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Characterization of aroma compounds of cold-pressed avocado oil using solid-phase microextraction techniques with gas chromatography–mass spectrometry

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Abstract

The main objective of this study was to investigate the aroma profile and some physicochemical properties of cold-pressed avocado oil. Headspace solid-phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) methods were used for the identification and quantification of aroma compounds of avocado oil. A total of 19 aroma compounds were detected in avocado oil. The aroma groups identified in avocado oil were aldehydes, terpenes, alkene, ketones, alkanes, and aromatic hydrocarbons. Alkanes were the dominant aroma group possessing 37.95% of total aroma concentrations in avocado oil. In avocado oil, tridecane and hexanal were the most abundant aroma compounds. Among the aroma compounds, (Z)-5-tridecene, biphenyl, pulegone, and β-curcumene were identified for the first time in avocado oil. Additionally, the free fatty acid and peroxide value of avocado oil were determined 0.82% and 10.19 meq O_2/kg oil, respectively.

Keywords: *Avocado oil, cold-press, aroma, HS-SPME, GC-MS antioxidant*

1.INTRODUCTION

Avocado (*Persea americana*) is a subtropical fruit that is a member of the Lauraceae family. This fruit is originated from Central America and cultivated in many parts of the world, especially in Mexico, the United States, and Chile (Woolf et al., 2009). The most commonly known cultivars of avocado are Hass and Fuerte (Qin and Zhong, 2016). Avocado fruit consists of three parts which are peel (15%), pulp (65%), and seed (20%) (Tan and Ghazali, 2019). The pulp of avocado is generally consumed as fresh when only ripened but it is also used as a sauce or drink (Guzmán-Gerónimo et al., 2008). This fruit has high oil content which is varied from 15% to 27% in the pulp, depending on the cultivar and the harvest time (Mahendran et al., 2019). Moreover, it contains many bioactive compounds such as polyphenols, carotenoids, and tocopherols (Qin and Zhong, 2016). Avocado has significant effects on health thanks to comprising these various compounds. Due to its high oil and rich nutritional content, positive effects on health, and short shelf life of mature avocado, oil has been obtained from avocado fruit. Unlike many vegetable oils, avocado oil is produced from the pulp, not the seed. Because the seed contains very little oil (2%) and some toxic substances such as hepatotoxic. Avocado oil has the highest proportion of monounsaturated fatty acids (58%) especially oleic acid, followed by saturated fatty acid (26%) and polyunsaturated fatty acid (16%) (Tan and Ghazali, 2019). The most common saturated and polyunsaturated fatty acids are palmitic acid and linoleic acid, respectively (Qin and Zhong, 2016). Avocado oil is usually used for cooking, as well as cosmetics and skincare products. (Haiyan et al., 2007).

Aroma is one of the most important characteristics considered while purchasing a product by a consumer. It is also notable as they contribute to the quality of the product. The combination of different aroma compounds such as terpenes, alcohols, aldehydes, ketones, and esters forms the aroma character of avocado oil. Terpene compounds are the main aroma groups in avocado fruit and also in avocado oil (Pereira et al., 2013). The aroma profile of avocado oil can vary depending on the quality of the raw material used, the cultivar, the oil extraction method, and the storage conditions after extraction (Woolf et al., 2009). Several methods such as cold-press, microwave, solvent, supercritical carbon dioxide extraction have been used for the extraction of aroma compounds (Moreno et al., 2003; Liu et al., 2021). In the current study, HS-SPME (Headspace solid-phase microextraction), which is a simple, quick, and inexpensive method, was used for the extraction.

There are studies on the aroma compounds of avocado fruit in the extant literature (Hausch et al., 2020; Mahendran et al., 2019; Galvao et al., 2016; Obenland et al., 2012; Guzmán-Gerónimo et al., 2008; López et al., 2004; Pino et al., 2000). However, there are a limited number of studies on the aroma profile of avocado oil. Moreno et al. (2003) investigated aroma compounds in avocado oil using different extraction methods (microwave-squeezing, microwave-hexane, hexane, and acetone). They found that a total of 36 aroma compounds and the least number of aroma compounds were identified with the microwave-squeezing method. Haiyan et al. (2007) analyzed the aroma compounds both in cold-pressed and refined avocado oil and they found that aroma profiles of these oils were qualitatively similar. Woolf et al. (2009) investigated the aroma compounds of avocado oils of different qualities (high, moderate, and poor). Therefore, the main objective of the present study was to investigate the aroma profile of cold-pressed avocado oil using the HS-SPME method and to determine the color properties, free fatty acids, and peroxide value of cold-pressed avocado oil.

