Effect of Variety, Time of Eating, and Fruit-to-Fruit Variation on Volatile Release during Eating of Tomato Fruits (*Lycopersicon esculentum*)

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Tomato fruits were eaten by one individual while expired air from the nose was sampled into an atmospheric pressure ionization-MS fitted with an air-sampling interface. The release of seven volatile compounds from four different types of tomato was followed, and the concentration of volatiles on the breath with time was determined. Different tomato types contained significantly different concentrations of each compound, resulting in a type-specific volatile "fingerprint". However, the temporal aspects of the release profile of each compound were consistent across all types and were specific for that compound, except for enzymically released compounds from Delice tomatoes. Three classes of compound were identified in terms of their release characteristics. Some compounds (e.g., isobutylthiazole) are formed during ripening and show rapid release, other compounds are formed by the lipid oxidation pathway when tissue is macerated and are released rapidly (hexenal) or more slowly (hexenol) depending on the enzyme reactions that form them.

INTRODUCTION

The volatile composition of tomatoes has been determined previously using solvent extraction and chromatographic techniques coupled with mass spectrometry (Petro-Turza, 1987) or by headspace analysis from above macerated fruits (Linforth et al., 1994a; Baldwin et al., 1991; Buttery et al., 1987; Kazeniac and Hall, 1970). Tomato volatiles can be classified into those compounds formed in the fruit during ripening (e.g., isobutylthiazole, 3-methylnitrobutane; Buttery and Ling, 1993) and those formed when the fruit is macerated either by cutting or by eating (the C6 products of the lipid oxidation pathway; Galliard et al., 1977).

Because the lipid oxidation pathway consists of a sequence of enzymes [lipase, lipoxygenase, lyase, isomerase, alcohol dehydrogenase (ADH); Petro-Turza, 1987; Riley et al., 1996; Bicsak et al., 1982], it is expected that volatiles will be released at differing rates determined by the number of enzymic steps required and the activity of specific enzymes. The first volatile compounds formed are the aldehydes [hexanal and (*Z*)-3-hexenal] derived from linoleic and linolenic acids, respectively. Isomerase can convert (*Z*)-3-hexenal to (*E*)-2-hexenal, and both aldehydes can potentially be converted to the corresponding alcohols by ADH.

Previously in our laboratory, changes in volatile profile during eating of tomatoes were measured using Tenax trapping of volatiles from the nose followed by GC/MS (Linforth et al., 1994a,b). This provided information on the pattern of volatile release during eating. However, with the Tenax method, it was necessary to sample volatiles from several tomatoes, eaten sequen-

tially, to obtain sufficient volatile compounds for GC/MS analysis. Measurement of fruit-to-fruit variation was therefore not possible, and the technique was also very time-consuming, which prevented adequate replication.

The release of volatiles from foods can be measured on a breath-by-breath basis in real time by direct introduction of breath into an atmospheric pressure ionization source of a mass spectrometer (API-MS) (Taylor and Linforth, 1996; Taylor, 1996). The technique draws air from the nose of the subject eating food, directly into the ionization chamber of the MS. Water vapor present in air acts as the chemical ionization agent for the volatiles present. Ions formed in the source are separated in a single-quadrupole MS with the result that resolution (1 amu) is entirely on a mass basis. This means that compounds producing ions with the same mass cannot be differentiated, including stereoisomers and positional isomers. The dead volume of the system is low so that effective real time analysis occurs with a usual sampling frequency of 0.1 Hz giving 50 data points on a normal 5 s breath cycle. The MS is usually operated in single ion monitoring (SIM) mode with the ions of choice preprogrammed into the MS. The sensitivity of the API-MS system depends on the type of volatile compound analyzed, but the typical lower limit of detection is 10–100 ppb (by volume). Some preliminary data on the volatiles found in expired air from the nose during eating of tomatoes have been presented previously (Linforth et al., 1996; Taylor and Linforth, 1996). These data showed temporal differences in volatile release with significant differences between the C6 aldehydes and alcohols.

