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Determining Specific Food Volatiles Contributing to PTR-MS Ion Profiles Using GC-EI-MS

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This work focused on developing a method to determine the volatile compounds that contribute to individual masses observed by PTR-MS in the headspace of a food product (e.g., cheese crackers). The process of interfacing a PTR-MS with a GC-MS (electron impact) through an existing sniffing port is outlined, and the problems faced in doing so are discussed. For the interface developed, linearity for both detectors working online for a wide range of concentrations of a selected compound (hexanal) was good ($R^2 = 0.88$). There was also a good correlation between the responses for both instruments (confidence interval for the slope between 0.56 and 1.18) over a range in concentrations despite the different ionization processes taking place. The application of our system (PTR-MS/GC-MS interface) to a real food system (cheese crackers) in which volatiles were isolated via purge and trap allowed the assignments of most of the PTR-MS masses to major volatile compounds in the samples. However, in this interface it is important to consider some limitations related to GC resolution, compound identification by EI-MS, PTR-MS sensitivity (and overloading), PTR-MS inlet requirements (ca. 20 mL/min), ion chemistry in the PTR-MS, and potentially changing sample composition over time, altering the contribution of a given compound to a specific ion. These issues are discussed.

KEYWORDS: PTR-MS; GC-EI-MS; food volatiles; food PTR-MS ion profile

INTRODUCTION

Since proton transfer reaction mass spectrometry (PTR-MS) was developed by Lindinger and co-workers for online trace gas analysis (*1*), its applications in different fields have been continuously growing. Among them, PTR-MS has been shown to be a powerful tool in food flavor applications, for example, in studies on in vivo aroma release (*2–5*) or in quality control (*6–11*). Moreover, PTR-MS data have been used for sensory predictions (*12, 13*).

Unfortunately, PTR-MS has been interfaced with a low-resolution MS and thus provides only unit mass resolution. Thus, on sampling food volatiles, one obtains an ion profile but little information on the compounds that contribute to a given ion in this profile. In most applications it is desirable to know what the PTR-MS is measuring. For example, knowing that a specific PTR-MS ion comes from a product of lipid oxidation (e.g., hexanal, off-flavor component) versus butyric acid (desirable cheese flavor component) permits a different interpretation of that data point.

One can distinguish between some isobaric compounds using different approaches. For example, obtaining the PTR-MS ion profile of individual pure compounds provides an ion spectrum that may offer unique secondary ions (fragments) that permit distinguishing between compounds or chemical classes (*14, 15*). Wyche et al. (*16*) have demonstrated the use of alternative reagent gases for PTR-MS to distinguish between aldehydes and ketones of equal mass. Variation of E/N (electric field strength/buffer gas number density), the observation of the isotopic abundance, or differences in the mobility of isomeric structures in the buffer gas are some strategies that have been proposed to help in the identification (*6, 17*); nevertheless, they are difficult to apply to complex aroma mixtures. The possibility of using other types of MSs instead of a quadrupole, which is currently used in the standard PTR-MS, has been also explored. For example, a proton-transfer ion trap mass spectrometer (PTR-IT-MS) (*18, 19*) and a time-of-flight mass spectrometer (PTR-TOF-MS) (*20*) have been used in place of the quadrupole. The latter system has been shown to have good mass resolution but low sensitivity compared to the standard PTR-MS (*21*).

An alternative approach to determine the compounds that contribute to a given PTR-MS ion profile of a complex sample is interfacing a PTR-MS with a GC-MS. This approach can provide the fraction of a PTR-MS ion that comes from a given aroma compound. This technique was initially applied to the analysis of atmospheric pollutants, a relatively simple mixture (*22–25*).

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Recently Lindinger et al. (*26*) have developed a PTR-MS/GC-MS system based on this principle, and they applied it in the analysis of coffee headspace. Although this is a very valuable technique, it requires some modifications to the PTR-MS, and the complex setup required can make it unaffordable for many laboratories. Thus, we have chosen to develop an alternative, easily affordable *online* PTR-MS/GC-MS interface using a sniffing port of a GC and standard PTR-MS. To illustrate this interface and issues in its development, we have used it in the assignment of volatiles to PTR-MS ions in the headspace of cheese crackers. We emphasize that our goal in this publication was not to assign all of the compounds found in the headspace of cheese crackers to PTR-MS ions, but to logically discuss the development of this interface with its strengths and weaknesses.

