Production of Volatile Compounds in Ripening Kiwi Fruit (*Actinidia chinensis*)

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The volatile components of kiwi fruit have been isolated by adsorption on Tenax GC. After desorption the mixture was analyzed by capillary gas chromatography–mass spectrometry. Of the 27 compounds identified, 2-hexenal was the major component in mature fruit but on further ripening ethyl butanoate began to dominate the profile. Lipoxidase inhibition with stannous [tin(II)] chloride confirmed that 2-hexenal was formed by lipid degradation.

This fascinating fruit is a native of China where it originally grew in the margins of the forest in the Yangtze Valley and was known as yang-tao. It was introduced into European gardens in 1900 and was first planted in New Zealand in 1909 where it was known as the Chinese Gooseberry, presumably because of the similarity of its interior texture to the American gooseberry. The sprawling deciduous climbing plant first attained commercial acceptance in New Zealand where two varieties are now grown: Hayward and Bruno. The fruit of both varieties is light brown with a "furry" skin and is 75–100 mm long. The fruit of the Bruno variety is narrow and cylindrical while that of the Hayward variety is more rounded. The interior of the fruit is bright green with tiny black seeds radiating from a central core.

One reason for the fruit's great commercial success is that it is reported to be very durable and stands up well to the long sea voyages required to supply markets in Europe, America, and Japan. However, the ripening process undoubtedly continues during transport, and considerable alteration in flavor is reported to occur.

While several reports have appeared dealing with the volatile flavors of the kiwi fruit, none have investigated the variation in components with ripening of the fruit. In the most recent report (Takeoka et al., 1986) 48 compounds were identified by GC/MS of a flavor isolate produced by vacuum distillation of blended pulp. However, methods involving steam distillation or distillation under reduced pressure have been criticized as potentially producing artifacts (Strauss et al., 1968). Furthermore, while the profiles produced by headspace methods often differ from those obtained by classical methods, they may more closely resemble the situation experienced by consumers of the fruit.

EXPERIMENTAL SECTION

Sample Preparation. Several batches of kiwi fruit were obtained (NZ Hayward, Australian Hayward, and Australian Bruno). Mature fruit were selected in all cases on the basis of their solids content (19% for Hayward and 14% for Bruno). For ripening trials fruit were allowed to ripen in air at 25 °C at a relative humidity of 65%. After a period of 3 days under these conditions, the fruit were still firm but the flesh was more juicy. These fruit are described in Table I as ripe. After a total of 7 days under these conditions, the skin of the fruit had developed a wrinkled appearance and the flesh was soft to the touch. Such fruit are described as very ripe.

Trapping of Volatiles. Four ripe fruit (250 g) free from any skin damage were selected from each batch for analysis. The peeled fruit were macerated in a glass blender equipped with stainless steel blades and a sealed glass lid with two glass necks. Two glass-lined stainless steel tubes (1.5 mm × 115 mm) packed with Tenax GC (5–10 mg, 60–80 mesh) were connected in series between the flask and a rotary vacuum pump. A nitrogen bleed was installed in the other neck of the vessel and purified nitrogen drawn over the surface of the fruit pulp and through the Tenax tubes at 15 cm^3/min for 2 h (1.8 dm^3 of N_2). The nitrogen line was equipped with a buffer vessel provided with a bleed to atmosphere that ensured that the pressure within the sample flask remained close to 1 atm during sampling. The tubes were conditioned before use by heating for 20 min at 250 °C with helium flow (5 cm^3/min). Every 15 min the blender was switched on for 30 s in order to break the cells and release the volatile components. Breakthrough was determined by connecting four tubes in series and measuring the amount of breakthrough into the third and fourth tube as a percentage of the amount trapped by the first two tubes. For all substances studied (with the exception of acetaldehyde) the breakthrough into the third tube was less than 2%. For acetaldehyde the breakthrough was about 10%, and so since only two tubes were used in this investigation, results for acetaldehyde cannot be regarded as quantitative. For quantitative estimation, a known quantity of *n*-decane was added as an internal standard before the fruits were blended. For enzyme inhibition studies, the whole ripe fruit were blended with 300 cm^3 of 0.01 M stannous chloride and the volatiles sampled immediately as described above. A control sample from the same batch of fruit was blended with 300 cm^3 of distilled water and sampled in the same manner. All analyses were performed in duplicate.