Work on tomato fruits is difficult because they are in a state of metabolic change with flavor changes occurring from day to day as they ripen, and there also appears to be considerable fruit-to-fruit variation (Gal-

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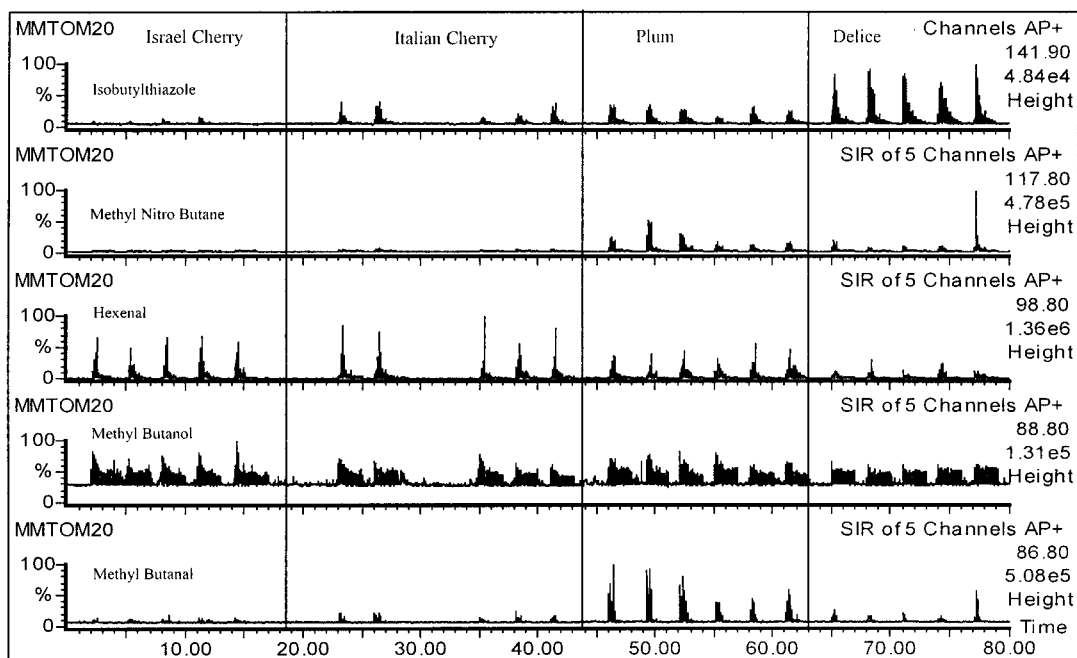
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Figure 1. Graph showing raw breath-by-breath data from the four types of tomato. Continuous sampling of volatile concentrations, tomatoes eaten in sequence, time *X*-axis, intensity *Y*-axis, single subject, five volatiles, ion detected and maximum peak height are shown. Time is expressed in minutes.

liard et al., 1977). Hence, it is necessary to perform many replicates and yet analyze samples over the shortest time. API-MS is a rapid technique (one analysis takes 3 min) so that adequate replication can be achieved over a short time period to minimize differences resulting from metabolic change. In this paper, the API-MS technique was applied to study volatile release from four types of tomato, to estimate the fruit-to-fruit variation within one type, and to compare the release characteristics of preformed volatiles (e.g., isobutylthiazole) with C6 aldehydes and a C6 alcohol formed by the lipid oxidation pathway.

MATERIALS AND METHODS

Procedures. A platform quadrupole mass spectrometer (Micromass, Altrincham, U.K.) operating in the API positive ion mode was fitted with a custom-built air-sampling interface (Linforth and Taylor, 1997). The operating parameters of the API source were optimized while headspace of each of the selected volatiles was continuously introduced. Since the API-MS system discriminates solely on the basis of molecular weight, it cannot differentiate positional isomers (e.g., 2- and 3-methylbutanal), stereoisomers such as (*E*)-2-hexenal and (*Z*)-3-hexenal, or the corresponding alcohols. For this reason the generic terms methylbutanal, hexenal, and hexenol are used in the results. The cone voltage was adjusted to give maximum sensitivity for the MH$^+$ ion or, in the case of hexenol, the MH$^+$ − H$_2$O ion. The compounds and the cone voltages were as follows: methylbutanal, 18; methylbutanol, 18; hexenal, 18; hexenol, 12; hexanal, 12; isobutylthiazole, 37; methylnitrobutane, 18. In SIM mode, the Micromass platform software allows a different cone voltage to be used with each ion monitored; thus, the conditions can be optimized for each analyte. For all compounds the corona pin voltage used was 4 kV.

