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Identification of volatile compounds in cultivars barker, collinson, fortuna and geada of avocado (*Persea americana*, Mill.) fruit

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Abstract

The objective of this paper was to determine the volatile profile of four cultivars - Barker, Collinson, Fortuna and Geada of avocado (*Persea americana,* Mill.) fruit and to perform a detailed study on the effect of volatiles extraction conditions. The best conditions for extraction for Collinson and Fortuna cultivars were by using a mixture of pentane and ethyl ether (2:1) as solvent for 80 min, while for Barker and Geada cultivars, the solvents were hexane and pentane-ether (2:1), respectively but for a shorter extraction period of 60 min. A total number of 158 compounds were separated in all 4 avocado cultivars, among them eighty-four volatile compounds were identified. The principal volatile compounds viz. hexanal, ethyl acetate, methyl dodecanoate, 2,5-dimethyl furan, 1,3-butanediol, 2-ethylphenol, 2-butanol, α–bergamotene, β–caryophyllene, (*E*)-2-decenal were common in all the 4 cultivars. (*E*)-2-pentenal which possesses fruity aroma was found only in the cultivar Fortuna while ethyl acetate possessing fresh fruity flavor was present in higher content in Collinson cultivar. Benzaldehyde which possesses characteristic fruity and nutty odor note was present at a higher concentration (4.3%) in only Geada cultivar and in traces in Barker cultivar but it was not detected in Collinson and Fortuna cultivars.

Keywords: avocado; aroma; quality; pulp; GC-MS.

Practical Application: The innovative nature of this work is that we have monitored the volatile profile of 4 varieties - Barker, Collinson, Fortuna and Geada largely cultivated in the northeast region of Brazil. There is no published work as yet on volatile composition of these varieties. The work also optimizes the conditions of extraction from these cultivars so that higher numbers of volatiles are captured. The differences in volatile compounds among the four varieties could contribute for their suitable usage as fresh or processed fruit.

1 Introduction

Flavor is one of the most important attributes of foods in determining consumer acceptance and there has been an increasing interest to study its impact on quality of a food product. In the case of tropical fruits, aroma constituting of volatile components is one of the most appreciated characteristics, which determines its quality and it is particularly sensitive to compositional alterations (Ibáñez et al., 1998; Barreto et al., 2013). In addition, tropical fruits are often inexpensive, extremely rich in vitamins and can be used in a wide range of food products (Pino et al., 2001). Among the tropical and subtropical fruits, the avocado (*Persea americana,* Mill.) is very much appreciated, and it occupies a prominent place in the market due to its nutritional value, especially fibers and lipids. Moreover the fruit has soft flavor and low sugar content (about 10 g/kg of pulp), and thus is even recommended for diabetic suffering people since it serves as high-energy food (Sinyinda & Gramshaw, 1998; Maitera et al., 2014), maintain skin elasticity (Athar & Nasir, 2005) and reduce the coronary heart risk (Berasategi et al., 2012).

Botanically, avocado fruit is classified in three varieties: West Indian, Mexican and Guatemalan races (Morton, 1987). The Collinson and Fortuna cultivars are Guatemalan x West Indian hybrids, while Barker and Geada are of West Indian race (Brasil, 1971; Medina, 1980). In Brazil, the fruit is classified in two groups (Brasil, 1971): the first group, in which Barker, Collinson and Geada cultivars are included, are suitable for long-distance transport and hence are commercialized as whole fruit, while the second group, in which Fortuna cultivar belongs, is delicate due to its consistency having soft pulp texture and it is more suited for oil extraction.

The physico-chemical composition of avocado fruit pulp and oil has been reported earlier (Turatti & Canto, 1985; Martinez et al., 1988; Lozano et al., 1993; Bora et al., 2001, Moreno et al., 2003; Berasategi et al., 2012, Mooz et al., 2012; Galvão et al., 2014; Indriyani et al., 2016). However, the information on the composition of volatile flavor constituents of avocado is limited. A total of 90 volatile compounds were reported to be present in an Australian cultivar Sherwil and the major compounds identified were pentanal, hexanal. (*E*)-2-hexenal, β-caryophyllene and α-copaene (Whitfield et al., 1980). Avocado fruits harvested in Mexico were analyzed by Yamaguchi et al. (1983) who

