API-IT-MS for Measuring Aroma Release from Dentifrice Products Using a Device To Simulate Tooth Brushing

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A mechanical tooth brushing device coupled to an atmosphere pressure ionization ion trap mass spectrometer (API-IT-MS) combination has been developed to study the influence of time and dilution on aroma release from a model dentifrice system. API-IT-MS response to nine commonly used dentifrice flavor components was initially studied. Linear regression models were developed based on an exponential dilution method (EDA) to permit quantification of these compounds. Good linear fits were generated for the majority of compounds (R^2 > 0.92). The threshold detection limits were also calculated, and they greatly depended on the type of aroma compound. A brushing device was then coupled to the API-IT-MS and used to monitor the release profile of three aroma components from a model dentifrice system at flavor concentrations ranging from 0.1 to 20 mg g^{-1}. Large differences in the aroma release patterns were observed for different compounds (limonene, menthone and cinnamic aldehyde) that depended on their physicochemical characteristics (vapor pressure and log P), and on additional factors such as aroma−matrix interactions. In addition, a linear increase in API-IT-MS response with increased flavor concentration up to 1 mg g^{-1} flavor was observed, while at higher concentrations, e.g. between 1 and 20 mg g^{-1}, a plateau in response was noticed. This suggests that at concentrations above 1 mg g^{-1} a transition from a purely dissolved state to an emulsified state occurred. This fact influenced the time-dependent characteristics of the release curve (I_{max} and t_{max}) for the three assayed flavor compounds.

INTRODUCTION

Aroma volatiles are essential in characterizing the flavor and olfactory character of consumer products, and are often the most important factor driving sensory perception. Aroma contributes to a product's uniqueness, drives consumer preference and can even contribute to the perception of efficacy (*1*). In the case of oral care products such as dentifrices, flavor is undoubtedly one of most important attributes influencing consumer acceptance and liking of a product.

Two factors are essential in determining the aroma driven sensory profile of a product: the concentrations of aroma compounds present and the degree to which these compounds are released from the product matrix. The latter is specifically what determines the perceived sensory quality of the product. The matrix composition and the spatial distribution of the key ingredients (macro- and microstructure) greatly influence the interactions between aroma compounds themselves, as well as interactions with matrix components, and therefore determine

aroma release (*2*). A large body of work has been conducted in the food science field to determine the effect of these interactions on aroma release from foods and on their effect on aroma perception (*3*). However, there is little related work published on cosmetics, toiletries and oral care products.

Generally speaking, a dentifrice gel is a complex matrix that contains water, humectants (e.g., sorbitol, glycerin, or propylene glycol) and an array of other ingredients including hydrocolloids, abrasives, surfactants, flavor compounds, salts and coloring agents. The complexity of the matrix and the chemical and physical nature of the various ingredients within it significantly influence the release of aroma compounds during brushing. Furthermore, the dynamic nature of the brushing process, i.e. the dilution of the dentifrice as well as the mechanical action that takes place, is also important and should be addressed when developing methods to quantify flavor release. Prior studies published on aroma release from dentifrices have focused on static headspace analysis techniques (*4−6*). For example, a study has been published on the suitability of SPME to sample the aroma compounds that remain in the oral cavity after tooth brushing (*1*). While static headspace analysis provides valuable

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Table 1. Aroma Compounds Studied for Their Performance in the API-MS Instrument and Some of Their Physicochemical Properties

| | | | | | physicochemical properties | |
compd	vol^a (μL)	CAS registry no.	MW	BP^{b,c} ($^\circ$C)	VP^{b,d} (mmHg 25 $^\circ$C)	log P^{b,e}
limonene	600	138-86-3	136	176	1.55	4.83
menthone	200	10458-14-7	154	207	0.37	3.05
menthol	50	89-78-1	156	212	0.0637	3.40
methyl salicylate	500	119-36-8	152	223	0.0343	2.55
carvone	200	99-49-0	150	279	0.103	2.71
cinnamic aldehyde	600	104-55-2	132	246	0.0289	1.9
anethole	550	104-46-1	148	234	0.070	3.39*
menthyl acetate	550	8948-5	198	227	0.0913	4
eugenol	500	97-53-0	164	253	0.0226	2.27

^{a} μL of each compound injected into the 500 mL flask. ^{b} Experimental values obtained from EPI Suite database from EPA (2000). The values with an asterisk are estimated values from KOWIN v.1.67 (included in the Software EPI Suite). ^{c} BP: boiling point. ^{d} VP: vapor pressure. ^{e} log K_{ow}: log partition coefficient octanol/water.

findings with regard to the behavior of aroma compounds within dentifrice matrices of various compositions, it fails to capture the dynamic nature of product usage in real time during brushing, since the effect of time, dilution and mechanical action are not taken into account. Therefore, a more appropriate methodology is needed to quantify these real-time effects.

Proton transfer reaction mass spectrometry (PTR-MS) and atmospheric pressure ionization mass spectrometry (API-MS) have successfully been employed for the analysis of aroma release during food consumption when coupled with artificial mouth devices (7−11) or directly *in vivo* (12−14). However, there are no published works on using use these techniques in aroma release studies from oral care products despite their value in helping to understand the key dynamic factors driving aroma release and to better predict aroma perception in real time. Therefore, this work has focused on the study of the reliability of atmosphere pressure ionization ion trap mass spectrometry (API-IT-MS) for the analysis of aroma release from a model dentifrice using an artificial "mouth" for simulating tooth brushing in real time.

MATERIALS AND METHODS

Aroma Compounds. Nine aroma compounds of varying physicochemical properties and representative of dentifrice flavoring blends were analyzed by API-IT-MS to determine the instrument sensitivity to them (e.g., determine detection thresholds and linear ranges). The aroma compounds and some of their physicochemical properties are shown in **Table 1**. All compounds were provided by the Colgate Palmolive Company (Piscataway, NJ) and were of a purity level higher than 90% by GC analysis.