MATERIALS AND METHODS

Samples. Two sets of baked snack cheese crackers (1 and 4 months of age) were purchased from a local grocery store. The samples were manufactured at the same production site and used the same product formulation. We have no information on the handling/storage of these products from manufacture to our analysis.

Chemical Compounds. All of the reference compounds [hexanol (98-01-1), phenylacetaldehyde (122-78-1), 2-furfural (98-01-1), furfuryl alcohol (98-00-0), 2-pentylfuran (3777-69-3), and 2-pentenal (764-39-6)] were purchased from Sigma Aldrich (Milwaukee, WI) in the highest purity available.

Design of the Interface PTR-MS/GC-MS. The GC effluent (ca. 2 mL) was split into two fractions: one fraction (ca. 1 mL/min) entered the EI-MS (flow controlled by tubing diameter and length used in transfer) inlet. The other column fraction (ca. 1 mL) went directly to the PTR-MS (Ionicon Analytik, Innsbruck, Austria) via an existing GC sniffing port. The sniffing port was slightly modified for this purpose to allow the addition of makeup gas (purified air at 18 mL/min). This mixture went through a deactivated silica capillary placed inside a heated metal tube (50 cm length) to the PTR-MS inlet. Mixing the GC effluent with pure air allowed an adequate gas volume to enter the PTR-MS drift tube to maintain the necessary operating pressure (2 mbar, manufacturer's recommendations). To avoid possible condensation of compounds along the transfer line that connected the GC-MS with the PTR-MS, this tube was heated with heating tape and two external heaters (200 °C).

The ion intensities were corrected by taking into account the ion intensities of masses 21 ($H_3{}^{18}O^+$) and 37 ($H_3O^+ - H_2O$) (indicative of the amount of reactant ions) and the transmission factors of each mass though the quadrupole according to reference (*2*). An E/N ratio of 123.4 Td was used.

An HP5890 series II gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a DB5 capillary column (30 m length, 0.25 mm i.d., and 0.25 μm film thickness; Supelco, Bellefonte, PA) and a flip-top inlet injector system (Agilent Technologies, Apple Valley, MN) were used in this study. The chromatographic conditions were as follows: initial oven temperature, 40 °C; 2 min hold; and a program rate of 5 °C/min to 250 °C (10 min hold).

Mass spectra were obtained using a 5970 HP mass spectrometer (Hewlett-Packard) scanning from *m*/*z* 29 to 350 amu at 2.9 scans/s. Compounds were identified by comparison with standard mass spectra (when available) and using the mass spectra libraries (Wiley and NIST database). Quantitative data were obtained by electronic integration of the TIC peak areas.

PTR-MS/GC-MS Operating Conditions. The PTR-MS dwell time was chosen by taking into consideration that longer times can afford greater sensitivity, but shorter times ensure resolution of GC peaks of common mass and improve GC resolution. Because data acquisition rate (short or long dwell times) has advantages and disadvantages, we chose to acquire data sets at both fast and slow acquisition rates (5 and 1 ms/amu) to determine the best acquisition rate.

Contrary to Lindinger et al. (*26*), we chose to use a narrow-bore GC column rather than a megabore column. A megabore column (i.d. = 0.53 mm) provides increased peak width and a high column capacity but at great cost in terms of chromatographic resolution. We chose peak resolution (small column diameter) versus capacity (large column diameter) knowing that the volume of column effluent would be inadequate for the PTR-MS and thus have to be diluted with a makeup gas. Breathing quality air (cylinder) was added to the column effluent to maintain the optimum pressure conditions (2 mbar) in the PTR-MS drift tube. Adding air also allowed the system to be operated with air instead of helium, which could alter the ion–molecule reaction kinetics (*25*).

As noted earlier, all of the transfer lines from the GC sniff port to PTR-MS inlet were wrapped with heating tape. Heating the transfer lines was absolutely necessary to reduce both the lag time between the detection of compounds in the two detectors (that would complicate peak assignment) and the number of cycles (or time) for a mass (PTR-MS) to reach baseline after a maximum signal (also increasing the noise in the system).

