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Breath-by-breath analysis of banana aroma by proton transfer reaction mass spectrometry

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Abstract

We report on the in vivo breath-by-breath analysis of volatiles released in the mouth during eating of ripe and unripe banana. The air exhaled through the nose, nosespace (NS), is directly introduced into a proton transfer reaction mass spectrometer and the time-intensity profiles of a series of volatiles are monitored on-line. These include isopentyl and isobutyl acetate, two characteristic odour compounds of ripe banana, and 2*E*-hexenal and hexanal, compounds typical of unripe banana. Comparing the NS with the headspace (HS) profile, two differences are outlined. First, NS concentrations of some compounds are increased, compared to the HS, while others are decreased. This indicates that the in-mouth situation has characteristics of its own—mastication, mixing/dilution with saliva, temperature and pH—which modify the aroma relative to an HS aroma. Second, we discuss the temporal evolution of the NS. While 2*E*-hexenal and hexanal steadily increase in the NS during mastication of unripe banana, no such evolution is observed in volatile organic compounds (VOCs) while eating ripe banana. Furthermore, ripe banana shows high VOC concentrations in the swallow breath in contrast to unripe banana. (Int J Mass Spectrom 223–224 (2003) 743–756)

Keywords: Banana; Aroma; PTR-MS; Breath-by-breath analysis

1. Introduction

Banana (*Musa sapientum* L.) is one of the most common tropical fruits, and one of Central America's most important crops. It is grown in all tropical regions and is one of the oldest known fruits. The main producer is India, followed by Brazil, Ecuador, Indonesia, the Philippines, China and Colombia. Since bananas are a staple food, they are of importance for domestic consumption, specially because they grow quickly and can be harvested the whole year round.

Currently 20% of total production enters world trade. From an economic perspective bananas compete with grapes for second place behind citrus fruits, both accounting for 13–14% of world fresh fruit production. Banana production has been increasing by 3% per year over the last decade [1].

From a consumer perspective, the most appealing features of banana are their flavour, nutrition or health aspects and convenience for consumption. Here, we are concerned with aroma, as it is perceived during

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eating. Analysing on-line, breath-by-breath the time evolution of a series of selected volatile compounds exhaled through the nose, we explore the release of banana volatiles as it occurs in the mouth. This approach, termed nosespace (NS) analysis, brings flavour research closer to the consumer and gives a more intimate view of the consumer's perspective of aroma.

In this article, we first give a short review of banana aroma. Considerable literature on this subject has been published, making banana a fruit whose volatile composition can be considered as known. We then discuss the headspace (HS) of ripe and unripe banana, measured by proton transfer reaction mass spectrometry (PTR-MS). This serves as a reference to the subsequent discussion of NS data. We report on the release of volatile organic compounds (VOCs) during eating of banana, and compare these to HS profiles. Finally, we discuss the dynamic aspects of the in-mouth aroma—its evolution over time during mastication. Ultimately, we wish to better understand the specificities of the in-mouth aroma experience, and how it differs from an HS aroma.

1.1. The aroma of banana

Extensive work on the volatile fraction of banana at various degrees of ripeness has resulted in the identification of approximately 250 compound [2]. Characteristic volatile esters, alcohols, acids and carbonyls are the four main compound classes that give strength and character to banana aroma, while amines and phenols also contribute.

1.1.1. Esters

With more than 100 compounds, esters predominate in the volatile fraction of banana. Among the esters, acetates are of particular importance. Due to their high concentrations in banana and low odour thresholds, they are characterisitic flavour compounds of banana. Specifically isopentyl acetate and isobutyl acetate, are known as the two most important impact compounds of banana aroma. They were quantified at 75 and 47 ppm, respectively, while their sensory thresholds are reported to be 2 and 50 ppb [3]. Both molecules elicit banana, pear and fruity sensory notes, while isobutyl acetate also has a slight rum note. Some other acetates of importance to banana are ethyl acetate (200 ppm), 2-pentyl acetate (10 ppm) [4], butyl acetate and hexyl acetate at ∼4 ppm. Two butanoates (ethyl and isopentyl) were quantified at ∼5 ppm while isopentyl isopentanoate was found at 3 ppm.

In 1993 Shiota [5] identified minor esteric compounds in banana that contribute to its flavour. In particular, he reported carboxylic moieties represented by acetate, butanoate, 3-methylbutanoate and pentanoate, and alkoxy moieties represented by unsaturated alcohols 4*Z*-hexenol, 4*Z*-hepten-2-ol, 4*Z*-octenol, 5*Z*-octenol and 4*Z*-decenol. While they were not quantified, sensory evaluation revealed a dominating fruity note and banana, melon, orange and mango undertones.

1.1.2. Alcohols

Alcohols are the second most important group of volatiles in banana extracts. Fifty-seven saturated and unsaturated alcohols were identified, mainly linear and branched molecules. The most abundant are isopentanol (1–12 ppm) [3], 2-pentanol (15 ppm) [6], isobutanol (8 ppm) [3] and hexanol (2 ppm) [3]. The only terpenic alcohol found to date is linalool.

1.1.3. Acids

Banana is rich in volatile organic acids. Thirty-five acids were reported, mainly linear compounds from C2 to C18 (saturated and unsaturated). Branched, hydroxy- or oxo- acids as well as two unsaturated acids with a terminal double bond have also been identified [4]: 6-heptenoic and 7-octenoic acid which are uncommon among fruits. No quantitative data are available except for acetic acid (5 ppm).

1.1.4. Carbonyls

The carbonyl group (aldehydes and ketones) is represented by approximately 30 compounds. Two aldehydes were quantified at high concentration: 2*E*-hexenal (18 ppm [4] and 32 ppm [6]) and hexanal (5 ppm [4] and 22 ppm [6]). This is in contrast to an earlier study in 1961 [7], which systematically reported higher values. Thus, 2*E*-hexenal was quantified

at 76 ppm, and 2-pentanone at 27 ppm. The buttery hydroxyketone, 3-hydroxy-2-butanone, was found at 20 ppm [6].