Exponential Dilution Analysis (EDA). The analytical characteristics (detection thresholds and linear range) of the API-IT-MS for all nine aroma compounds were determined using exponential dilution analysis (EDA). This methodology is based on the dilution of volatile compounds confined in a flask (in the gas state) when a constant gas flow is applied. The dilution of the volatile compounds follows an exponential decay curve in which it is possible to calculate the concentration of the compounds in the exiting gas stream at any time using eq **1**:

$$C_t = C_0 \, e^{-(F/V)t} \tag{1}$$

where C_t is the concentration of volatile compounds at time t; C_0, the initial volatile concentration in the exponential dilution flask; F, the flow rate of the dilution gas; V, the volume of exponential dilution flask; and t, time after starting the dilution.

To perform EDA analysis a gas-phase mixture of aroma compounds was prepared by injecting known amounts of each volatile compound (**Table 1**) into a 500 mL sealed flask through a rubber septum by using a gas syringe. Volatilization was enhanced by using a stirring bar and heating the flask in a water bath at 40 °C. After 3 h of equilibration, 5 mL of headspace was injected into a second sealed flask (serving as the exponential dilution chamber) of 274.5 mL of volume through a septum. To minimize the possibility of condensation in the syringe or flask, the
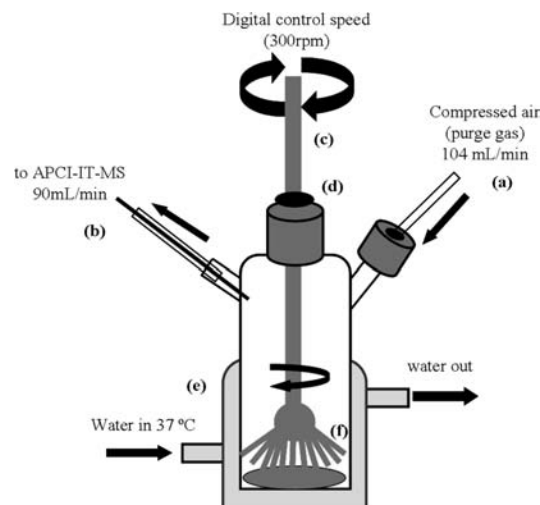

Figure 1. Device for simulating tooth brushing: (**a**) purge gas entrance; (**b**) outgoing gas with volatile compounds to the API-IT-MS; (**c**) brush connected to an electric lab mixer; (**d**) septum to avoid gas leaks; (**e**) water-jacketed glass flask; and (**f**) dentifrice slurry.

syringe was warmed before sampling and the dilution flask was maintained at 40 °C. After 1 min of equilibration, a constant air flow was applied to the exponential dilution flask (104 mL/min). The sample exiting the exponential dilution chamber was directed into the API-IT-MS through a sample transfer line. Ideal gas law equations were used to predict the initial volatile concentration injected into the exponential dilution chamber and the dilution rate.

Device for Simulating the Brushing Conditions. To simulate the dynamic conditions experienced during tooth brushing, a special device was built (**Figure 1**). This device consists of a water-jacketed glass flask with three orifices: the first permits clean air entry into the flask to purge the sample (104 mL/min), a second is the purge gas outlet which is connected though a heated transfer line (90 °C) to an adapted API-IT-MS inlet (*15*), and a third for the introduction of a brush connected to an electric lab mixer (simulating brushing) with digital speed control. This last orifice is firmly sealed around the brush shaft with a septum to avoid leaks from the flask. During the experiment setup, the brush assembly is removed and the dentifrice sample is added to the apparatus using a 20 mL plastic syringe containing 15 mL of dentifrice gel. Then 30 mL of deionized water is added to the apparatus and the brush is introduced into the flask and connected to the mixer. This device was not designed to simulate exactly what occurs in the mouth but to provide a device that can reproduce the dynamic effects of dilution and mechanical action, associated with a brush dissolving a paste in a small amount of liquid with continued agitation over a period of 3 min.

Dentifrice Model Systems. A model dentifrice containing sorbitol (500−600 g kg^{-1}), polyethylene glycol (10−50 g kg^{-1}), carboxymethylcellulose (5−10 g kg^{-1}), sodium saccharine (1−5 g kg^{-1}), sodium fluoride

Table 2. Theoretical Models for the Prediction of Concentration (C) and Estimated Models for the Prediction of Ion Intensity in the API-IT-MS Measurement (I) Based on the Exponential Decay Equation ($y = y_0 + y_1\, e^{-kt}$) for Nine Representative Aroma Compounds of Dentifrices

compd	targeted ion	C, theoretical model	I, estimated model	R^2	RSD[a]	minimal detection instrument response = $y_0 + 3 \times$ RSD
limonene	137	$10588.5\, e^{-0.379t}$	$24287.4 + 1865100\, e^{-0.5041t}$	0.981	32131	120680
menthone	155	$1017.0\, e^{-0.379t}$	$41060.8 + 628068\, e^{-0.4311t}$	0.989	10810	73620
menthol	157	$229.6\, e^{-0.397t}$	$17152.2\, e^{-0.05t}$	0.317	3860	11580
methyl salicylate	153	$12944.6\, e^{-0.379t}$	$8858.5 + 1028000\, e^{-0.5818t}$	0.985	17717	62000
carvone	151	$1117,7\, e^{-0.397t}$	$12851.9 + 121038\, e^{-0.1342t}$	0.843	11865	48448
cinnamic aldehyde	133	$13331.5\, e^{-0.379t}$	$9926.7 + 149434\, e^{-0.3198t}$	0.946	6404	29139
anethole	121	$10575.2\, e^{-0.379t}$	$3874.2 + 46380.9\, e^{-0.4338t}$	0.948	2900	12573
menthyl acetate	199	$7668.2\, e^{-0.379t}$	$16336.9 + 60535.2\, e^{-1.5190t}$	0.577	4883	30695
eugenol	165	$716.4\, e^{-0.379t}$	$8678.0 + 10873.2\, e^{-0.0962t}$	0.305	4320	21638

[a] RSD = residual standard deviation; API-IT-MS response in counts per second (cps).

