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In Vitro and *in Vivo* Release of Aroma Compounds from Yellow-Fleshed Kiwifruit

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Comparisons were made between the aroma volatiles of the yellow-fleshed kiwifruit, "Hort16A", at two different stages of eating ripeness: firm and soft. The firm fruit contained a small number of aroma compounds that the soft fruit did not contain. In general, however, the largest difference between the two firmness categories was in the levels of esters, with the soft fruit containing higher concentrations and a larger number of esters than the firm fruit. *In vitro* analysis directly after maceration using atmospheric pressure chemical ionization mass spectrometry (APCI−MS) showed the relative importance of the most intense aromas between fruit at the two different firmness stages and was used to compare the release rates of aromas. A comparison of the aroma concentrations from gas chromatography mass spectrometry (GC−MS) and APCI−MS headspace analyses showed that the APCI−MS headspace showed less bias toward enzymatically generated lipid degradation compounds. A GC-sniffing study showed that many of the most intense compounds, acetaldehyde, hexanal, ethyl butanoate, and (*E*)-2-hexenal but not ethanol, showed odor activity in macerated fruit. In addition, dimethyl sulfide (DMS), a volatile present at very low levels in the fruit, also appeared to be an important contributor to the odor. *In vivo* analyses also showed much higher levels of aroma compounds in the soft fruit compared to the firm fruit, with evidence of persistence of some compounds, including DMS. There were a number of similarities between the breath profiles of the two panelists, which confirmed the importance of DMS in "Hort16A" aroma.

KEYWORDS: Kiwifruit; *Actinidia chinensis*; APCI−MS; GC−MS; breath analysis; dimethyl sulfide

INTRODUCTION

The aroma of the green-fleshed kiwifruit cultivar, "Hayward" (*Actinidia deliciosa* (A. Chev.) Liang et Ferguson var. deliciosa "Hayward") has already been well-studied (*1*−*3*). A second cultivar of kiwifruit (*A. chinensis* Planch. var. chinensis "Hort16A"), with a very different volatile aroma profile from that of "Hayward", was commercialized in New Zealand in 1995. This fruit, which is marketed as ZESPRI GOLD, has yellow flesh and is described as having sweet, banana, and blackcurrant-like flavors (*4*). Differences in the sensory evaluations of these fruit are partly due to the different proportions of the same volatile components and are also due to the presence of cultivar-specific impact compounds. In general, "Hayward" is sensorily characterized by C6 aldehydes and alcohols, with some esters produced upon ripening. In contrast, "Hort16A" is characterized less by "green/grassy" aromas and more by fresh tropical notes. However, previous studies of kiwifruit flavor in which the tissue has been macerated and the aroma released in the headspace (HS) collected over periods of 20−30 min (*1*, *5*) provide little information on the volatile profile likely to be experienced by a consumer. During maceration, cellular disruption allows enzymes and substrates, previously separated in different compartments of the cells, to interact and consequently liberate aroma compounds. The development of aromas by this mechanism has been well-documented (*6*, *7*), and although this lipid degradation process occurs to some degree during mastication in the mouth, the extent of the reaction is highly time-dependent (*1*, *7*, *8*). To avoid the bias in the aroma profile toward these lipid degradation compounds, a rapid technique for measuring the changes in volatile components is required.

Atmospheric pressure chemical ionization mass spectrometry (APCI−MS) (*9*) and proton-transfer reaction mass spectrometry (PTR−MS) (*10*, *11*) are techniques that allow the changes in the HS concentrations of released aromas to be followed and quantified in real time. They have been used to study the release of key volatile compounds from fruit and vegetables as a function of time (*12*−*14*). Although these experiments were conducted *in vitro*, using dynamic HS measurements, the results provide information on the rate of volatile release directly after maceration, which can aid in understanding the temporal release

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of volatiles during eating. Most APCI−MS and PTR−MS publications describe the use of these instruments to analyze changes in the concentrations of aromas exhaled from the nose, providing rapid and detailed information on the breath-by-breath release of volatile compounds (*9*, *15*, *16*). Breath-by-breath profiles are thought to be very similar to those sensed by the olfactory epithelium and are the most accurate method available for monitoring real-time aroma release while eating. However, one of the tradeoffs for temporal resolution is that compounds that fragment to give the same mass under chemical ionization conditions (isobaric compounds) cannot be resolved. This is perhaps one of the reasons that much of the real-time release work has been carried out using model systems with well-defined volatile components (*16*−*18*) rather than applied to the release of volatiles from real food products (*12*−*14*).

The aim of this work is to gain more information on the aroma profile of "Hort16A" kiwifruit as presented to and appreciated by the consumer. In particular, APCI−MS is used to obtain an understanding of the temporal effects of aroma release *in vitro* and *in vivo*. Traditional aroma studies with gas chromatography mass spectrometry (GC−MS) are based on the collection and preconcentration of aromas over long time periods and do not take into account the fact that the rate of release of aromas may change over time, particularly those released from living biological tissues. "Hort16A" kiwifruit share a number of aroma components in common with the "Hayward" variety, and it may be important to define more exact ratios of all of the major odor-active components of each fruit to be able to reconstruct more authentic natural flavors representing these two varieties. This is more difficult when the method of sampling introduces a bias in the ratios of aromas present, such as long sampling times. In addition, understanding the sequence of the aroma release may be key for the reconstruction of natural flavors because the temporal aspects of volatile release have been shown to be related to aroma perception (*16*). This research may aid in the development of more authentic "Hort16A"-type flavors.

MATERIALS AND METHODS

"Hort16A" Kiwifruit Samples. The "Hort16A" kiwifruit were sourced from a commercial orchard in Te Puke, New Zealand, in an attempt to minimize the variability of the fruit. Throughout transportation to the laboratory in the U.K., the kiwifruit were held between 0 and 2 °C and were then stored at 4 °C until required. At 24 h prior to sampling, the fruit were removed from refrigeration and allowed to come to ambient temperature. Firmness measurements were carried out with a hand-held Effegi penetrometer. A small disc of skin was removed from the cheek of the fruit (midway between the calyx end and the stem end) using a vegetable peeler. The penetrometer fitted with a 8 mm plunger was pushed into the fruit to the prescribed depth on the tip, and the measurements of the rupture pressure on the gauge were recorded as kgf. This measurement was carried out on two positions on each fruit, on opposite cheeks.