1.1.5. Amines and phenols

Amines and phenol derivatives constitute two other chemical classes encountered in banana with less than 10 individual chemicals for each class. Only eugenol was quantified (1–3 ppm) [3,6].

Besides the flavour profiles per se, some investigations focused on changes in volatiles during ripening. At the beginning of the sixties, Hultin and Proctor [7] monitored 10 volatile compounds during ripening—one acid, three alcohols, three esters, one aldehyde and two ketones—from the green to the over-ripe stage. Ethyl alcohol was found to strongly increase during ripening. To a lesser extent isopentyl alcohol and its corresponding acetate also increased. Later, Macku and Jennings [8] followed 17 volatile compounds during ripening (12 esters, 4 alcohols and 1 ketone). They observed an increase until the onset of peel browning, before they started to decrease at high maturation (over-ripe). The only exception was ethyl acetate, which increased continuously even into late senescence.

Tressl and Drawert [9] described biogenetic pathways for the formation of banana volatiles. They proposed three main pathways: (i) conversion of amino acids into methyl-branched alkyls and acyl compounds of esters or into methyl-branched alcohols, (ii) fatty acid metabolism leading to the production of acids, esters, alcohols and ketones and (iii) enzymatic degradation of linoleic and linolenic acid into aldehydes and ketoacids.

1.2. NS analysis

The aroma (odour) of food products is related to VOCs released during eating/drinking that reach the olfactory epithelium in the upper part of the nose. When flavour active compounds interact with olfactory receptors, a sensory perception is triggered.

VOCs can reach the olfactory epithelium from two different directions. Either they are sniffed through the nose, via the orthonasal pathway and enter the nostrils from the front. Alternatively, they reach the olfactive receptors through the oral cavity and the pharynx via the retronasal pathway. Orthonasal aroma is associated with the odour perceived from food held in front of the nose (sniffing). In contrast, when food is eaten, mastication, shearing, salivation, temperature and pH changes lead to release of aromatic compounds that reach the olfactory epithelium through the retronasal path.

The significance of NS analysis stems from the fact that it brings flavour analytics closer to the real eating experience. From a consumer's perspective, what really matters are those aromatic compounds that are released in the mouth and can be perceived during eating and drinking, rather than the total content on VOCs in food products. Aroma perception during eating is typically a dynamic phenomenon that evolves in intensity and profile. Once food is swallowed, some aromatic compounds persist in the breath air, fading slowly with time, and contributing to the after-odour. In summary, in-mouth aroma has various aspects of its own that are characteristic of the eating situation. Considering that release, evolution and fading of aroma are central to a pleasurable eating experience, it is critical to integrate these aspects in the evaluation of the aromatic performance of food products.

The NS technique applied here samples air exhaled through the nose as food is being consumed. The first real-time breath-by-breath analysis dates back to 1988 [10]. In the mid-90s, Linforth et al. coupled atmospheric pressure ionisation mass spectrometry to an NS sampling device, and made significant contributions to the advancement of this field [11–13]. Recently, we published on the NS analysis of coffee using PTR-MS [14]. Here, we report on the in-mouth aroma while eating banana.

2. Material and methods

2.1. HS analysis

The HS of banana was measured in the set-up shown in Fig. 1. Pieces of peeled banana were placed in the

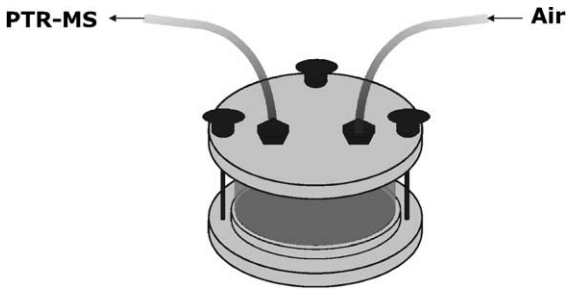

Fig. 1. Experimental set-up to measure the HS of banana.

glass vial with a metal cover having two tubings on the top. Through one tubing, HS air was sampled at a rate of 18 $cm^3\,min^{-1}$ and introduced into the drift tube of the PTR-MS. The second tubing was left open for laboratory air to enter and replace the sampled volume. The glass vial had a diameter and a height of 10 and 5 cm, respectively.

2.2. In vivo breath-by-breath NS analysis

NS air was sampled via two glass tubes fitted into the nostrils (Fig. 2). The separation and diameter of the tubes were adapted individually to allow the person to breathe comfortably. The air from both tubes was combined and a small fraction (18 $cm^3\,min^{-1}$) was introduced into the drift tube of the PTR-MS. The nosepiece was heated to 38 °C, while the tubings were at 45 °C, to prevent condensation along the sampling line.

2.3. PTR-MS

The PTR-MS technique has been extensively discussed in a series of review papers [15–18]. Briefly, it combines a soft, sensitive and efficient mode of chemical ionisation (CI), adapted to the analysis of trace VOCs, with a mass filter. In this study, 18 $cm^3\,min^{-1}$ gas was continuously introduced into the drift tube (CI cell). The drift tube contained besides buffer gas, a controlled ion density of H_3O^+. VOCs that have proton affinities larger than water (proton affinity of H_2O: 166.5 $kcal\,mol^{-1}$) are ionised by proton transfer from H_3O^+, and the protonated VOCs are mass analysed. The ion source produces nearly exclusively H_3O^+ ions (>98%), that are extracted and transferred into the drift tube.