Another parameter that was important to take into consideration was the acquisition mode, which also is related to the dwell time. Operating in baragraph mode as opposed to the MID mode (scanning specific masses) is required when the masses of interest are unknown or if the study objective is to obtain a mass fingerprint of the product headspace (*7, 8, 10, 11*, among others). Previous studies using a GC-PTR-MS (*22–25*) or PTR-MS/GC-MS interface (*26*) have been performed using the MID acquisition mode, thus selecting specific masses to be monitored. In our work, the PTR-MS was operated in the baragraph mode, scanning all masses between 20 and 220 amu, because we did not know a priori the masses of the key volatiles in our sample. This is an important point because the main objective of our interface was not only to be used as a confirmation tool of some major compounds in the sample but to try to identify the maximum number of compounds from a complex extract. As expected, the sensitivities of both acquisition modes were very different; for example, the signal (*m*/*z* 83) from the headspace of a solution of hexanal in water ($1.5 \times 10-5$ μg/L) was undistinguishable from the system noise (1×10^2 cps) when operated in the baragraph mode but >10 times more intense when operated in the MID mode (1.1×10^3 cps).

Sample Preparation for Assigning Compounds to PTR-MS Masses. Once we had our PTR-MS/GC-MS interface established, our next task was to test it using a real food product. Ideally, we wanted to use the PTR-MS to directly sample a food headspace and then be able to monitor how the headspace changed in the food over time. This requires the PTR-MS/GC-MS interface to determine what compounds actually changed with time. We could not simply add a sample of product headspace to the PTR-MS/GC-MS: the headspace was much too dilute to afford any MS identifications. Thus, we had to find a means to concentrate the product headspace and then inject it into the PTR-MS/GC-MS. This concentration step undoubtedly would result in a sample that was somewhat different from the product headspace: it is well recognized that all isolation methods will produce an isolate differing from the true product headspace. Yet, there is no alternative to this step, so we must accept that when we assign a given mass to various compounds (perhaps several compounds contribute to a single PTR-MS mass), we will likely be in error to some extent.

Preliminary work was performed to compare different headspace concentration techniques (data not shown) for use. On the basis of our results, purge and trap (P&T; Tenax) was chosen as the most suitable method to obtain high volatile recovery from the samples.

The procedure we used is briefly described. Fifty grams of ground, whole crackers was placed in a special purge flask (274.5 mL volume), and the flask was flushed with purified nitrogen gas (40 mL min^{-1}) for 30 min at 45 °C, trapping the purged volatiles in a glass Tenax-filled GC liner (100 mg of Tenax TA 60/80, Supelco). The volatile compounds trapped on the Tenax trap were desorbed (in splitless mode) in the GC injection port (250 °C) for 10 min (facilitated by the flip-top GC injection port closure). During this time the compounds were cryofocused at the beginning of the GC column with liquid nitrogen to reduce band broadening. Separation of volatiles was performed in the same column and chromatographic conditions describe above.

PTR-MS/GC-MS Sample Analysis. During the first 10 min of analysis (corresponding to the desorption and cryofocusing of volatiles

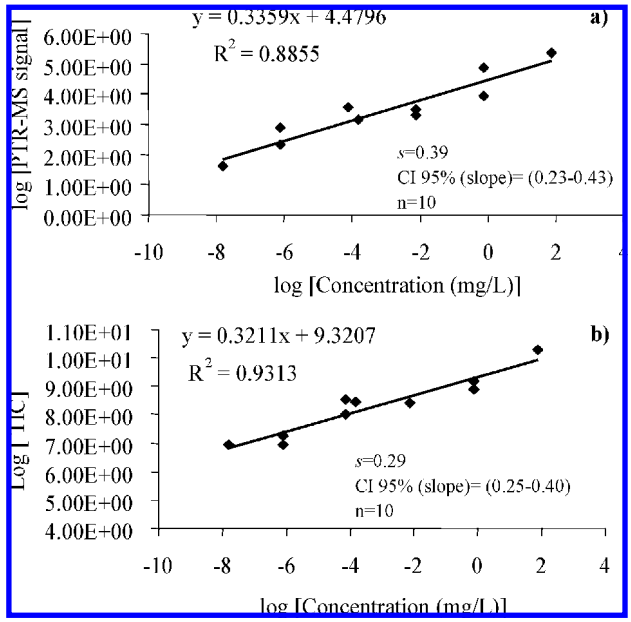

Figure 1. Relationship between the PTR-MS signal (**a**) (ion 83) and the TIC areas (**b**) of hexanal from GC-MS. The figures include the results of the linear regression analysis: R^2 (determination coefficient), *s* (standard error), CI (95% confidence interval for the slope), *n* (number of observations).

in the GC-MS), the PTR-MS monitored the background of the system (corresponding to the mixture of carrier gas from the GC column and pure air, necessary to maintain the required pressure in the PTR-MS drift tube). When desorption and cryofocusing were completed, the GC run started, and we recorded the PTR-MS cycle number. This is needed to relate the GC retention time to a specific PTR-MS cycle number. All of the samples were analyzed in triplicate.