(2.43 g kg^{-1}), tetrasodium pyrophosphate (1−5 g kg^{-1}), water (80−200 g kg^{-1}), silica abrasives (200−250 g kg^{-1}), sodium lauryl sulfate (10−20 g kg^{-1}) and betaine (10−15 g kg^{-1}) was prepared by Colgate using a high shear blender traditionally used in the industry. This model dentifrice was used to study the relationship between the API-IT-MS response and the aroma concentration in dentifrices under conditions simulating tooth brushing.

A model flavoring mixture was added to the model dentifrice at various concentrations. This mixture contained equal proportions of a subset of three of the nine model aroma compounds (cinnamic aldehyde, limonene and menthone). These three specific compounds were chosen based on their physicochemical properties, which span the ranges of interest in terms of log P and volatility. Six different aroma concentrations were tested in the dentifrice model systems (0.1, 0.5, 1, 5, 10, and 20 mg g^{-1}). All were prepared by dilution of a 20 mg g^{-1} sample.

API-IT-MS Sampling and Operating Conditions. Once the sample and water were in the device and the brush was sealed on the apparatus, the purge gas flow was opened (104 mL/min) and the mixer turned on (300 rpm). An API-IT-MS system (LC-Q ion trap, Finnigan MAT/ThermoQuest, San Jose, CA) instrument coupled to a venturi inlet system (15) was used for analyzing volatiles released from the tooth brushing device. All the samples were analyzed in triplicate.

API-IT-MS operating conditions were as follows: vaporizer temperature, 400 °C; capillary temperature, 150 °C; capillary voltage, 15 V, corona discharge needle voltage, 6 kV; plasma current, 5 μA,; sheath gas, nitrogen; pressure, 80 arbitrary units (5.7 L min^{-1}); auxiliary gas, nitrogen; pressure, 60 arbitrary units (7.5 L min^{-1}); flow rate of sample into the source, 90 mL min^{-1}. The instrument worked in positive ion and full scan mode with a scan rate of 0.03 s per mass.

While data were collected for 3 min, only data on aroma released between 0 and 2.5 min were used in calculations. The aroma release curves represent the intensity of one ion/compound from which different parameters were calculated to compare aroma release between different samples. The parameters calculated were I_{max} (maximum intensity of release) and AUC (the area under the curve), which represents the total release of one compound during the time frame t = 0 to 2.5 min. The values (I_{max} and AUC) corresponding to the parent ions (MH$^+$) of all the aroma compounds were used in the data analysis except for anethole, for which fragment m/z 121 was used. Xcalibur Software v.1.2 from Finnigan Corp. was used for data acquisition and analysis.

Statistical Analysis. For EDA experiments the first order exponential decay model (e.g., $y = y_0 + y_1\, e^{-kt}$) was used to calculate the theoretical concentrations to compare to the API-IT-MS response versus time. In the model, y_0 is the offset, y_1 is the amplitude and k is the decay constant. Nonlinear regression analysis was used to estimate the parameters of the model. A first estimation of the value of the detection limit for each aroma compound was obtained as the concentration corresponding to a value of the API-IT-MS response equal to the offset plus 3 times the residual standard deviation ($y_0 + 3 \times$ RSD) obtained from a nonlinear regression fit. The linear range we report is not literally accurate in the sense that the instrument generally gave a linear response at the highest concentration tested in the EDA analysis so that the upper limit noted here is generally not the true upper limit of the instrument, but the upper limit of *our testing* for linearity.

Linear regression analysis was used to estimate the linear relationship between AUC and I_{max} extracted from the aroma release curves using the tooth brushing device and the percentage of aroma compound in the model dentifrice systems. In all cases, STATISTICA program for Windows version 7.1 (StatSoft Inc., 2005, www.statsoft.com), was used for data processing.

RESULTS AND DISCUSSION

Analytical Performance of API-IT-MS for Representative Dentifrice Aroma Compounds. To obtain reliable quantitative data with an API-IT-MS, the determination of detection thresholds and linearity of the instrument response for compounds is an essential step. It has been shown that different types of API-MS instruments (or source inlets) can result in different sensitivities and linear ranges for the same aroma compounds (15−17). Therefore, it was necessary to calculate the detection thresholds and the response linearity of the API-IT-MS instrument used for the analysis of the aroma compounds selected. This was also useful in that API-IT-MS data for the aroma compounds typically used in cosmetics, toiletries and, in particular, dentifrices is scarce in the literature. These compounds have specific physicochemical characteristics of low volatility and high hydrophobicity (see **Table 1**) that can be problematic when utilizing this technique.

As noted earlier, linearity and detection thresholds were determined using an exponential dilution method (EDA). The advantages of this approach compared with the conventional serial dilution of a standard solution (or several standards) used in other works (15, 16) is that, in the latter, the number of concentration points necessary to cover the range of concentration of interest is limited by the time-consuming nature of the process. However, the use of EDA for calibration purposes has been shown to provide a means of continuously varying analyte concentration as a well-defined function of time (18). Theoretical models representing the decay in the concentration of aroma compounds based on the dilution rate applied in the exponential flask were calculated. Ideal gas law equations were used to predict the initial volatile concentration injected into the exponential dilution chamber and the dilution rate.