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identified 52 volatile compounds, the main compounds being C_6 alcohols and aldehydes (hexanol, (*E*)-2-hexenol, (*E*)-3-hexenol, (*Z*)-3-hexenol, (*E*)-2-hexenal. Pino et al. (2000) analyzed volatile constituents of two cultivars (California and Haas) of avocado and reported the presence of 35 compounds, most of these being terpene types such as (*E*)-nerodilol, β-caryophyllene, β-pinene, trans-α-bergamotene and β-bisabolene. Working with another cultivar Moro grown in Cuba, Pino et al. (2004) reported the principal volatile compounds being (*Z*)-nerodilol, (*E,E*)-2,4-decadienal, (*E,E*)-α-farnesene, β-caryophyllene, caryophyllene oxide and α-copaene. Obenland et al. (2012) studied the influence of maturity and ripening on aroma volatiles of Haas cultivar of avocado and reported an 85% decline in the amount of hexanal from firm to fully ripe fruit. Forty three compounds were found to be present in cultivar Fuerte of avocado fruit cultivated in Egypt (El-Mageed, 2007). Volatile profile of avocado pulp of 3 cultivars Simmonds, Booth 7 and Monroe were monitored during the ripening of fruit as affected by 1-methylcyclopropene (Pereira et al., 2013). However, to the best of our knowledge, no data are yet published on some largely grown avocado cultivars in Brazil.

The main cultivars of avocado grown In Brazil, are Fortuna, Hass, Fuerte, Geada and Yard. The Experimental Station of Itambé (IPA - region in the Pernambuco, Brazil) recommends the cultivation of Collinson, Barker, and Fortuna cultivars especially in the northeast region of Brazil where these are largely grown. Thus the objective of this work was to initially obtain volatile extracts, following a modified simultaneous distillation and extraction technique, from avocado pulp of different cultivars viz. Barker, Collinson, Fortuna and Geada by varying the extraction conditions such as use of different solvents and extraction time in order to evaluate the number of compounds present in these volatile extracts followed by identification and quantification of the volatile compounds present in the pulps of different cultivars.

2 Materials and methods

2.1 Materials

Avocado fruits of four cultivars, Barker, Collinson, Fortuna and Geada were obtained from a farm located in Goiana city in the Pernambuco state of Brazil. The fruits were transported to the laboratory in the city of João Pessoa in the standard cardboard boxes currently used for export packaging and did not have any application whatsoever of inhibitor or accelerator for the control of maturation. Fruits free from any apparent skin damage were selected for analysis. The ripe mature fruit, after being washed with distilled water, was cooled to 2 °C. The skin and kernel were separated manually by using a stainless steel knife and the pulp macerated. The solvents and authentic standard flavor compounds were of more than 99.5% purity and belonged to the firms, Merck and Sigma-Aldrich, respectively.

2.2 Volatiles isolation

The volatile compounds from the pulp of avocado fruit were extracted by simultaneous distillation and extraction technique using a modified procedure Likens and Nickerson's apparatus (Likens & Nickerson, 1964). The extraction conditions were initially optimized by varying the parameters such as: solvent (hexane or a mixture of pentane and ethyl ether in proportion 2:1) and extraction period (40, 60, 70 or 80min), with the objective to obtain a large number of compounds on chromatographic analysis. The weight of pulp (150 g), the volume (200 mL) of water added and the volume (20 mL) of the solvent were fixed. The extracts were concentrated to a final volume of 0,3 mL under flow of nitrogen gas (Narain & Galvão, 2004).

2.3 High resolution gas chromatography/mass spectrometry

A combined system of Varian gas chromatograph (GC 3800) coupled with mass spectrometer (Saturn 2000R) and its processing workstation was used. Two microliters of the concentrated volatile extract was injected in the column in split (1:20) mode. Capillary GC investigations were carried out on a 30 m (length) x 0.25 mm (internal diameter) polyethylene glycol innophase bondable polar capillary column (HP-INNOWax; 0.25 µm film thickness; Hewlett Packard, Inc., Palo Alto, USA). The carrier gas was helium (99.995% pure) and column head pressure was maintained at 11.5 psi having a flow rate of 1 mL/min. The oven temperature was programmed: initiation at 30°C for 5 min, increased at 7 °C/min to 100 °C, maintained at 100 °C for 5 min, increased at 1 °C/min to 130°C, followed by a later increase of 10 °C/min to 195 °C wherein it was maintained for 45 min. The temperatures of the injection port and the GC/MS interface were 190 °C and 210 °C, respectively. The mass spectrometer was operated in the electron ionization mode with an electrical energy of 70 eV and an ion source temperature of 250 °C. The mass spectrum was scanned between 33 and 450 atomic mass units at 0.3 sec interval.

2.4 Compounds identification

The linear retention index (LRI) values for unknowns were determined based on retention time and index data obtained by analyzing a series of normal alkanes (C_6-C_{25}). Volatile components were positively identified by matching their RI values and mass spectra with those of standards, also run under identical chromatographic conditions in the laboratory. The identification was also based on matching an unknown mass spectrum with the spectra available on the NIST (National Institute of Standards and Technology, USA) and mass spectral data system or the literature (Jennings & Shibamoto, 1980; Adams, 1995; Kondjoyan & Berdagué, 1996).