Determination of Linearity between GC-MS and PTR-MS. For the determination of the linearity of the PTR-MS and GC-MS, a solution of hexanal in Milli-Q water (76 mg/L) was prepared and from this different dilutions (between 76 and 0.76×10^{-6} mg/L) were prepared and analyzed. For analysis, the series of hexanal dilutions were purged with N_2 (40 mL/min) for 10 min, and the stripped hexanal was collected in a Tenax trap. The Tenax trap was desorbed in the injection port of the GC following the same protocols as explained above.

Direct PTR-MS Headspace Sampling. Twenty-five grams of crackers was placed in a 100 mL Mason jar and covered. After 1 h at room temperature, the capillary inlet of the PTR-MS was introduced in the headspace of the jar (always in the same position) through a pierced aluminum lid. Sampling was carried out for about 20 cycles (10 min). The PTR-MS was operated at an inlet flow of 19 mL/min that resulted in a pressure of 2 mbar in the drift tube. Immediately prior to sample analysis, we sampled the room air to determine background. The PTR-MS was operated in the baragraph mode, scanning all masses between 20 and 220 amu.

Statistical Analysis. Linear regression analysis was performed to compare the responses between the PTR-MS and GC-MS when working online. A two-sample Student's *t* test (at 95% significance level) was used to compare the TIC areas and PTR-MS responses in the two sets of samples (1.5 and 4 months). Statgraphics Centurion XV (Statpoint, Inc., 2005) was used for data processing.

RESULTS AND DISCUSSION

Linearity and Correlation between the GC-MS and PTR-MS Responses. To determine the linearity of the PTR-MS and GC-MS signals when both instruments were interfaced, solutions of hexanal in water at different concentrations were extracted by P&T and analyzed using the PTR-MS/GC-MS interface. **Figure 1a** shows the relationship between hexanal concentrations in the sample series and the PTR-MS signal for the fragment *m*/*z* 83 (major ion from hexanal). These results show excellent linearity between sample concentration and PTR-MS signal ($R^2 = 0.88$). As hoped, this is equivalent to the relationship found between sample concentrations and the hexanal TIC areas in the GC-MS, $R^2 = 0.89$ (**Figure 1b**). It is of value to note that both signals were linear over nearly a 10^{10} change in hexanal concentration (in water).

Linear regression analysis was also applied to determine the correlation between PTR-MS and GC-MS signals. For *m*/*z* 83, the equation obtained was as follows: log PTR-MS signal (*m*/*z* 83) = 5.26 + 0.87 log TIC, with $R^2 = 0.84$, a residual standard deviation (*s*) of 0.44, and a 95% confidence interval for the slope of 0.56–1.18. For *m*/*z* 101 the regression obtained was as follows: log PTR-MS signal (*m*/*z* 83) = −4.29 + 0.87 log TIC, with $R^2 = 0.76$, $s = 0.54$, and a 95% confidence interval for the slope of 0.47–1.21. It is interesting to note that for both ions the 95% confidence intervals for the slopes included the value $b = 1$, showing a good agreement between both signals despite the different ionization processes that take place in the two instruments, electron impact in GC-MS and chemical ionization with water in the PTR-MS.

Table 1. Occurrence of Ions in the Headspace of the Sample Attributed to the Cheese Crackers by Direct PTR-MS

	% signal[a]			% signal	
ion (*m*/*z*)	mean	± sd	ion (*m*/*z*)	mean	± sd
35	0.1	0.03	75	0.5	0.07
41	1.8	0.11	77	0.2	0.01
42	0.3	0.10	79	0.5	0.03
43	15.5	0.39	83	1.3	0.07
44	0.6	0.05	84	0.1	0.03
45	8.4	0.27	85	0.4	0.02
47	4.1	0.02	86	0.1	0.02
48	0.9	0.16	87	6.3	0.59
49	0.2	0.04	88	0.4	0.06
51	0.4	0.04	89	1.9	0.12
57	1.7	0.11	90	0.1	0.03
58	0.5	0.03	91	0.1	0.05
60	0.5	0.09	97	0.1	0.03
61	18.8	0.58	99	0.1	0.04
62	0.5	0.14	101	0.4	0.09
63	0.5	0.15	103	0.1	0.03
64	0.0	0.01	104	0.2	0.04
65	0.4	0.07	105	0.1	0.04
69	20.2	0.69	107	0.1	0.02
70	1.2	0.14	114	0.2	0.05
71	0.5	0.02	118	0.0	0.02
72	0.1	0.01	119	0.1	0.00
73	9.0	0.35	165	0.1	0.01
74	0.5	0.03			
total	86.6			13.4	

[a] Percent of total ion signal of each of the 47 ions found in the headspace of the sample ($n = 3$).