Table 2 shows the theoretical models calculated for the decay in the concentration of each aroma compound as a function of dilution time. The constant in each model represents the ratio between the gas flow into the dilution chamber and its volume (F/V). **Figure 2a** shows an example of the theoretical values corresponding to the concentration of limonene versus time. **Table 2** also includes the results corresponding to the nonlinear regressions applied to estimate the experimental API−IT-MS response versus time and the statistical parameters to judge the adequacy of the fit, specifically, the coefficient of determination
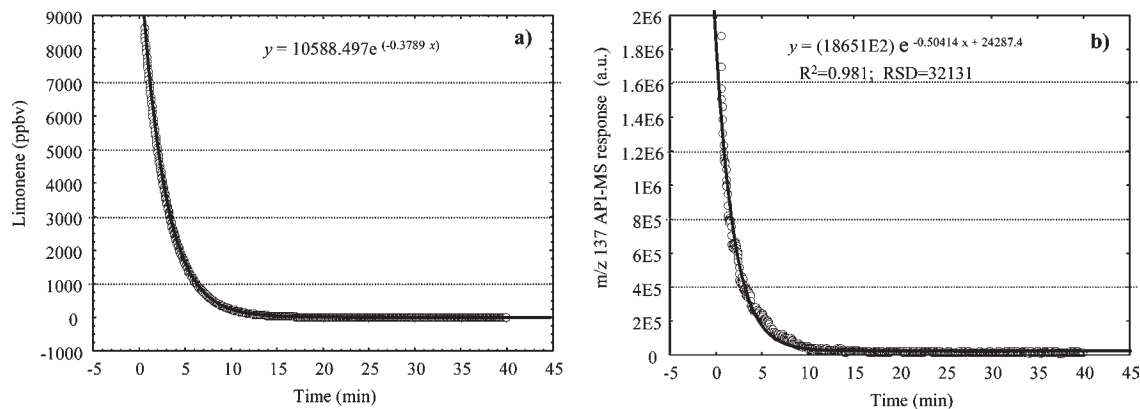
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$$y = 10588.497e^{(-0.3789\,x)}$$

a)

$$y = (18651E2)\,e^{-0.50414\,x + 24287.4}$$

$$R^2 = 0.981;\ RSD = 32131$$

b)

Figure 2. Plots corresponding to the theoretical model for the prediction of the concentration of limonene (on the left) and the experimentally estimated model for the prediction of the API-IT-MS intensity of the mass *m/z* 137 (limonene) (on the right).
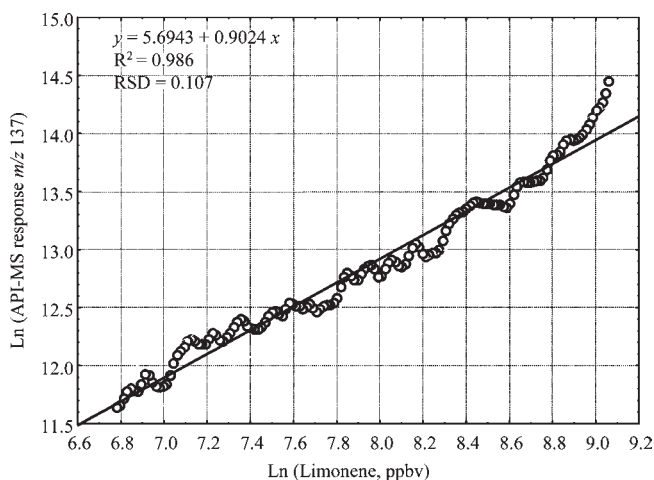

$$y = 5.6943 + 0.9024\,x$$
$$R^2 = 0.986$$
$$RSD = 0.107$$

Figure 3. Relationship between instrument response (*y*) and gas phase concentration (*x*) obtained by linear regression EDA data for limonene monitoring *m/z* 137.

(R^2) and the residual standard deviation (RSD). In addition, **Figure 2b** shows the API-MS response versus time experimentally obtained for the ion *m/z* 137 corresponding to limonene.

As is shown in **Table 2**, the estimated model showed, in general, adequate fits for most of the aroma compounds. In this sense, R^2 was > 0.9 for masses *m/z* 137, 155, 133, 153, and 121, corresponding to limonene, menthone, cinnamic aldehyde, methyl salicylate, and anethole. The fit for carvone (*m/z* 151) resulted in a lower but yet acceptable $R^2 = 0.84$. In all of these cases, the decay rates for the intensity followed a similar trend to those predicted in the theoretical models for the concentration decay. However, the fits obtained for menthol, menthyl acetate and eugenol (*m/z* 157, *m/z* 153 and *m/z* 165) were very poor, with $R^2 < 0.6$ in all three cases. This was mainly because of the low signal that these compounds generated in the API-IT-MS instrument. Low vapor pressure, incomplete volatilization in the dilution chamber, or condensation in the volatilization flask or the analytical instrument itself could have been potential causes for the poor response observed.

The exponential dilution models for the concentration of volatile compound (theoretical) and for the API-IT-MS response (experimentally measured) were plotted as Ln(API-MS ion intensity) versus Ln(concentration), and a linear regression analysis was performed. An example of the linear regression calculated for the intensity of mass *m/z* 137 versus the concentration of limonene is shown in **Figure 3**. A similar analysis was

conducted for all nine flavor compounds (**Table 3**). The linear model obtained for each aroma compound, and the linear range limits (highest and the lowest aroma concentration in the gas phase) and the detection limits (ppb) extracted from the API-IT-MS responses, are summarized in **Table 3**. As noted in Materials and Methods, the upper limits reported are not necessarily the true upper limits of instrument linearity but only represent the upper limit tested: We expect that the true upper limit would be significantly above that determined in testing. Issues of assuring that all of a given aroma compound is volatilized in the EDA flask and there was no condensation in the apparatus at any point limited the upper concentrations of testing. We acknowledge that in some instances we were working at concentrations exceeding our assurance of instrument linearity. However, the work of Buffo et al. (*15*) has shown the signal of this API-IT-MS increases well beyond the upper limit of linearity so we would definitely see an increase in instrument response, albeit nonlinear which would confound absolute quantification, at higher concentrations.

