

CHARACTERIZATION OF VOLATILE AROMA CONSTITUENTS IN AVOCADOS (*PERSEA AMERICANA* MILL) GROWN IN SRI LANKA

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ABSTRACT

The volatile components of avocados were isolated by Tenex GC and analysed by capillary Gas Chromatography linked with Mass Spectrometry. A total of 73 components were detected as avocado volatiles, of which 70 compounds comprising 99% of the isolates were positively identified. Sixteen of the identified compounds have not been previously been reported as avocado volatiles including butanal, oct-1-ene, dimethyl cyclohexane, nonan-2-one and tetradecanoic acid. In ripe avocados, C-6 alcohols and aldehydes were the major group of volatiles with trans-hex-3-en-1-ol as the most abundant constituent of 22.04%. The C-6 aldehyde, cis-hex-2-enal accounted to 6.66% of the aroma isolates in the ripe fruits. Terpenoids were the predominant class of constituents in unripe avocados. One monoterpene, limonene (2.97%) and ten sesquiterpenes were identified, β -caryophyllene being the major terpene (14.28%) followed by β -franscene (10.92%) and α -cubebene (10.2%) as the main hydrocarbons. In an olfactory assessment at odour port during GC, six aroma compounds were described possessing the characteristics green flavour of avocado. The C-6 alcohols and aldehydes were significant components of aroma together with several compounds deriving from fatty acid precursors. The oxidative decomposition of carotenoids leads to the formation of terpenoids in unripe avocados.

Key words: aroma volatiles, avocado, flavour, odour port analysis, terpenoids

INTRODUCTION

The avocado (*Persea americana* Mill) belongs to the family *Lauraceae* and is one of the major fruit crops in the world. It is native to central America but is grown in all tropical and subtropical regions of the world. During the past decade, the demand for this fruit has increased significantly as a result of increased consumer awareness of fruit's dietary value and improved fruit quality resulting from implementation of maturity standards and improved storage and transportation facilities. In Sri Lanka, avocado is grown in central high lands most often as seedlings among crop plants on estates and in back gardens. The avocado seasons normally exists from April to July when the fruits are relatively abundant. Avocado is a favorite article of diet in Sri

Lanka and is mainly consumed in the form of fresh fruit. It is also used as an ingredient in salads and tortillas with lemon juice, pepper and salt.

Avocado is an oleaginous fruit and the lipid content in the mesocarp varies from 12.4 - 24.2% at mature ripe stage (Nagalingam, 1994). Because of the high oil content, the fruits have the highest energy value of any fruit. The high oil content also contributes to the consistency and the special taste of the fruit. Unlike the other fruits, the avocado is high in fat, protein, vitamins and minerals but low in sugars; therefore it can be recommended as a high energy food for the diabetic (Swisher *et al.*, 2001). Knowledge of the identities of the constituents which are responsible for avocado flavour is important for the control of flavour, both in the fresh and processed products. The high oil content of the fruit has attracted special attention with respect to composition of fruit but relatively little work has been done on the composition of the volatile constituents of avocado fruit and therefore the present study was undertaken to characterize the volatile aroma components of avocados.

MATERIALS AND METHODS

Avocado Samples

Fresh, locally harvested Avocado fruits cv. *Hass* were obtained from a commercial grower at Kandy, Sri Lanka. Fruits were washed with water and dried in the air before use. Fruits were ripened in a humidity control cabinet at 20°C and the RH of 90-94%. To obtain such humidity level, the air pump was used to bubble the air through water in a tray. Each day, the stages of ripening of fruits were determined subjectively by applying gentle pressure to the fruit held the palm of the hand. Categories were hard, soft and ripe. These stages were confirmed by texture measurement using Steven's Compression Response Analyzer using 50 kg load cell. Fruits at different ripening stages were halved longitudinally and the seeds removed. Hard fruits were peeled and the flesh put through a food shredder. For soft and ripe fruits, the pulp was scrapped and homogenized for 5 min.