Application of PTR-MS/GC-MS for the Identification of Compounds Responsible for Ions Detected by PTR-MS in a Food Headspace. A large number of ions were found in the PTR-MS output using P&T sampling of cheese crackers. Thus, we chose to do some preliminary work to permit focusing our efforts on ions truly of interest to us. Basically, we directly sampled the cracker headspace after 1 h of equilibration at room temperature with the PTR-MS versus a blank sample. In this manner, we found 47 ions that could uniquely be assigned as originating and being detectable from the cracker product (**Table 1**). Our subsequent work focused on identifying the volatiles in the product headspace that contributed to each of these 47 ions and then monitoring each selected PTR-MS ion as a function of product age.

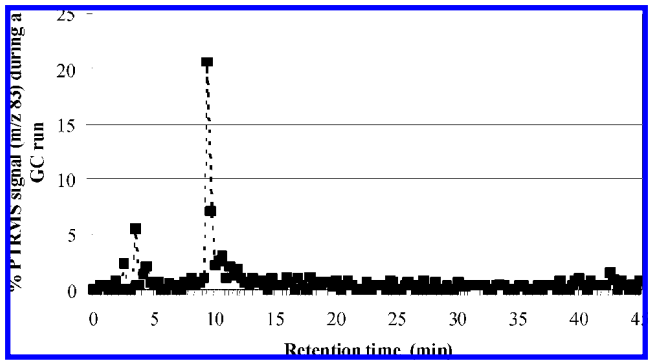

Figure 2. Example showing the percentage of signal corresponding to *m*/*z* 83 during a GC run (note that the maximum percentage at 9.4 min corresponds to the retention time of hexanal).

Using the PTR-MS/GC-MS system, we calculated the contribution of each compound identified by GC-MS to each of the 47 selected PTR-MS ions. The contribution of each ion was expressed as percentage of signal at one specific GC run time compared to the intensity of the ion in the whole PTR-MS/GC-MS run. **Figure 2** shows graphically an example of the PTR-MS signal of ion *m*/*z* 83 during a PTR-MS/GC-MS run. An examination of this figure shows that the largest *m*/*z* 83 signal occurred at a GC run time of 9.4 min. Not surprisingly, this compound was identified as hexanal. There are two other compounds that also contributed to mass 83: one at about 2.5 and the other at ca. 3.0 min. Other than these three compounds, the remainder of the ion count came as background accumulated over the entire GC run.

The percentages of ion intensities contributed by each volatile compound in the cracker headspace aroma isolate were calculated. **Table 2** presents the assignment of GC-MS compounds to specific PTR-MS ions, taking into consideration only those that contributed >10% of the ion intensity. In this way we tried to ensure a correct identification avoiding other interference signals (background noise, compound carry-over, inadequate peak resolution, etc.). In retrospect, the background of the system could have been lower using Teflon rings in the drift tube instead of the Viton rings as was used in this experiment (*26*). Although the three repetitions for the same sample have been treated independently, **Table 2** shows the final averaged results. Despite the high dilution of the sample before reaching the PTR-MS, of the 47 ions that were selected as representative of the sample headspace by PTR-MS (**Table 2**), most showed intensities >10% in the PTR-MS/GC-MS experiment. Also, the major ions determined by direct PTR-MS of the sample headspace and P&T PTR-MS/GC-MS were similar, lending credibility to the methodology. This is particularly significant because the data from the PTR-MS/GC-MS was of a P&T aroma isolate as opposed to direct headspace sampling of the product.