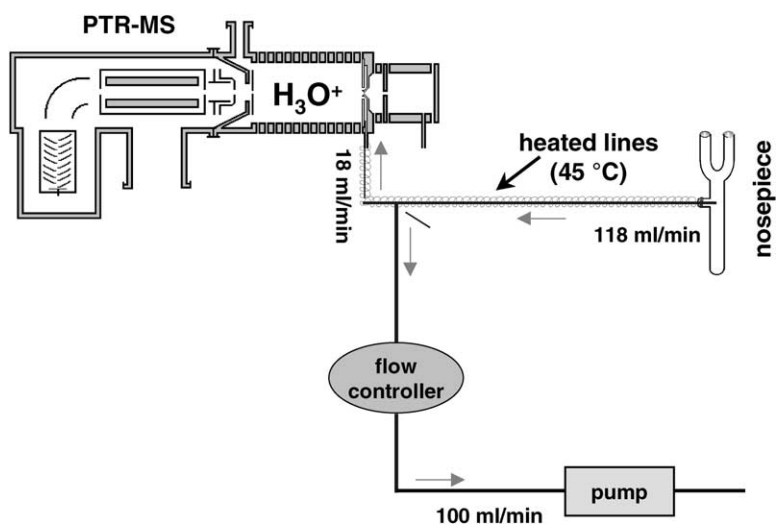

Fig. 2. Experimental set-up for breath-by-breath NS analysis while eating banana. The air exhaled during food consumption is sampled via the nosepiece and a small fraction introduced into the PTR-MS for on-line VOC analysis.

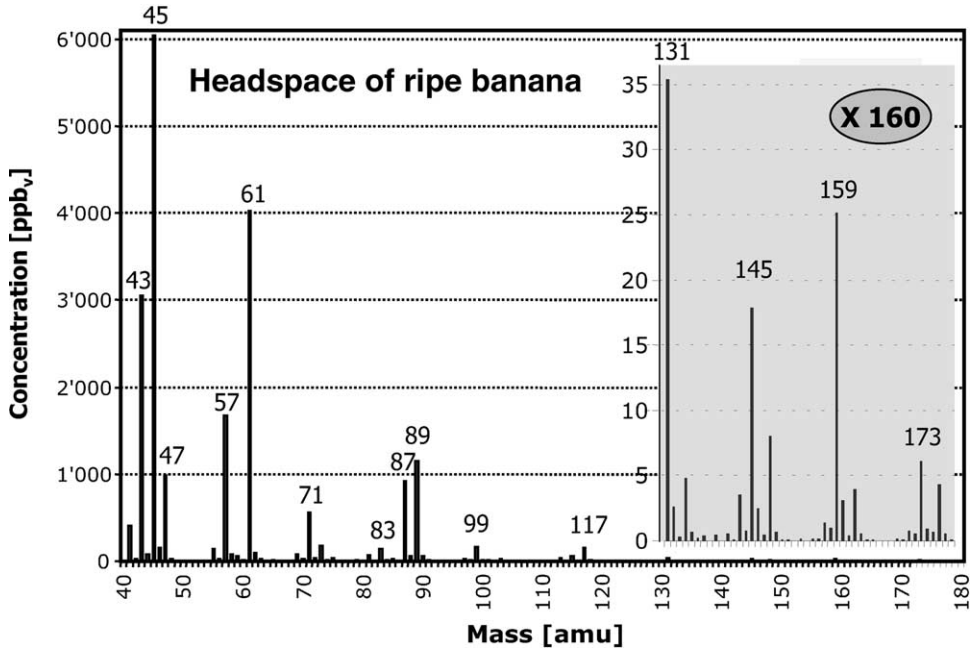

Fig. 3. PTR-MS HS spectra of ripe banana on a linear intensity scale. The spectrum is dominated by a series of ion masses. The assignment of many of these ion signals is given in Table 2.

3. Results

3.1. The HS of ripe banana

In a first series of experiments, the PTR-MS HS of ripe and unripe banana were measured over a mass range from 20 to 180 amu. Fig. 3 shows a typical spectrum of a ripe banana. In the mass range below 130 amu, the spectrum is dominated by a series of ions—m/z = 45, 61, 87, 89, 99 and 117. Above 130 amu, the ion intensities are magnified by a factor 160. We see various prominent ion peaks, most notably m/z = 131, 145, 159 and 173. To assign these ions, two different sources of information were used. On the one hand, a vast literature exists on VOCs and their concentrations in banana (see Section 1). This allows a first assignment of the prominent ion peaks. In addition, we have measured PTR-MS spectra of many pure VOCs in order to assess whether they fragment upon ionisation [19]. While many VOCs do not fragment, we have collected in Table 1 the fragmentation patterns of a series of compounds that show significant break-up.

As reported in previous publications [15,19], protonating a hydroxyl group by proton transfer from H_3O^+ in the drift tube of the PTR-MS may lead to loss of H_2O from the protonated parent ion. Hence, several alcohols fragment in PTR-MS, showing a prominent ion signal at $[M + H - H_2O]^+$. Isopentanol (MW = 88), does not give any ion intensity at the parent mass $[M+H]^+$. Fragments appear at m/z = 71, corresponding to loss of H_2O $[M + H - H_2O]^+$, at m/z = 43 $[C_3H_7]^+$ and m/z = 41 $[C_3H_5]^+$. We have no data on pure isobutanol but expect the protonated parent peak to partially fragment via H_2O loss (m/z = 57). The isomer *n*-butanol gives exclusively $[M + H - H_2O]^+$, analogous to isopentanol.