The statistical estimators of the adequacy of the fit (R^2 and RSD) were also tabulated. Good linear fits were generated for the majority of the compounds analyzed, including limonene, menthone, cinnamic aldehyde and methyl salicylate ($R^2 > 0.92$), as well as anethole and carvone ($R^2 = 0.84$ and 0.73 respectively). Menthyl acetate showed an acceptable R^2 (0.87) although the linear range was very narrow, probably due to a very high detection limit (> 4000 ppb), the highest of all the aroma compounds studied here. Linear fits were not generated for menthol and eugenol, since, as shown before, the detection of these compounds by API-IT-MS was very poor. The low vapor pressure of these compounds was likely the limiting factor for the application of EDA and API-IT-MS for calibration purposes.

The detection limits greatly depended on the type of aroma compound under analysis. In this case, interestingly, the detection limits for menthone and carvone were very low, even lower than those calculated for much more volatile common food flavor compounds (e.g., ethyl butyrate or *cis*-hexenol) using the same instrument but a different calibration procedure (*15*). Limonene showed a detection limit of 244 ppb that is very similar to that calculated for other much more volatile compounds such as benzaldehyde (210 ppb) in the above-mentioned study. Therefore, in the case of aroma compounds with higher vapor pressures, the calibration using EDA gave very good results in terms of sensitivity. The rest of the compounds showed detection limits between 400 and 700 ppb.

Except for menthyl acetate, the linear ranges were about three orders of magnitude. The upper limits for linearity were approximately 10,000 ppb (except for menthone and carvone), which are

Table 3. Linear Calibration Models y = Ln(API-MS intensity) versus x = Ln(concentration), Detection Limits and Linear Ranges of Nine Representative Aroma Compounds of Dentifrices

compd	linear calibration model	R^2	RSD[a]	linear range (ppbv[b])	detection limit (ppbv[c])
limonene	$y = 5.6943 + 0.9024x$	0.986	0.107	244–8103	244
menthone	$y = 7.6426 + 0.8063x$	0.979	0.102	66–1096	66
menthol					
methyl salicylate	$y = 1.3822 + 1.2947x$	0.975	0.168	665–10938	665
carvone	$y = 9.4771 + 0.3374x$	0.824	0.456	20–1096	20
cinnamic aldehyde	$y = 5.5811 + 0.6625x$	0.924	0.185	403–13359	403
anethole	$y = 2.7054 + 0.8631x$	0.842	0.292	665–9897	665
menthyl acetate	$y = -9.92 + 2.3573x$	0.874	0.171	4023–8103	4023
eugenol					

[a] RSD = residual standard deviation. [b] The upper limit only corresponded to that tested in the EDA experiment. [c] Concentration in the gas phase expressed as part per billion by volume.
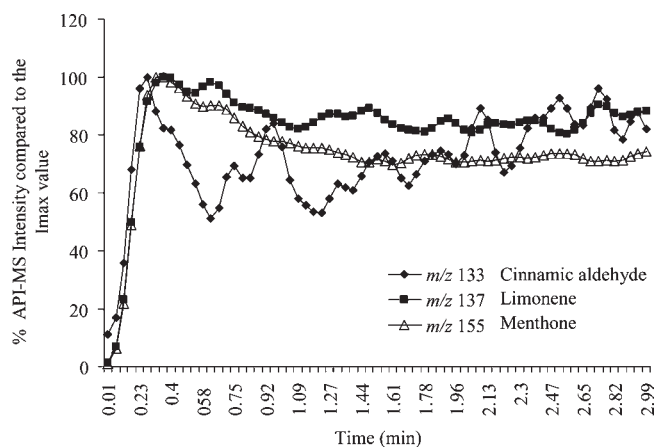
Figure 4. Aroma release curves (intensity vs time) from a dentifrice flavored with 1 mg g^{-1} of an equal mass mixture of three model aroma compounds.

similar to those described for API-MS and other food volatile compounds such as ethyl butyrate and *cis*-3-hexenol in water (*14*).

Aroma Release under Tooth Brushing Conditions. The study of aroma release in real time can be used to describe the release of flavor components from toothpastes during brushing and to establish the factors driving flavor release as influenced by product composition. In order to study aroma release from dentifrices, a tooth brushing device was built and coupled to the API-MS (as explained in Materials and Methods). The apparatus was not necessarily designed to replicate the exact process of tooth brushing but rather to reproducibly reflect the nonequilibrium conditions associated with brushing: for example, the release of flavors from the dentifrice upon dilution in water (saliva in the mouth) under mechanical action (a rotating brush).

A model dentifrice composition flavored with a blend of equal amounts of three of the nine flavor compounds described in the previous section, namely, cinnamic aldehyde, limonene and menthone, was used to check the performance of the device. The aroma compounds were chosen to be representative of the range of compounds usually used in dentifrice formulations (in terms of functional groups, hydrophobicity and volatility) and also because of the ability to monitor them via API-MS.