For the eating experiments, one person ate portions of the tomatoes (20 g) while resting one nostril at one end of a plastic tube (12 mm × 50 mm). The tidal flow of air from the nostril passed back and forth through the tube. Part of this airstream was sampled into the API source (30 mL/min) through a capillary tube (0.53 mm i.d.), inserted through the wall of the plastic tube at right angles to the direction of flow. As the subject breathed out, expired air was sampled but, on inspiration, laboratory air was sampled.

Four types of tomato (Delice, Italian plum, Italian cherry, and Israeli cherry) were obtained from Mack Multiples (Paddock Wood, U.K.). Portions of tomato were cut from intact fruit, weighed (20 ± 1 g), and placed in the mouth of a subject with minimal delay (typically 30 s). The tomato portions were eaten according to a fixed protocol; the tomato was chewed for 30 s, and then a swallow was allowed. Further chewing and mouth cleansing took place from 30 to 60 s, when the remaining tomato solids were swallowed. Normal breathing continued for a further 60 s, with no swallowing allowed.

The data obtained from the runs were processed in the following manner. First, the peak heights for each breath were obtained. These data were then smoothed using a weighted average algorithm and then normalized [expressed as a percentage, relative to the maximum intensity (I_{max})].

The tentative identification of the major compounds by API-MS was confirmed by Tenax trapping of volatiles from the headspace above tomato homogenate, followed by gas chromatography/mass spectroscopy (GC/MS) (Hewlett-Packard 5890; Fisons MD800 mass spectrometer).

Statistical Analysis. Data for the different volatile components were analyzed by one-way analysis of variance using the least significant differences of means (LSD) technique on Genstat 5 (Lawes Agricultural Trust, Rothamsted Experimental Station, U.K.). Comparisons were made at the 5% level of significance. Variation between samples was expressed as percentage coefficient of variation (CV%; (standard deviation × 100)/mean).

RESULTS AND DISCUSSION

Varietal Differences. The tomatoes (Delice, plum, cherry) were chosen to represent the wide range that can be found commercially and also to provide a comparison between cherry tomatoes grown in different countries (Israeli and Italian). The fruits were eaten as described under Materials and Methods, while air was sampled from the nostril of the person eating the fruits. This method measures the volatile profile close to the olfactory epithelium under eating conditions. For comparisons of tomatoes, data from only one person

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Table 1. Peak Height at Maximum Intensity (I_{max}) for the Selected Volatiles Present in Nosespace during Eating of Tomatoes (20 g Portions)

	hexenal	methylbutanal	methylnitrobutane	methylbutanol	hexenol	isobutylthiazole	hexanal
			(a) Israeli Cherry Tomatoes				
rep 1	522063	19407	2876	39108	48172	1885	0
rep 2	435993	24269	2619	13236	21848	1663	0
rep 3	639619	30183	1730	20577	38025	2567	0
rep 4	845096	32627	2286	34296	62759	4161	0
rep 5	618986	27500	3301	33317	64755	1178	0
mean	612000	26800	2560	28100	47100	2290	0
SD	137000	4630	532	9640	16000	1040	0
CV%	22	17	21	34	34	45	
			(b) Italian Cherry Tomatoes				
rep 1	721117	60406	10402	25944	16363	15997	1950
rep 2	580231	39796	14094	21539	18202	14243	1078
rep 3	895700	38165	7072	40876	12900	5485	910
rep 4	412538	47577	14773	11420	22139	7218	427
rep 5	637688	48436	11323	12610	8595	13304	2503
mean	649000	46900	11500	22500	15600	11200	1370
SD	159000	7900	2770	10700	4620	4130	749
CV%	25	17	24	48	30	37	55
			(c) Plum Tomatoes				
rep 1	330179	254851	90161	31164	11211	12903	1797
rep 2	366554	282762	184883	32198	17403	14557	1430
rep 3	390813	208492	111497	24903	12069	9644	734
rep 4	261747	55414	59518	14896	10618	11796	1528
rep 5	457912	175527	74167	19748	5232	9211	1150
mean	361000	195000	104000	24600	11300	11600	1330
SD	65000	79200	44000	6610	3880	2000	362
CV%	18	41	42	27	34	17	27
			(d) Delice Tomatoes				
rep 1	127478	68951	67920	11693	6579	28944	2014
rep 2	224408	52384	35514	11522	7547	41113	682
rep 3	101556	66229	45733	9471	8678	36497	587
rep 4	278448	36936	42592	6479	6159	33453	406
rep 5	105594	142249	245766	6913	10786	42289	1728
mean	167000	73400	87500	9220	7950	36500	1080
SD	71200	36300	79900	2210	1660	4920	655
CV%	42	49	91	24	21	14	61