2.5 Statistical analysis

All determinations were obtained from triplicate measurements and results are expressed as means ± standard deviations. All results were processed using the SAS software (SAS Institute, Cary, NC) Version 9.1.3. Significant differences between the mean values of different characteristics were determined by applying Tukey's test for multiple comparisons at the probability of 5% ($p \leq 0.05$).

3 Results and discussion

Based on the analysis of total ion chromatograms of the volatile extracts obtained from pulps of different cultivars (Collinson, Barker, Fortuna and Geada) of avocado extracted with different solvents, Table 1 summarizes the data on number of peaks in different chromatograms and its quantitative representation classified according to the peak area.

The data presented in Table 1 reveal the capture of a larger number (84) of volatile compounds when the extraction was performed using the pulp of cultivar Collinson, with the solvent mixture pentane-ether (2:1) for 80 min. However, for Barker and Geada cultivars a better extraction was obtained after 60 minutes, using hexane and pentane-ether (2:1) as a solvent, respectively. The best condition for extraction from Fortuna cultivar was the one realized for 80min, regardless of the solvent, when it was possible to separate 50 volatile compounds. It was also observed that increasing the extraction time from 40 to 80 min did not result in any major change in number of compounds in the extract obtained from Collinson and Geada cultivars. The extraction time which showed better efficiency in capturing the volatiles from Barker and Geada cultivars was 60min which is lower than that for Fortune and Collinson varieties (80 min). Chaintreau (2001) reported that the higher the lipid content of food matrix greater is the time required for volatiles extraction as lipid-containing matrix strongly increases the required time. Thus, the ideal conditions of extraction for a large number of volatile compounds depend on the cultivar of avocado and its lipid content (Galvão et al., 2014).

Table 2 lists the volatiles compounds identified in pulp of different cultivars - Collinson, Barker, Fortuna and Geada of avocado along with their characteristic odor notes.

A total number of 158 compounds were separated in all 4 avocado cultivars (Collinson, Barker, Fortuna and Geada), out of which 65 compounds were positively identified, 25 tentatively identified and 68 compounds could not be identified mainly due to the lack of standard organic compounds.

Table 1. Distribution of number and peak area of volatile components in chromatograms resulting from different extracts obtained by varying extraction conditions of pulp from different cultivars of avocado fruit.

Number	Cultivar	Extraction conditions		Total number of peaks	According to the peak area		
		Solvent	Time (min.)		> 10%	1 to 10%	< 1%
1	Collinson	*n*-hexane	40	13	3	9	1
2			60	17	3	9	5
3			70	14	4	6	4
4			80	19	3	2	14
5		Pentane-ethyl ether	40	17	2	15	-
6			60	12	2	10	-
7			70	61	3	4	54
8			80	84	2	9	73
9	Barker	*n*-hexane	40	41	2	15	24
10			60	82	2	13	67
11			70	58	1	16	41
12			80	43	3	14	26
13		Pentane-ethyl ether	40	18	2	12	4
14			60	9	2	5	2
15			70	55	3	5	47
16			80	17	4	4	9
17	Fortuna	*n*-hexane	40	19	2	5	12
18			60	24	2	17	5
19			70	30	2	9	19
20			80	50	2	25	23
21		Pentane-ethyl ether	40	25	2	9	14
22			60	37	2	8	27
23			70	32	3	10	19
24			80	50	4	12	34
25	Geada	*n*-hexane	40	30	2	10	18
26			60	52	2	21	29
27			70	44	3	15	26
28			80	53	1	12	40
29		Pentane-ethyl ether	40	36	1	21	14
30			60	82	1	26	55
31			70	66	2	20	44
32			80	60	2	5	54

Table 2. Volatile compounds present in pulp of different cultivars (Collinson, Barker, Fortuna and Geada) of avocado along with their characteristic odor notes.

