JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Characterization of the Bound Volatile Extract from Baby Kiwi (*Actinidia arguta*)

Coralia V. Garcia,[†,§] Siew-Young Quek,[*,†] Ralph J. Stevenson,[†,#] and Robert A. Winz[§]

[†]Food Science Programme, School of Chemical Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand

[§]The New Zealand Institute for Plant and Food Research, Private Bag 92169, Auckland 1142, New Zealand

[#]Auckland Cancer Society Research Centre, The University of Auckland

ABSTRACT: The glycosidically bound volatile fraction of baby kiwi (*Actinidia arguta*) was studied. Glycosidic precursors were isolated from juice by adsorption onto an Amberlite XAD-2 column. After enzymatic hydrolysis with Rapidase AR2000, the released aglycones were analyzed by GC-MS. Alcohols, terpenoids, and benzenoids were the most abundant compound classes. Aromatic compounds and norisoprenoids showed the highest concentrations. Major compounds were 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (Furaneol), benzyl alcohol, 3-hydroxy-β-damascone, hexanal, and (*Z*)-3-hexen-1-ol. Precursors of aroma compounds including benzoic acid, cinnamic acid, and coniferyl alcohol were also found. Eugenol, raspberry ketone, and 4-vinylguaiacol were identified for the first time in the fruit of an *Actinidia* species. The high concentration of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone in bound form (95.36 μg/kg) is particularly interesting and justifies further investigation.

KEYWORDS: *Actinidia arguta*, baby kiwi, glycosides, aroma, volatiles, Furaneol

INTRODUCTION

Actinidia arguta (Sieb et Zucc.) Planch. ex Miq. var. *arguta* is widespread in northeastern Asia, being found in Siberia, Korea, Japan, and northern China. This species has a high resistance to cold, which is why it is sometimes called "hardy kiwifruit". Other common names for this fruit are "baby kiwi" and "kiwi berry".[1,2]

The fruit are small and hairless, weighing 5−15 g, with edible skin. *A. arguta* tends to be sweeter than *Actinidia deliciosa* or *Actinidia chinensis*, but has the notable disadvantage of a much shorter storage and shelf life. Commercially available cultivars include 'Ananasnaya' in the United States and Chile, 'Kosui' in Japan, 'Chiak' in Korea, and 'Hortgem Tahi', 'Hortgem Toru', 'Hortgem Wha', and 'Hortgem Rua' in New Zealand.[1]

Several studies have been carried out on the volatile composition of *A. deliciosa* [3−6] and *A. chinensis*,[6−8] but there is a scarcity of reports on *A. arguta*.[9]

The aroma of baby kiwi has been described as banana, floral, fruit candy, grassy, green, melon, and tropical.[9] It is because of this rich aromatic flavor that *A. arguta* is an attractive kiwifruit species, despite its short storage life and difficulty in growing.[2]

The volatile composition of *A. arguta* fruit has been revealed by using headspace and solvent extraction techniques. The main compounds were identified as the esters ethyl butanoate and ethyl hexanoate, followed by several lipid-derived volatiles, such as (*E*)-2-hexenal, hexanal, and (*Z*)-3-hexenal. From the solvent extract, additional major compounds including camphor and methyl and ethyl benzoate were detected.[9] A study applying GC-MS/O identified the odor-active compounds in *A. arguta*, which included fruity esters such as ethyl butanoate and methyl benzoate, the caramel-like 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (Furaneol), several green-smelling compounds such as 1-octen-3-one, (*E*)-2-hexenal, pentanal, and hexanal, and some odorants with floral notes.[10] Interestingly, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone has been reported in *A. arguta*, as tentatively identified, in only one paper,[10] notwithstanding its apparent contribution to the aroma of this fruit, which could be explained by the difficulty in isolating it.[11]

It is known that aroma volatiles also occur in glycosylated form in fruit. The proportion of glycosidically bound volatiles is usually greater than that of free volatiles, making them an important potential source of flavor compounds. Alcohols, terpenoids, norisoprenoids, volatile organic acids, and shikimic acid derivatives are known to occur as glycosides. The odorous aglycones may be released from the sugar moiety during maturation, processing, and storage or by enzymes, acids, or heat. Glycosidically bound volatiles have been reported in a number of fruits, including grape, apricot, mango, and passionfruit, among others,[12,13] but there is currently only one study on the glycosidically bound volatiles from kiwifruit of the 'Hayward' cultivar[14] and no information on bound volatiles from other *Actinidia* species, including *A. arguta*.

The objective of this study is to identify the bound volatile composition of *A. arguta* fruit to determine the presence of potential flavor compounds.

MATERIALS AND METHODS

Plant Material. *A. arguta* (Sieb et Zucc.) Planch. ex Miq. var. *arguta* 'Hortgem Tahi' fruits were harvested during March 2010 from the Plant and Food Research orchard in Te Puke, New Zealand, and kept in cool storage at 2 °C until required. Fruits were kept in storage for under a month. At 24 h prior to sampling, fruits were taken out of storage, repacked into kiwifruit trays covered with a polyliner, and held at 20 °C until ripe.

Received: April 12, 2011
Revised: June 24, 2011
Accepted: June 25, 2011
Published: June 25, 2011

The fruits were analyzed at the eating-ripe stage, having an average flesh firmness of 1.28 Instron (0.13 kgf) and a soluble solids content of 15 °Brix.

The flesh firmness was evaluated using an Instron model 4301 compression tester (Instron, Canton, MA). A hand-held refractometer model N-20E (Brix 0−20%, ATAGO, Tokyo, Japan) was used to measure soluble solids content.