Extraction of Aroma Compounds from "Hort16A" for Analysis by Gas Chromatography. "Hort16A" kiwifruit were peeled, cut in half longitudinally, and then pulped (*5*). Three half fruit were pulped and mixed together, and the aroma compounds were sampled from the homogeneous pulp. The aromas were sampled with Chromosorb 105 cartridges and were then analyzed by GC−MS in the same manner as detailed in Matich et al. (*5*).

Identification of Odor-Active Aroma Compounds of "Hort16A". Volatiles were sampled from the fruit HS, at the same time as the APCI−MS analyses, for GC-sniffing experiments and for confirmation of the compounds responsible for the APCI−MS ions observed. In both cases, four "Hort16A" kiwifruit were peeled and cut in half longitudinally, and halves of each fruit were blended together using a commercial blender (Phillips, type HR2810/A). A subsample of the pulp (approximately 60 g) was then weighed into a 250 mL Schott bottle. The Schott bottle lid contained an inlet and outlet port. Dried air was introduced at a flow rate of 45 mL min^{-1} through the inlet and exited through a Tenax TA cartridge fitted at the outlet. The HS was collected for 5 min. The Tenax TA cartridges were desorbed at 150 °C with cryofocussing at the injector end of a capillary column into a gas chromatograph (HP5890). The column effluent was split 1:1 between a Fisons MSD mass spectrometer and an odor port for the GC sniffing. The effluent went to the MSD only for confirmation of compounds present. The volatiles were separated using a 30 m × 0.32 mm BP1 capillary column (SGE, Milton Keynes, U.K.) with a 1 μm film thickness. The oven temperature was initially held at 40 °C, increased at 5 °C min^{-1} to 100 °C, and then finally increased at 10 °C min^{-1} to 210 °C. The MS detector was held at 250 °C. Mass spectra (*m*/*z* 28−320) were collected and analyzed using Mass Lab (MicroMass, Manchester, U.K.) software. Identifications were based on mass spectra and calculation of retention indices. Further identification and verification of retention indices was based on a comparison with authentic reference compounds (see **Table 1** for a list). The heated transfer line of the odor port was held at 150 °C. Two panelists were used for the GC-sniffing experiments; one was presented with five replicates of fruit, and the other was presented with three replicates of fruit. Panelists were given a timer, which was started at the same time as the GC−MS run, and were asked to write down the time at which an odor was noticed and any characteristics of that odor, if recognized.

Instrumental Aroma Analyses Using APCI−MS. *Static HS Analysis of Kiwifruit Samples.* The whole peeled kiwifruit were placed inside a modified commercial blender (Phillips, type HR2810/A) as described in Boukobza et al. (*12*). For static HS measurements, 2 of the 3 apertures were sealed. The third aperture was used to sample the HS at a flow rate of 10 mL min^{-1} into the APCI−MS source. The tissue was macerated (3−5 s) in this blender, while the HS was sampled. The HS above the fruit macerate was sampled for a further 5 min after maceration. The ion current intensities at specific time points were later converted to HS concentrations (ppbv) using a calibration method based on authentic reference compounds as described in Taylor et al. (*19*).

Dynamic HS Analysis of Kiwifruit Samples. The dynamic HS analyses were carried out on an initially whole but peeled kiwifruit using the system as described in Boukobza et al. (*12*), except that the HS was sampled at a flow rate of 5 mL min^{-1} and was diluted with a dried air gas flow (∼100 mL min^{-1}). The HS was sampled for a total of 5 min after maceration.

Breath-by-Breath Analysis. Two panelists were asked to inhale, place a portion of kiwifruit (∼20 g) in their mouth, and then exhale through their nose into the APCI−MS at time, $t = 0$. Panelists were asked to continue breathing (through their nose) and chewing in a regular pattern with their mouth closed, until the fruit was gone. The gas flow rate into the mass APCI−MS was 30 mL min^{-1}. Several exhalations were monitored during the eating process, such that changes in concentrations of a number of volatile compounds were measured in each discrete breath. Panelists consumed 4 portions of kiwifruit of each firmness for replication. Panelists were also asked to conduct "background" breaths at regular intervals during the analysis. This was a control, whereby the panelist breathed into the APCI−MS without any fruit in their mouth. For the breath-by-breath analyses, the ions monitored were modified from those chosen for the dynamic HS analyses based on the GC-sniffing results. Six ions [representing acetaldehyde, hexanal, hexenal, ethyl propanoate, ethyl butanoate, and dimethyl sulfide (DMS)] were monitored in batches of 3 ions along with acetone. Hexenal represents both (*Z*)-3-hexenal and (*E*)-2-hexenal, isobaric compounds that cannot be distinguished using this method. Acetone is a metabolite naturally present in expired breath and is a useful marker to ensure that panelists are breathing regularly and can provide information on when panelists have swallowed (*19*). For the breath analyses, hexanal was monitored on *m*/*z* 101, instead of *m*/*z* 83, the ion on which it was measured in the HS analyses. The total ion chromatogram (TIC), i.e., all ions in the range *m*/*z* 40−210, was also monitored.

Data Manipulation and Statistical Analyses. The chromatograms generated in the MassLynx software (Micromass, Manchester, U.K.) were integrated so that peak heights and corresponding times could be extracted. These data were exported into Microsoft Excel and further processed using *t* tests, *F* tests, and analysis of variation (ANOVA).

Table 1. APCI–MS Ions (13) Used *in Vitro* and *in Vivo* To Study "Hort16A" Aroma and the Source of the Authentic Reference Compounds Used for Identification and Quantification