Isolation of Volatiles

A weighed quantity of 300 g sample from 3 avocados was transferred to an aroma isolation apparatus with a screw top containing both an inlet and outlet tubes. The flask was placed in a water bath at 40°C. A SGC trap, containing Tenax-GC (polymer of 2, 6 diphenyl-*P*-phenylene) as the absorbent, was connected to the outlet and a oxygen free nitrogen was connected to the inlet. The nitrogen was passed over the pulp at a flow rate of 40ml/min for 1 hour and the avocado volatiles were swept onto the trap. The inlet was also connected to a tube containing an absorbent (charcoal) to prevent volatiles from the gas supply reaching

the trap. At the end of collection time, the flask was removed and the trap was connected directly to the nitrogen supply at the same flow rate for 5 min to remove the moisture. A blank isolate was also prepared using an empty isolation apparatus. Alkanes standards (C_6 to C_{25}) were used in the measurement of retention indices of the standard and the compounds separated from avocados. Ethanol, ethyl acetate, hexanal, nonanal, hexan-1-ol, hex-2-en-1-ol, hex-3-en-1-ol and β -pinene were purchased from BDH Chemicals, UK and β -caryophyllene, α -cubebene, β -franscene and α -humulene from Aldrich Chemical Ltd, UK.

Gas Chromatography Analysis of Volatiles

A Perkin-Elmer Sigma 3B equipped with flame ionization detector (FID) was used for routine gas chromatographic analysis. A fused silica capillary column of 60 m x 0.32 mm I.D., coated with 1 μ m film thickness of 95% dimethyl siloxane + 5% phenyl siloxane (SE 52/54) bonded phase was used to separate the volatile components. The trap was placed directly in the injection port of the gas chromatograph and its contents were desorbed with the helium carrier gas at the flow rate of 1.5 ml/min for 5 min onto the front end of the capillary column. The column was cooled to 0°C with liquid CO_2 which entered the oven through a metal tube, surrounding the column. After desorption the oven was heated rapidly to 30°C, followed by heating at 3°C/min to 250°C. The column temperature was maintained at 250°C until the completion of the separation. The injector port and the detector temperature were maintained at 250°C and 260°C, respectively. A Hewlett Packard model-3390A reporting integrator was used to determine the peak area during routine analysis. Linear retention indices for the volatile components were calculated by chromatographing n-alkanes (C_6 to C_{25}) mixed with the samples. Linear retention indices for the authentic aroma compounds were similarly determined.

Gas Chromatographic-Odour Port Analysis

Aromas of the separated components were assessed at the column outlet during the chromatographic separation using the technique of GC-Odour port assessment. Chromatographic effluents were split in a ratio of 1:1 by means of the outlet splitter containing a two fold vespel ferrule, such that two equal lengths of deactivated fused silica (0.3 x 0.32 mm I.D.) led from the ferrule, one length of the GC detector and the other length of the external sniffing port. The odours of the separated components were described by two assessors experienced in descriptive analysis of odours. Triplicate assessments were performed for each ripening stages of avocados.

Gas Chromatographic-Mass spectrometric Analysis of Volatiles

Volatile components were identified as far as possible by Gas Chromatography – Mass Spectrometry

analysis using a Kratos MS 80RFA Mass Spectrometer linked on line to a Kratos DS 90 data processing system coupled to a Carlo-Erba 5300 Gas Chromatography. The same GC conditions were used in this instrument. The significant operating parameters were: ionization voltage - 70eV; Emission current - 100 μ A; Ion source temperature - 200°C; Accelerating voltage - 4KV; Resolution – 1000; Scan speed – 1s/decade; Scan time – 0.2s.

Component Identification and Quantitative Assessment

Interpretation of Mass Spectra was done by computerized data matching, using libraries on the GC-MS data systems (Eight Peak Index of the Mass Spectra) and by manual comparisons with published mass spectra. Identifications were confirmed by comparing the Linear Retentive Indices (LRI) or Kovats Index of each component. The mass spectra and retention indexes of compounds which were not available, were matched, where possible with values recorded in the literature.

Quantitative data were derived from the TIC monitor obtained during GC – MS and for trace components by extrapolation from integrator (Hewlett Packard 3370B) data obtained from the GC-FID chromatogram recorded during routine GC analysis. Triplicate analyses were performed.

RESULTS AND DISCUSSION

Components identified in headspace concentrates from avocado samples at three ripeness stages are listed in Table 1. Overall 73 components were detected as avocado volatiles, of which 70 compounds comprising 99% of the samples were positively identified. The three unidentified compounds were present in the sample in such low amounts that no mass spectrum could be recorded. Changes in individual volatiles during ripening were also assessed. In ripe fruits, the isolates were dominated by C-6 alcohols and aldehydes but terpenes were the abundant classes of volatiles in unripe fruits.