Gas Chromatography–Mass Spectrometry. A Carlo Erba Fractovap 4160 gas chromatograph equipped with a 50 m × 0.23

Table I. Volatile Components Identified in Kiwi Fruit

peak no.	compound	KI	amount, % mature	amount, % ripe	amount, % very ripe
1	acetaldehyde	363	1.5	6.8	0.1
2	2-propanone	530		0.2	
3	ethyl ethanoate[a]	595	3.9	4.7	1.6
4	methyl propanoate	610	0.9	0.2	
5	1-penten-3-one	650	2.0	0.3	
6	3-pentanone	672		0.7	
7	ethyl propanoate	685		0.4	
8	methyl butanoate[a]	705	2.0	25.4	11.1
9	*dimethyl disulfide	728	1.0		
10	*2-methyl-2-butenal	730		0.3	
11	hexanal[a]	778	6.2	0.9	0.2
12	ethyl butanoate[a]	790	0.6	14.2	69.4
13	ethyl 2-butenoate	825		0.2	0.1
14	2-hexenal[a]	832	76.1	25.5	7.1
15	(*E*)-3-hexen-1-ol[a]	840		0.2	0.1
16	*(*Z*)-3-hexen-1-ol	845		0.2	0.1
17	2-hexen-1-ol[a]	852	1.3	2.0	0.2
18	1-hexanol[a]	860	0.4	2.0	0.2
19	propyl butanoate	880		0.4	0.2
20	ethyl pentanoate	882		0.3	0.2
21	methyl hexanoate	905		1.5	1.0
22	*2-methylpropyl butanoate	935		0.3	0.2
23	butyl (or 1-methylpropyl) butanoate	978		0.4	0.3
24	ethyl hexanoate[a]	985	2.9	9.5	6.1
25	octanol	1061		0.2	
26	methyl benzoate[a]	1078	0.4	1.8	1.2
27	ethyl benzoate	1154	1.0	1.9	1.2

[a] Previously identified. See text.

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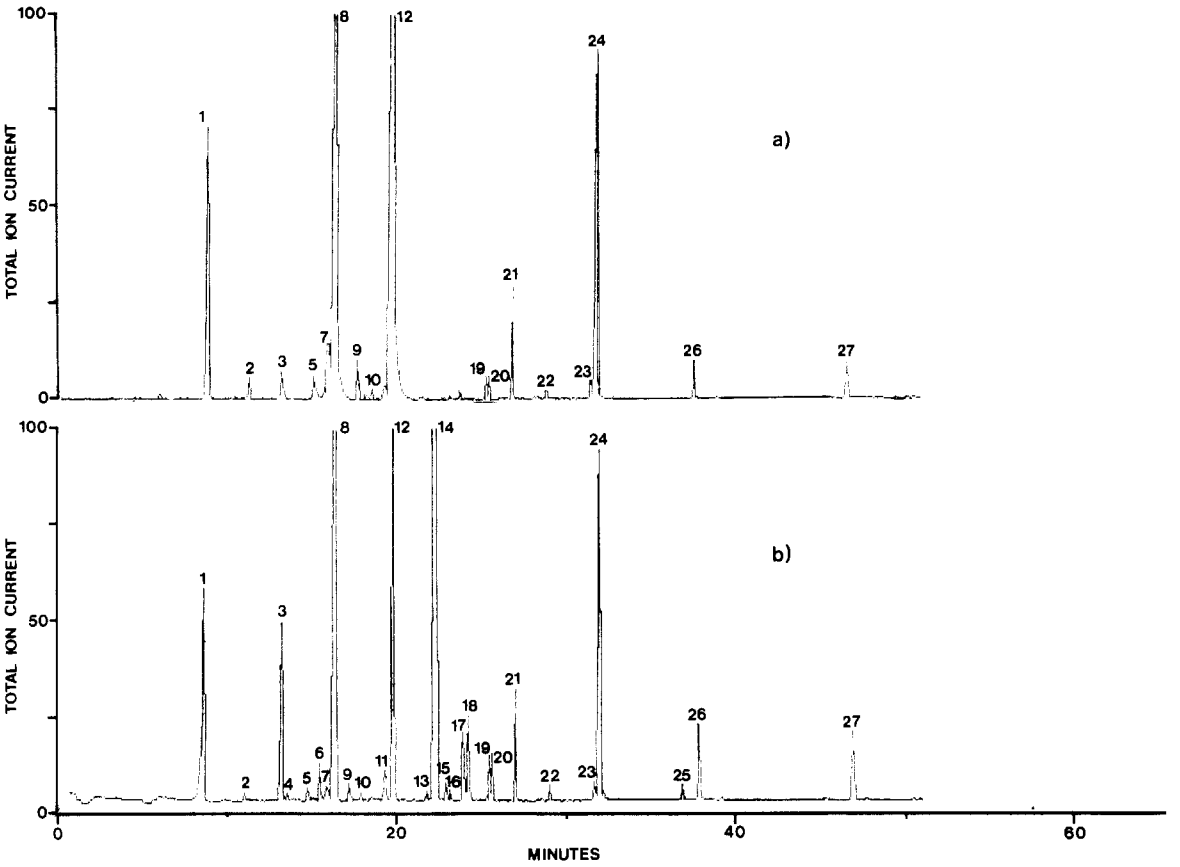