Standards and Solvents. All chemicals used were of analytical grade or better. Methanol was purchased from Merck (Darmstadt, Germany); diethyl ether, acetone, and pentane were obtained from Scharlau (Barcelona, Spain). Pentane was redistilled before use.

The C7−C30 saturated alkanes standard mixture, for calculating retention indices, was purchased from Supelco (Bellefonte, PA).

Pure volatile standards were obtained from the following suppliers: 4-vinylguaiacol (2-methoxy-4-vinylphenol) from Endeavor (Daventry, U.K.); 2-pentanol, eugenol, benzoic acid, acetic acid, methyl salicylate, cinnamic acid, geraniol, benzyl alcohol, isovaleric acid, and butanol from VWR (Poole, U.K.); cyclohexanone, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, (*E*)-2-hexenal, 2-phenylethanol, benzaldehyde, 3-(methylthio)-1-propanol, raspberry ketone (4-(4-hydroxyphenyl)butan-2-one), coniferyl alcohol, 1-octen-3-ol, 2-ethylhexanol, *p*-hydroxyphenethyl alcohol, hexanal, hexanol, vanillin, isoamyl alcohol, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, linalool, nerol, α-terpineol, and furfuryl alcohol from Sigma-Aldrich (St. Louis, MO); *cis*-linaloloxide and β-damascenone from Shiono Koryo Kaisha (Osaka, Japan).

Isobutanol, 3-pentanol, 3-methyl-2-butenal, cyclopentanol, prenol (3-methyl-2-buten-1-ol), 3-octanol, *trans*-carveol, (*E*)-8-hydroxylinalool, and *trans*-isoeugenol were obtained from the standards collection at Plant and Food Research.

Extraction and Hydrolysis of Glycosidic Precursors. The glycosidic precursors were isolated by adsorption onto an Amberlite XAD-2 column (Supelco), according to the method of Young and Paterson[14] with some modifications. The 55 mL column was prepared at least one day prior to use and by washing sequentially with 110 mL each of Milli-Q water, acetone, water, 3 M HCl, and water until chloride free. The column was then stored in water at room temperature until use.

Fruits (2.3 kg) were cut in half, and the juice was extracted with a BJE 200 juicer (Breville, Australia), followed by pressing in a wine press (Medio model, Ferrari Group, Italy) lined with cheesecloth. The procedure was done at 4 °C in a cold room. The juice was clarified by vacuum filtration through Celite 545 (VWR, Haasrode, Belgium) in a Büchner funnel.

The clear juice obtained was passed down the previously prepared Amberlite XAD-2 column, followed by elution with 160 mL each of water, pentane and methanol, in that order. The water and pentane eluents were discarded. The methanol fraction, containing the glycosidic aroma precursors, was collected and then divided into four portions and evaporated to dryness in a rotary evaporator and further dried under vacuum at room temperature for 2 h. The dried glycosidic extracts obtained were kept at −20 °C until required.

The citrate/phosphate hydrolysis buffer was prepared by dissolving 4.2 g of anhydrous citric acid (AppliChem, Darmstadt, Germany) and 2.84 g of anhydrous Na_2HPO_4 (Scharlau) in 100 mL of Milli-Q water and adjusting to pH 5 with 4 M NaOH, as described by Young and Patterson.[14]

Each portion of the dried glycosidic extract (one-fourth of the total) was dissolved in 2 mL of buffer and extracted three times with 1 mL of pentane to remove residual free volatiles.

Enzymatic hydrolysis was carried out using Rapidase AR2000 (DSM Food Specialties, Delft, The Netherlands), which is a commercial enological preparation of several *Aspergillus niger* glycosidase activities used to release grape aromatic compounds. Rapidase AR2000 was dissolved in hydrolysis buffer and used at a concentration of 74.4 mg enzyme/kg pulp. After the addition of 30 μL of a 0.1 mg/mL solution of cyclohexanone in ether as internal standard, the tube was closed and placed in a dry bath (AccuBlock D1200, Labnet, Woodbridge, NJ) at 37 °C for 48 h. Three replicates and a control (no enzyme added) were prepared. The hydrolysate was cooled over ice, and the released aglycones were extracted five times with 0.80 mL of a mixture of pentane/ether (1:1 v/v). The extract was dried over anhydrous Na_2SO_4, preconcentrated in a rotary evaporator, over an ice bath, and then further concentrated under a gentle stream of nitrogen to a final volume of 100 μL.

Analysis of Volatile Compounds. Analysis of the bound volatile extract was performed on a Shimadzu QP2010 Plus gas chromatograph−mass spectrometer (Kyoto, Japan) equipped with a CombiPAL autosampler (CTC Analytics, Switzerland). The column used was a Stabilwax (Restek, Bellefonte, PA) (30 m length × 0.25 mm i.d. × 0.25 μm film thickness). Helium was used as the carrier gas (column flow = 1.5 mL/min). A 1 μL sample was injected in split mode (split ratio = 5). The operating conditions were as follows: injector, 250 °C; ion source, 230 °C; interface, 250 °C. The GC oven temperature was programmed at 50 °C for 2 min, increased at 5 °C/min to 150 °C, at 10 °C/min to 200 °C, and at 20 °C/min to 247 °C, and then held at 247 °C for 10 min. The total analysis time was 39.35 min. The mass spectrometer's source was operated in electron impact ionization mode. Compounds were analyzed in SCAN mode, and the total ion current (TIC) in the range m/z 40−400 was acquired.

Kovats indices were obtained by injecting a C7−C30 straight-chain alkane mixture, under the same conditions as the analyzed samples, and using the retention index feature of the software to calculate them.