compound id by GC–MS	main ion and ion fragments at a cone voltage of 14 V[a]	potential interferences for the main ion	commercial source of authentic standard
acetaldehyde	45[b,c] 71 (25) 89 (22) 61 (10)		Sigma-Aldrich (Dorset, U.K.)
dimethyl sulfide	63[c]	acetaldehyde adduct	Sigma-Aldrich (Dorset, U.K.)
ethanol	47[b] 93 (95)	ethyl butanoate fragment	Sigma-Aldrich (Dorset, U.K.)
ethyl butanoate	117[b,c] 89 (30) 47 (30) 59 (15)	methyl pentanoate, propyl propanoate, butyl acetate, propyl formate, other branched esters	Firmenich (Geneva, Switzerland)
ethyl hexanoate	145[b]	butyl butanoate, other branched esters	Sigma-Aldrich (Dorset, U.K.)
ethyl pentanoate	131[b]	methyl hexanoate, propyl butanoate, butyl propanoate, pentyl acetate, hexyl formate, other branched esters	Firmenich (Geneva, Switzerland)
ethyl propanoate	103[b,c]	methyl butanoate, propyl acetate, butyl formate, other branched esters	Firmenich (Geneva, Switzerland)
eucalyptol	137[b] 101[c]	many monoterpenes, methyl benzoate	Sigma-Aldrich (Dorset, U.K.)
hexanal	83[b] (54) 99 (42) 117 (16)	hexenol, pentadienal	Sigma-Aldrich (Dorset, U.K.)
hexanol	85[b] 83 43 (20)	pentenal	Sigma-Aldrich (Dorset, U.K.)
hexenal	99[b,c]		Sigma-Aldrich (Dorset, U.K.)
methyl propanoate	89[b]	ethyl acetate, ethyl butanoate fragment, acetaldehyde dimer	Sigma-Aldrich (Dorset, U.K.)

[a] Where there is more than one ion listed, the main ion used for monitoring is underlined. Values in parentheses next to ion fragments denote the percentage intensity of the fragment ion compared to the main ion; no number indicates 100%. [b] Ions monitored for *in vitro* experiments. [c] Ions monitored for *in vivo* experiments.

Post-ANOVA, Fisher's least significant difference test was used to determine the minimum difference between comparisons that could be considered statistically significant. Statistical significance is quoted at the level $\alpha = 0.05$.

RESULTS AND DISCUSSION

Flavor Profiles of "Hort16A" Using GC−MS. It is well-known that the volatile components, as well as the sensory properties, of climacteric fruit, such as kiwifruit, are markedly affected by fruit ripeness (*20*). The eating firmness range of "Hort16A" is approximately 0.5−1.0 kgf (*21*). However, the range commonly presented to consumers as ripe is 0.6−0.8 kgf (*4*). Above this, the fruit are rather firm, and below 0.5 kgf, the fruit are soft and easily damaged. The volatile compounds collected from the HS above macerated "Hort16A", using "purge and trap" sampled onto Chromosorb cartridges, are presented in **Table 2**. The first set of data is from macerated kiwifruit in the firmness range of 0.71−0.82 kgf. Fruit with values lying in this range are termed "firm" throughout this paper. The second ripeness level occurs toward the overripe end of the eating firmness range (0.45−0.57 kgf) and is referred to as "soft" fruit. Low levels of 6-methyl-5-hepten-2-one, methyl pentanoate, pentadecane, 1-methyl-4-isopropylbenzene (*p*-cymene), β-pinene, and sabinene were found in the firm fruit but not in the soft fruit. **Table 2** shows that the concentration of most of the esters, as with many fruits, increased with increased fruit ripeness. In the soft fruit, butyl propanoate, ethyl but-2-enoate, ethyl pentanoate, ethyl propanoate, methyl butanoate, and propyl butanoate were found, whereas they had not been identified in the firm fruit at all. All other components were in common between the fruit at the two firmness categories, albeit at different levels (see **Table 2**). Lipid oxidation products, such as (*E*)-2-hexenal and hexanal, were recorded at higher levels than the esters in both firm and soft fruit. These compounds are formed when the tissue is disrupted and the lipid degradation enzymes mix with the lipids from different cellular compartments; the extent of this reaction is time-dependent. As the volatiles listed in **Table 2** were preconcentrated onto Chromosorb resin over a period of 20 min, it is unclear whether these levels of the lipid oxidation products would contribute significantly to the aroma experienced by a consumer while eating a piece of kiwifruit (i.e., over 5−15 s).

Correlating APCI−MS Ions to the Compound Identified in "Hort16A" by GC−MS. The APCI−MS scanned all ions in the range of *m*/*z* values from 40 to 210 to survey the major volatiles released into the HS above macerated "Hort16A". Measuring the static HS of the kiwifruit macerate *in vitro* using a direct sampling method, such as APCI−MS (*22*), provides an intermediate between HS sampling by GC−MS and breath-by-breath analysis by APCI−MS. The static HS measurement using the APCI−MS allows for a larger amount of material to be sampled than in breath analysis while monitoring the rapid changes in aroma immediately after maceration over relatively short periods of time (i.e., measurements can be made every 11 ms). **Table 1** lists the most intense ions measured from this HS analysis, the volatiles likely to be represented by these ions and some potential (isobaric) interfering compounds from kiwifruit. Duplicate GC−MS analyses of the HS of three fruit from the same batch trapped onto Tenax TA provided a qualitative measure of the volatile compounds released from that batch of fruit to enable a correlation of the ions to specific volatile compounds. All assignments were made based on the compounds that had the highest concentration in GC−MS studies. The tradeoff for being able to study the release of the

Table 2. Compounds Identified in "Hort16A" Kiwifruit by GC–MS at Two Different Ripeness Levels Measured by Firmness (Firm and Soft)

	firmness 0.71–0.82 kgf (firm)		firmness 0.45–0.57 kgf (soft)	
compound id by GC–MS	concn in ng/g FW	std. dev.	concn in ng/g FW	std. dev.
4-methyl-3-penten-2-one	1.1	1.1	3.8	2.6
6-methyl-5-hepten-2-one	1.6	0.4	0.0	0.0
acetaldehyde	8.4	3.1	23.4	22.6
acetic acid	16.1	3.9	5.5	8.7
acetone	11.0	3.5	8.1	2.0
butanal	1.3	1.7	4.4	0.4
butyl propanoate	0.0	0.0	1.5	2.5
1-methyl-4-isopropylbenzene	1.7	0.3	0.0	0.0
decanal	10.4	6.4	5.2	2.8
ethanol	595.5	423.3	320.2	132.2
ethyl but-2-enoate	0.0	0.0	0.5	0.5
ethyl acetate	1.1	0.2	3.9	0.3
ethyl benzoate	0.8	0.8	0.6	0.5
ethyl butanoate	3.6	0.6	9.1	2.3
ethyl octanoate	0.6	1.1	1.4	2.5
ethyl pentanoate	0.0	0.0	0.3	0.6
ethyl propanoate	0.0	0.0	2.4	1.7
ethyl benzaldehyde	1.4	0.2	1.9	1.4
eucalyptol	4.0	0.8	2.6	1.6
geranyl acetone	2.9	0.8	3.0	1.2
hexanal	22.0	3.8	14.6	1.0
hexanol	3.1	0.1	1.3	0.2
(*E*)-hex-2-enal	76.2	11.2	43.1	10.6
(*E*)-hex-2-enol	7.3	0.6	4.4	1.0
methanol	0.4	0.7	0.5	0.8
methyl benzoate	0.1	0.2	2.5	1.3
methyl butanoate	0.0	0.0	39.8	6.0
methyl pentanoate	0.3	0.6	0.0	0.0
methyl propanoate	3.3	1.3	4.6	1.3
octanal	2.6	1.1	1.3	1.3
pent-1-en-3-ol	1.1	0.9	1.1	0.1
pentadacane	3.6	3.1	0.0	0.0
pinene, β-	0.4	0.7	0.0	0.0
propyl butanoate	0.0	0.0	0.3	0.6
sabinene	0.5	0.5	0.0	0.0
tetradecane	21.5	9.3	6.6	6.0
toluene	2.3	0.6	4.1	0.3