In avocados, the levels of C-6 alcohols and aldehydes remarkably increased during ripening. Three C-6 alcohols were present at high concentration in ripe fruits. The predominant alcohol is *trans*-hex-3-en-1-ol which accounts 22.04% in the ripe fruits. The geometric isomer, *trans*-hex-2-en-1-ol is present at about 17.43%. In unripe fruits, these two components are present at 7.71% and 4.21% respectively. No work appears to have been published on the changes in volatile constituents during ripening of avocados. However, only two reports are available on the volatile components of avocados. Yamaguchi (1989) reported that *trans*-hex-2-en-1-ol and hexan-1-ol comprised 58.95% and 9.82% in the ripe avocado isolate but the variety of the avocado examined was not mentioned. They also reported the presence of low levels of

hexanal (0.39%) and *trans*-hex-2-enal (1.61%) in their isolates. This is in contrast with our findings where hexanal was present 5.51% and *trans*-hex-2-enal was not identified as a component of the aroma isolate. Compared to hexanal and *cis*-hex-2-enal, the concentration of *trans*-hex-3-enal was found to be low at 0.37% in ripe fruits. According to the work of Kazeniak and Hall (1990), the formation of some of the *cis*-hex-2-enal is through *trans*-hex-3-enal by the action of enzyme *isomerase*. It is well known that this type of double bond shift occurs readily. Because of this double bond shift, it is difficult to get an accurate analysis of the relative amount of each compound as it occurs in the avocado.

The quantity and quality of C-6 alcohols and aldehydes is dependant on the amount of oxygen in the sample, enzyme activity, extent of comminution, isolation procedure and the extent of heat. Therefore, some of the aldehydes may be transformed to alcohols by enzymatic action. Since blending effectively disintegrates the avocado tissues, the natural enzymes and their substrates are intimately mixed. In addition, an excess air is whipped into the mixture during blending the pulp. The combination of these factors would be expected to greatly favour the development of flavour compounds by enzymatic oxidation (Whitefield *et al.*, 1980).

The domination of aroma isolates from ripe avocado, by C-6 alcohols and aldehydes appears to be unique among tropical fruits. These two classes of compounds accounted over 65% of the total aroma isolates and the characteristics flavour come from the C-6 alcohols and aldehydes. In wide range of plant tissues, the carbonyl compounds are derived from the reaction of fatty acids with oxygen and lipoxygenase enzyme. The dominant compounds identified in the ripe avocado aroma are reported to be derived from fatty acid degradation. Avocads are known to be rich in oil. The average oil content of unripe and ripe fruits used in this study was 15.4% and 21.1% respectively. Several authors examined the formation of C-6 alcohols and aldehydes from unsaturated fatty acids. (Stone *et al.*, 1999 and Galliard *et al.*, 2001).

Investigating the fatty acid composition of lipids in *Hass* avocados, the unsaturated fatty acids such as oleic acid - 70.2%, linoleic acid - 10.1% and linolenic acid - 1.03% were found to prevail in the ripe fruits (Mahendran and Thireganathan, 1998). On maceration of tissues, enzymatic decomposition of lipid proceeds very rapidly. Kazeniak and Hall (1990) showed that in the presence of H⁺ and under the influence of heat, *cis*-hex-3-enal is transformed into *trans*-hex-2-enal isomer. These aldehydes may be converted to C-6 alcohols by the enzyme *alcohol dehydrogenase* during storage and ripening of fruits.

Table 1: Aroma Compounds of Avocado (*Persea americana* Mill)