Identification of the compounds was achieved by comparing their mass spectra and retention times with those available in the GC-MS libraries (NIST08, FFNSC1.3) and the Compound Database of Plant and Food Research or published in the literature (NIST Chemistry WebBook, http://webbook.nist.gov/chemistry/). When available, authentic standards were used for a positive identification. Compounds for which no standards were available are reported as tentatively identified.

Semiquantification of aroma compounds was obtained from the total TIC and was performed by comparing the peak areas of the volatiles in the sample to the peak area of an internal standard of cyclohexanone. Response factors were determined by the analysis of solutions containing known concentrations of the reference compounds with cyclohexanone as internal standard. When the authentic standard for a compound was not available, the response factor of a representative compound was used as follows: 1-hexanol for alcohols, linalool for terpenoids, (*E*)-2-hexenal for aldehydes, acetic acid for carboxylic acids, vanillin for phenolic compounds, and β-damascenone for norisoprenoids.

RESULTS AND DISCUSSION

The glycosidically bound volatile compounds of *A. arguta* were released by enzymatic hydrolysis using Rapidase AR2000. This commercial enzyme preparation contains several glycosidase activities, including β-glucosidase (5.6 nkat/mg), α-arabinofuranosidase (9.2 nkat/mg), α-rhamnosidase (0.32 nkat/mg), and β-apiosidase (1.08 nkat/mg), which has the advantage of hydrolyzing most glycosides of aroma compounds including *O*-β-D-glucosides and *O*-diglycosides. Aglycones are released from diglycosides by first breaking the sugar−sugar bond by the action of an exoglycosidase, followed by the hydrolysis of β-D-glucoside by β-glucosidase, which releases the corresponding aglycone and glucose.[12]

A total of 89 compounds were detected in the bound volatile extract of ripe *A. arguta* fruit. There were few compounds present in the control sample (without enzyme). However, small

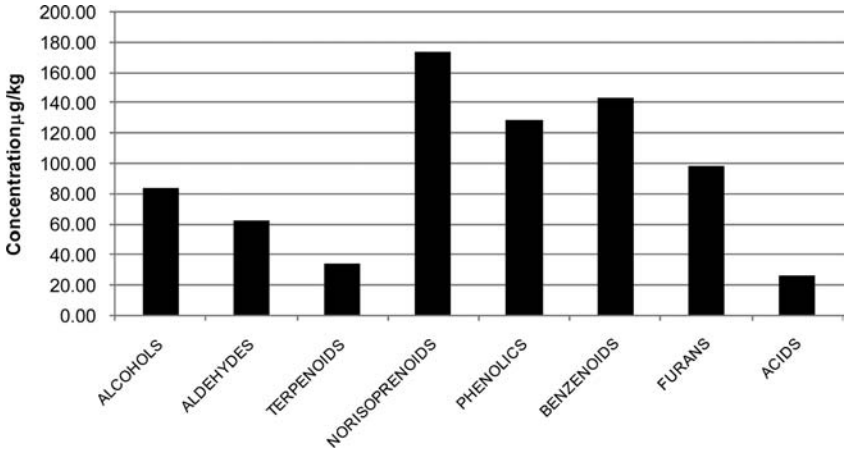

Figure 1. Levels of bound aroma volatiles of ripe *A. arguta* fruit.

amounts of (*E*)-2-hexenal, benzyl alcohol, 2-phenylethanol, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, 3-hydroxy-β-damascone (tentatively identified), and some carboxylic acids were found, which can be explained by the acidity of the buffer (pH 5.0), because flavor glycosides are also known to be hydrolyzed by acid.[15]

Alcohols (22 compounds), terpenoids (17 compounds), and benzenoids (20 compounds, including 14 phenols) were the most numerously represented compound classes, whereas norisoprenoids and aromatic compounds were the most abundant (Figure 1). Compounds with the highest concentrations were 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, benzyl alcohol, 3-hydroxy-β-damascone (tentatively identified), hexanal, and (*Z*)-3-hexen-1-ol. On the other hand, the free volatile compounds of *A. arguta* have been reported to mainly consist of esters, alcohols, and aldehydes, with ethyl butanoate, ethyl hexanoate, (*E*)-2-hexenal, hexanal, and (*Z*)-3-hexenal being the main contributors.[9] On the basis of HS-SPME analysis and GC-O odor intensity, Yang et al. identified ethyl butanoate, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (tentatively identified), 1-penten-3-one, pentanal, hexanal, (*E*)-2-hexenal, 1-octen-3-ol, linalool, terpinen-4-ol, and α-terpineol as important contributors to the aroma of the 'Ananasnaya' cultivar. On a 0−15 Osme scale, most of these compounds were described as having moderate odor intensities,[3−6] except for ethyl butanoate and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, which had high odor intensities of 10 and 9, respectively.[10]

The compounds isolated in the bound volatile extract of *A. arguta* are shown in Table 1.

The concentration of C6 alcohols and aldehydes as free volatiles varies according to the cultivar, ripening stage, and isolation technique, with samples analyzed by headspace showing the highest levels. The reported levels of these compounds as free volatiles were generally higher than the bound levels obtained in the present study[9] (Plant and Food Compound Database, data not published).