volatiles from fruit in real time is that, with the current technologies available, it is impossible to resolve all of the issues with interference of isobaric ions (*23*, *24*). Only ions that could be confidently assigned to major components of "Hort16A" aroma were monitored (see **Table 1**).

In total, 11 ions, representing some of the most highly concentrated volatiles in "Hort16A" were chosen for monitoring using APCI−MS (see **Table 1**). The compounds relating to these ions were individually dissolved in water at low levels (<1 ppm) to confirm that they showed minimal (if any) fragmentation/interferences, at a cone voltage of 14 V (see **Table 1**).

Real-Time Release of Key Compounds Immediately after Maceration. Static HS release profiles of selected volatiles are shown in **Figure 1** for both firm and soft fruit. A total of 5 of the 11 volatiles monitored are shown as an example of the profiles. These profiles show that low levels of volatiles were released from the whole peeled fruit prior to maceration in the blender (points S_i and F_i in **Figure 1**), mainly the result of tissue disruption caused by peeling the fruit. Immediately after maceration of the firm fruit (point F_{ii} in **Figure 1**), the intensity of 7 of the 11 ions monitored increased, although at different rates. Hexanol, ethyl propanoate, ethyl pentanoate, and ethyl hexanoate did not increase significantly above background levels in these fruit (only ethyl propanoate is shown in **Figure 1** as an example). This is perhaps not surprising for the esters, because apart from ethyl butanoate, these compounds only reach significant levels in ripened fruit. Eucalyptol showed a rather unusual HS profile (**Figure 1**), in that the concentration prior to maceration when the fruit was whole but peeled increased faster than after maceration. The reason for this was not investigated, but this finding may suggest that terpene compounds collect mostly in cells located in the outer pericarp, close to the skin, rather than being distributed throughout the inner pericarp.

Maceration of soft fruit as shown in **Figure 1** showed that all 11 volatiles were at levels distinguishable above the background. Ethanol (and acetaldehyde, data not shown) reached equilibrium levels much faster than any of the other volatiles, almost immediately after maceration in both firm and soft fruit.

The collection of the static HS over a 5 min period allowed for the calculation of HS concentrations at a number of time points both before and after maceration at time, $t = 0$. Concentrations are presented in **Table 3**. The time point $t = -30$ represents 30 s prior to maceration, i.e., whole but peeled fruit. **Table 3** shows that ethanol, followed by acetaldehyde, was at the highest concentration at both stages of fruit ripeness. In firm fruit, these compounds were followed by hexenal and hexanal, but in the soft fruit, ethanol and acetaldehyde were followed by esters. This is clearly different from GC studies (as detailed in **Table 2**), where lipid degradation compounds continued to dominate the aroma profile of soft fruit. Although the lipid degradation products were still key components of the static HS profile, these data showed less bias toward them than previous reports and are likely to be a more realistic profile of the ratios of the aromas that the consumer perceives.

A comparison of the real-time HS concentrations of firm fruit with the soft fruit (see **Table 3**) showed that, although the concentration of ethanol, acetaldehyde, and the esters increased markedly as the fruit ripened, the hexenal, hexanol, and hexanal concentrations were not substantially different between firm and soft "Hort16A".

Eating is a dynamic process that involves the dilution of volatile compounds in a tidal air flow as part of retronasal aroma delivery (*25*). The blender apparatus was modified to include a gas flow flushing through the HS of the blender prior to sampling by MS. Although the profiles were similar to those obtained under the static HS conditions, in general, the dilution effect of the gas flow resulted in much lower HS concentrations, such that only the ions for acetaldehyde, ethanol, hexanal, hexenal, and ethyl butanoate could be distinguished from the background profile in firm fruit. In the soft fruit, eucalyptol and methyl propanoate were additionally above background levels. The dynamic HS release curves for a number of these volatiles, which have been normalized to the maximum intensity obtained up to time $t = 5$ min, from both firm and soft fruit are plotted in **Figure 2**. There was a good correlation between the replicates of each volatile (labeled 1 and 2 for soft and 3 and 4 for firm fruit).

The change in concentration of the compounds over time directly after maceration may indicate relative rates of release in the mouth. Although the maximum concentration of ethanol was different depending upon the fruit ripeness (as shown by the difference in ion intensity shown in **Figure 1**), the rate of release of ethanol was essentially the same from both firm and soft fruit (as can be seen in both **Figures 1** and **2**). Ethanol was not only at the highest maximum concentration, but it also released fastest from macerated "Hort16A", with the maximum rate of release occurring within the first 10 s after maceration.