Figure 1. Capillary gas chromatogram of kiwi fruit volatiles obtained by trapping the headspace on Tenax GC with subsequent desorption (250 °C). Temperature programmed from 30 °C (2 min isothermal) to 200 °C at 2 °C/min on a 50 m × 0.25 mm (i.d.) BP-1 column. Key: (a) treated with 0.01 M stannous chloride; (b) treated with distilled water. The peak numbers correspond to the numbers in Table I.

mm (i.d.) BP-1 column (SGE Scientific; bonded silicone phase) was employed. The trapped volatiles were desorbed from each pair of tubes simultaneously in a modified SGE unijector at 250 °C for 2 min (flow rate 5 cm^3/min) and cryogenically focused at −50 °C on the first 0.6 m of the column with liquid nitrogen as coolant. The column was then temperature-programmed as follows: 30 °C hold 2 min and then to 200 °C at 2 °C/min. Helium carrier gas was used at a flow rate of 1.5 cm^3/min (30 °C). The injector temperature was 220 °C. The exit from the gas chromatograph was connected directly to the source of a Kratos MS-25 mass spectrometer operating in the electron-impact mode at an ionization voltage of 70 eV and scanning continuously at 1 s/decade. The transfer line was held at 220 °C while the ion source block was maintained at 180 °C. The data were acquired with use of a Kratos fast preprocessor interface and processed with DS-55 software. Chromatograms were obtained as plots of total ion current (TIC) versus time (Figure 1).

Mass spectral identifications were initially made by matching against the NBS library, but confirmation of identification was made, where possible, by comparison of mass spectra and Kovats indices with those of authentic samples and with published data (Ryhage and von Sydow, 1963; Jennings and Shibamoto, 1980). Those components for which no authentic samples were available are marked with an asterisk in Table I and can be regarded as only tentative identifications.

RESULTS AND DISCUSSION

Figure 1b shows a typical chromatogram of headspace volatiles from macerated kiwi fruit, and the components identified are listed in Table I, which also shows a comparison between the relative amounts of volatile constituents and the changes occurring during ripening. The compounds marked *a* have been previously detected in kiwi fruit (Takeoka et al., 1986) by vacuum distillation of the blended pulp. The major components detected by us in the headspace are identical with those observed previously. However, many of the minor components detected are biochemically related to those observed previously. For example, in common with Takeoka, we observe the methyl and ethyl esters of butanoic acid, but the propyl and butyl esters of this acid are also present in our samples. Hexanoic and pentanoic acids are esterified with different alcohols in our samples, and we observe ethyl benzoate as well as methyl benzoate while Takeoka reports only the latter.