2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone has been identified as a key odorant for strawberry and pineapple[16] and is known to occur in glycosylated form in strawberry,[17] pineapple,[18] raspberry,[19] tomato,[20] and blackberry.[21] This compound is also known as Furaneol, pineapple ketone, or strawberry furanone and is characterized by a strong sweet, caramel-like odor[16] and a very low odor threshold (0.04 ppb in water), indicating its importance as an odorant.[22] A carbohydrate, 6-deoxy-D-fructose, is considered to be the key precursor in the biosynthesis of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone and its glycoside.[11]

Another furan, mesifuran (2,5-dimethyl-4-methoxy-3(2*H*)-furanone), has an odor described as fermented fruit, and its odor threshold (0.03 ppb) is also very low. Both 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone and mesifuran have been reported as important odorants in strawberry.[11] Mesifuran is a derivative of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone and is generated through methylation by an *O*-methyltransferase.[23]

Mesifuran has been reported as a free volatile in solvent extracts of *A. arguta* fruit, but at a low concentration of 5.65 μg/kg (Plant and Food Compound Database, data not published), which contrasts with the high concentration of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone found as bound volatile in this experiment (95.36 μg/kg). Yang et al. reported 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone as free volatile in *A. arguta*, but did not determine its concentration because of the lack of sensitivity of HS-SPME for this compound.[10]

The high concentration of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone in bound form justifies further studies on the *A. arguta* aroma glycosides, because there is an obvious source of potential flavor stored within the fruit cells.

Benzyl alcohol and 2-phenylethanol were also detected in significant amounts in bound form (69.48 and 18.88 μg/kg, respectively); 2-phenylethanol is reported for the first time in *A. arguta* fruit. Benzyl alcohol has sweet, flowery notes, whereas 2-phenylethanol has been described as honey, spice, and rose; both have high odor thresholds of 10000 and 750−1100 ppb, respectively.[22,24]

Some aromatic compounds such as benzoic acid, cinnamic acid, and coniferyl alcohol are important precursors of odorous compounds, including benzoate esters and eugenol,[13,25] and were detected in the present study, although they have not been reported as free volatiles in kiwifruit, except for a low amount of benzoic acid in *A. chinensis* (Plant and Food Compound Database, data not published). Eugenol, raspberry ketone, and 4-vinylguaiacol were found for the first time in the fruit of an *Actinidia* species. Eugenol is the compound responsible for the aroma of cloves and has a low odor threshold of 6−30 ppb; 4-vinylguaiacol has a similar clove-like smell and a very low odor threshold (3 ppb), whereas raspberry ketone, as the name indicates, has a strong raspberry aroma and an odor threshold of 100 ppb.[22,24]

Table 1. Compounds Found in the Bound Volatile Fraction of Ripe *A. arguta* Fruit

compound	RI	concn (μg/kg)	SD	ID method[a]
2-butanol[c]	1022	5.17	0.57	MS, RI
hexanal	1086	54.92	1.59	MS, RI, Std
isobutanol	1091	2.26	0.22	MS, RI, Std
3-pentanol	1107	0.45	0.02	MS, RI, Std
2-pentanol	1119	0.97	0.02	MS, RI, Std
3-penten-2-one[c]	1128	0.13	0.03	MS, RI
3-ethyl-3-buten-2-one[c]	1133	0.11	0.02	MS
butanol	1143	0.31	0.02	MS, RI, Std
1-penten-3-ol[c]	1160	0.59	0.02	MS, RI
2,3-dehydro-1,8-cineole[c]	1189	0.57	0.09	MS
3-methyl-2-butenal	1204	1.54	0.08	MS, RI, Std
isoamyl alcohol	1208	13.25	0.26	MS, RI, Std
(*E*)-2-hexenal	1221	4.51	0.06	MS, RI, Std
3-methyl-3-buten-1-ol[c]	1250	1.25	0.02	MS, RI
cyclopentanol	1302	0.21	0.03	MS, RI, Std
prenol	1323	4.64	0.21	MS, RI, Std
3-methyl-1-pentanol[c]	1328	0.09	0.01	MS, RI
hexanol	1354	1.27	0.10	MS, RI, Std
4-hydroxy-4-methylpentan-2-one[c]	1363	3.72	1.97	MS, RI
(*Z*)-3-hexen-1-ol	1386	41.96	2.75	MS, RI, Std
4-methyl-3-penten-1-ol[c]	1390	0.16	0.02	MS, RI
3-octanol	1395	0.38	0.03	MS, RI, Std
(*E*)-2-hexen-1-ol	1408	3.70	0.41	MS, RI, Std
2-methyl-3-hexanol[c]	1437	0.48	0.03	MS
(*Z*)-linalool oxide	1442	0.04	0.01	MS, RI, Std
1-octen-3-ol	1454	4.85	0.42	MS, RI, Std
acetic acid	1471	9.90	0.51	MS, RI, Std
2-ethylhexanol	1492	0.76	0.08	MS, RI, Std
benzaldehyde	1526	1.45	0.10	MS, RI, Std
unknown	1539	0.19	0.07	
2,3-butanediol[c]	1542	0.13	0.02	MS, RI
linalool	1551	0.16	0.02	MS, RI, Std
4-hydroxy-5-methyl-2-hexanone[c]	1578	0.34	0.03	MS
isobutyric acid[c]	1586	0.63	0.15	MS, RI
furfuryl alcohol	1666	3.03	0.13	MS, RI, Std
isovaleric acid	1686	1.98	0.75	MS, RI, Std
α-terpineol	1698	1.17	0.15	MS, RI, Std
1,2-pentanediol[c]	1706	2.12	0.19	MS
3-ethylbenzaldehyde[c]	1709	0.39	0.03	MS
3-(methylthio)-1-propanol	1720	0.36	0.03	MS, RI, Std
α-phellandren-8-ol[c]	1729	0.98	0.12	MS
4,5-octanediol[c]	1735	0.98	0.13	MS
4-hydroxy-4-octanone[c]	1748	10.18	0.48	MS
pentanoic acid[c]	1755	2.22	0.05	MS, RI
methyl salicylate	1777	6.85	0.64	MS, RI, Std
nerol	1802	0.46	0.11	MS, RI, Std
geraniol	1852	2.21	0.29	MS, RI, Std
p-cymen-8-ol[c]	1855	2.64	0.36	MS, RI
hexanoic acid	1864	8.51	1.41	MS, RI, Std
(*E*)-carveol	1870	0.28	0.04	MS, RI, Std
benzyl alcohol	1883	69.48	5.46	MS, RI, Std
2-phenylethanol	1915	18.88	2.13	MS, RI, Std
(*E*)-2,6-dimethyl-3,7-octadiene-2,6-diol[c]	1951	0.67	0.01	MS, RI
δ-octalactone[c]	1971	0.88	0.15	MS, RI