2. MATERIALS AND METHODS

2.1. Materials and chemicals

The cold-pressed avocado oil was obtained from a local supplier in Antalya province in Turkey in the year of 2021. The oil sample in the amber glass bottle was stored in a cool and dark place until analysis.

In the present study, purified water was used from the Millipore-Q water purification system (Millipore Corp., Saint-Quentin, France). Aroma standard (4-nonanol) was supplied from Sigma Aldrich (Steinheim, Germany). Acetic acid, chloroform, sodium sulfate, potassium iodide, diethylether and potassium hydroxide were obtanied from Merck (Gernsheim, Germany). All chemicals and solvents were analytical and chromatography grade purity.

2.2. Methods

2.2.1. Physicochemical analyses

Color properties (L^*, a^*, and b^*) of the avocado oil were obtained using a color meter (HunterLab Color-Quest, Hunter Associates Laboratory, Inc., Reston, VA, USA). L^*, a^*, and b^* color parameters were represented lightness, redness-greenness, and yellowness-blueness, respectively (Guclu et al., 2021). Free fatty acids and peroxide value of the avocado oil were determined as described by American Oil Chemists' Society (1997). All the analyses were carried out in triplicate.

2.2.2. Extraction of aroma compounds

The extraction of aroma compounds was performed using SPME. The SPME fibre was used with 50/30 µm carboxen/divinylbenzene/polydimethylsiloxane (CAR/DVB/PDMS; Supelco, Bellefonte, Pennsylvania, USA) coated fused-silica fibre. The fibre was exposed to the headspace of a 20 ml capped vial, which contained 3 g of avocado oil, 5 µl of internal standard (4-nonanol in ethanol, 8.3 µg/100 ml). The fibre was conditioned in the GC injector port following instructions from the manufacturer. The avocado oil sample was incubated in place for 15 min at 60°C and during this time oil sample was agitated at 250 rpm. After this incubation, fibre was desorbed into the GC-MS injector at 250°C for 5 min (in splitless mode 0.8 min) (Kafkas et al., 2006). The analyses were carried out in duplicate.

2.2.3. GC-MS analysis of aroma compounds

Aroma compounds of avocado oil were analyzed by gas chromatography-mass spectrometry (GC-MS). GC-MS system consists of an Agilent 7890B GC equipped with a flame ionization detector (FID) and Agilent 7010B Network Mass Selective Detector (MSD). Aroma compounds were separated on a DB-Wax column (60 m x 0.25 mm, 0.25 µm thickness; J&W Scientific, Folsom, CA). A total of 2 µl of the extract was injected each time in pulsed splitless mode at 40 psi for 0.5 min. The FID and injector were set at 280 and 270°C, respectively. Helium (carrier gas) flow rate was 1.5 ml/min. The oven temperature program was first increased from 50 to 200°C at a rate of 5°C/min and then to 260°C at 8°C/min and 5 min hold time at 260°C. The MSD was operated in scan mode at 2.0 scan/s, with an electronic impact ionization energy of 70 eV, a mass range of m/z 30–300 a.m.u. The GC-MS interface and ionization source temperatures were set at 250 and 180°C, respectively. The aroma compounds were identified by comparing their mass spectra with those in Wiley 9, NIST 14 mass spectral data libraries, and an in-house library created with the use of alkane standards (C8-C32). Additionally, the identification and quantification of each aroma compound were done with the use of 4-nonanol as internal standards. Then, the means and standard deviations were computed for the data from the GC analysis in duplicate (Keskin et al., 2021).