were used to minimize variation as it has been shown previously that the eating pattern of one person is quite consistent (Haring, 1990). The breath-by-breath traces obtained for the five selected volatiles from five replicates of the four types are shown in Figure 1 (six replicates for plum). Even from these raw data, the differences between the types can be seen, although it is also evident that the replicates within one type (the fruit-to-fruit variation) vary considerably. For instance, the traces for methylbutanal in the plum tomatoes show considerable variation among the six fruits sampled. The fact that signal intensity returned to the baseline between each eating event demonstrated that there was no carry-over of volatiles from one sample to another.

The raw data from Figure 1 were analyzed further to derive parameters that might allow comparison between types. Since the breath-by-breath traces are the instrumental equivalent of time−intensity (TI) sensory traces, parameters used in TI data analyses were used [for overview, see Cliff and Heymann (1993)]. The concentration of volatiles at maximum intensity (I_{max}) was measured, as the flavor intensity may be proportional to the maximum concentration of flavor volatile. In addition, the total amount of volatiles delivered during eating was estimated by adding all of the peak

heights together over the time of eating (cumulative peak height).

Table 1 shows the I_{max} values for the seven volatiles. Within one tomato type, the fruit-to-fruit variation (CV%) ranged from 17 to 91% for individual compounds, although, as can be seen from Table 1d, this latter value resulted from one unusually high value. Cumulative peak heights from the same samples were also calculated, and similar variation was observed (data not shown). The cumulative method of data analysis was selected because combining the values over the whole time course might be expected to reduce variation compared to taking a single time value as occurs with I_{max}. However, mean CV% were 33 and 31% for the I_{max} and cumulative peak height, respectively, suggesting no significant difference in variability between the parameters.

The existence of such variation in tomato fruits is important, as quality assurance tests are frequently based on the mean quality obtained from a representative sample. Consumers, however, judge quality on individual tomato fruits and, although the mean quality of a tomato crop may be adequate, if variability is high, a significant proportion of the tomatoes may be below the minimum standard acceptable to consumers. The

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Table 2. Comparison of I_{max} Data and Mean Total Cumulative Height Data for the Four Tomato Types[a]

| volatile	Israeli cherry	Italian cherry	plum	Delice
	(a) I_{max} Data			
hexenal	[a]612000	[a]649000	[b]361000	[c]167000
methylbutanal	[a]26800	[a]46900	[b]195000	[a]73400
methylnitrobutane	[a]2560	[a]11500	[b]104000	[b]87500
methylbutanol	[a]28100	[a]22500	[a]24600	[b]9220
hexenol	[a]47100	[b]15600	[b]11300	[b]7950
isobutylthiazole	[a]2290	[b]11200	[b]11600	[c]36500
hexanal	[a]0	[b]1370	[b]1330	[b]1080
	(b) Mean Total Cumulative Height Data			
hexenal	[a]3250000	[ab]3130000	[b]2540000	[c]1100000
methyl butanal	[a]187000	[a]236000	[b]1010000	[a]294000
methylnitrobutane	[a]10800	[a]56000	[b]833000	[c]530000
methylbutanol	[a]161000	[a]117000	[a]168000	[b]24300
hexenol	[a]381000	[b]155000	[b]103000	[b]80000
isobutylthiazole	[a]11100	[b]85100	[b]105000	[c]263000
hexanal	[a]0	[b]8170	[b]7280	[b]5820

[a] Values in one row with different superscripts are significantly different at the $p < 0.05$ level.

greater the variation, the greater the number of substandard tomatoes. Examination of individual tomatoes can provide an estimation of this variability. API is a rapid method whereby the volatile content of individual tomatoes can be measured quantitatively and contrasts with conventional techniques that typically require batch quantities to obtain analyses.