dx.doi.org/10.1021/jf201469c |*J. Agric. Food Chem.* 2011, 59, 8358–8365

Table 1. Continued

compound	RI	concn (μg/kg)	SD	ID method[a]
(*E*)-2-hexenoic acid[c]	1990	1.70	0.58	MS, RI
perilla alcohol[c]	1999	0.31	0.07	MS, RI
phenol[c]	2017	0.86	0.07	MS, RI
2,4-dimethylphenethyl alcohol[c]	2024	0.95	0.06	MS
2,5-dimethyl-4-hydroxy-3(2*H*)-furanone	2041	95.36	5.17	MS, RI, Std
octanoic acid[c]	2075	1.90	0.45	MS, RI
1,7-octadien-3-ol[c]	2087	1.86	0.08	MS
3-acetoxy-4-(1-hydroxy-1-methylethyl)-1-methylcyclohexene[c]	2126	4.26	0.15	MS
(*E*)-1-(ethenyloxy)-3,7-dimethyl-2,6-octadiene[c]	2167	5.16	1.00	MS
eugenol	2178	1.02	0.27	MS, RI, Std
4-vinylguaiacol	2209	6.00	0.61	MS, RI, Std
(*Z*)-8-hydroxylinalool[c]	2278	2.18	0.21	MS, RI
(*E*)-8-hydroxylinalool	2317	6.03	0.60	MS, RI, Std
8-hydroxy-carvotanacetone[c]	2343	3.07	3.10	MS
geranic acid[c]	2356	4.10	2.21	MS, RI
(*E*)-isoeugenol[b]	2365	9.84		MS, RI, Std
2,3-dihydrobenzofuran[c]	2411	2.08	0.74	MS, RI
benzoic acid	2478	26.85	3.26	MS, RI, Std
3-hydroxy-β-damascone[c]	2551	56.43	5.90	MS, RI
vanillin	2597	11.51	1.67	MS, RI, Std
phenylacetic acid[c]	2603	25.12	3.64	MS, RI
3-oxo-α-ionol[c]	2658	40.63	5.04	MS, RI
3-oxo-7,8-dihydro-α-ionol[c]	2738	21.44	3.83	MS, RI
3-hydroxy-7,8-dihydro-β-ionol[c]	2757	26.87	3.85	MS, RI
4-(hydroxymethyl)-2-methoxyphenol[c]	2831	1.40	1.39	MS
4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one[c]	2862	4.87	0.25	MS
o-hydroxyphenethyl alcohol[c]	2867	4.21	0.61	MS
cinnamic acid	2911	36.47	5.61	MS, RI, Std
homovanillic acid[c]	3008	5.41	0.38	MS, RI
raspberry ketone	3023	0.81	0.37	MS, RI, Std
p-hydroxyphenethyl alcohol	3038	31.83	1.52	MS, RI, Std
rhododendrol[c]	3113	1.08	0.02	MS
4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-2-cyclohexen-1-one[c]	3213	23.92	2.06	MS
hexahydro-3-(2-methylpropyl)-pyrrolo[1,2-*a*]pyrazine-1,4-dione[c]	3316	2.75	0.72	MS
coniferyl alcohol	3441	11.02	1.03	MS, RI, Std

[a] Identification methods: MS, RI, Std (authentic standard = positive ID). RI was calculated on a Stabilwax column. [b] Detected only in one of the replicates. [c] Tentatively identified.

A small number of acids were found, probably bound as glycosidic esters. Fatty acids have been reported to be important components of the bound volatiles of mango.[26]

There were a number of norisoprenoid compounds tentatively identified in the bound volatile extract, including 3-hydroxy-β-damascone, 3-oxo-α-ionol, 3-oxo-7,8-dihydro-α-ionol, and 3-hydroxy-7,8-dihydro-β-ionol, which are reported for the first time in *A. arguta*. These compounds, with the exception of 3-hydroxy-β-damascone, are being reported for the first time in an *Actinidia* species. Interestingly, norisoprenoids have not been found in *A. arguta* free volatile extracts. 3-Hydroxy-β-damascone is a major component of grape glycosides;[27] this flavorless compound is related to β-damascenone, a potent odorant.[22] The labile (3*S*,9*R*)- and (3*S*,9*S*)-megastigma-6,7-diene-3,5,9-triol have generated these compounds upon heating under acidic conditions and are considered to be the progenitor compounds. The suggested biosynthetic pathway generates both β-damascenone and 3-hydroxy-β-damascone as products.[28] The aroma of 3-oxo-α-ionol is described as spicy;[24] interestingly, this compound has been described as an insect pheromone.[29] The odor thresholds for 3-oxo-7,8-dihydro-α-ionol and 3-hydroxy-7,8-dihydro-β-ionol have not been reported, and it is thus not possible to evaluate their contribution to the aroma.