3. RESULTS AND DISCUSSION

3.1. Physicochemical properties

The mean values of the physicochemical analysis of avocado oil are shown in Table 1. As seen in Table 1, the free fatty acid and peroxide value of avocado oil were determined 0.82% and 10.19 meq O_2/kg oil, respectively. The free fatty acid and peroxide values are generally related to the quality characteristics of oil and a good quality oil usually is of less free fatty acidity (Amanpour et al., 2019). In a previous study by Woolf et al. (2009), it was proposed that the free fatty acid value should be between 0.8 and 1% in virgin avocado oil for international quality standards. According to this recommendation, the free fatty acid value (0.82%) of avocado oil was between these limits in the present study. Regarding Codex Standard for Named Vegetable Oils, the peroxide value must be up to 15 meq O_2/kg oil for cold-pressed and virgin oils (CODEX, 2019). According to this, the peroxide value of avocado oil was below the limit of Codex in the current study. And also, considering the legal limits by EEC Regulations (1991), these values of avocado oil were below the peroxide number (20 meq O_2/kg) and fatty acidity (2%) limit specified for extra virgin olive oil. The results of the free fatty acid and peroxide value were also similar to previous studies. In a previous study by Moreno et al. (2003) free fatty acid and peroxide values of avocado oils were determined as 0.14-2.85% and 3.70-12.74 meq O_2/kg oil, respectively. In another study, free fatty acid was found to be 0.29-0.38% in avocado oil by different extraction methods (Tan et al., 2018). In an earlier study, free fatty acid of extra virgin avocado oil was detected range of 0.03–2.69% (Green and Wang, 2020).

Table 1. Physicochemical properties of avocado oil

Analysis	Avocado oil
Free fatty acid (oleic acid %)	0.82 ±0.04
Peroxide value (meq O_2/kg oil)	10.19 ±0.84
Color properties	
*L**	31.09 ±0.04
*a**	-0.35 ±0.01
*b**	42.61 ±0.02

Values are the mean of three replications with ± SD

Color is an important property influencing the visual acceptance of the oil by the consumer. *L*, a**, and *b** color values of the avocado oil were determined as 31.09, -0.35, and 42.61, respectively. In a previous study by Tan et al. (2017), they found that *L*, a**, and *b** values of avocado oil with different origins were between 28.44-31.97, 6.54-10.54, and 44.08-60.71, respectively. In another study, Tan et al. (2018) investigated color values of avocado oil extracted using various methods. According to the results, *L**, *a**, and *b** color values were determined between 1.29-43.99, -0.71-11.25, and 1.09-73.45, respectively.

3.2. Identification of aroma compounds in avocado oil

The aroma compounds and their amounts in avocado oil are presented in Table 2. As seen in Table 2, a total of 19 aroma compounds were determined in avocado oil including terpenes (5), aldehydes (4), alkanes (3), ketones (3), aromatic hydrocarbons (3), and alkene (1). Although alkanes had fewer compounds according to terpenes or aldehydes, they are the most abundant group in quantity. After the alkanes, aldehydes and alkene were the major aroma group in avocado oil in the current study. The total amount of aroma compounds was quantified as 16060.40 mg/kg in avocado oil.

Table 2. Aroma compounds of cold pressed avocado oil (mg/kg)

No	LRI	Compounds	Concentration (mg/kg)	Identification
		Terpenes		
1	1189	D-Limonene	620.94 ±13.09	LRI, MS, std
2	1458	α-cubebene	247.44 ±9.32	LRI, MS, std
3	1585	Caryophyllene	389.95 ±49.84	LRI, MS, std
4	1665	Pulegone	118.68 ±12.32	LRI, MS, std
5	1728	β-curcumene	350.93 ±46.45	LRI, MS, std
		Total	1727.94	
		Aldehydes		
6	1078	Hexanal	2053.81 ±97.48	LRI, MS, std
7	1181	Heptanal	206.03 ±5.33	LRI, MS, std
8	1287	Octanal	544.63 ±18.66	LRI, MS, std
9	1390	Nonanal	1119 ±76.73	LRI, MS, std
		Total	3923.47	
		Alkene		
10	1340	(Z)-5-Tridecene	1869.97 ±74.28	LRI, MS, std
		Total	1869.97	
		Ketones		
11	1175	2-heptanone	916.38 ±6.23	LRI, MS, std
12	1275	2-octanone	407.08 ±1.44	LRI, MS, std
13	1493	2-Decanone	121.99 ±31.37	LRI, MS, std
		Total	1445.45	
		Aromatic Hydrocarbons		
14	1410	Durene	364.63 ±21.63	LRI, MS, std
15	1416	Isodurene	606.61 ±53.71	LRI, MS, std
16	1996	Biphenyl	27.11 ±1.62	LRI, MS, std
		Total	998.35	
		Alkane		
17	1100	Undecane	955.81 ±42.67	LRI, MS, std
18	1200	Dodecane	1406.9 ±64.38	LRI, MS, std
19	1300	Tridecane	3732.5 ±511.03	LRI, MS, std
		Total	6095.21	
		General Total	**16060.40 ±457.09**	

LRI retention indices on DB-WAX column. Concentration mean values based on two repetitions as mg/kg. Identification methods of identification; LRI (linear retention index), Std (chemical standard); MS (mass spectra)

Alkanes were the major aroma group with a concentration of 6095.21 mg/kg and represented 37.95% of the overall aroma compounds. Tridecane has the highest concentration among alkane aroma compounds. Similar to our findings, undecane, dodecane, and tridecane were detected in avocado oils obtained by different extraction methods (Moreno et al., 2003). In a previous study, these three compounds were detected in some studied avocado cultivars (Pereira et al., 2013). In another study by Mahendran et al. (2019), evaluating the aroma profile of ripe avocado fruits packaged in a modified atmosphere, dodecane was detected in trace amounts.