From the data of Table 1, the amounts of individual volatiles can be compared and tested statistically. Table 2 shows the mean amounts of volatiles in the four types with differences tested with one-way ANOVA. Because the amounts of each compound are presented in terms of peak height, it must be remembered that comparison between compounds is inappropriate as the response factors for each compound were not determined. Thus, it is not valid to compare the peak areas for methylbutanal and isobutylthiazole and extrapolate these differences to actual amounts. Comparison of the amounts of each compound in the four types, however, is valid, and the data show that hexenal was highest in the cherry tomatoes with significantly different amounts in plum and Delice (Table 2a). Table 2b contains mean values and statistical analysis of the cumulative height data. The statistical differences are identical (bar one) to the I_{max} data in Table 2a, demonstrating again the linkage between these two parameters.

Inspection of the other volatiles demonstrates that there were significant differences for all seven compounds among the four types. Surprisingly, there were significant differences between the two cherry tomato batches for three of the selected compounds, which might be due to the differing growing conditions in the countries of origin in addition to other factors (transport conditions, age). Plum tomato was unusual in its high methylbutanal and 3-methylnitrobutane contents, and Delice had a high isobutylthiazole content. Each type had a unique ratio of the various volatiles, which could be taken to be a signature or fingerprint for that particular type. The differences in I_{max} for the seven compounds between types are clearly shown in Figure 2, and these differences may translate into sensory differences, which may be detected by consumers.

Variation with Time of Eating. The time courses of the release of isobutylthiazole, hexenal, and hexenol from the plum tomatoes are compared in Figure 3. The data from the five replicates were pooled and smoothed.
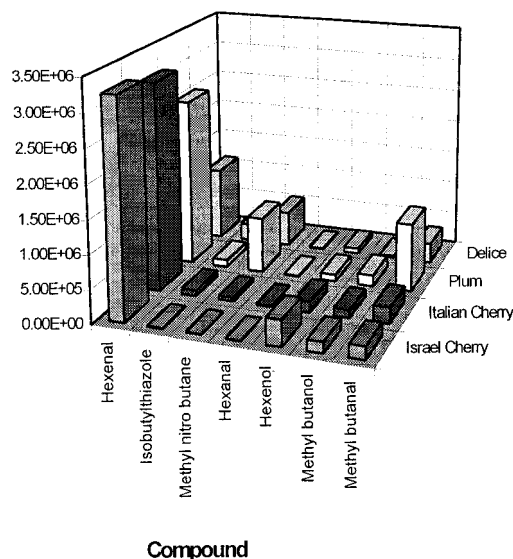
Figure 2. Comparison of I_{max} data for the four types of tomatoes.
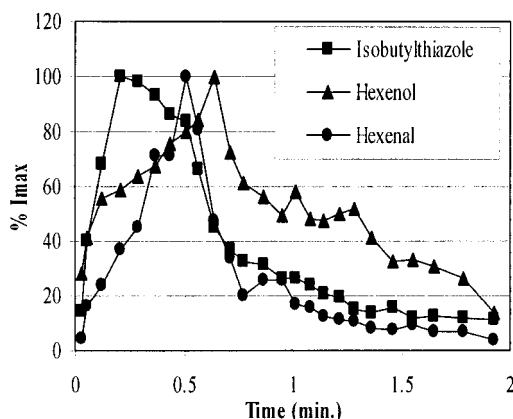
Figure 3. Release of isobutylthiazole, hexenal, and hexenol (5 replications) from plum tomatoes.

For easier comparison, the amounts of each compound have been adjusted to 100% at T_{max}. Isobutylthiazole is present in the intact tomato in the active form and is released first. API cannot distinguish hexenal isomers, and it is not possible to examine the action of isomerase enzymes, which convert (Z)-3-hexenal to (E)-2-hexenal. Therefore, the signal for hexenal in Figure 3 represents the sum of the (Z)-3 and (E)-2 isomers. However, the enzymic production of hexenal and hexenol is shown, the former increasing in concentration in the breath as a result of lipase, lyase, and lipoxygenase activity. Hexenol reached a maximum significantly later even though there is just one enzyme step (ADH) between the aldehyde and the alcohol. The appearance of the volatiles at different times suggested a difference in the relative reactivity of the individual enzyme reactions. Lipase, lyase, and lipoxygenase seemed to be rapid, but the ADH step was significantly slower, perhaps due to its pH optimum being around neutrality (Longhurst et al., 1990), whereas the pH of the tomato macerate is acid.