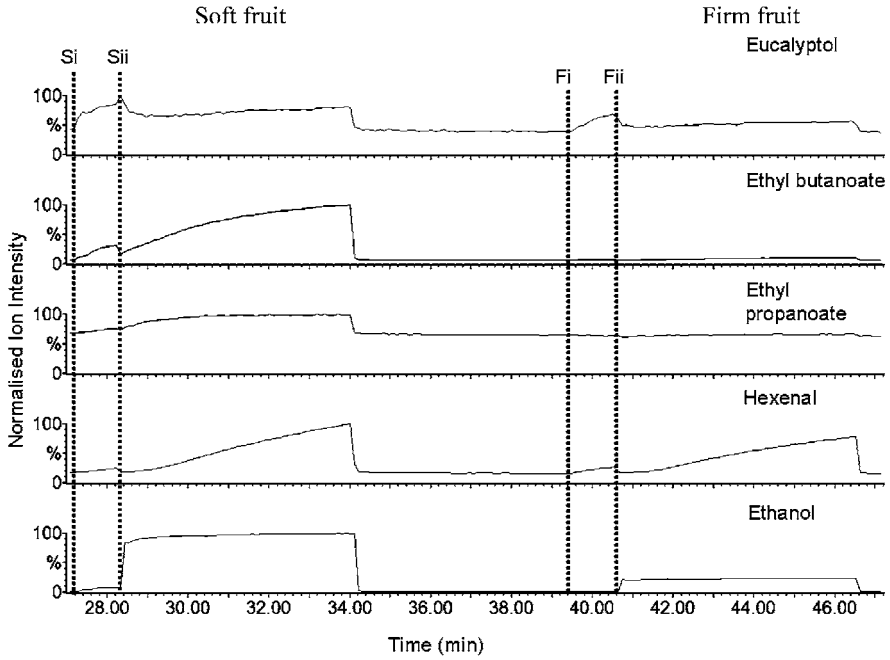

Figure 1. Static HS release of a number of compounds from soft fruit (S, on left) and firm fruit (F) "Hort16A". The dotted lines mark when the blender was initially connected to MS for two samples (S_i and F_i) and also the point at which maceration began for 5 s in the blender (S_{ii} and F_{ii}).

Table 3. Concentration of "Hort16A" Compounds per 1 g of Fruit in Static HS at Different Time Points before and after Maceration at $t = 0$ for Eating Ripe and Overripe Fruit

static HS concn after maceration (ppbv/1 g of FW)	firm fruit					soft fruit				
compound	$t = -30$	$t = 0$	$t = 30$ s	$t = 1$ min	$t = 5$ min	$t = -30$	$t = 0$	$t = 30$ s	$t = 1$ min	$t = 5$ min
acetaldehyde	2.2	2.9	7.4	7.6	12.0	12.3	18.5	69.6	71.4	69.3
ethanol	3.1	9.0	152	170	196	45.8	52.2	1107	1146	1226
ethyl butanoate	0.59	0.26	0.23	0.59	1.7	7.0	5.9	11.2	18.6	44.4
ethyl hexanoate								0.02	0.09	0.41
ethyl pentanoate	0.05	0.06	0.00	0.01	0.03	0.01	0.00	0.03	0.05	0.19
ethyl propanoate						0.25	0.30	0.94	1.6	2.0
eucalyptol	0.30	0.19	0.07	0.10	0.22	0.62	0.87	0.33	0.34	0.56
hexanal	0.87	1.20	1.29	1.93	9.9	0.53	1.10	2.20	2.59	12.3
hexanol					0.26	0.04	0.27	0.18	0.29	1.10
hexenal	1.7	0.45	0.65	3.7	28.2	1.5	2.0	1.0	3.5	29.9
methyl propanoate			0.01	0.34	1.2	3.8	5.1	8.2	11.3	19.7

The rate of release of acetaldehyde was similar to that for ethanol and was also not markedly different between firm and soft fruit. The rates of release of hexanal and hexenal were also essentially the same from firm and soft fruit. However, these compounds showed the lowest initial rate of release. For hexanal, there appeared to be a lag of almost 1 min before the maximum rate of release was obtained. For hexenal, this lag time was slightly reduced. Interestingly, ethyl butanoate and methyl propanoate (in soft fruit) also seemed to show a small lag in release of approximately 10 s, after which the maximum rate was obtained. Ethyl butanoate, identified in both firm and soft fruit but at a much higher level in the riper fruit, did show different rates of release between the fruit of different firmness.

These *in vitro*-based experiments highlighted the low levels of volatiles present in firm "Hort16A" (with the exception of ethanol and acetaldehyde) and showed the importance of minimizing the sampling time after maceration of kiwifruit tissue to prevent bias in the aroma profiles. The *in vitro* experiments also clearly showed that a number of the main components released at different rates. A fine balance in the release rates of volatiles, such as ethyl butanoate, hexenal, and hexanal, may be the key to a recognizable characteristic aroma. For example, although these volatiles are common components between "Hayward" (*1*−*3*) and "Hort16A", it may be that the balance in release rates of the many components contributes to the difference in perceived aroma. The importance of the rate of release will be much greater for those components that have a higher concentration to threshold ratios.

Odor Activity of "Hort16A" Volatiles Using GC Sniffing. Observations from the *in vitro* experiments suggested that some of the most intense components of "Hort16A" aroma were released in large quantities over very short time periods after maceration. However, it is well-known that it is not necessarily the most concentrated compounds that characterize the odor. Although the aroma-active components in "Hayward" kiwifruit essence and puree have been determined (*3*) using gas chromatography−odor port analysis (GC−O), no such work has been published on "Hort16A" fruit. Preliminary GC-sniffing experiments were conducted to determine whether any of these components present in high quantities were odor-active. Two panelists were used, one with 3 replicates of pulped "Hort16A" samples and the other with 5 replicates of pulped "Hort16A" samples. Panelist one identified 5 compounds that had the same retention times in all three GC-sniffing runs. These were