The release of these three flavor compounds was monitored following the procedure outlined in Materials and Methods, and the aroma release curves obtained are shown in **Figure 4**. This figure illustrates the changes in instrument response (by comparison of the intensity at each time in relation to the I_{max}) across model compounds reflecting differences in volatile release to each

compound. As can be seen, although the three compounds reached the maximum intensity almost at the same time (above 0.4 min), differences were observed in the release pattern during the simulated brushing. The release of cinnamic aldehyde (mass m/z 133) was the lowest of three compounds. In addition, a drop in the release was observed immediately after the compound reached the I_{max}. However, also observed was an increase in its intensity of release halfway through the "brushing", reaching, at the end, very similar values to its I_{max}. Interestingly, the release pattern observed for the other two compounds was greatly different. Although the absolute value of menthone release (m/z 155) was higher than that of limonene (m/z 137) (data not shown), both compounds showed similar release patterns. A slight difference is that menthone release decreased initially before reaching a constant value while limonene release decreased very little over time maintaining a value close to its maximum release.

The main factor controlling the release of the aroma compounds was the rate of mass transfer across the air:dentifrice slurry interface. Mass transfer rate of a volatile component across a gel:air interface is primarily dependent upon the vapor pressure differential across the interface and any physical barriers to its diffusion to or across the interface. Therefore, when considering the effect of various dentifrice ingredients on flavor release (volatiles), their effect on both the vapor pressure of the individual matrix components and various flavor compounds in it as well as on the diffusion process within the matrix should be taken into account. The interactions between the compounds can affect their vapor pressures, as well as the diffusion rate to the interface and as a result will impact the rate of mass transfer across the air interface. With respect to the diffusion of components within the matrix, the main physical barrier consideration would be the rate of dispersion into the aqueous phase upon dilution. For example, the flavor release from a dentifrice composed of hydrocolloids that slowly disperse/dissolve in water would be expected to be initially lower than one that quickly dissolves/disperses upon dilution. Chemical and spatial interactions between the matrix and the flavor components also need to be considered.

When considering the release of three model flavor compounds (limonene, menthone and cinnamic aldehyde) from the same dentifrice matrix (**Figure 4**), the differences observed in instrument response are likely the result of a combination of the abovementioned factors. First, the impact of vapor pressure differences across the compounds is considered. In their pure state, limonene and menthone have much higher vapor pressures than cinnamic aldehyde (**Table 1**), and this same trend seems to hold true in the dentifrice system as well, since the instrument response was much lower for cinnamic aldehyde than for limonene and menthone. Likewise, the hydrophobic character of each of these compounds

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(i.e., log P, **Table 1**) also plays a role, as the more hydrophobic ingredients are driven out of the slurry and into the gas phase in greater quantity, upon dilution. The results from our experiments are somewhat consistent with this, since again, cinnamic aldehyde, for which the instrument response was the lowest, is far less hydrophobic than the other two compounds. However, if we compare the release curves for limonene versus menthone (**Figure 4**), the results clearly indicated that there are additional factors to be considered in the release of these flavors from a dentifrice matrix. Limonene was released to a lower extent than menthone (data not shown), however, based on vapor pressures (pure state) and log P alone, limonene should have been released in a greater amount. However, this compound showed a constant release during brushing, while menthone, that was more released in absolute intensity (data not shown), was progressively being less released along brushing (**Figure 4**). Interactions between matrix ingredients and various flavor components are likely to have changed the vapor pressure of these compounds relative to their pure state. Changes in viscosity of the medium have also been shown to have an effect of flavor release from food systems (*19, 20*) and are likely to play a role here as well. One also notes that, if the volatile compounds exceed their solubility limits in the dentifrice matrix, the formation of flavor emulsion droplets occurs, and this may also impact the dynamics of flavor release (*21*).

We propose that the differences in the mass transfer rate observed across our volatiles were mainly caused by differences in vapor pressure between volatiles. This vapor pressure differential is a result of the inherent volatility of the compounds, their solubility limits in the dentifrice matrix, and interactions (physical or chemical interactions) with the dentifrice components For example, chemical interactions between aroma compounds containing carbonyl groups, such as aldehydes and polysaccharides and hydrocolloids, have been previously described in different food products (*22, 23*). Furthermore, physical entrapment of high log P compounds by certain network-forming hydrocolloids such as starch, xanthan and carrageenan have been also previously described (*19, 20, 24*). More recently, Potineni and Peterson (*25*) have shown in chewing gums that some polyalcohols, such as sorbitol (an ingredient of our dentifrice systems) or glycerine, can interact with cinnamic aldehyde forming hemiacetals thereby modifying aroma release.

The effects of flavor concentration and ingredient interactions on aroma release are explored in the following sections.

Influence of Flavor Concentration on API-IT-MS Response. To be able to use API-IT-MS to quantitatively monitor the release of aroma compounds from dentifrices, we must know the effect of aroma compound concentration on API-IT-MS response. While that requirement seems too obvious to be stated, the concern is with detection limits and linearity in instrument response: very high levels of flavorings are used in dentifrices. According to the classical partitioning mechanism that governs the release of aroma compounds dissolved in a matrix, the theoretical equation for the release of volatiles into a flowing gas is given as (*26*)

$$dm/dt = hD\ Agc[c_e^i(t) - c_e(t)] \qquad (2)$$

In this equation, dm/dt is the rate of mass transfer from the matrix to the gas phase, hD is the overall mass transfer coefficient, Agc is the surface area of gas/matrix interface; $c_e^i(t)$ is the concentration of aroma compound at gas/matrix interface (gas phase) and $c_e(t)$ is the concentration of aroma compound in the continuous phase of the matrix.