Table 3 shows the T_{max} data for the plum tomatoes. T_{max} values for individual compounds that are formed during ripening (e.g., isobutylthiazole, 0.26 min) were significantly earlier ($p < 0.05$) than those generated by enzymic action during maceration (e.g., hexenal, 0.51 min). In this latter group, significant differences in T_{max}

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Table 3. T_{max} Data (Minutes) for the Selected Volatiles from Plum Tomatoes

	hexenal	isobutylthiazole	methylnitrobutane	hexanal	hexenol	methylbutanol	methylbutanal
rep 1	0.46	0.29	0.29	0.49	0.63	0.29	0.22
rep 2	0.67	0.29	0.48	0.49	0.66	0.40	0.32
rep 3	0.50	0.20	0.20	0.66	0.66	0.44	0.13
rep 4	0.51	0.31	0.22	0.68	0.77	0.38	0.38
rep 5	0.44	0.22	0.37	0.60	0.60	0.37	0.35
mean	[a]0.52	[b]0.26	[b]0.31	[ac]0.58	[c]0.66	[b]0.37	[b]0.28
SD	0.08	0.04	0.10	0.08	0.06	0.05	0.09
CV%	15	15	32	14	9	13	32

[a] Values in one row with different superscripts are significantly different at the $p < 0.05$ level.

Table 4. Comparison of T_{max} Data for Release of Volatiles from the Four Tomato Types

volatile	Israeli cherry	Italian cherry	plum	Delice
hexenal	[a]0.45	[a]0.44	[a]0.52	[b]0.33
isobutylthiazole	[a]0.25	[a]0.26	[a]0.26	[a]0.21
methylnitrobutane	[a]0.34	[a]0.21	[a]0.31	[a]0.24
hexanal	N/A	[a]0.52	[a]0.58	[b]0.34
hexenol	[b]0.43	[b]0.58	[b]0.66	[a]0.18
methylbutanol	[a]0.21	[a]0.18	[b]0.37	[a]0.20
methylbutanal	[a]0.24	[a]0.18	[a]0.28	[a]0.22

[a] Values in one row with different superscripts are significantly different at the $p < 0.05$ level.

values were observed for hexenal and hexenol ($p < 0.05$), but the T_{max} for hexanal was between these values and not significantly different from either of them. The hexanal–hexenol T_{max} values agree with the expected sequence of volatile release given the enzymic pathway and sequence that is responsible for their production. The CV% values for T_{max} are lower than for the I_{max} data, showing that the compounds are more consistently released at the mean times shown. A defined eating protocol reduced variation due to eating patterns, resulting perhaps in consistent T_{max}, whereas tomato variation will be responsible for the large differences experienced in I_{max}.

Temporal profiles of volatiles were consistent between types of tomato (Table 4), with the exception of the Delice tomatoes. Thus, it appears that while the intensity of the volatiles may result in subtle differences in taste between types of tomato, differences in velocity of release may also be important. Indeed, Delice was perceived by the subject to have a different flavor from the other types investigated. This result emphasizes the importance of the differences in texture and firmness of the various tomato types (cherry tomatoes are relatively hard, whereas Delice are very soft), which may be responsible for the temporal differences in volatile release. Another factor that may be responsible for the early increase in volatile concentration in Delice could be the presence in the intact fruit of higher than expected concentrations of the active flavor compounds, hexanal, hexenal, and hexenol.

Conclusions. The data presented show considerable variation in nosespace volatile concentration both among tomatoes of the same type and among different types. However, it was found that the time release behaviors of volatiles from all tomato types were similar, with the exception of Delice. From this finding one might speculate that, in these tomato samples, it is the different concentrations of the volatiles that are responsible for the different perceived flavors rather than a temporal difference in release due to the different textures of the fruits. However, with only a limited number of volatiles analyzed and no data on the

differences in nonvolatile content (e.g., sugar/acid ratios), this hypothesis needs further work to confirm its veracity.

The differences in I_{max} may be used to build up a volatile fingerprint that is unique for each type, although it may also depend on the growing regime. The data were obtained by measuring nosespace rather than conventional headspace measurements. Nosespace may be more relevant in the consideration of how changes in flavor compound concentrations affect perception of the aroma, but combined sensory and nosespace analysis would be required to investigate this aspect further. The data presented here demonstrate that rapid analysis by API-MS in the nose may provide a screening tool to judge volatile content.

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