Some acetates also show fragmentation. Ethyl acetate gives up to 60% as the protonated parent ion $[M + H]^+$, while 35% of the ion intensity appears at m/z = 61. This corresponds to the protonated carboxylic moiety. Ethyl butanoate undergoes a similar

Table 1
Fragmentation of pure compounds, following chemical ionisation by proton transfer in PTR-MS

Compounds	Parent *m/z*	Mass spectral abundances at [*m/z*] (in %)													
		41	43	55	57	61	71	81	83	85	89	99	101	117	145
Isopentanol	88	15	46				39								
Ethyl acetate	88		5			35					60				
Ethyl butanoate	116	1	5				2				24			67	
Hexyl acetate	144	4	27		5	53				8					3
Hexanal	100			22					73				5		
2*E*-Hexenal	98				54			13				33			

The table gives the percentage at the respective ion masses.

fragmentation in which the dominating peak is $[M + H]^+$. The most important fragment is the protonated carboxylic moiety (24% of total signal). Minor ions, corresponding to fragmentation of the carbon chain, also appear. Moving to hexyl acetate, the protonated parent ion accounts only for 3% of the total ion signal, while the dominant ion peak corresponds to the protonated carboxylic moiety at $m/z = 61$ (53%).

The third group of compounds known to partially fragment in PTR-MS, are the aldehydes [15,19]. They often fragment by H_2O loss from the protonated parent. In the case of hexanal, this characteristic fragment accounts for 73% of the total ion signal, corresponding to an ion signal at $m/z = 83$, and only 5% appear at the parent mass ($m/z = 101$). In the case of the 2*E*-hexenal, the parent ion represents 33%. Yet, the main fragment does not correspond to water loss (only 13% at $m/z = 81$), but appears at $m/z = 57$. This fragment can only be explained by assuming an internal rearrangement of the molecular ion.

From this short overview, we see that some VOCs show significant fragmentation despite the use of one of the softest ionisation modes. Many of the compounds not specifically discussed here have either been included in former publications [15,19], or show little or no fragmentation. A more extensive study on ionisation-induced fragmentation in PTR-MS is in progress (unpublished research).

Based on measured fragmentation patterns and published VOC composition of banana, Table 2 gives an assignment for the most prominent HS compounds. Many of the key aroma compounds of banana are readily observed by PTR-MS.

3.2. Unripe vs. ripe banana

The HS composition of banana strongly evolved during ripening. Fig. 4 compares the HS spectra of unripe and ripe banana on a logarithmic intensity scale. In addition, Table 3 summarises the HS concentrations of the most prominent ion peaks. The HS of unripe and ripe banana differed in various aspects. The most obvious difference is the overall lower HS concentrations in unripe relative to ripe banana. Furthermore, ripe banana had a higher fraction of high molecular weight VOCs. Finally, we noticed some specific differences in profile composition.

A dominant ion of unripe banana in Fig. 4 is $m/z = 83$. This compound decreased (by one order of magnitude) during ripening. On the basis of known fragmentation patterns, this ion is attributed to a hexanal fragment (loss of H_2O from protonated parent; $[M + H - H_2O]^+$). Two other compounds appearing at $m/z = 99$ and 81 also decreased during ripening. These two ions are mainly attributed to 2*E*-hexenal.

In ripe banana, the concentration of a number of HS VOCs is strongly increased relative to unripe banana. In particular, a series of ions with masses larger that 100 appeared in the spectrum. All these compounds can be assigned and are flavour active. Table 2 gives their assignment together with their odour notes. The most significant are isobutyl acetate at $m/z = 117$ and isopentyl acetate at $m/z = 131$, two flavour

Table 2
Tentative identification of VOCs in the HS of banana

Protonated mass (amu)	Compounds	Formula	Odour descriptors
173	Isopentyl isopentanoate	$C_{10}H_{20}O_2$	Apple, sweet
159	Isopentyl butanoate	$C_9H_{18}O_2$	Apricot, banana, pineapple
145	Hexyl acetate	$C_8H_{16}O_2$	Pear, estery
131	Isopentyl acetate	$C_7H_{14}O_2$	Banana, pear
117	Isobutyl acetate	$C_6H_{12}O_2$	Banana, rum
101	Hexanal	$C_6H_{12}O$	Grassy, green
99	2*E*-Hexenal	$C_6H_{10}O$	Apple, leafy, fruity
89	Ethyl acetate	$C_4H_8O_2$	Banana, ethereal, pineapple
	Pentanol	$C_5H_{12}O$	Ethereal, fruity, yeasty
87	2,3-Butandione	$C_4H_6O_2$	Buttery, creamy
	2-Pentanone	$C_5H_{10}O_2$	Ethereal, fruity
83	Hexanal (fragment)	C_6H_{10}	–
81	2*E*-Hexenal (fragment)	C_6H_8	–
71	2-Methyl-2-propenal	C_4H_6O	–
	Pentanol (fragment)	C_5H_{10}	–
69	Isoprene	C_5H_8	–
61	Acetic acid	$C_2H_4O_2$	Vinegar, sour, pungent
	Hexyl acetate (fragment)	$C_2H_4O_2$	–
	Ethyl acetate (fragment)	$C_2H_4O_2$	–
57	2-Propenal	C_3H_4O	–
	Butanol (fragment)	C_4H_8	Apple, ethereal, medicinal
	Hexyl acetate (fragment)	C_4H_8	–
	Hexanol (fragment)	C_4H_8	–
	2*E*-Hexenal (fragment)	C_3H_4O	–
47	Ethanol	C_2H_6O	Ethereal, fresh
45	Acetaldehyde	C_2H_4O	Ethereal, ethanolic, yoghurt
43	Acetic acid (fragment)	C_2H_3O	–
	Hexyl acetate (fragment)	C_3H_6	–
	Ethyl acetate (fragment)	C_2H_3O	–
	Hexanol (fragment)	C_3H_6	–

compounds that are considered to give the banana fruit its characteristic aroma.

In contrast to the majority of the HS compounds, whose concentrations increased during ripening, a few compounds decreased. Two such compounds are shown in Fig. 5: 2*E*-hexenal and hexanal. 2*E*-Hexenal has a typical green odour note and is known to be emitted from ripening plants, among others as a defence to wounding [20].