No significant differences were observed between the various varieties of fruit studied. The profile for the mature fruit is dominated by the presence of 2-hexenal as observed by Takeoka. However, dramatic changes were found to occur as the fruit was allowed to ripen further at room temperature. The changes in the total amounts of volatile substances, reported as relative peak areas, are shown in Figure 2. Total volatiles increased in a sigmoidal manner similar to that observed for ripening bananas (Mattei and Paillard, 1973). While the amounts of most volatile components increased only slightly during ripening, there was a dramatic increase in the amount of ethyl butanoate after 2–4 days (at which stage the outer skin had developed a distinct wrinkled appearance). This 7-fold increase in ethyl butanoate was accompanied by a fall in the amount of 2-hexenal, the minimum in the 2-hexenal level corresponding with the increase in ethyl butanoate level (Figures 3 and 4). Since ethyl butanoate has a threshold level of around 15 ppb (Lindsay et al., 1969), increases in the concentration of this component would have a dramatic effect on flavor.

The production of several short-chain aldehydes has been reported to occur when cucumber tissues are ruptured (Flemming et al., 1968), and it is a well-established fact

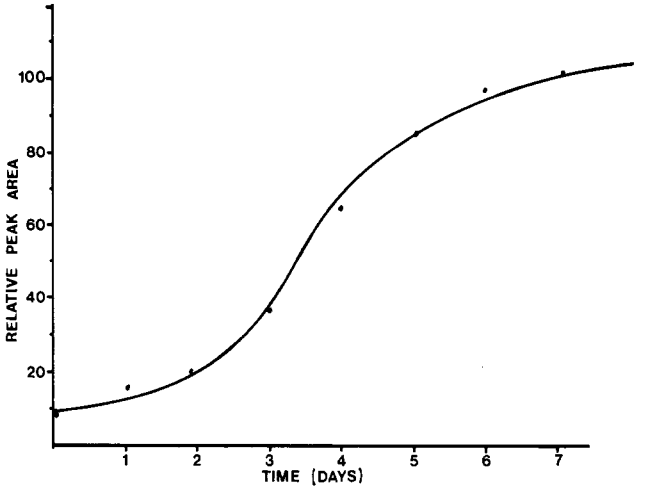

Figure 2. Total volatiles in ripening kiwi fruit.

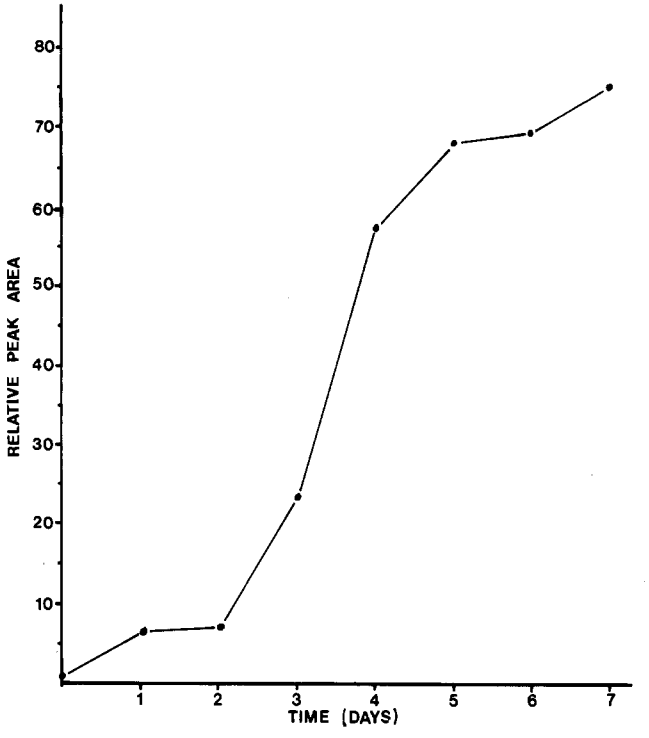

Figure 3. Levels of ethyl butanoate in ripening kiwi fruit.