Terpenoids found in bound form include linalool, (*Z*)- and (*E*)-8-hydroxylinalool, nerol, geraniol, and prenol, among others. 8-Hydroxylinalool has been reported in glycosidic form in several species of fruit, whereas linalool, geraniol, and nerol have been detected as bound volatiles in kiwifruit.[14] Linalool, nerol, and geraniol are floral compounds; linalool has a low odor threshold of 6 ppb, whereas that of geraniol is 40−75 ppb and that of nerol is 300 ppb. Prenol is a simple terpenoid having an herb-like aroma.[22,24] 8-Hydroxylinalool is a product of the hydroxylation of linalool and a precursor of lilac aldehydes and alcohols.[30]

The bound volatile composition of *A. arguta* differs from that of *A. deliciosa* 'Hayward', the common green kiwifruit, as shown

Table 2. Major Compounds Found in the Bound Volatile Extracts of Ripe *A. arguta* and *A. deliciosa* 'Hayward'[a]

A. arguta			*A. deliciosa* 'Hayward'[b]		
compound	concn (μg/kg)	odor threshold (ppb)[c]	compound[d]	concn (μg/kg)	odor threshold (ppb)[c]
2,5-dimethyl-4-hydroxy-3(2*H*)-furanone	95.36	0.04	benzaldehyde	142.2	350−3500
benzyl alcohol	69.48	10000	(*E*)-2-hexenal	138.5	17
3-hydroxy-β-damascone[d]	56.43	na[e]	hexanal	20.7	4.5−5
hexanal	54.92	4.5−5	3-octanol	11.3	na
(*Z*)-3-hexen-1-ol	41.96	70	2-methylbutanal	8.6	0.35
3-oxo-α-ionol[d]	40.63	na	hexanol	5.4	2500
cinnamic acid	36.47	na	2-phenylethanol	4.9	750−1100
p-hydroxyphenethyl alcohol	31.83	na	geraniol	2.9	40−75
3-hydroxy-7,8-dihydro-β-ionol[d]	26.87	na	butanol	2.3	500
benzoic acid	26.85	na	1-(2-hydroxy-5-methylphenyl)ethanone	2.3	na
phenylacetic acid[d]	25.12	10000	β-damascenone	2.1	0.002
4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-2-cyclohexen-1-one[d]	23.92	na	neral	1.6	30
3-oxo-7,8-dihydro-α-ionol[d]	21.44	na	geranial	1.6	32
2-phenylethanol	18.88	750−1100	unknown	1.6	
4-vinylguaiacol	6.00	3	2-hydroxybenzaldehyde	1.5	na
1-octen-3-ol	4.85	1			

[a] Compounds with concentrations over the odor threshold are also included. [b] Adapted from ref14. [c] Values in water, from refs 22 and 33. Value for 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone from ref 11. [d] Tentatively identified by MS and/or RI, without the use of authentic standards. [e] na = not available.

Table 3. Aroma Components of Ripe *A. arguta* and *A. deliciosa* 'Hayward' Identified in the Free Volatile Extract by GC-MS-O[a]

A. arguta[b]		*A. deliciosa* 'Hayward'[c]	
compound	descriptor	compound	descriptor
ethyl butanoate	fruity, sweet	(*E*)-2-hexenal	green, marzipan, sweet
2,5-dimethyl-4-hydroxy-3(2*H*)-furanone[d]	caramel	1-penten-3-one	herbal, kiwifruit-like
1-penten-3-one	cut grass	hexanal	green, crushed leaves
(*E*)-2-hexenal	green, fruity	ethyl 2-methylpropanoate	melon, bubblegum
1-octen-3-ol	mushroom	dimethyl trisulfide	garlic, rotten
pentanal	green, earthy	(*E*)-3-hexenol	herbal, green
hexanal	cut grass	1-octen-3-one	mushroom
methyl benzoate	fruity, sweet		
ethyl benzoate	floral, celery		
pentanol	tropical fruit		

[a] The main compounds ranked by odor intensity (determined by Osme value in *A. arguta* and time−intensity profiling in 'Hayward'). [b] Adapted from ref 10; compounds were identified by MS, RI, and odor. [c] Adapted from ref 31; compounds were identified by MS, RI, and odor. [d] Tentatively identified by RI and odor.

in Table 2. The major compounds in the bound volatile extract of *A. arguta* were 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, benzyl alcohol, 3-hydroxy-β-damascone (tentatively identified), and hexanal, with 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, hexanal, 4-vinylguaiacol, and 1-octen-3-ol having concentrations above their odor thresholds. The major bound volatile compounds from 'Hayward' reported by Young and Paterson[14] were benzaldehyde, (*E*)-2-hexenal, and hexanal, with (*E*)-2-hexenal, hexanal, 2-methylbutanal, and β-damascenone having concentrations above their odor thresholds. The levels of bound volatiles reported in the present study are generally much higher than what was reported for 'Hayward'. This difference in composition was reflected in the perceived aroma of the hydrolysates, as the sample from *A. arguta* had a strong sweet, pineapple-like smell, whereas the sample from 'Hayward' was reported to have a smell resembling apple.[14]

A comparison of the odor-active compounds of *A. arguta* versus those of *A. deliciosa* 'Hayward' is shown in Table 3. Yang et al. identified ethyl butanoate, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (tentatively identified by odor and RI), 1-penten-3-one, (*E*)-2-hexenal, 1-octen-3-ol, pentanal, hexanal, methyl benzoate, and pentanol as the most important compounds for the aroma of *A. arguta*.[10] On the other hand, Frank et al. determined that the seven most important compounds for the aroma of 'Hayward' are (*E*)-2-hexenal, 1-penten-3-one, hexanal, ethyl 2-methylpropanoate, dimethyl trisulfide, (*E*)-3-hexenol, and 1-octen-3-one.[31] It is noticeable that sweet-smelling compounds are predominant in *A. arguta*, whereas green-smelling compounds predominate in 'Hayward', which agrees with the sensory descriptions of these fruits. In particular, the presence of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone as one of the most important odorants for *A.*

arguta is of interest, as we found this compound to be in high concentration in glycosylated form. Hexanal, 1-octen-3-ol, and (*E*)-2-hexenal were reported as odorants by Yang et al.[10] and also found in the bound volatile portion of *A. arguta* in the present study.