3.3. NS measurements

Fifteen different VOCs were measured simultaneously while eating unripe and ripe bananas. In Fig. 5, we show a selection of six VOCs. Isopentyl and isobutyl acetate are two important flavour impact compounds of ripe banana. Neither were detected in unripe banana. In contrast, the C6-aldehydes, 2*E*-hexenal and hexanal, are both typical green banana compounds

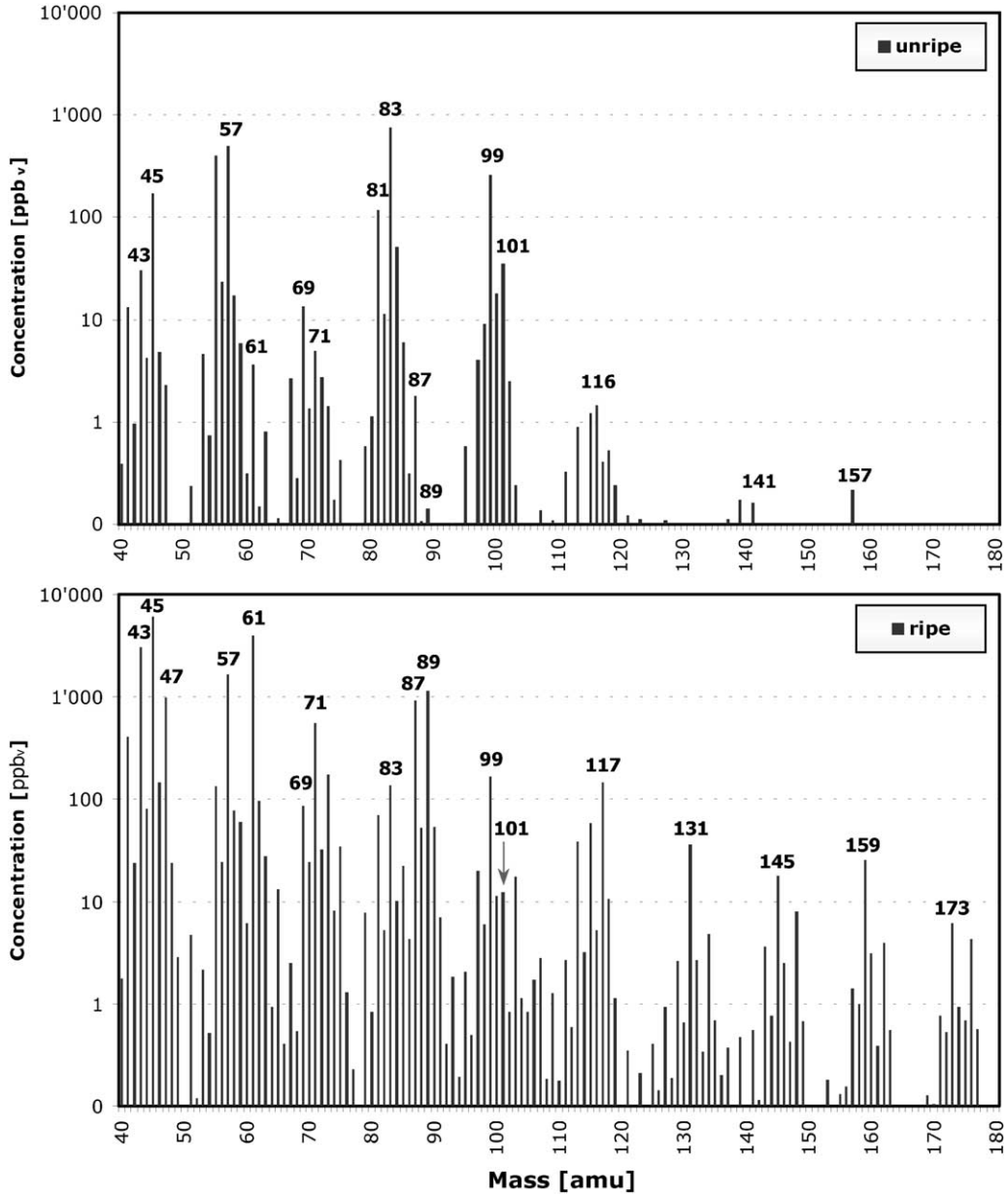

Fig. 4. PTR-MS HS spectra of unripe and ripe banana on a logarithmic intensity scale.

and absent in ripe banana NS. Finally, we also include $m/z = 89$ and 87. Both correspond to the superposition of two compounds: mass $m/z = 89$ is a superposition of ethyl acetate and isopentanol, while $m/z = 87$ has contributions from 2,3-butandione and 2-pentanone.

The time domain from 0 to 10 min on each trace corresponds to breath-by-breath analysis while eating an unripe banana. The traces plotted from 10 to 19 min correspond to ion signals at the same masses, but while eating a ripe banana.