While seldom explicitly stated, there is an upper limit to the validity of this equation to quantitatively predict aroma release
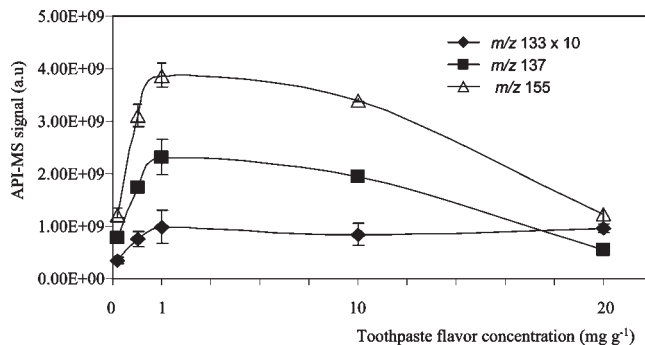

Figure 5. Relationship between flavor concentration in the model dentifrice and API-IT-MS response using the brushing device (notice that for a better view, the intensity of mass *m/z* 133 has been increased 10×).
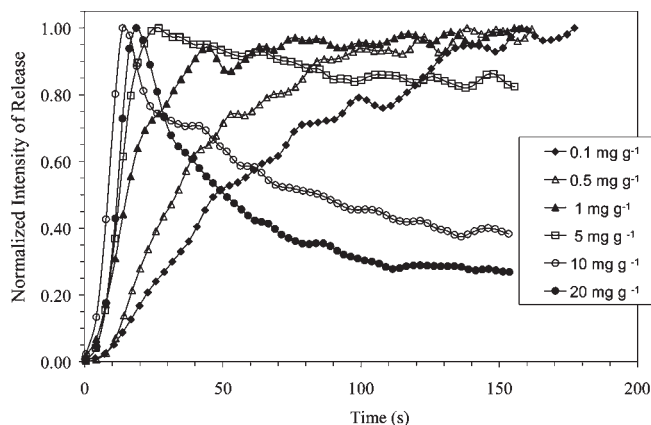

Figure 6. Real time release of limonene from a model dentifrice matrix at various flavor mixture concentrations ranging from 0.1 to 20 mg g^{-1}.

into a flowing gas phase. When the continuous phase of a model food (dentifrice) system becomes saturated with a given volatile, we would expect there to no longer be a linear relationship between the concentration of a volatile compound in a matrix and release into a flowing gas stream. At concentrations above solubility limits, one would expect emulsion formation to occur assuming adequate shear is provided to the system. An emulsion would have the same vapor pressure as the pure substance/ mixture, and thus, release would no longer be concentration dependent. Therefore, we could expect a linear relationship between the API-IT-MS response with increasing aroma compound concentrations in the dentifrices only until saturation of the aqueous phase.

The above hypothesis was tested using model dentifrice systems aromatized with different flavor concentrations (0.1 to 20 mg flavor mixture [equal wt mixture of all three compounds] g^{-1} model dentifrice) using the artificial brushing device. The results of this experiment are shown in **Figure 5**. To facilitate comparing the results, the intensity of mass *m/z* 133 (cinnamic aldehyde) was increased 10× in this figure. As indicated by the results, there was a linear increase in MS response with increased flavor concentration for all compounds for concentrations up to 1 mg g^{-1} flavor. However, at higher concentrations, between 1 and 20 mg g^{-1}, a plateau in response was observed. It appears that there was a reduction in instrument response in the case of *m/z* 155 (menthone) and *m/z* 137 (limonene) at the highest concentrations within the 2 min time frame of the experiment. At ca. 1 mg g^{-1} flavor the aqueous phase (in the dentifrice:water slurry) may be already saturated and the aroma compounds likely form a second, noncontinuous phase (an emulsion). Adding more aroma
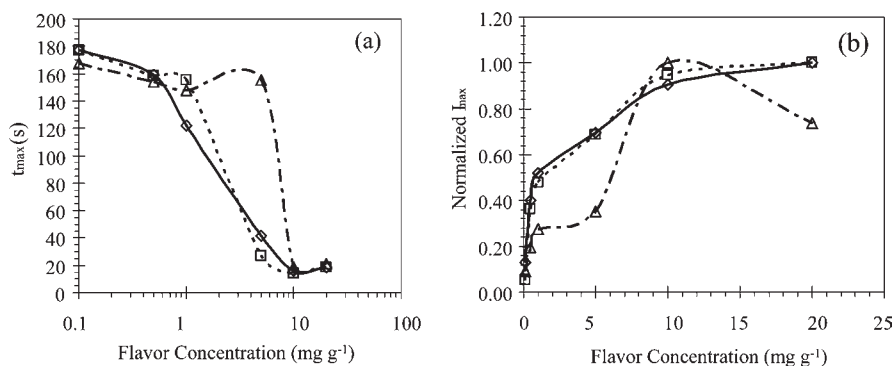
Figure 7. Effect of flavor concentration on (**a**) t_{max} and (**b**) I_{max} for limonene (◇), menthone (□) and cinnamic aldehyde (△). I_{max} normalized using highest value for each component.

to the system would not increase the concentration in the continuous phase but rather is likely to affect the emulsion properties (e.g., droplet number and size). Differences in the release of flavor components from droplets versus dissolved flavors have been observed and suggest that the release from droplets occurs via a mechanism that differs from the classical partition mechanism established for dissolved flavors (*21*). The existence of flavor droplets (i.e., emulsion) may influence the time-dependent characteristics of the release curve, more specifically the maximum intensity of release, I_{max} and the time at which this maximum occurs, t_{max}. As demonstrated by Taylor and co-workers (*21*), when flavor emulsion droplets are present, the shape of the real-time release profile is significantly different from the gradual release profile observed when the flavors are dissolved. More specifically, the maximum flavor intensity (I_{max}) increases and the time at which the maximum occurs (t_{max}) decreases. Furthermore, the larger the droplets, the higher the I_{max}.