Compound	Retention (Time min)	LRI †	% Relative abundance *			Odour § quality
			Unripe	Soft	Ripe	
Acetaldehyde	2.57	368	0.78	2.04	3.94	Fragrant, floral
Ethanol	5.23	512	5.51	6.21	7.44	Sweet
2-Propanol	7.82	522	0.49	0.41	0.21	Yeasty, fruity
Acetone	8.07	527	tr	tr	0.20	
Diethyl ether	8.36	530	tr	tr	tr	Stale grass
Penta-1-3-diene	9.25	540	tr	tr	tr	
Methyl acetate	10.40	543	0.44	0.91	1.21	Floral, sweet
2-Methyl propanal	12.49	550	0.24	0.74	0.83	
2-Methyl pentane	14.80	557	0.45	0.40	0.01	Roasted cereal
3-Methyl pentane	14.97	562	1.14	1.01	0.18	
Propanol	15.21	569	1.12	1.17	1.29	Buttery
Unknown	15.60	577	tr	tr	0.09	
Di acetal	15.97	584	0.12	0.14	0.17	Buttery
Butanal	16.81	592	0.34	0.41	0.54	
Hexane	17.05	598	0.18	0.32	0.35	Fruity
2-Methyl furan	17.24	606	tr	tr	0.10	
Ethyl acetate	18.12	612	1.22	1.91	2.44	Sweet
Methyl cyclopentane	18.64	619	0.10	0.15	0.19	
2-Methyl-1-propanol	19.04	628	0.21	0.20	0.07	Fragrant
3-Methyl butanal	19.51	651	0.29	0.17	0.08	
2-Methyl butanal	20.01	660	tr	tr	tr	Sweet (mango)
2, 4-Dimethyl pentane	20.43	674	0.22	0.32	0.35	
Pentanal	20.74	689	0.09	0.26	0.32	Pungent
3-Pentanone	21.45	691	0.10	0.41	0.60	
3-Pentanol	21.53	692	0.12	0.12	0.14	Fragrant, caramel
2-Ethyl furan	23.06	700	tr	tr	tr	
3-Methyl butanol	23.27	722	0.21	0.41	0.77	Floral fragrant
3-Hydroxy-butan-2-one	24.33	731	0.12	0.19	0.26	
2-Methyl butanol	25.46	733	0.29	0.51	0.72	Chemical solvent
Pentan-1-ol	26.16	760	tr	tr	tr	
Toluene	26.30	767	0.24	0.20	0.01	Green, fruity
Oct-1-ene	26.69	792	0.18	0.24	0.29	
<i>Trans</i> -hex-3-enal	27.05	798	0.12	0.19	0.37	Green, grassy
Hexanal	27.14	799	1.82	3.31	5.51	
Dimethyl cyclohexane	27.14	801	0.19	0.36	0.40	Green, grassy
Octane	27.36	809	0.31	0.56	0.59	
Trimethyl cyclohexane	27.61	812	0.04	0.26	0.30	Green, grassy
Furfural	27.70	817	0.21	0.35	0.42	

Compound	Retention Time (min)	LRI [†]	% Relative abundance*			Odour [§] quality
			Unripe	Soft	Ripe	
<i>Cis</i> -hex-2-enal	30.59	847	2.38	4.29	6.66	Green, fruity
<i>Trans</i> -hex-3-en-1-ol	30.63	856	7.71	19.60	22.04	Green, floral
<i>Trans</i> -hex-2-en-1-ol	31.49	860	4.21	13.76	17.43	Green, fruity
Hexan-1-ol	32.45	866	3.04	10.47	13.71	Green, fragrant
Heptanal	33.98	884	0.22	0.61	0.81	Caramel, buttery
Nonane	34.20	898	0.20	0.19	0.16	Sweet.
Nonan-2-one	36.27	927	0.19	0.17	tr	Fragrant
5-Methyl furfural	36.89	939	0.22	0.47	0.49	
β -Pinene	37.32	949	2.69	1.48	tr	
Ethyl benzene	38.04	968	0.41	0.99	1.08	
2-Methyl-5-hepten-2-one	40.86	990	0.21	0.15	0.04	Musty
Octanal	41.07	991	0.37	0.51	0.74	Sweet, fruity
Tri methyl benzene	41.17	1002	0.11	0.15	0.17	
Limonene	43.29	1036	2.97	1.71	tr	Sweet, fragrant
Unknown	43.71	1042	0.05	0.07	0.11	
Nonanal	47.28	1087	1.32	2.14	2.90	Fruity (peach)
Decanal	54.20	1186	1.42	1.51	1.74	Orange peel.
Dodecane	56.24	1200	tr	tr	tr	
α -Cubebene	67.31	1269	10.20	4.64	0.24	
α -Copaene	69.21	1386	4.86	0.71	tr	
β -Farnesene	69.94	1402	10.92	0.41	tr	
β -Caryophyllene	70.42	1421	14.28	7.20	0.12	Slight burnt.
β -Gurjunene	72.38	1476	4.57	tr	tr	
Calarene	73.04	1504	3.28	1.20	0.11	
δ -Cadinene	75.24	1524	3.74	1.11	tr	
Tetra decanal	76.04	1582	0.21	0.14	0.09	Oily, fatty, rancid
α -Humulene	76.21	1624	2.07	1.00	0.12	Fruity (guava)
δ -Elemene	77.40	1729	0.76	0.70	0.12	Leathery
Unknown	79.04	1803	tr	0.04	0.09	
Tetradecanoic acid	81.92	1894	0.02	0.14	0.17	Slight oily, waxy.
Nonadecane	82.24	1909	0.21	0.14	tr	
Eicosane	83.74	1986	0.17	0.12	0.11	Musty / mouldy
3-Methyl octadecane	84.87	1993	0.02	0.07	0.09	
δ -lactone	86.01	2018	tr	tr	0.02	
Heneicosane	87.56	2039	0.04	0.20	0.22	

* Relative percentage of total peak area.