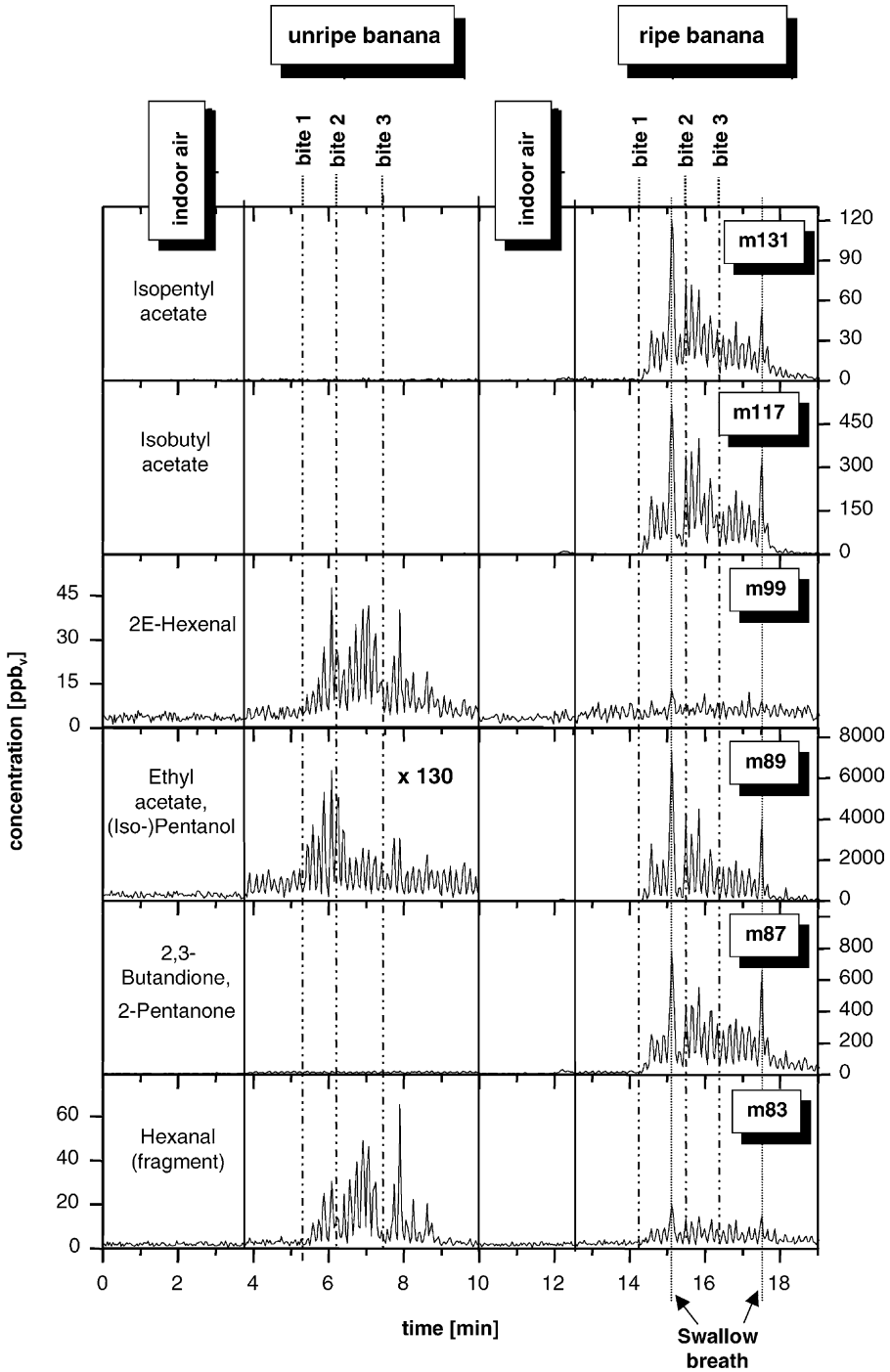

Fig. 5. Breath-by-breath, NS spectra of six selected masses while eating unripe (left traces) and ripe (right traces) banana.

Table 3
HS concentrations of the most prominent ion peaks for unripe and ripe banana

Protonated mass (amu)	HS concentration of banana (ppbv)	
	Unripe	Ripe
131	<1	215
117	5	580
99	400	58
89	5	5500
87	9	1350
83	505	90
81	133	28
71	12	2700
69	63	135
61	43	11700
57	610	5350
47	105	6750
45	1870	9900
43	88	11150
33	380	1450
Σ	4228	56956

The values included in the table are the mean of five measurements (the average standard deviation is about 20%). These include measurements on (i) the top end-piece of banana, (ii) banana cut in four pieces, (iii) the HS of the middle piece alone, (iv) smashed banana and (v) banana wounded by 14 stitches. In all cases the HS concentrations are normalised to 3 g of banana. We have investigated these five HS profiles to explore whether, e.g., the end-piece or wounded bananas differ in HS composition. The differences we observe among the HS profiles will be discussed in a forthcoming publication.

3.3.1. Unripe banana

From 0 to 3.75 min, the nosepiece sampled air from the laboratory. We see low ion intensities, indicating that laboratory air did not significantly contribute to the background signal at these masses. Then, the nosepiece was placed into the nostrils and the air exhaled through the nose monitored breath-by-breath by PTR-MS. We observed a slight increase in signal intensity at $m/z = 87$, 89 and 99, relative to laboratory air. But these intensities remained low. Then, after about 5 min, the assessor took a first of three bites from an unripe banana. We immediately observed a distinct breath-by-breath signal on three of the ion traces shown in Fig. 5. These are 2-hexenal at mass $m/z = 99$, hexanal at $m/z = 83$ and a mixture of ethyl acetate and isopentanol at $m/z = 89$.

While eating a banana, the person breathes regularly through the nose. With each breathing cycle we saw an increase in ion intensity as the assessor exhaled through the nose followed by a decrease when inhaling.

3.3.2. Ripe banana

The time section from 10 to 19 min gives the breath-by-breath ion traces during eating of a ripe banana. From 10 to 12.6 min the nosepiece sampled laboratory air. Then the nosepiece was placed into the nostrils of the assessor and the breath air monitored for the next 2 min, without banana in the mouth. On all masses included in Fig. 5, neither the laboratory air nor the breath air of the assessor gave any significant background intensity. The assessor then took a first bite from a ripe banana and a series of volatiles appeared in the air sampled during the exhalation. Again the signal intensity of the volatiles increased and decreased with the rhythm of the breathing cycles of the assessor. For ripe banana, the assessor took three consecutive bites, while swallowing prior to each new bite. Finally, after about 17 min the third bite was swallowed, and the NS spectrum was recorded for two more minutes, with no banana left in the mouth of the assessor. The volatiles that remain in the breath air compose the after-odour.