Class	Compound	RI	Area (%)				Odor attribute
			Collison	Barker	Fortuna	Geada	
Alcohols	2-propanol	954	+	+	ND	ND	Bitter taste
	octen-3-ol[4,7,10]	960	+	+	ND	ND	Very strong, sweet, mushroom
	2-methyl -2-butanol	1014	+	+	ND	ND	Ethereal fusel notes, fermented & yeasty
	2-butanol	1038	1.68 ± 0.09[a]	1.44 ± 0.25[a]	+	+	Oily, wine-like, fusel-alcoholic note
	2-methyl-1-propan-1-ol[2*]	1099	+	ND	ND	ND	Sweaty ethereal,
	n-butanol[2]	1116	+	ND	ND	1.26 ± 0.11	Wine, apple, banana
	3-methyl-1-penten-3-ol	1127	+	ND	ND	+	Winey, cognac, whisky, fruity, green notes
	isoamyl alcohol[2*]	1195	+	+	ND	ND	Breathtaking, alcoholic odor;
	2-methyl-1-butanol[13]	1203	+	ND	+	+	Ethereal, fusel oil, fermented winey notes
	n-amyl alcohol[2]	1218	+	+	+	1.07 ± 0.22	Alcoholic-breathtaking, fusel-like odor
	2-hexanol	1228	+	+	+	+	Weak, fruity, fatty
	3-methyl-3-buten-1-ol[13]	1249	+	+	ND	ND	Fruity, green
	2-penten-1-ol[11]	1305	+	+	ND	ND	Fresh, ethereal, fruity-green, citrus,
	hexanol[2,9,11,13]	1322	+	+	+	+	
	4-methyl-1-pentanol	1345	+	ND	ND	ND	Oily green-fruity, herbaceous,
	(*E*)-3-hexen-1-ol[2,3,9]	1359	+	+	ND	ND	Green, grass, leafy odor; bitter, grassy-fatty notes
	2-octanol	1413	ND	+	ND	+	Weak green, floral-leafy, musty odor and taste Strong, penetrating, fatty-green, grassy unripe fruit odor
	2,3-butanediol	1547	+	ND	4.24 ± 0.88	+	Weak ethereal, herbal;
	1-octanol[2,3,7,10*]	1560	ND	ND	1.04 ± 0.31[b]	2.51 ± 0.55[a]	Grassy, plastic, solvent
	1,2-propane-diol*	1604	+	ND	ND	ND	Sweet taste
	α–terpineol[2]	1657	+	+	+	1.00 ± 0.19	Sweet, floral (lilac), lime odor;
	1,3-butanediol	1693	+	2.37 ± 0.57[b]	5.19 ± 1.02[a]	1.10 ± 0.17[c]	Solvent for flavors
	nonanol	1706	+	ND	ND	1.01 ± 0.89	Fruity-green,waxy, soapy,
	benzyl alcohol[2,11]	1821	+	ND	ND	ND	Faint, sweet, fruity aroma; sweet taste
	2-phenyl ethanol[2,11]	1890	+	+	ND	+	Rose, perfumery
	Tridecanol	1957	ND	+	ND	+	Very faint, waxy-woody and sweet-fruity odor; waxy taste
	2-ethyl phenol[2*]	2088	+	1.12 ± 0.33[a]	1.44 ± 0.08[a]	1.42 ± 0.71[a]	Sweet tarry phenolic

Different superscript letters in the same row denote significant differences ($p < 0.05$); Standard deviation (SD) <0.01 is reported as 0.01 (n=3); + Compound detected but its area was less than 0.5%; ND: Not detected; * Tentative identification; [1]Reported in pulp of Australian hybrid cultivar "Sherwil" (Whitfield et al., 1980); [2] Reported in pulp of Mexican cultivar (Yamaguchi et al., 1983); [3] Reported in avocado leaves of Guatemalan and West Indian races (King & Knight, 1992); [4] Reported in pulp of fruit obtained from Israel (Sinyinda & Gramshaw, 1998); [5] Reported in pulp of California and Hass cultivars (Pino et al., 2000); [6] Reported in pulp of Mexican cultivar (Moreno et al., 2003); [7] Reported in pulp of Mexican cultivar (Lopez et al., 2004); [8] Reported in pulp of Moro cultivar from Cuba (Pino et al., 2004); [9] Reported in pulp of Fuerte cultivar from Egypt (El Mageed, 2007); [10] Reported in pulp of Hass cultivar from Mexico (Guzmán-Gerónimo et al., 2008); [11] Reported in oil of pulp of Australian cultivar (Haiyan et al., 2007); [12] Reported in pulp of Hass cultivar from Mexico (Obenland et al., 2012); [13] Reported in pulp of West Indian cultivar from Florida, USA (Pereira et al., 2013).

Table 2. Continued...