that 2-hexenal is normally formed as a result of enzymic activity (lipoxygenase and hydroperoxide lyase) on linoleic and linolenic acids (Drawert et al., 1966). The fruits used here were found to contain an average of 0.8% triglyceride material, and that this was the source of 2-hexenal in the present situation was confirmed by analysis of the headspace volatiles produced on maceration of the mature fruit in the presence of stannous [tin(II)] chloride, a known inhibitor of lipoxygenases (Josephson et al., 1984). The presence of the inhibitor markedly suppressed the formation of 2-hexenal (as shown in Figure 1a) but had little effect on the ester components.

The increase in the amount of ethyl butanoate is probably unrelated to the decrease in concentration of 2-hexenal, as the two components arise from different biosynthetic pathways. The change in 2-hexenal level is presumably related to a change in concentration or activity of one of the enzyme systems involved. In fact, lipoxygenase activity has been reported to decrease in grapes at later stages of maturity (Crouzet et al., 1984). On the other hand, it has also been shown (Yabumoto et al., 1978) that total ethyl ester levels in the headspace vapors of the

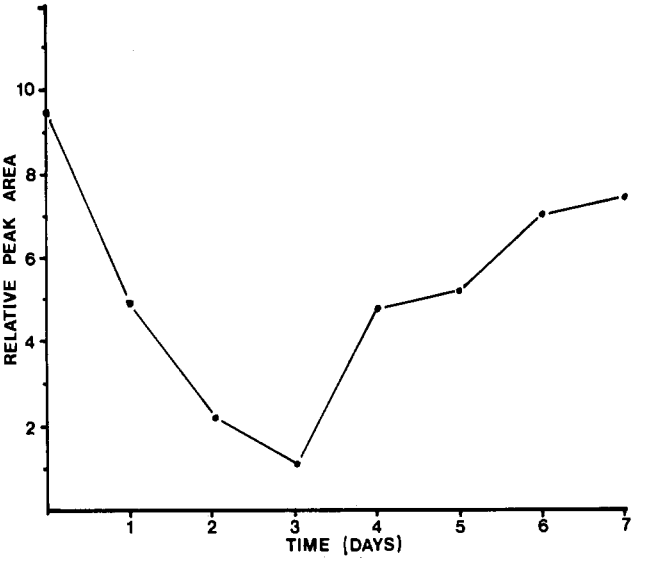

Figure 4. Levels of 2-hexenal in ripening kiwi fruit.

cavity in musk melons increase for up to 8 days after harvest.

Registry No. Acetaldehyde, 75-07-0; 2-propanone, 67-64-1; ethyl ethanoate, 141-78-6; methyl propanoate, 554-12-1; 1-penten-3-one, 1629-58-9; 3-pentanone, 96-22-0; ethyl propanoate, 105-37-3; methyl butanoate, 623-42-7; dimethyl disulfide, 624-92-0; 2-methyl-2-butenal, 1115-11-3; hexanal, 66-25-1; ethyl butanoate, 105-54-4; ethyl 2-butenoate, 10544-63-5; 2-hexenal, 505-57-7; (*E*)-3-hexen-1-ol, 928-97-2; (*Z*)-3-hexen-1-ol, 928-96-1; 2-hexen-1-ol, 2305-21-7; 1-hexanol, 111-27-3; propyl butanoate, 105-66-8; ethyl pentanoate, 539-82-2; methyl hexanoate, 106-70-7; 2-methylpropyl butanoate, 539-90-2; butyl butanoate, 109-21-7; 1-methylpropyl butanoate, 819-97-6; ethyl hexanoate, 123-66-0; 1-octanol, 111-87-5; methyl benzoate, 93-58-3; ethyl benzoate, 93-89-0.

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