4. Discussion

A series of publications discussed the HS composition of banana (see Section 1). We believe that most banana VOCs are known today. Furthermore, the compounds contributing to the aroma of banana at various degree of ripening have been quantified and characterised in terms of olfactory note and impact.

Recently, banana HS has been examined by direct injection mass spectrometry of HS gas using chemical ionisation. Spanel and Smith [21] investigated the HS of banana by the selected ion flow tube (SIFT)-MS method. SIFT-MS can be considered as the precursor of the current PTR-MS method and was originally developed by Ferguson and his colleagues [22] to

investigate ion–molecule reactions in the gas phase. While the SIFT-MS technique has been instrumental in determining a large number of gas phase reaction kinetics, it is less appropriate for the quantitative analysis of complex VOC mixtures. The SIFT-MS spectra of banana HS suffers from strong fragmentation and clustering. In particular, there is no trace of the key aroma compound isopentyl acetate.

Taylor and Linforth [13] measured banana HS using APCI-MS. The spectra are more straightforward to interpret, mainly because of reduced fragmentation and minimal clustering. Taylor and Linforth discussed their work in relation to the first study by Smith et al. and gave an improved interpretation of the direct injection MS data. In spite of the much improved quality of the HS spectra in APCI-MS, in comparison to SIFT-MS, it appears that relative intensities of compounds do not reflect geniune HS concentrations. The two dominant peaks of the HS, as measured by APCI-MS, are isopentyl acetate ($m/z = 131$) and isopentyl butanoate ($m/z = 159$). In contrast, compounds in the lower mass range, like ethyl acetate, who are known to be more abundant that isopentyl acetate and isopentyl butanoate, appear with relatively low intensities.

Here, we revisit the HS of banana, now using the PTR-MS technique. In contrast to Spanel et al., we observe a much reduced fragmentation and clustering (similar to Taylor et al.). This allows us to assign all key flavour compounds, as shown in Figs. 3 and 4 and Tables 2 and 3. What is peculiar in the spectra of Spanel et al. is the sequence of dominant ion signals at $m/z = 121$, 135, 149, 163 and 177. In the ripe banana profile shown in Fig. 3, and in the results reported by Taylor et al., the sequence of prominent ion peaks is 117, 131, 145, 159 and 173, all compounds that can be clearly assigned (Table 2). What this shift of four mass unit in the data of Spanel et al. is due to, is unclear.

We have analysed the PTR-MS HS of unripe and ripe banana and are able to assign the majority of the ion signals. The particularity of the banana fruit is that many key flavour compounds are present in the HS at appreciable concentrations and can hence be observed as strong ion peaks in PTR-MS.

In addition to analysing the HS, here we have gone one step further and also investigated on-line 15 volatiles released during eating banana. Six ion traces are shown in Fig. 5. This allows us to compare the HS volatile composition with the in-mouth profile. First, we observe some differences of the NS volatile composition relative to the HS, indicating that the retronasal aroma is different from the orthonasal one. In addition, the volatile profile in the mouth is not static but evolves with time. This is a specificity of in-mouth aroma, that cannot be investigated by traditional HS approaches, but which may be important to the sensory experience while eating.

In Table 4, we show the concentrations measured during NS and HS experiments. The HS concentration included in the table correspond to the mean of five measurements. In the case of NS data, the intensity varied strongly from breath-to-breath, and it seems a priori difficult to summarise in one single number the NS concentration for a given compound (ion intensity). For the purpose of comparison with HS data, we neglect here the dynamic aspects of the in-mouth situation and instead used Table 4 for the breath signal with the highest concentration. The HS concentrations reported in Table 4 are the average of five repetitions: the standard deviations are about 20% of the reported values.

Assuming that the NS aroma profile is identical to the HS (apart from for the overall intensity), one would expect that the ratio HS/NS should be the same for all masses. The concentrations in the HS and NS spectra would hence only differ by a factor that is identical for all masses. Deviations could then only be attributed to volatiles that are present in the breath air (endogenous source, i.e., from human metabolism) but absent in the HS. (Such endogenous volatiles will overlap on the in-mouth profile originating from the food, and modify the in-mouth profile originating from to the food.) We have determined these ratios for unripe and ripe banana and included the values in the two final columns of Table 4. For most of these ratios we obtain values in the range 5–10. This mean that on average, NS intensities are 10–20% of HS intensities.

Table 4
NS and HS concentrations unripe and ripe banana, for a series of VOCs, as measured by PTR-MS

Protonated mass (amu)	Concentrations (ppbv)				Ratio HS/NS	
	Unripe NS	Unripe HS	Ripe NS	Ripe HS	Unripe	Ripe
131	<1	<1	120	215	–	1.8
117	<1	5	52	580	>5	11.2
99	40	400	5	58	10	11.6
89	40	5	7300	5500	0.125	0.8
87	3	9	770	1350	3	1.8
83	60	505	20	90	8.4	4.5
81	5	133	<1	28	26.6	>28
71	5	12	1670	2700	2.4	1.6
69	<1	63	70	135	>63	1.9
61	40	43	9000	11700	1.1	1.3
57	30	610	2300	5350	20.3	2.3
47	35	105	2700	6750	3	2.5
45	275	1870	4320	9900	6.8	2.3
43	25	88	7050	11150	3.5	1.6
33	50	380	180	1450	7.6	8.1

The HS concentration are the mean of five separate experiments, as outlined in Table 3. A series of NS experiments were performed. Large variations in absolute intensity from experiments to experiments, and from breath-to-breath, were observed, but the overall pattern discussed here is reproduced throughout the various experiments. In stead of taking means, the concentrations in table are based on one specific and representative example. We included the concentration for the exhalation with the highest intensity as representative for each respective masses. In the last two columns we also report the ratios of HS/NS concentrations for ripe and unripe banana.