In the aroma compounds of avocado oil, aldehydes were quantitated for the second-highest amount of the total aroma compounds (3923.47 mg/kg). Hexanal was the main aldehyde in avocado oil and generally associated with green and fatty notes given by this compound. Aldehyde compounds are generally formed as a result of the oxidation of fatty acids. In other words, hexanal is derived from linoleic acid, while octanal and nonanal are derived from oleic acid (Haiyan et al., 2007). In a review article, it was reported that avocado oil contains high chlorophyll content and can be photo-oxidized when exposed to light and/or oxygen (Qin and Zhong, 2016).

In previous studies, similar to our results, hexanal, heptanal, octanal, and nonanal have been reported in avocado oil (Moreno et al., 2003; Liu et al., 2021). These compounds have also been detected in avocado fruits with different cultivars in earlier studies (Guzmán-Gerónimo et al., 2008; Pereira et al., 2013; Galvao et al., 2016; Hausch et al., 2020). In a study examining the volatile profiles of both cold-pressed and refined avocado oil, hexanal and nonanal were detected in both oil samples (Haiyan et al., 2007). In another previous study, hexanal, octanal, and nonanal were determined as aroma-active compounds in avocado fruit providing fatty or oily odor (Hausch et al., 2020). Of all aroma compounds determined in avocado oil, alkene compound, (Z)-5-tridecene, was another significant aroma group after alkanes and aldehydes. In the present study, (Z)-5-tridecene was identified and quantified for the first time in avocado oil. It has been detected in the strawberry, *Capsicum* species in previous studies (Debnath et al., 2020; Yang et al., 2020).

In the current study, monoterpene (D-limonene and pulegone) and sesquiterpene (α-cubebene, β-caryophyllene, and β-curcumene) compounds were identified in avocado oil. The total concentration of terpenes was 1727.94 mg/kg. D-limonene was the most abundant compound followed by β-caryophyllene and pulegone. Similarly, Moreno et al. (2003), reported that α-cubebene and β-caryophyllene were detected in avocado oil. In a previous study by Pereira et al. (2013), limonene, α-cubebene, and β-caryophyllene have been determined in avocado fruits of different maturity and it was stated that the amount of sesquiterpene aroma compounds decrease during the ripening period of avocado fruits. Pulegone and β-curcumene were detected for the first time in avocado oil in the current study. Pulegone has been detected in the oils of pennyroyal, savory, and peppermint in previous studies (Coelho et al., 2012; Sellamuthu et al., 2013). In the present study, three aromatic hydrocarbons, durene, isodurene, and biphenyl, were identified in avocado oil. Among other aroma groups, aromatic hydrocarbons composed the least concentration (998.35 mg/kg) of the total amount of aroma compounds. In a prior study by Guzmán-Rodríguez et al. (2020), durene was identified in avocado leaves. In the present study, biphenyl compound was identified for the first time in avocado oil. This compound has been also determined in quinoa porridge and foxtail millet in earlier studies (Zhang et al., 2019; Li et al., 2021) but not in avocado fruit or oil.

4. CONCLUSIONS

In this work, aroma compounds, free fatty acid, peroxide value, and color properties of cold-pressed avocado oils were examined. As a result of the aroma analysis, a total of 19 aroma compounds were identified and quantified in avocado oil. Four of these aroma compounds, (Z)-5-tridecene biphenyl, pulegone, and β-curcumene were detected for the first time in avocado oil in the current study. Alkanes were the dominant aroma group followed by aldehydes and alkene. Tridecane and hexanal were the most abundant aroma compounds in avocado oil with a concentration of 3732.5 mg/kg and 1869.97 mg/kg, respectively. In addition, the free fatty acid and peroxide value of avocado oil were determined 0.82% and 10.19 meq O_2/kg oil, respectively. It has been concluded that the different physicochemical properties and aroma profile of avocado oil may be due to the variety of raw materials used, growing and harvesting conditions, and different extraction methods.

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