05\)) on the fourth day of maturation. This change in volatility was observed in both cases of the extraction techniques (Figure 3).

![Figure 3. The variation of volatility during three ripening stages.](image-url)

The abundance of hydrocarbons and terpenoids was examined by multivariate analysis of variance in comparison with the ripening stages. The Scheffe post hoc test was used to investigate which pairs of means were significant, and detailed results are presented in Table 2. It is evident that 21 of 44 total volatiles were statistically significant (\(p < 0.05\)).

### Table 1. Cont.

| Volatile Compounds                     | CAS Number | RT \(^{a}\) | RI \(^{b}\) | Soxhlet Breaking | Ripe | Overripe | UAE Breaking | Ripe | Overripe |
|----------------------------------------|------------|-------------|------------|------------------|------|----------|--------------|------|----------|
| Aldehydes                              |            |             |            |                  |      |          |              |      |          |
| nonanal                                | 124-19-6   | 17.7        | 1104       | 0.08            | 0.11 | 0.07     | 0.08          | 0.16 | 0.02     |
| Ketones                                |            |             |            |                  |      |          |              |      |          |
| octan-2-one                            | 111-13-7   | 10.6        | 907        | 0.56            | 0.28 | 0.48     | 0.27          | 0.12 | 0.19     |
| Others                                 |            |             |            |                  |      |          |              |      |          |
| trans-decahydronaphthalene            | 493-02-7   | 16.1        | 1057       | 0.34            | 0.10 | 0.25     | 0.27          | 0.02 | 0.27     |
| (15,45)-1,6-dimethyl-4-propan-2-yl-1,2,3,4-tetrahydrogenaphthalene (calamene) | 72897-55-4 | 30.8        | 1519       | 0.13            | 0.24 | 0.19     | 0.13          | 0.23 | 0.22     |

\(^{a}\) RT: Retention time (min); \(^{b}\) RI: Experimental retention index.
Three volatiles significantly differed between the ripening stages, including 1-methyl-4-(propan-2-ylidene)cyclohex-1-ene; 1-methyl-4-(prop-1-en-2-yl)benzene (p-cymene); and (1S,8aR)-4,7-dimethyl-1-propan-2-yl-1,2,3,5,6,8a-hexahydronaphthalene (δ-cadinene).

Table 2. The statistically significant volatile compounds between ripening stages.

| No. | Volatile Compounds                                                                 | Ripening Stages in Pairs          | p-Value |
|-----|-----------------------------------------------------------------------------------|-----------------------------------|---------|
| 1   | 2,6-dimethyloctane                                                                | Breaking Ripe Overripe Ripe       | 0.021   |
| 2   | 4-methylnonane                                                                     | Breaking Ripe Overripe Ripe       | 0.066   |
| 3   | 2-methylnonane                                                                     | Breaking Ripe Overripe Ripe       | 0.047   |
| 4   | 1-methyl-2-propylcyclohexane                                                      | Breaking Ripe Overripe Ripe       | 0.064   |
| 5   | decane                                                                            | Breaking Ripe Overripe Ripe       | 0.046   |
| 6   | butylcyclohexane                                                                  | Breaking Ripe Overripe Ripe       | 0.002   |
| 7   | 10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0]undecane                               | Breaking Ripe Overripe Ripe       | 0.037   |
| 8   | 2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (α-pinene)                                 | Breaking Ripe Overripe Ripe       | 0.408   |
| 9   | 7-methyl-3-methyleneocta-1,6-diene (β-myrcene)                                      | Breaking Ripe Overripe Ripe       | 0.486   |
| 10  | 1-methyl-4-(prop-1-en-2-yl)cyclohex-1-ene (D-limonene)                              | Breaking Ripe Overripe Ripe       | 0.133   |
| 11  | 1-isopropyl-4-methylcyclohexa-1,4-diene (γ-terpinene)                               | Breaking Ripe Overripe Ripe       | 0.000   |
| 12  | 1-methyl-4-(prop-2-ylidene)cyclohex-1-ene                                          | Breaking Ripe Overripe Ripe       | 0.001   |
| 13  | 1-methyl-4-(prop-1-en-2-yl)benzene (p-cymene)                                      | Breaking Ripe Overripe Ripe       | 0.033   |
| 14  | (1R,9S,Z)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene (β-caryophyllene) | Breaking Ripe Overripe Ripe       | 0.053   |
| 15  | (1E,4E,8E)-2,6,6,9-tetramethylycloundeca-1,4,8-triene (humalene)                   | Breaking Ripe Overripe Ripe       | 0.000   |
| 16  | (1R,4aS,8aS)-7-methyl-4-methylene-1-propan-2-yl-2,3,4a,5,6,8a-hexahydro-1H-naphthalene (γ-cadinene) | Breaking Ripe Overripe Ripe       | 0.036   |
| 17  | (1S,8aR)-4,7-dimethyl-1-propan-2-yl-1,2,3,5,6,8a-hexahydrone (δ-cadinene)           | Breaking Ripe Overripe Ripe       | 0.005   |
| 18  | 4-isopropyl-1,6-dimethyl-1,2,3,4,4a,7-hexahydrophanthalene (δ-cadinene)             | Breaking Ripe Overripe Ripe       | 0.021   |
| 19  | (1S)-4,7-dimethyl-1-propan-2-yl-1,2,3-dihydrophanthalene (α-calacorene)             | Breaking Ripe Overripe Ripe       | 0.008   |
| 20  | trans-decahydronaphthalene                                                         | Breaking Ripe Overripe Ripe       | 0.026   |
| 21  | (15,4S)-1,6-dimethyl-4-propan-2-yl-1,2,3,4-tetrahydronaphthalene (calamene)         | Breaking Ripe Overripe Ripe       | 0.009   |