One might expect significant changes in the headspace profiles of these two sampling methods (*27*). Only seven ions (*m*/*z* 35, 48, 60, 65, and 91) selected in the headspace of the product by

Table 2. Retention Times of the Most Representative Ions of the Headspace of the Crackers (1.5 and 4 Months Old) during a PTR-MS/GC-MS Run and Tentative Assignment of These Ions to Compounds That Eluted at the Same Retention Time in GC-MS

		% *S*[b]					% *S*		
RT[a] PTRMS	ion *m*/*z*	1.5 months	4 months	tentative ID	RT PTRMS	ion *m*/*z*	1.5 months	4 months	tentative ID
1.87	**45**	10	18	acetaldehyde		69	20	14	
1.98	**47**		12	ethanol		70	10	15	
	72	26	33			85	16 ± 0.8	16	
	73	32 ± 20	54 ± 8			97	45	13 ± 11	
	74	24	23			101	18	12	
	75		10			104	11	-	
	89	49 ± 14	45 ± 20		8.57	71		14 ± 0.7	1-pentanol
						86		6.7 ± 3	
	90	29 ± 10	27 ± 20			83	45 ± 20	79 ± 7.4	
	43					84	23 ± 15	72 ± 3	
	61	17 ± 3	16 ± 6			99		10	
	62	9 ± 7	13 ± 4			**101**		40 ± 8	
	41	53 ± 21	67 ± 30			118		33 ± 10	
	42	9	46 ± 7			119		12	
	44		53 ± 14		11.5	**97**		7 ± 3	furfural
	45		38 ± 1			119		12 ± 4	*cis*-3-hexanal
	49		42 ± 20		12.4	64		11 ± 1.6	xylene
	57	24	41 ± 20			77		13	
	58	40 ± 20	69 ± 18		13	97		9 ± 0.1	heptanal
	61	8 ± 2	38 ± 1.7		13	105		13 ± 3	methional
	62		35 ± 5		15.5	**107**		17 ± 9	benzaldehyde
	63		20 ± 5			114		10	
	64		11 ± 0.2			118		10	
	69	66 ± 20	74 ± 20			**165**	23 ± 5.7	13 ± 4.6	TBBQ
	70		74 ± 20						
	71	26	21						
	79	10	37 ± 2.8						
	85	38	25 ± 20						
	86	42	28 ± 15						
	87	75 ± 2	60 ± 20						
	88		53 ± 20						
	103	11 ± 0.6							
	104	23	40 ± 20						
	105	15	31 ± 14						

[a] Retention time (min) corresponding to the PTR-MS-GC-MS run. [b] % *S* is the percent signal intensity (average ± standard deviation) of one ion at one specific retention time compared with the total contribution of the same ion during the whole PTR-MS/GC-MS run (see the text for more detail). If no standard deviation is shown, the value corresponds to one determination. The ion corresponding to the protonated compound ($M - H^+$) is given in boldface.