Class	Compound	RI	Area (%)				Odor attribute
			Collison	Barker	Fortuna	Geada	
Aldehydes	butanal[9]	887	ND	3.58 ± 0.20	ND	ND	Cocoa, fruity, malt-like green odor
	2-methyl butanal[11]	905	+	+	1.84 ± 0.66	+	Musty, cocoa, coffee, nutty
	pentanal[1,7,10,11,12]	947	+	1.72 ±	+	+	Strong, acrid, pungent odor; chocolate
	2-methyl 2-butanal[2,13*]	970		+			Strong, green, fruit
	hexanal[1,2,3,4,6,7,9,10,11,12,13]	1080	30.28 ± 4.89[a]	29.35 ± 1.88[a]	25.96 ± 4.55[a]	3.53 ± 0.51[b]	Oily, avocado
	(*E*)-2-pentenal	1131	+	ND	1.63 ± 0.14	ND	Fruity, apple-like
	(*E*)-2-hexenal[1,2,7,9,10,11,12]	1219	+	ND	ND	+	Herbal, sweet
	octanal[1,4,6,7,11]	1278	+	+	ND	+	Fatty-fruity odor; sweet, citrus-orange-fatty taste
	nonanal[1,4,6,11,12]	1388	+	+	+	+	Fatty-floral-rose, waxy odor
	benzaldehyde[1,2,10,11,12]	1445	ND	+	ND	4.27 ± 0.28	Almond, fruity, powdery, nutty
	(*E,E*)-2,4-heptadienal[11,11,12*]	1482	ND	ND	6.39 ± 1.07	+	Pungent, cinnamon, spicy odor; fatty green
	(*E*)-2-nonenal[4,7,10*]	1525	+	+	ND	+	Oily, solvent
	(*E*)-2-decenal[7,10]	1622	2.02 ± 0.59[a]	+	1.06 ± 0.67[a]	+	Sweet, oily
Alkanes & Alkenes	2-methyl heptane	941	4.80 ± 0.86[a]	ND	ND	5.43 ± 1.18[a]	
	(*E*)-2-octene	833	+	6.98 ± 1.07[a]	1.16 ± 0.09[b]	ND	Gassy
Aromatics	toluene[9,11]	1031	+	+	ND	ND	Sweet, pungent odor
	p-xylene[2*]	1138	+	+	3.76 ± 0.23	ND	
	o-xylene[2,4*]	1189	+	+	+	ND	
	propyl benzene	1198	+	+	+	+	
	1,3,5-trimethylbenzene[11]	1241	+	ND	ND	ND	Sweet
	p-cymene	1266	+	ND	ND	ND	Strong, characteristic, terpene odor
	1,3-diethyl-benzene	1299	+	+	+	ND	
	naphthalene[2*]	1717	ND	+	+	1.22 ± 0.03	Musty taste
Esters	isopropyl formate	843	+	3.13 ± 0.91	+	ND	Sweet
	ethyl acetate[5,9]	887	19.80 ± 1.31[a]	7.41 ± 1.25[b]	2.04 ± 0.17[c]	+	Ethereal, sharp, wine-brandy like odor
	isopropyl acetate	913	ND	1.30 ± 0.53	+	+	Sharp, fruity, ethereal odor; sweet apple-like taste
	methyl butanoate[9]	989	+	+	ND	1.43 ± 0.60	Sweet, ethereal fruity odor; apple-like taste
	isoamyl formate[2*]	1054	ND	+	ND	ND	Sharp, green estery, apple and waxy
	octyl acetate	1475	+	ND	ND	1.05 ± 0.84	Green, waxy, fruity-floral citrus odor; citrus taste
	benzyl acetate[2]	1696	+	+	ND	ND	Sweet, floral, fruity odor of jasmin and gardenia, fruity taste
	methyl dodecanoate	1802	1.25 ± 0.45[b]	5.79 ± 0.61[a]	6.33 ± 0.62[a]	5.07 ± 1.07[a]	Waxy, creamy fatty taste
	linalyl hexanoate*	1837	ND	+	ND	ND	Green, dry-fruity odor; pineapple-pear taste
	propyl dodecanoate	1897	ND	+	ND	1.15 ± 0.33	Waxy, soapy and floral
	methyl-(*E*)-cinnamate[2*]	2050	ND	+	+	+	Fruity-balsamic odor; sweet fruity

Different superscript letters in the same row denote significant differences ($p < 0.05$); Standard deviation (SD) <0.01 is reported as 0.01 (n=3); + Compound detected but its area was less than 0.5%; ND: Not detected; * Tentative identification; [1]Reported in pulp of Australian hybrid cultivar "Sherwil" (Whitfield et al., 1980); [2] Reported in pulp of Mexican cultivar (Yamaguchi et al., 1983); [3] Reported in avocado leaves of Guatemalan and West Indian races (King & Knight, 1992); [4] Reported in pulp of fruit obtained from Israel (Sinyinda & Gramshaw, 1998); [5] Reported in pulp of California and Hass cultivars (Pino et al., 2000); [6] Reported in pulp of Mexican cultivar (Moreno et al., 2003); [7] Reported in pulp of Mexican cultivar (Lopez et al., 2004); [8] Reported in pulp of Moro cultivar from Cuba (Pino et al., 2004); [9] Reported in pulp of Fuerte cultivar from Egypt (El Mageed, 2007); [10] Reported in pulp of Hass cultivar from Mexico (Guzmán-Gerónimo et al., 2008); [11] Reported in oil of pulp of Australian cultivar (Haiyan et al., 2007); [12] Reported in pulp of Hass cultivar from Mexico (Obenland et al., 2012); [13] Reported in pulp of West Indian cultivar from Florida, USA (Pereira et al., 2013).

Table 2. Continued...