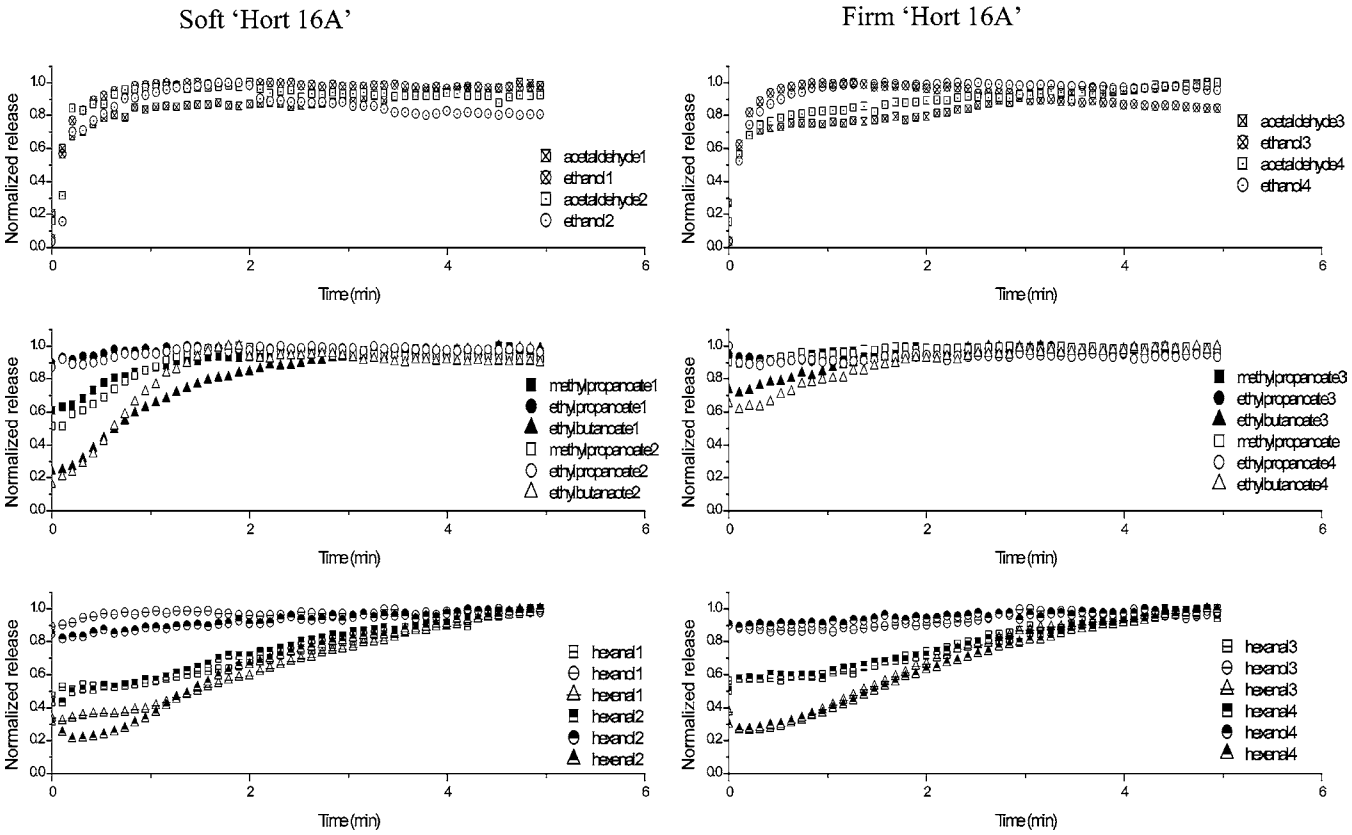

Figure 2. Dynamic release profiles of soft and firm "Hort16A" fruit.

identified by a comparison to the MS library as acetaldehyde, DMS, hexanal, ethyl butanoate, and (*E*)-2-hexenal. The retention times and mass spectra were confirmed by a comparison to authentic standards. The odor descriptors for these compounds can be seen in **Table 4**. Panelist two also identified all of the same compounds as panelist one, often using similar descriptors.

These experiments showed that indeed many components at the highest concentrations also contributed to the characteristic odor of "Hort16A". In terms of highly odiferous components present at very low levels in "Hort16A", the GC-sniffing results provided another potential target aroma to monitor in the *in vivo* experiments using the APCI−MS. DMS is an example of a component that is found at relatively low levels in the fruit but makes a large contribution to the odor. DMS was described as having sulfur, onion-like aromas. It is likely that the reason that DMS was not identified in the previous GC studies is due to the way in which the Chromosorb cartridges were dried with N_2 (g) prior to analysis to remove excess water from the resin, a process which is not required when using Tenax TA traps.

Although both panelists identified many other odoriferous compounds in the HS, the descriptors used to identify the compounds were not always identical, suggesting that more rigorous GC-sniffing experiments should be carried out to gain a full understanding of the odor-active components of "Hort16A" flavor.

Release of "Hort16A" Volatiles during Eating. Previous studies of real-time aroma release in the mouth have shown that the largest variability can come from between panelists; hence, data from the two panelists were considered independently.

Six ions were monitored in the *in vivo* analyses, but only those for acetaldehyde and DMS were measurable in the nose while consuming firm "Hort16A" fruit for panelist one. The concentrations of the other volatiles in each discrete breath were not significantly different from those obtained from the background breath (i.e., prior to consumption of the fruit), suggesting that many of the volatiles in firm fruit were below or close to the limit of detection (~1 ppbv) for this type of instrumentation. This observation confirms the results seen in the *in vitro* studies and emphasizes the low volatile levels found in real food systems. Acetaldehyde and DMS reached average (±1 standard error) maximum breath concentrations of 17.4 (±1.6) and 2.2 (±0.24) ppbv, respectively, for approximately 20 g of fruit.

As observed in the HS experiments, the maximum concentrations in the nose were significantly higher in the soft fruit compared to the firm fruit for most compounds. Although the levels of DMS in both firm and soft fruit were significantly higher than the background, they were not significantly different for the firm and soft fruit. The average maximum in-nose concentrations for the soft fruit for both panelists are given in **Table 5**. These results were clearly different from those obtained from the static HS experiments, where acetaldehyde was at the highest concentration *in vitro*. However, it has already been shown that the ratio between concentrations measured in the HS and in the nose is not straightforward and is instead highly dependent upon specific physicochemical properties of the aroma compound (*26*).

The average maximum intensity of each volatile per exhalation while eating fruit was compared to background breath levels for each volatile. **Figure 3** shows the raw intensity of ion 101 (hexanal) over 12 discrete breaths of panelist one as an example. In the inset of **Figure 3**, the raw intensity of ion 101 is plotted over time for the four individual replicates of soft fruit. The average intensities of all compounds, except for DMS, were at a maximal level in the second exhalation and then diminished as breathing continued (and as the fruit was cleared from the mouthspace). The average intensity of DMS was largest in the first breath. Statistical analysis showed that, for all compounds,

Table 4. Compounds Identified in GC-Sniffing Analysis of "Hort16A" Macerated by Two Panelists

compound identified by GC sniffing	odor descriptor[a] panelist one	frequency of identification (out of 3)	odor descriptor[a] panelist two	frequency of identification (out of 5)
acetaldehyde	ethereal, chemical (2), solvent	3	burnt, alcoholic (2), sweet (4), fruity	4
dimethyl sulfide	sulfur (2), onion	3	sulfur, off-smell, Hort16A (2), fruity, chemical (2), almond (2), apricot, foul	5
hexanal	apple, green (2), fruity, candy	3	chemical (2), sweet (2), green (2), phenolic (2), almond	5
ethyl butanoate	fruity (3)	3	chemical (3), sweet (3), vomit/sickly (2), green/grass (2), aldehyde (2), fruity	5
(*E*)-hex-2-enal	fruity, green, plastic, sweet, almond	3	rubber (3), green (2), cinnamon and apple, pungent, sweet, phenolic	5

[a] The values in parentheses after a descriptor indicate the number of times that the descriptor was used in separate replicates.