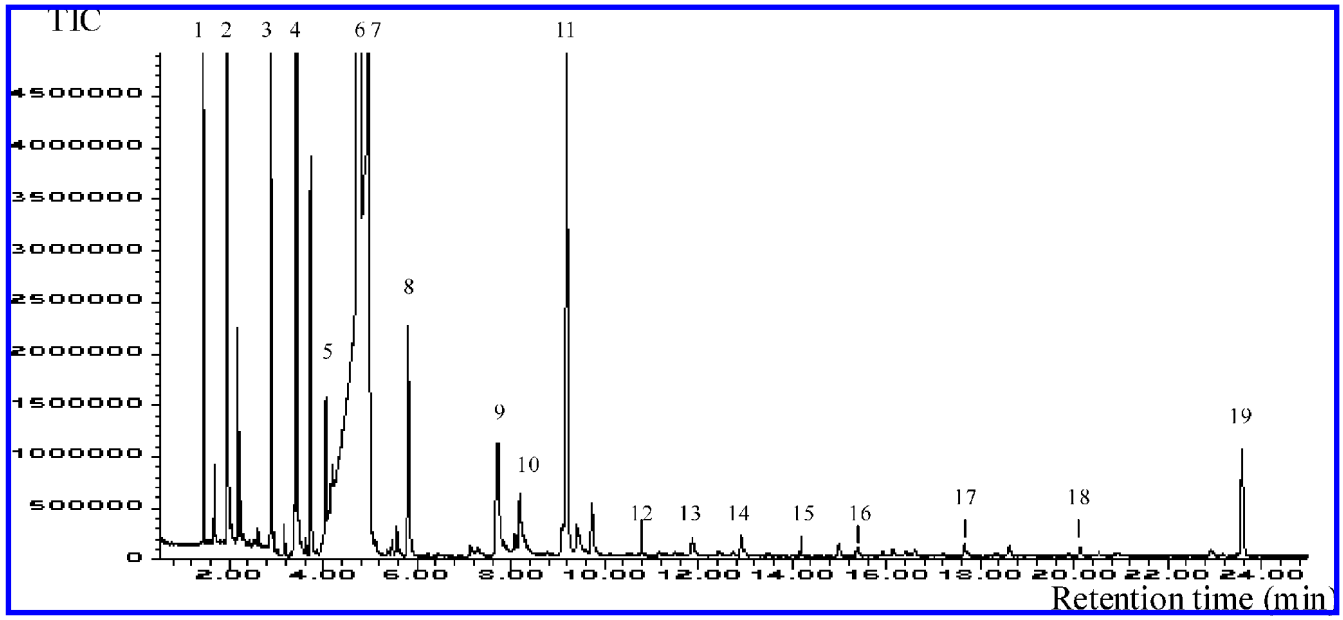

Figure 3. Example of a P&T TIC chromatogram of baked crackers showing some of the compounds tentatively identified: acetaldehyde (1), methanol (2), isobutyraldehyde (3), ethyl acetate (4), acetic acid (5), 3-methylbutanal (6), 2-methylbutanal (7), 1-penten-3-ol (8), 1,2-propanediol (9), 1-pentanol (10), hexanal (11), furfural (12), xylene (13), heptanal (14), methional (15), benzaldehyde (16), octanal (17), nonanal (18), *tert*-butylbenzoquinone (19).

direct headspace showed intensities lower than 10%, although their intensities determined by direct-PTR-MS were also extremely low (data not shown).

The early eluting peaks of the GC-MS run (1.8–4 min) corresponded to very volatile compounds (e.g., acetaldehyde, ethanol, isobutyraldehyde, and ethyl acetate). The dominant masses detected corresponded in general to the molecular ion of each compound [e.g., *m/z* 45 (acetaldehyde), *m/z* 47 (ethanol), *m/z* 73 (isobutyradehyde), *m/z* 89 (ethyl acetate)]. Their corresponding isotopic ions, as expected, were found in lower concentrations (*17*).

When two compounds were poorly resolved by GC, compound assignments were very difficult. For example, acetic acid (*m/z* 61) and 3- and 2-methylbutanal (*m/z* 87) eluted very closely and also, as we commented before, acetic acid tailed greatly in the GC column (**Figure 3**). This resulted in a continuous background of acetic acid ions at the same time where the other two compounds eluted. The high concentration of these compounds in the extracts together with poor resolution of the early eluting compounds resulted in finding a large number of fragments between 4.3 and 4.7 min that were problematic to assign. Most corresponded to nonspecific alkane fragments from the loss of water and fragmentation (e.g., *m/z* 45, 57, 71, etc.). Although these reactions are highly probable with alcohols (*14*), we have also observed similar spectra for aldehydes. For instance, the loss of a water molecule (−18 amu) from aldehydes such as pentanal, hexanal, and heptanal gave ions of *m/z* 69, 83, and 97, respectively.

Regarding 1-penten-3-ol and 1-pentanal, we did not observe the M + 1 ion (*m/z* 87) for either of these compounds. However, we did find the ion *m/z* 69 at the expected retention time of these compounds. This fragment has been found to be more abundant than the molecular ion for 1-penten-ol (*22*) but also has been shown as the main fragment produced from pentanal (*14*). Because we did not find a fragment *m/z* 57 (typical of alcohols) and considering the higher concentration of the aldehyde in the samples compared with the alcohol, the ion *m/z* 69 likely corresponds with the 1-pentanal.

Although the MH^+ ion was most abundant for some compounds, for most compounds, fragments of the parent were the most abundant. In some cases, such as pentanol (MW = 88) and heptanal (MW = 114), the fragments *m/z* 71 and 97, respectively, were the only evidence that these compounds were in the samples. Buhr et al. (*14*) showed different fragmentation patterns for the isomers 1-pentanol and 2-pentanol; they observed a high proportion (>80%) of fragment *m/z* 71 for 2-pentanol.

Hexanal was one of the largest peaks in the chromatograms. Although we observed the MH^+ ion (*m/z* 101), the most abundant fragment was *m/z* 83. This fragment has been repeatedly shown to be the most abundant ion in the determination of hexanal by PTR-MS (*14, 22, 28*). Although observed only in the 4-month-old samples, some heavier ions were also observed, for example, *m/z* 119. This ion could be an artifact. It may be the protonated diol produced from hexanal by acid-catalyzed hydration in the drift tube favored by the high concentration of acetic acid in these oldest samples. This mechanism could also explain the formation of fragment *m/z* 104 from other aldehydes such as 2- and 3-methylbutanal and pentanal. The formation of the enolate anion from hexanal could be responsible for the *m/z* 99 ion that we observed during the elution of hexanal.