Class	Compound	RI	Area (%)				Odor attribute
			Collison	Barker	Fortuna	Geada	
Furans & Derivatives	tetrahydrofuran	851	ND	10.37 ± 1.07	+	ND	Ether-like
	3-methyl furan	856	+	+	1.07 ± 0.01	ND	
	2-methyl furan	862	ND	0.89 ± 0.15	1.41 ± 0.06	ND	Sweet-gassy, ether-like, solvent
	2,5-dimethyl furan	944	8.66 ± 0.30[a]	2.97 ± 0.52[b]	2.33 ± 0.91[b]	2.33 ± 0.06[b]	Sweet-gassy, solvent, metallic-burnt, spicy
	2-ethyl furan	950	ND	+	ND	+	Strong, sweet-ethereal, burnt brown musty odor
	furfural[2,4,7,10,11]	1456	+	+	ND	ND	Sweet, coffee
Ketones	2-hexanone[2]	1094	+	ND	2.03 ± 0.28	ND	Fruity, ketonic, banana, yeasty, cheese, dairy notes
	cyclohexanone[2*]	1288	ND	+	ND	ND	Minty, acetone
	2-decanone	1490	2.05 ± 0.15	+	+	ND	Orange-like, floral odor
Others	γ-nonalactone[2]	2043	ND	+	1.15 ± 0.08	+	Strong, fatty, coconut
	pyridine[2,4*]	1162	+	+	1.01 ±0.01	+	Salty taste; Strong, amine-like, fishy, burnt odor
	acetoin[2*]	1257	+	ND	ND	ND	Sweet, buttery, creamy, milky, fatty
	acetophenone*	1643	ND	+	ND	1.43 ± 0.09	Sweet, pungent, harsh
	p-methyl acetophenone	1763	+	3.09 ± 0.81[a]	1.38 ± 0.37[b]	1.52 ± 0.21[b]	Harsh, but sweet, floral-hay odor; sweet, cherry-berry taste
	2,5-dimethyl pyrazine[11]	1321	ND	+	ND	+	Cocoa roasted, nuts, roast beef, woody, grass, medical
	ethyl pyrazine[11]	1339	+	+	ND	+	Musty, nutty, buttery, peanut odor
	3-methylpyrrolpyrazine*	1989	ND	+	ND	+	
Terpenes	myrcene[3,4,5,7,10,12,13*]	1149	+	+	ND	1.17 ± 0.31	Herbal, sweet
	β-pinene[3,5,7,10,11]	1121	+	+	1.48 ± 0.01	+	Sweet, lemon
	limonene[3,4,5,7,9,10,12,13]	1183	+	+	1.04 ± 0.22	+	herbal, sweet
	3-carene[11]	1233	+	+	ND	ND	Fresh, harsh, turpentine Strong, fatty odor
	α-cubebene[1,3,4,5,9,13*]	1430	+	+	ND	ND	Mild woody balsamic
	α-copaene[1,4,5,6,7,8,9,10,13*]	1465	ND	+	ND	+	Woody, spicy, sweet
	linalool[2,5,10*]	1533	+	+	ND	ND	Sweet floral
	β-caryophyllene[1,3,4,5,6,7,8,9,10,13]	1585	+	3.03 ± 0.11[c]	5.05 ± 0.39[b]	14.51 ± 2.55[a]	Woody, spicy, dry odor;
	α-humulene[5,13*]	1675	ND	+	ND	+	Mild woody with earthy weak spice notes
	α-bergamotene[5,9,13*]	1755	3.15 ± 0.01[b]	+	2.56 ± 0.19[c]	4.63 ± 0.02[a]	Woody, warm, tea
	γ–selinene*	1804	+	ND	ND	+	Herbal
	(*E*)-nerolidol[5]	2017	ND	ND	3.04 ± 0.22	+	Faint, woody-floral, slight green Orange-Rose, waxy, sweet odor

Different superscript letters in the same row denote significant differences ($p < 0.05$); Standard deviation (SD) <0.01 is reported as 0.01 (n=3); + Compound detected but its area was less than 0.5%; ND: Not detected; * Tentative identification; [1]Reported in pulp of Australian hybrid cultivar "Sherwil" (Whitfield et al., 1980); [2] Reported in pulp of Mexican cultivar (Yamaguchi et al., 1983); [3] Reported in avocado leaves of Guatemalan and West Indian races (King & Knight, 1992); [4] Reported in pulp of fruit obtained from Israel (Sinyinda & Gramshaw, 1998); [5] Reported in pulp of California and Hass cultivars (Pino et al., 2000); [6] Reported in pulp of Mexican cultivar (Moreno et al., 2003); [7] Reported in pulp of Mexican cultivar (Lopez et al., 2004); [8] Reported in pulp of Moro cultivar from Cuba (Pino et al., 2004); [9] Reported in pulp of Fuerte cultivar from Egypt (El Mageed, 2007); [10] Reported in pulp of Hass cultivar from Mexico (Guzmán-Gerónimo et al., 2008); [11] Reported in oil of pulp of Australian cultivar (Haiyan et al., 2007); [12] Reported in pulp of Hass cultivar from Mexico (Obenland et al., 2012); [13] Reported in pulp of West Indian cultivar from Florida, USA (Pereira et al., 2013).