In conclusion, compounds with aromatic rings, derived from the shikimate pathway, as well as C_{13}-norisoprenoids, derived from the degradation of carotenoids,[13,32] showed the highest concentrations. According to other papers, norisoprenoids, in particular 3-hydroxylated and 3-oxo derivatives, such as the ones found in this study, are abundant components of the bound volatile portion of fruits. Norisoprenoid glycosides have been considered to be important flavor precursors, although further degradation is often needed after enzymatic release to generate odorous compounds.[32] There is also a great diversity in the structures of shikimate derivatives reported as glycosides, including important odorants such as eugenol, 4-vinylguaiacol, and vanillin, which were also found in this study. Several compounds reported as important odorants for *A. arguta* were found in bound form, including 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, hexanal, 1-octen-3-ol, and (*E*)-2-hexenal. In particular, the high level of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone in bound form constitutes an interesting point for further research.

AUTHOR INFORMATION

Corresponding Author

*Phone: +64-9-373-7599, ext. 85852; Fax: +64-9-373-7422. E-mail: sy.quek@auckland.ac.nz.

Funding Sources

This work was financed by a New Zealand International Doctoral Research Scholarship to C.V.G. and by NZIRRS + NZFRST Grant C11X1007.

ACKNOWLEDGMENT

We thank Ross Atkinson and Mindy Wang for valuable discussions and Adam Matich for the gift of reference compounds.

REFERENCES

(1) Nishiyama, I. Fruits of the *Actinidia* genus. *Adv. Food Nutr. Res.* **2007**, *52*, 294–324.

(2) Williams, M. H.; Boyd, L. M.; McNeilage, M. A.; MacRae, E. A.; Ferguson, A. R.; Beatson, R. A.; Martin, P. J. Development and comercialization of 'baby kiwi' (*Actinidia arguta* Planch). *Acta Hortic. (ISHS)* **2003**, *610*, 81–86.

(3) Takeoka, G. R.; Guntert, M.; Flath, R. A.; Wurz, R. E.; Jennings, W. Volatile constituents of kiwi fruit (*Actinidia chinensis* Planch.). *J. Agric. Food Chem.* **1986**, *34*, 576–578.

(4) Young, H.; Paterson, V. J.; Burns, D. J. W. Volatile aroma constituents of kiwifruit. *J. Sci. Food Agric.* **1983**, *34*, 81–85.

(5) Bartley, J. P.; Schwede, A. M. Production of volatile compounds in ripening kiwi fruit (*Actinidia chinensis*). *J. Agric. Food Chem.* **1989**, *37*, 1023–1025.

(6) Wang, M. Y.; MacRae, E.; Wohlers, M.; Marsh, K. Changes in volatile production and sensory quality of kiwifruit during fruit maturation in *Actinidia deliciosa* 'Hayward' and *A. chinensis* 'Hort16A'. *Postharvest Biol. Technol.* **2011**, *59*, 16–24.

(7) Friel, E. N.; Wang, M.; Taylor, A. J.; MacRae, E. A. In vitro and in vivo release of aroma compounds from yellow-fleshed kiwifruit. *J. Agric. Food Chem.* **2007**, *55*, 6664–6673.

(8) Günther, C. S.; Matich, A. J.; Marsh, K. B.; Nicolau, L. (Methylsulfanyl) alkanoate ester biosynthesis in *Actinidia chinensis* kiwifruit and changes during cold storage. *Phytochemistry* **2010**, *71*, 742–750.

(9) Matich, A. J.; Young, H.; Allen, J. M.; Wang, M. Y.; Fielder, S.; McNeilage, M. A.; MacRae, E. A. *Actinidia arguta*: volatile compounds in fruit and flowers. *Phytochemistry* **2003**, *63*, 285–301.

(10) Yang, E.; Zhao, Y.; Qian Michael, C. Effect of edible coating on volatile compounds of hardy kiwifruit during storage. In *Flavor and Health Benefits of Small Fruits*; American Chemical Society: Washington, DC, 2010; Vol. 1035, pp 79−94.

(11) Siegmund, B. K.; Leitner, E. Furaneol and mesifuran in strawberries − an analytical challenge. In *Expression of Multidisciplinary Flavour Science*; Blank, I., Wüst, M., Yeretzian, C., Eds.; ZHAW Züricher Hochschule für Angewandte Wissenschaften: Winterthur, Switzerland, 2010; pp 537−540.

(12) Sarry, J. E.; Günata, Z. Plant and microbial glycoside hydrolases: volatile release from glycosidic aroma precursors. *Food Chem.* **2004**, *87*, 509–521.

(13) Reineccius, G. *Flavor Chemistry and Technology*, 2nd ed.; Taylor and Francis: Boca Raton, FL, 2006.

(14) Young, H.; Paterson, V. J. Characterisation of bound flavour components in kiwifruit. *J. Sc. Food Agric.* **1995**, *68*, 257–260.