Two notable exceptions are $m/z = 69$ and 57. In our case, we know that the intensity at $m/z = 69$ is partly due to isoprene coming from the breath air (probably related to the isoprenoid biosynthetic pathway) [23], and that its concentration can vary significantly depending on human metabolism [24,25]. In addition, isoprene formation in plants is know to occur via elimination of pyrophosphate from dimethylallyl diphosphate, giving rise to a series of hydrocarbons—among others isoprene [26]. Hence, the observed isoprene originates from two distinct sources, of which the endogenous source (human metabolism) varies with blood cholesterol level [25]. We, therefore, exclude this mass from our discussion; $m/z = 57$ is a mass where a large number of fragments overlap (see Table 2). In an ongoing study on fragmentation of VOCs in PTR-MS, we observed some modulation in the fragment patterns with changes in relative humidity of the gas being analysed (unpublished research). Since NS air is more humid than the HS of banana, we believe that the HS/NS ratio at $m/z = 57$ is biased because it is composed mainly of fragments, and that this fragmentation might differ in the HS relative to the NS.

A large number of these ratios are in the range 5–10, indicating that for many of the investigated compounds, concentrations in the HS are 10–5 higher that in the breath air. But we also see a series of masses where the ratios deviate appreciably from this 10 to 5 range. Low ratios, as in the case of $m/z = 87$, 71, 61 and 47 indicate that the volatility is increased in the mouth relative to the other VOCs. In contrast, an HS/NS ratio of larger than 20 at $m/z = 81$ is indicative of a decreased relative volatility in the mouth compared to the HS situation. We interpret these deviations as a manifestation of the specificity of the in-mouth situation, which modulates the aroma of banana, relative to the HS aroma.

The other element that is characteristic to the in-mouth situation is the dynamic nature of aroma as measured by NS sampling. In the case of unripe banana, we observe a gradual increase in the volatile concentrations with each exhalation for the C6-aldehydes, indicating that progressively more

volatiles are released in the mouth while unripe banana is being masticated. C6-aldehydes ($m/z = 99$ and 83) are known to be formed and released from ripening plants when wounded by lipoxygenase-dependent conversion of α-linolenic acid [20]. With increasing severity of damage to the unripe banana (mastication), we observe that more and more of these compounds are released. Our data do not allow to differentiate between hexenal and hexanal formed as a defence to wounding and amounts already present and increasingly released during mastication (or a superposition of both). After three breathing cycles, the person swallows the piece of banana.

As the assessor took a second bite, we observed again a gradual increase in volatile concentrations with each breathing cycle, for hexenal and hexanal. In contrast, the concentration at $m/z = 89$ was rather low and did not reach the intensities observed at the first bite. In fact, it was barely above the level in the breath air. After about six breathing cycles the assessor swallowed the second bite and immediately took a third one. We again observed a gradual increase in the aldehydes, while the ion intensity at $m/z = 89$ remained low. It seems that the compound(s) observed at mass 89, ethyl acetate and isopentanol, are particularly abundant at the tip of the banana.

During eating of a ripe banana, we observe a whole set of different compounds. Most importantly, we monitored breath-by-breath the two key aroma compounds of banana: isobutyl and isopentyl acetate. 2*E*-Hexenal and hexanal, typical of the ripening fruit have nearly disappeared in the NS, indicating that the ripening process is complete. In contrast to the unripe banana, we did not note any gradual increase in released intensity with subsequent breathing cycle. The intensities remained approximately constant for the three first exhalations, before the assessor swallowed the piece of banana. The subsequent swallow breath showed very strong ion intensities at all masses [27]. The second bite led to in-breath intensities similar to the first bite. Yet, the swallow breath following the second bite appeared less intense than after the first swallowing. The third bite was again very similar to the first. While the breath-by-breath intensity did not change as long as the banana was in the mouth, a very prominent swallow peak was again observed. After having swallowed the final (third) bite the ion intensities gradually decreased, with some weak after-odour remaining until the end of the experiment.

5. Conclusions

The HS profiles of ripe and unripe banana was analysed by PTR-MS and compared to previous reports, using either SIFT-MS [22] or APCI-MS [13]. While our results agree with those obtained by APCI-MS, many discrepancies remain with the SIFT-MS results. Furthermore, the PTR-MS and APCI-MS data closely fit with the literature on VOCs of banana, allowing to assign most ion peaks to known VOCs.

The in-mouth aroma of ripe and unripe banana were analysed by on-line sampling of the air exhaled through the nose while eating unripe and ripe bananas, and mass analysing the VOCs by PTR-MS. Six selected compounds are discussed. Two compounds are characteristic of the aroma of ripe banana—isopentyl acetate at $m/z = 131$ and isobutyl acetate at $m/z = 117$—while two others are characteristic of ripening banana—2*E*-hexenal and hexanal. The breath-by-breath temporal release pattern revealed various dynamic elements that are characteristic of the eating situation. While eating unripe banana we observed a gradual increase in 2*E*-hexenal and hexanal with mastication, until swallowing. Either they already pre-exist and are progressively released in ever higher concentrations, or they are formed during mastication. No particularly high concentrations were observed in the exhaled air just after swallowing (swallow breath).

Eating a ripe banana, we observed isopentyl acetate and isobutyl acetate, compounds characteristic of banana aroma. In contrast, VOCs characteristic of unripe banana were largely absent. No gradual increase was observed with mastication, as occurred in unripe banana. In contrast, very prominent swallow peaks were observed.

In-mouth aroma analysis via on-line breath-by-breath monitoring of volatiles exhaled through the

nose, is a first step in our effort to bring analytical sciences closer to the world of the consumer. By better understanding how, when and in which dose food aroma is delivered to our olfactory senses during eating, we ultimately expect to improve the aroma impact of food products.

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