The loss of the two methyl groups from xylene (MW 106) is likely the origin of the ion *m/z* 76 because it appeared in the PTR-MS trace at the same GC retention time as xylene. Although we also found the ion *m/z* 107 in the PTR-MS profile, this ion appears more likely to come from benzaldehyde because it was found at the same GC retention time (15.5 min) as benzaldehyde. Nevertheless, both compounds could contribute to the *m/z* 107 signal when the product headspace is directly sampled, as has been already suggested (*24*). The PTR-MS ion, *m/z* 114, was found at the elution time of nonanal. This ion may have arisen from the loss of the carbonyl group, giving the alkyl fragment C8. Although the loss of carbonyl groups in the drift tube has never been reported before, this could be possible. A recent review about the measurements of VOCs by

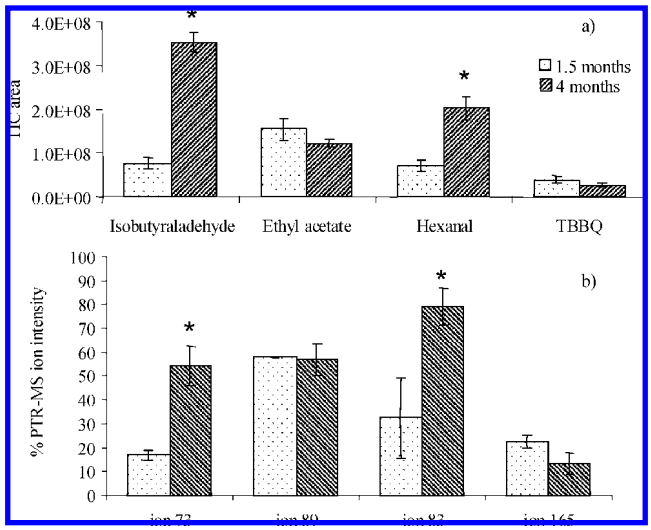

Figure 4. Influence of sample age on the PTR-MS signal (**a**) and GC-MS TIC (**b**). The results correspond to the average of three repetitions. The error bars represent ± one standard deviation. Asterisks denote significant difference at 95% confidence level (Student' *t* test).

PTR-MS noted the lack of knowledge dealing with the fragmentation reactions that take place in the drift tube (*21*).

We also detected the ion *m*/*z* 165 at the same retention time that *tert*-butylbenzoquinone (TBBQ) eluted. TBBQ is the primary oxidation product of *tert*-butylhydroquinone (TBHQ), an antioxidant commonly used to slow rancidity in edible oils (*29, 30*).

It is interesting to note that new ions and a general increase in the intensities of them were observed in the older samples (**Table 2**). We assume that this is due to the formation of new compounds, mainly an increase in aldehydes likely originating from lipid oxidation.

It appears that the fragmentation pattern in the PTR-MS also depended on the concentration of volatiles in the sample; that is, some fragments appeared only when compounds were present in higher concentrations. Although it is difficult to directly relate a single ion to a single volatile compound due to the complexity of the sample headspace, for those compounds in high concentrations, we observed, in general, good agreement between the increase in the intensity of their PTR-MS ions and an increase in TIC area that was determined by GC-MS. For example, in **Figure 4**, it can be seen that isobutyraldehyde and hexanal showed significant increases in the TIC area of the 4-month-old samples versus the younger samples: similar trends were found for the PTR-MS signal of the corresponding ions *m*/*z* 73 and *m*/*z* 83. Likewise, ethyl acetate and TBBQ did not show significant differences in their TIC areas or in their corresponding main PTR-MS ions *m*/*z* 89 and *m*/*z* 165.

In conclusion, the results of this work show that interfacing a PTR-MS/GC-MS can be used to assign a given compound (or compounds) present in the headspace of a food product with specific PTR-MS ions. In the setup presented in this work, interfacing a PTR-MS with a GC-MS via an existing sniffing port is relatively easy and does not require any specific equipment. However, the task is not simple. A number of compromises must be made in sample isolation and in the setup and operation of the instruments. We are plagued by limitations in volatile isolation and its biases: adequate GC resolution, compound identification by EI-MS, PTR-MS sensitivity (and overloading), PTR-MS inlet requirements (ca. 20 mL/min), ion chemistry in the PTR-MS, and potentially changing sample composition over time, altering the contribution of a given compound to a specific ion. (Note that we did not deal with this latter issue in this work.)

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