Among the identified components in all avocado cultivars were 27 alcohols, 13 aldehydes, 11 terpenes, 11 esters, 9 aromatics, 6 furans and 3 ketones. Ninety volatile compounds were identified among all the four cultivars, while 22 compounds were common in all cultivars. However, thirty-four compounds were identified for the first time in this work on avocado pulp. Among them some important compounds from fruity/floral/sweet aroma standpoint are: 3-methyl-1-penten-3-ol (green, fruity), 2-octanol (floral), 1,2-propane-diol (sweet), nonanol (fruity), tridecanol (sweet-fruity), (*E*)-2-pentenal (fruity), isopropyl formate (sweet), isopropyl acetate (fruity), octyl acetate (fruity-floral, waxy), linalyl hexanoate (fruity), propyl dodecanoate (floral), 2-decanone (floral), acetophenone (sweet), *p*-methyl acetophenone while those with fatty/oily/waxy notes are: 2-propanol (bitter), 2-butanol (oily), 2-hexanol (fatty, fruity), 4-methyl-1-pentanol (oily), methyl dodecanoate (waxy, fatty).

An earlier work (Whitfield et al., 1980) reported a maximum of 90 volatiles compounds identified in avocado pulp. However, in the present study, the higher number of compounds recorded could be related to two factors such as selection of specific avocado varieties and the method of extraction employed when compared to other studies. Furthermore, the experimental conditions were also optimized which contribute to the achievement of better results. Although there was such difference seen in the number of peaks, the majority classes of compounds in all published work were seen to be those of terpenes and aldehydes. All terpenic compounds identified in this work were previously reported. However, among the 13 compounds belonging to aldehydes class found in this study, only one compound reported first time in this work was (*E*)-2-pentenal, which possesses fruity aroma and was found only in the cultivar Fortuna.

Among the principal volatile compounds, the compounds such as hexanal, ethyl acetate, methyl dodecanoate, 2,5-dimethyl furan, 1,3-butanediol, 2-ethylphenol, 2-butanol, α-bergamotene, β-caryophyllene, (*E*)-2-decenal were common in all the 4 cultivars.

The major compounds identified in avocado pulp of cultivar Fortuna according to their distribution were hexanal (26%), (*E,E*)-2,4-heptadienal (6.4%), methyl dodecanoate (6.3%), 1,3-butanediol (5.2%), β−caryophyllene (5.1%), 2,3-butanediol (4.2%), *p*-xylene (3.8%), (*E*)-nerolidol (3.0%) and α−bergamotene (2.6%) while for the Geada cultivar the compounds were β−caryophyllene (14.5%), 2-methyl heptane (5.4%), methyl dodecanoate (5.0%), α−bergamotene (4.6%), benzaldehyde (4.3%), hexanal (3.5%) and octanol (2.5%). Collinson cultivar constituted of main compounds viz. hexanal (30.3%), ethyl acetate (19.8%), 2,5-dimethylfuran (8.7%), 2-methyl heptane (4.8%), α−bergamotene (3.1%), (*E*)-2-decenal (2.0%) and 2-butanol (1.7%) while pulp of Barker cultivar contained hexanal (29.3%), tetrahydrofuran (10.4%), ethyl acetate (7.4%), (*E*)-2-octene (7.0%), methyl dodecanoate (5.8%), butanal (3.6%), isopropyl formate (3.3%), *p*-methyl acetophenone (3.1%), β−caryophyllene (3.0%), 2,5-dimethyl furan (3.0%) and 1,3-butanediol (2.4%).

Among the volatile compounds identified in this study, hexanal represented over 25% of the total chromatogram in Fortuna, Collinson and Barker cultivars, this result is coherent with most of the work published (Yamaguchi et al., 1983; Guzmán-Gerónimo et al., 2008, Pereira et al., 2013; Obenland et al., 2012). Hexanal (linoleic acid derivative) has been reported to be a volatile compound present in avocado pulp together with [E] 2-hexenal, octanal and nonanal. This compound is one the most abundant volatiles present in avocado and is mostly associated with low maturity fruit. The changes that occur in this compound also are known to characterize a green or grassy aroma. One of the clearest associations of aroma volatiles with flavor is related to the changes that occur in hexanal, 2-hexenal and 2,4-hexadienal, three aldehydes which characterize for a green or grassy aroma which is associated with ripening (El-Mageed, 2007, Pereira et al., 2013; Obenland et al., 2012).