Table 5. Average Maximum In-Nose Concentrations of "Hort16A" Volatiles in Overripe Fruit Eaten by Two Panelists

compound	panelist one in-nose concn (ppbv)	standard error	panelist two in-nose concn (ppbv)	standard error
acetaldehyde	39.9	4.7	95.7	17.0
dimethyl sulfide	2.4	0.38	1.7	0.08
ethyl propanoate	2.3	0.3	9.4	1.7
ethyl butanoate	66.4	4.2	188.9	24.1
hexanal	10.3	0.28	12.7	0.71
hexenal	0.5	0.05	0.6	0.10

except ethyl butanoate, there was no significant difference in the average intensities of the first and second breaths, including DMS. For ethyl butanoate, the second breath was significantly larger than the first.

Persistence of Volatiles in the Breath. The soft fruit also showed markedly higher concentrations in total breath volatiles, measured using TIC (data not shown), compared to the background breath, even after the fruit had cleared from the mouthspace (usually around the third to fourth breath). The TIC levels remained significantly higher for the soft fruit even after the eighth breath; this sustained level of breath volatiles showed the persistence of some compounds (*27*). Identification of these compounds is vital for understanding the aftertaste or aftersmell of a food product (*28*). DMS showed the greatest persistence after consuming soft fruit; the breath concentration of the soft fruit only returned to the level of the background breath after breath number seven. Ethyl butanoate, ethyl propanoate, and hexenal were also significantly higher, at least until breath number five (well after the time after which the fruit was assumed to have been swallowed). These volatiles in a specific combination may be responsible for "Hort16A" aftertaste. DMS, which has not been reported as a key aroma contributor to "Hayward" kiwifruit, is likely to be one of the key components in differentiating these varieties.

Time Taken To Reach Maximum In-Nose Intensity while Eating "Hort16A". Volatiles are released from the nose in discrete exhalations, rather than continuously; therefore, instead of calculating a rate of release for each volatile, the time taken to reach maximum intensity *in vivo* was calculated. The effect of ripeness on the time taken to reach maximum intensity ($T_{I,max}$) could only be considered for DMS and acetaldehyde, because these were the only compounds detected in both firm and soft fruit. There was no significant difference in $T_{I,max}$ for DMS or acetaldehyde between fruit of different ripeness. However, in both firm and soft fruit, DMS had the shortest $T_{I,max}$. In the soft fruit, the $T_{I,max}$ of DMS was followed by hexanal, ethyl

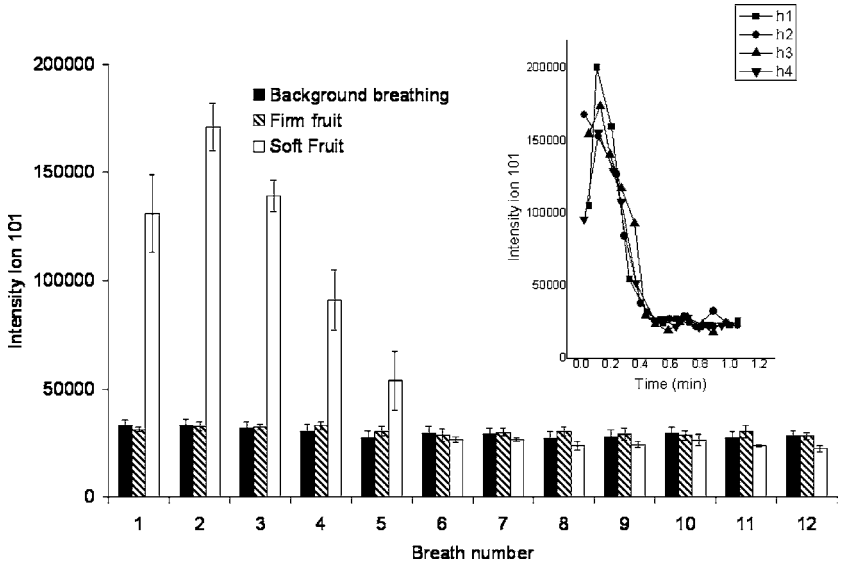

Figure 3. Average intensity of ion 101 (hexanal) over 12 breaths while consuming firm and soft Hort16A (error bars are 1 standard error). (Inset) Individual release profiles (measured as peak height, *h*) of four replicates of soft fruit.

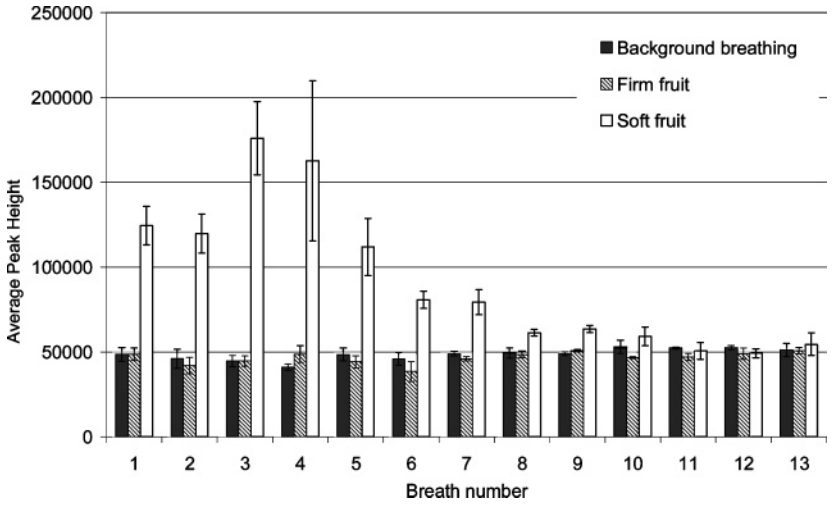

Figure 4. Peak heights for hexenal in each breath of panelist two (error bars are 1 standard error) when eating firm and soft fruit compared to background breathing.