Values are means of 3 replications correlated to nearest 0.01%, if <0.001% then quoted as trace (tr).

[†] Linear Retention Indices (LRI) calculated for the SE52/54 capillary column of the GC-MS system.

[§] By GC-Odour port assessment.

Alcohols, a part from C-6 compounds, accounted for about 8% and 11% of the isolates obtained from the unripe and ripe fruits respectively. Similarly, total aldehydes proportions of the isolates other than C-6 compounds accounted about 5% and 12% in the unripe and ripe fruits respectively. The level of acetaldehyde increased from 0.78% to 3.94% during ripening of avocados. The concentration of ethanol was higher than that of acetaldehyde suggesting a rapid conversion of acetaldehyde to ethanol by enzymatic action. Apart from acetaldehyde, nonanal and decanal are reputed to be important volatile constituents in avocado. Moreover, 2-methyl propanal, butanal, 3-methyl butanal, 2-methyl butanal, pentanal, heptanal, and octanal were also identified. The total amount of aldehydes concentration of avocado increased with progressive ripening. The amino acid profile of avocado has been reported by (Ahmed and Barmore, 1983) and strecker degradation of alanine, valine, leucine and isoleucine explains the formation of acetaldehyde, 2-methyl propanal, 2-methyl butanal and 3-methyl butanal, respectively. Many of the aldehydes identified in this study can be related to the major groups of the non-volatile constituents found in fruits. The straight chain saturated and unsaturated aldehydes are fairly typical of those formed from oxidative degradation of the fatty acids. These are common to many fruits although each fruit appears to have its own relative proportions of the different volatile constituents.

Aliphatic ketones account less than 2% in the ripe and unripe isolates. Acetone, 3-pentanone, 3-hydroxybutan-2-one, nonan-2-one and 2-methyl-5-hepten-2-one were identified in both ripe and unripe fruits. The number of esters identified in this study is limited. The level of esters is nearly 4% and 2% in the ripe and unripe fruits respectively. Methyl acetate and ethyl acetate are the only two esters identified from both isolates. These two esters commonly occur in aroma volatiles of many tropical fruits including avocados (Kader, 1989). As the endogenous level of acetaldehyde and ethanol increased, the concentration of methyl acetate and ethyl acetate were found to increase during ripening. The levels of these two esters remained high at edible ripe stage. This may indicate the biosynthesis of these two esters continues with progressive ripening, as happened with other esters in apples treated with carboxylic acids. Apples were found to produce carboxylic esters from added aliphatic volatile alcohols and acids apparently by absorbing the alcohols and acids from the surrounding atmosphere and converting them into esters which were released into the atmosphere again. (De Potter *et al.*, 2003). As an effect of non-enzymatic browning reaction between the reducing sugars and amino acids upon heating, volatile carbonyl compounds are formed and these may highly affect the character of aroma. In avocado, these compounds were identified as furfurals, 2-methyl furan and 2-ethyl furan.

The most important aroma constituents of unripe avocados are terpenes. These compounds comprise over 60% of the total volatiles. In ripe fruits these compounds are present at a total level less than 1%. One

monoterpene hydrocarbon, limonene (2.97%) and ten sesquiterpene hydrocarbons were identified from both ripe and unripe isolates. The major sesquiterpene hydrocarbon identified in this study was β -caryophyllene which accounts 14.28% in the unripe fruits and the levels decreased to 0.12% with progressive ripening (Figure: 1). Stevens *et al* (1999) reported that β -caryophyllene was the major sesquiterpene hydrocarbon component identified from guava. Softening results in a considerable relative decrease in the concentrations of α -cubebene and β -farnescene. Other terpene hydrocarbons which have been identified to contribute aroma of unripe avocados were α -copaene (4.86%), β -gurjunene (4.57%), calarene (3.28%), δ -cadinene (3.74%) and α -humulene (2.07%). Young *et al* (1999) found no sesquiterpenes in ripe avocado mesocarp extracted under the reduced pressure, but found 2-methyl-2-butanal, dimethyl formaldehyde and methanol which were not found in this study.