(15) Williams, P. J.; Strauss, C. R.; Wilson, B.; Massy-Westropp, R. A. Use of C18 reversed-phase liquid chromatography for the isolation of monoterpene glycosides and nor-isoprenoid precursors from grape juice and wines. *J. Chromatogr., A* **1982**, *235*, 471–480.

(16) Buechi, G.; Demole, E.; Thomas, A. F. Syntheses of 2,5-dimethyl-4-hydroxy-2,3-dihydrofuran-3-one (Furaneol), a flavor principle of pineapple and strawberry. *J. Org. Chem.* **1973**, *38*, 123–125.

(17) Mayerl, F.; Näf, R.; Thomas, A. F. 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone glucoside: isolation from strawberries and synthesis. *Phytochemistry* **1989**, *28*, 631–633.

(18) Wu, P.; Kuo, M. C.; Zhang, K. Q.; Hartman, T. G.; Rosen, R. T.; Ho, C. T. Analysis of glycosidically bound 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone in pineapple. *Perfum. Flavor.* **1990**, *15*, 51–53.

(19) Pabst, A.; Barron, D.; Etievant, P.; Schreier, P. Studies on the enzymic hydrolysis of bound aroma constituents from raspberry fruit pulp. *J. Agric. Food Chem.* **1991**, *39*, 173–175.

(20) Krammer, G. E.; Takeoka, G. R.; Buttery, R. G. Isolation and Identification of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone glucoside from tomatoes. *J. Agric. Food Chem.* **1994**, *42*, 1595–1597.

(21) Du, X.; Finn, C. E.; Qian, M. C. Bound volatile precursors in genotypes in the pedigree of 'Marion' blackberry (*Rubus* Sp.). *J. Agric. Food Chem.* **2010**, *58*, 3694–3699.

(22) Leffingwell odor and flavor detection thresholds in water (in parts per billion), http://www.leffingwell.com/odorthre.htm (June 16, 2011).

(23) Wein, M.; Lavid, N.; Lunkenbein, S.; Lewinsohn, E.; Schwab, W.; Kaldenhoff, R. Isolation, cloning and expression of a multifunctional *O*-methyltransferase capable of forming 2,5-dimethyl-4-methoxy-3(2*H*)-furanone, one of the key aroma compounds in strawberry fruits. *Plant J.* **2002**, *31* (6), 755–765.

(24) Acree, T.; Arn, H. Flavornet, http://www.flavornet.org/flavornet.html (April 12, 2011).

(25) Koeduka, T.; Fridman, E.; Gang, D. R.; Vassão, D. G.; Jackson, B. L.; Kish, C. M.; Orlova, I.; Spassova, S. M.; Lewis, N. G.; Noel, J. P.; Baiga, T. J.; Dudareva, N.; Pichersky, E. Eugenol and isoeugenol, characteristic aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 10128–10133.

(26) Lalel, H. J. D.; Singh, Z.; Tan, S. C. Glycosidically-bound aroma volatile compounds in the skin and pulp of 'Kensington Pride' mango fruit at different stages of maturity. *Postharvest Biol. Technol.* **2003**, *29*, 205–218.

(27) Günata, Z.; Wirth Jérémie, L.; Guo, W.; Baumes Raymond, L. Norisoprenoid aglycon composition of leaves and grape berries from Muscat of Alexandria and Shiraz cultivars. In *Carotenoid-Derived Aroma Compounds*; American Chemical Society: Washington, DC, 2001; Vol. 802, pp 255−261.

(28) Suzuki, M.; Matsumoto, S.; Mizoguchi, M.; Hirata, S.; Takagi, K.; Hashimoto, I.; Yamano, Y.; Ito, M.; Fleischmann, P.; Winterhalter, P.; Morita, T.; Watanabe, N. Identification of (3*S*,9*R*)- and (3*S*,9*S*)-megastigma-6,7-dien-3,5,9-triol 9-*O*-β-D-glucopyranosides as damascenone

progenitors in the flowers of *Rosa damascena* Mill. *Biosci., Biotechnol., Biochem.* **2002**, *66*, 2692–2697.

(29) Ishida, T.; Enomoto, H.; Nishida, R. New attractants for males of the Solanaceous fruit fly *Bactrocera latifrons*. *J. Chem. Ecol.* **2008**, *34*, 1532–1535.

(30) Kreck, M.; Püschel, S.; Wüst, M.; Mosandl, A. Biogenetic studies in *Syringa vulgaris* L.: synthesis and bioconversion of deuterium-labeled precursors into lilac aldehydes and lilac alcohols. *J. Agric. Food Chem.* **2002**, *51*, 463–469.

(31) Frank, D.; O'Riordan, P.; Varelis, P.; Zabaras, D.; Watkins, P.; Ceccato, C.; Wijesundera, C. Deconstruction and recreation of 'Hayward' volatile flavour using a trained sensory panel, olfactometry and a kiwifruit model matrix. *Acta Hortic. (ISHS)* **2007**, *753*, 107–119.

(32) Winterhalter, P.; Skouroumounis, G. Glycoconjugated aroma compounds: occurrence, role and biotechnological transformation. In *Biotechnology of Aroma Compounds*; Berger, R., Babel, W., Blanch, H., Cooney, C., Enfors, S., Eriksson, K., Fiechter, A., Klibanov, A., Mattiasson, B., Primrose, S., Rehm, H., Rogers, P., Sahm, H., Schügerl, K., Tsao, G., Venkat, K., Villadsen, J., von Stockar, U., Wandrey, C., Eds.; Springer: Berlin, Germany, 1997; Vol. 55, pp 73−105.

(33) Rychlik, M.; Schieberle, P.; Grosch, W. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants*; Deutsche Forschungsanstalt für Lebensmittelchemie: Garching, Germany, 1998.