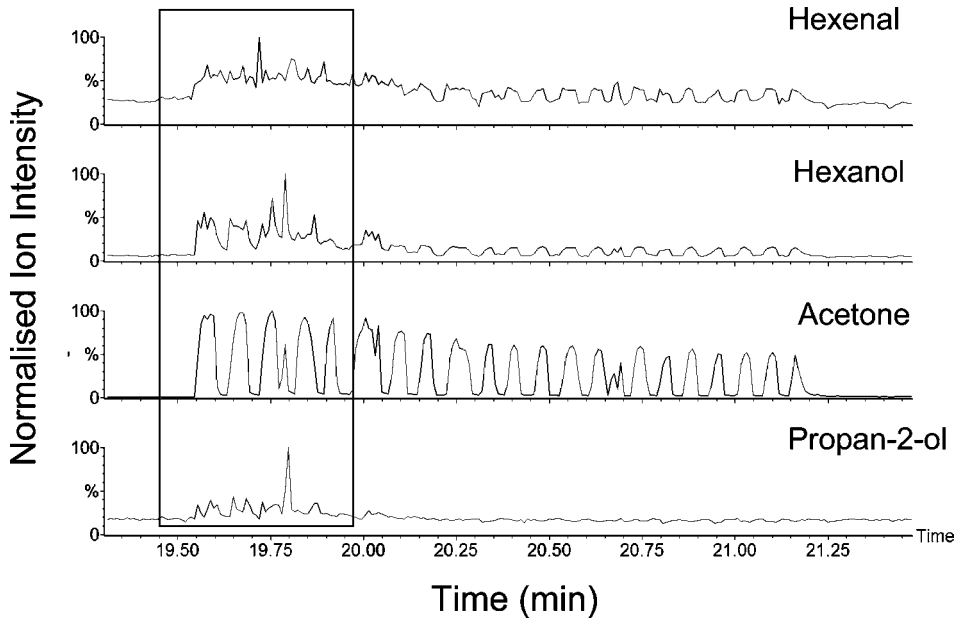

Figure 5. Swallowing action in the raw data (e.g., at 19.80 min in breath number 3), which causes an increase in all volatiles during and immediately after the swallow.

butanoate, hexenal, acetaldehyde, and then ethyl propanoate. The $T_{l,max}$ values of DMS and acetaldehyde in the firm fruit were significantly different. This was also the case for the soft fruit. In addition, in the soft fruit, the $T_{l,max}$ value of DMS was also significantly shorter than that of hexenal and ethyl propanoate. Although the odor activity of each volatile is likely to be more important than the total concentration reached, it is likely that these different rates of release will play a role in defining the characteristic odor sensed while eating a piece of kiwifruit.

Comparing Breath Profiles of Two Panelists. Interestingly, the average maximum in-nose concentration of DMS, hexanal, and hexenal from the soft fruit was very similar for the two panelists. Panelist two, however, had much higher maximum in-nose concentrations of the esters (see **Table 5**). This difference could be attributed to panelist two receiving slightly riper fruit than the first panelist or may be due to the specific interaction of the esters with the different oral mucosa. The saliva of each panelist is likely to contain different amounts of salivary proteins and salts. Salivary mucin has previously been show to decrease the volatility of mid-chain-length esters (*28*, *29*).

In general, the average concentrations of each volatile per breath for the second panelist were more variable across replicates than for the first panelist. This is likely because of different mastication patterns of the two panelists. For example, it was observed that panelist one swallowed the fruit after relatively few chews, whereas panelist two masticated the fruit longer before swallowing. Mastication not only pumps air (enriched with volatiles) out of the mouth and into the nose but has also been shown to cause fluctuations in the nasal airflow rate (*30*). Also, for panelist two, only DMS showed significantly higher ion intensities in firm fruit than in the background breath, compared to both DMS and acetaldehyde for panelist one (data not shown). Similar to panelist one, all volatiles were significantly larger than the background for soft fruit.

Volatiles in the breath of panelist two generally took longer to return to the background than panelist one; i.e., maximal levels were observed after four or five breaths compared to one

or two for panelist one (**Figure 3** shows an example for panelist one, and **Figure 4** shows an example for panelist two). There are a number of potential reasons for this situation. It may simply be that panelist two took longer to swallow the fruit bolus, or it may be the result of less interaction between the volatiles and the oral mucosa of panelist two. In addition, an increased rate of respiration has been shown to be important in the decrease of the gas-phase volatile concentration (*31*), and it seems reasonable to assume that different people naturally respire at different rates. It can also be seen (**Figure 5**) that panelist two swallowed regularly throughout the breath-sampling period (at breath numbers three and six). Swallowing has been shown to be followed by an exhalation that transports volatiles from the pharynx to the nasal cavity (*30*).

These results combined show the large difference in volatile levels between fruit of different firmness from within the eating ripeness range. Volatile release from firm fruit is at very low levels both in *in vitro* and *in vivo* experiments and is difficult to monitor reliably without some form of preconcentration. Aroma release from soft fruit, however, is at relatively higher levels and is more suitable for these types of experiments. That said, the inherent variability in this type of experiment, which deals with real living systems (both fruit and humans), means that only very large differences meet the criteria of statistical significance, particularly if the number of replicates tested is relatively small. The ability to monitor aroma release from fruit directly after maceration or during mastication is, however, very desirable. First, these results indicate the relative importance of compounds derived from lipid degradation compared to those that are endogenous. It is also useful to follow the release of the major compounds in the nose to gain knowledge on the components that are likely to be involved in aftertaste. Although this work confirmed that there are elements of the eating process that are highly individual, there were a number of similarities between panelists. The maximum in-nose concentrations of hexanal, DMS, and hexenal were remarkably similar, suggesting that, although the time taken for maximum release differs between panelists, approximately the same amount of aroma was delivered. Finally, this study shows that, although the most intense volatiles from "Hort16A" kiwifruit were observed in the nose during eating, one of the volatiles present at low levels in the fruit plays a more important role. DMS was perceived as odor-active by both panelists and behaved similarly in terms of the speed of release in the nose and in persistence in the breath.

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