According to Haiyan et al. (2007), hexanal arises from linoleic acid, whereas octanal and nonanal are oleic acid derivatives. Hexanal levels were generally lower in avocado in which there was a higher oleic acid content which was also observed in our results. These results correspond to the physical and chemical parameters presented earlier, as well as to some studies on avocado oil (Haiyan et al., 2007; Villa-Rodríguez et al., 2011; Galvão et al., 2014).

The volatile compounds methyl dodecanoate, (*E,E*)-2,4-heptadienal, hexanal, γ-nonalactone, (*E*)-2-decenal, 2-hexanol, nonanal, 2-octanol, 4-methyl-1-pentanol, 2-butanol and (*E*)-2-nonenal are known for fatty, waxy and oily notes. Among all cultivars studied in this work, most of these compounds were present in greater amounts in the avocado pulp from Fortuna cultivar which possessed higher fat content (16%) as reported earlier (Galvão et al., 2014).

Among the compounds detected in all cultivars of avocado 22 compounds were present only in Collinson cultivar while 13 compounds were in Barker cultivar, and only one compound each pertained to Fortune and Geada cultivar. However, most of these compounds could not be identified. Among the specific compounds which could be identified, 2-methyl-1-propan-1-ol (traces), 4-methyl-1-pentanol (traces), 1,2-propane-diol (traces), benzyl alcohol (traces), 1,3,5 trimethylbenzene (traces), *p*-cymene (traces) and acetoin (traces) were in Collinson cultivar while butanal (3.58%), 2-methyl 2-butanal (traces), isoamyl formate (traces), linalyl hexanoate (traces) and cyclohexanone (traces) were in Barker cultivar.

The difference in concentration of some volatile compounds among the cultivars could be explained by several factors including the lipid concentration of the pulp for example the high concentration of hexanal in Fortuna cultivar could be related to the higher lipid content (16 g/100 g) of this pulp. Ethyl acetate was present in higher content in Collinson cultivar and this compound has an appreciative and fresh fruity flavor which could be responsible for high acceptability of this cultivar.

The aromatic profile of Barker cultivar was quite similar to that of Collinson variety which could be related primarily due to the almost same lipid content of these pulps, being 12% and 13%, respectively. Fourteen volatile compounds viz. 2-propanol, octen-3-ol, 2-methyl butanol, isomayl alcohol, 2 hexanol, 3-methyl 3-buten-1-ol, 2-penten-1-ol and 3 hexen-1-ol, toluene, (*E,E*)-2,4-heptadienal, benzyl acetate, furfural, 3-carene, α-cubebene, α-copaene and linalool were common in these cultivars. However, these compounds were not found in the cultivars of Fortuna and Geada.

Among the fruits of the West Indian race (Barker and Geada), 4 compounds such as 2-octanol, tridecanol, benzaldehyde and propyl decanoate were present only in these cultivars However in hybrid (West Indian vs Guatemala) fruits belonging to Collison and Fortuna cultivars the compounds viz.2,3-butanediol and (*E*)-2-pentenal were specific and presente in both these cultivars.

The most prominent difference among the cultivars was in their total terpenes content which was 20% in Geada and lower concentrations of 3, 3.15 and 13% in Collison, Barker and Fortuna cultivars, respectively. Although Geada cultivar is characterized for its low lipid content (3g/100g) and early maturing of fruit (Gayet, 1995), it has the highest terpenes which characterizes from aroma standpoint this cultivar to be very different from other 3 cultivars. Benzaldehyde which possesses characteristic fruity and nutty odor note was present at a higher concentration

(4,3%) in only Geada cultivar and in traces in Barker cultivar but it was not detected in Collinson and Fortuna cultivars.

Among all these cultivars, the cultivars Geada and Fortuna cultivars are largely produced and commercialized in Brazil (Francisco & Baptistella, 2005; Cabia et al., 2014). According to Souza (2008), Brasil is a country where avocado production is mainly for the domestic market where Fortuna cultivar occupies good produtivity, good resistance to diseases and its higher pulp yield (Campos, 1984). However, this cultivar is considered to be of low aroma intensity and is mostly utilized for oil extraction while the cultivar Geada is much appreciated for its pleasing aroma.

4 Conclusions

This work studied the volatile composition of Barker, Collinson Geada and Fortuna cultivars of avocado fruit. The ideal conditions of extraction for capture of a large number of volatile compounds depend on the cultivar of avocado. The higher concentrations the some volatile compounds in Fortuna cultivar was related to higher lipid content. Hexanal was the main compound for Barker, Collinson and Fortuna cultivars while β−caryophyllene was for Geada cultivar. The aromatic profile of Barker cultivar was quite similar to that of Collinson variety which could be related primarily due to the almost same lipid content of these pulps. Benzaldehyde which possesses characteristic fruity and nutty odor note was present at a higher concentration (4.3%) in only Geada cultivar and in traces in Barker cultivar but it was not detected in Collinson and Fortuna cultivars.

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