The normal flavour of avocado fruit is usually described as bland with a green and peppery after taste. The characteristics green flavour is due to presence of C-6 alcohols and aldehydes which comprises over 65% of the total aroma isolates in ripe fruits. The likely cause of peppery flavour is at present unknown but atleast three of the sesquiterpenes hydrocarbons, β -caryophyllene, α -cubebene and α -copaene could impart a distinctive flavour to the rather bland tasting flesh of the avocado. Some of the identified volatiles are derived from the non-lipid precursors. The oxidative decomposition of carotenoids leads to the formation of terpene compounds.

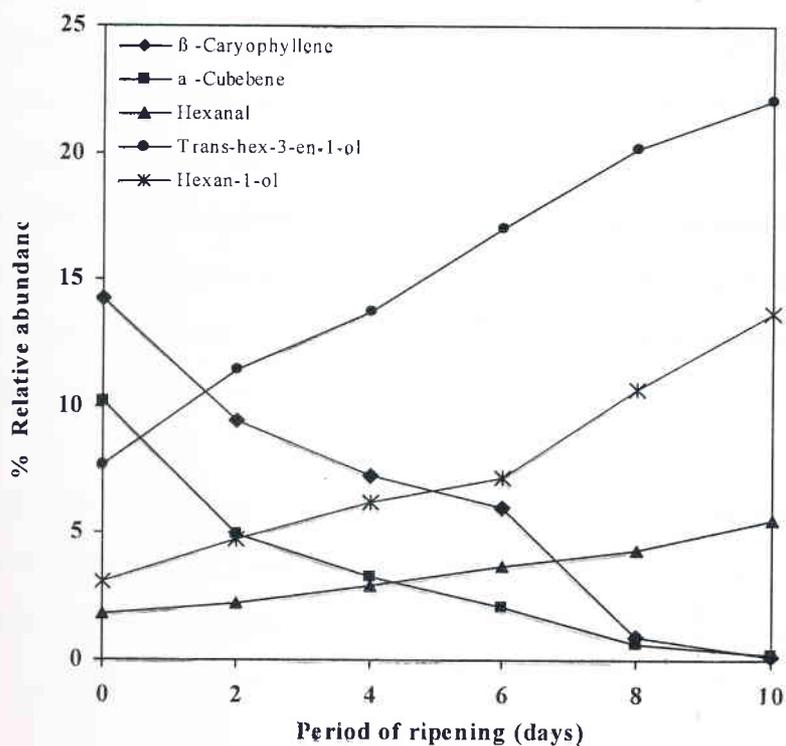


Figure 1: Changes in pre-dominant volatile constituents during ripening of avocados at 20°C

The carotenoid content of avocados cv. *Hass* was determined recently as 18µg/g fresh fruit. Onyewu and co-workers (1996) surveyed derivatives of carotenoids (particularly β-carotene) formed upon heat treatment and identified one of them as toluene. Avocado contains phospholipids, glycolipids and triacylglycerol which are rich in linoleic acid and linolenic acid. On rupture of cells, these fatty acids are released by the action of *acyl hydrolases* and *phospholipase D* and are further converted by lipoxygenase into the 9- and 13-hydroperoxides of both linoleic and linolenic acids. The volatile cleavage product is hexanal when 13-hydroperoxy linoleic acid is the substrate while *cis*-hex-2-enal is produced on cleavage of 13-hydroperoxy linolenic acid. Previous work in this (Young *et al.*, 1999) had established that homogenization of avocado tissue caused the conversion of linoleic and linolenic acid to volatile carbonyl fragments.

In this study, as expected, a large number of volatile compounds were obtained in addition to terpenes, especially compounds relating from lipid degradation products which included alcohols and aldehydes. Partly because of the enzymatic procedures instantaneously occurring upon the maceration of the fruit, it is difficult to establish which of the volatile components were present originally in the fruit and which developed during the comminution and homogenization. In addition to the compounds identified, avocado extracts contained a number of related higher molecular weight compounds which are probably complexes of substituted phenols and studies to identify these continue.

CONCLUSIONS

The characteristic green flavour of avocado is due to the presence of C-6 alcohols and aldehydes which comprises over 65% of the total aroma isolates at ripe stage. Terpenes are the important aroma constituents of unripe avocados and expected to contribute a peppery after taste. A greater number of alcohols and carbonyl compounds identified in aroma isolates are classic volatiles produced derived from fatty acid oxidation and degradation. Olfactory assessments of odour port during GC six peaks were described as possessing significant green note of avocado flavour.

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