a Based on observed means. b The mean difference is significant at the 0.05 level.
There is a lack of published data relating to the differences between the variations of volatile components of avocado oil and the ripening stages of avocado fruit. The majority of literature reports have focused on the aroma of avocado fruit; however, a limited number of studies have investigated the aroma of avocado oil [25,33–37]. Nonetheless, similar results can be drawn from the study of avocado fruit volatiles. Pereira [42] reported that the sesquiterpenes of avocado fruit decreased during ripening. Moreover, similar results have been reported in other climacteric fruits, in which series of changes in metabolic biosynthesis occur during storage ripening [43]. In particular, the monoterpens in mango fruits (Mangifera indica L. “Kensington Pride”) have increased on the fourth day of ripening and decreased afterwards [43].

Furthermore, in the same fruit, the monoterpenes, sesquiterpenes, and aromatics were determined at a higher total amount in ripened mango compared with the unripe and overripe stages [44]. In another study by Zidi on figs (Ficus carica L.) [45], β-caryophyllene and D-limonene increased significantly from the unripe to the ripe stage and were suppressed in the fully ripe stage.

Ethylene is well-known to control the storage duration and rate of ripening of climacteric fruits. A potential hypothesis is that the rise could be linearly correlated with ethylene synthesis. Several studies reported that climacteric fruits including apple (Malus domestica Borkh.) [46], tomato (Solanum lycopersicum L.) [47], and mango [48] undergo a rapid production of terpenes which depends on the response of ethylene. Thus, ethylene plays a key role in the metabolic events of volatiles during ripening [49]. Nevertheless, numerous underlying processes are yet to be delineated and merit additional study [50]. Another possible interpretation of the suppression of terpenoids over the later ripening stages (over-ripe) could be correlated with the presence of terpenoid hydroperoxides. The mesocarp of avocado fruit contains idioblastic cells that contain oil sacs and sesquiterpene hydroperoxides [7,51,52]. During maturation or enzymatic reaction, a degradation of a primary wall of the parenchyma cells occurs, which releases the oil from the idioblastic cells, and then the released hydroperoxides act on the terpenoids.

4. Conclusions

In summary, this work shows the analysis of avocado oil extracted from the Zutano variety by two (SE and UAE) techniques. In this context, petroleum ether volatile fractions were estimated over three ripening stages (breaking, ripe, and overripe) using HS-SPME–GC–MS. The Zutano variety, which is cultivated in the Crete region, gave a fragrant oil which has not been previously studied. This cultivar is characterized from seven main volatile compounds, including 2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (α-pinene), 1-methyl-4-(prop-1-en-2-yl)cyclohex-1-ene (D-limonene), 4,10-dimethyl-7-propan-2-yltricyclo[4.4.0.01,5]dec-3-ene (α-cubebene), (1S,6S,7S,8S)-1,3-dimethyl-8-propan-2-yltricyclo[4.4.0.02,7]dec-3-ene (α-copaene), (1R,9S,Z)-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene (β-caryophyllene), 2,6,6-dimethyl-6-(4-methylpent-3-enyl)bicyclo[3.1.1]hept-2-ene (α-bergamotene), and (1E,4E,8E)-2,6,6,9-tetramethylcycloundeca-1,4,8-triene (humulene). The analyzed fractions consisted of a high content of terpenoids with an average relative abundance of over 61.7%. ANOVA revealed that the extraction methods did not have statistically significant differences between their qualification and semi-quantification results. In contrast, the application of MANOVA between the ripening stages and some volatiles indicated that the p-values were lower than 0.05. Even though ripening is one significant factor that affects volatiles, additional research is required to approve the above results. This study could form the foundation for additional research on the impact of ethylene and the metabolism of avocado oil volatiles.

Author Contributions: Conceptualization, M.X. and C.S.P.; methodology, M.X., L.V., E.H.K., P.-K.R. and C.S.P.; software, M.X., L.V., E.H.K. and P.-K.R.; validation, M.X. and E.G.; formal analysis, E.G.; investigation, M.X., E.G., L.V. and E.H.K.; resources, E.G.; data curation, M.X. and E.G.; writing—original draft preparation, M.X.; writing—review and editing, L.V., E.H.K., P.-K.R., C.S.P. and P.A.T.; visualization, C.S.P. and P.A.T.; supervision, C.S.P.; project administration, C.S.P. and
P.A.T.; funding acquisition, E.G. and P.-K.R. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** The authors would like to thank Ioannis Lathourakis and Antonios Galanis for the sponsorship of *Persea americana* Mill. “Zutano” variety avocados from the Greek island of Crete, Chania.

**Conflicts of Interest:** The authors declare no conflict of interest.

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