In the dentifrice matrix, the effect of flavor concentration on the release profile shows a transition at the saturation concentration of 1 mg g^{-1} that is consistent with the transition from a purely dissolved state to an emulsified state. **Figure 6** shows the release profile for limonene as a function of total flavor concentration in the matrix. At flavor concentrations below 1 mg g^{-1} the release is gradual, whereas above this saturation concentration the release quickly increases to a maximum and then decreases. The same was observed for menthone and cinnamic aldehyde. In the case of cinnamic aldehyde, the saturation point occurred at a higher concentration of 5 mg g^{-1}, due to its lower log P and volatility and hence increased solubility in the matrix compared to limonene and menthone. I_{max} and t_{max} values for each of the three flavor compounds were quantified from the real-time release profiles (**Figure 7**). A sharp decrease in t_{max} was observed above the saturation point (**Figure 7a**). The maximum intensity plot as a function of flavor concentration showed two distinct slopes followed by a plateau (**Figure 7b**). The initial increase in I_{max} can be attributed to the increase in the aroma compound concentration dissolved in the continuous phase, while the slope following the point of inflection, in the concentration range 1−10 mg g^{-1}, may be attributed to an increase in the size of the emulsion droplets (*21*). Cinnamic aldehyde, having a much lower log P and vapor pressure and a much higher boiling point than menthone and limonene, shows slightly different release behavior as flavor concentration increases.

From the findings described in the later portion of this work, it is generally concluded that the flavor release of aroma compounds from a dentifrice matrix changes with increasing concentration, even at concentrations that are above the solubility limits of the compounds in the matrix. Quantifying the relation-

ship between instrument response and concentration of aroma in dentifrice systems then becomes a futile exercise since any linear regression models that may be calculated to relate instrument response to the actual aroma concentration in the matrix will only be valid within the linear portion of the aroma release curve, essentially between 0 and 1 mg g^{-1} flavor. These concentrations are much lower than what is typically used in a commercial dentifrice. The qualitative description of flavor release at higher concentrations is in practice of greater value.

Conclusion. In this work, a mechanical brushing device coupled with API-IT-MS has been developed which mimics the influence of time and dilution on the subsequent aroma release from dentifrices. This combination of instruments allows the profiling of the real-time release of most of the typical aroma compounds used in dentifrices under nonequilibrium conditions. This technique may only be used quantitatively for components that are present in concentrations below the solubility limit in the matrix. At concentrations above this limit, nonlinear instrument responses are observed due to a saturation of the dentifrice continuous phase. In this case, the methodology developed may be used qualitatively and comparatively, but without the possibility to calculate actual concentrations of the components that are being released. The composition of the dentifrice matrix and interactions between matrix ingredients and the flavor components are likely to affect the saturation concentrations for various compounds, therefore influencing the flavor release profile. Such interactions are the focus of future work in this area.

LITERATURE CITED

(1) Payne, R.; Puchalski, A. E.; Labows, J. SPME applications in consumer products. In *Flavor, Fragrance and odor analysis*; Marsili, R., Ed.; Marcel Dekker, Inc.: Rockford, IL, 2002; pp 277−295.

(2) Cayot, N.; Dury-Brun, C.; Karbowiak, T.; Savary, G.; Voilley, A. Measurement of transport phenomena of volatile compounds: A review. *Food Res. Int.* **2008**, *41* (4), 349–362.

(3) Guichard, E. Interactions between flavor compounds and food ingredients and their influence on flavor perception. *Food Rev. Int.* **2002**, *18* (1), 49–70.

(4) Labows, J. N. Surfactant Solubilization Behavior Via Headspace Analysis. *J. Am. Oil Chem. Soc.* **1992**, *69* (1), 34–38.

(5) Robinson, R. S.; Tavss, E. A.; Santalucia, J.; Carroll, D. L. Flavor Characterization of Dentifrices Using Equilibrium Headspace Gas-Chromatography. *J. Chromatogr., A* **1988**, *455*, 143–149.

(6) Tavss, E. A.; Wiet, S. G.; Robinson, R. S.; Santalucia, J.; Carroll, D. L. Flavor Characterization of Dentifrices Using Kinetic Headspace Gas-Chromatography. *J. Chromatogr., A* **1988**, *438* (2), 273–280.

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(7) Hansson, A.; Giannouli, P.; Van Ruth, S. The influence of gel strength on aroma release from pectin gels in a model mouth and in vivo, monitored with proton-transfer-reaction mass spectrometry. *J. Agric. Food Chem.* **2003**, *51* (16), 4732–4740.

(8) Savary, G.; Lafarge, C.; Doublier, J. L.; Cayot, N. Distribution of aroma in a starch-polysaccharide composite gel. *Food Res. Int.* **2007**, *40* (6), 709–716.

(9) van Ruth, S. M.; Buhr, K. Influence of mastication rate on dynamic flavour release analysed by combined model mouth/proton transfer reaction-mass spectrometry. *Int. J. Mass Spectrom.* **2004**, *239* (2−3), 187–192.

(10) van Ruth, S. M.; O'Connor, C. H.; Delahunty, C. M. Relationships between temporal release of aroma compounds in a model mouth system and their physico-chemical characteristics. *Food Chem.* **2000**, *71* (3), 393–399.

(11) van Ruth, S. M.; Roozen, J. P. Influence of mastication and saliva on aroma release in a model mouth system. *Food Chem.* **2000**, *71* (3), 339–345.

(12) Mei, J. B.; Reineccius, G. A.; Knighton, W. B.; Grimsrud, E. P. Influence of strawberry yogurt composition on aroma release. *J. Agric. Food Chem.* **2004**, *52* (20), 6267–6270.

(13) Taylor, A. J.; Besnard, S.; Puaud, M.; Linforth, R. S. T. In vivo measurement of flavour release from mixed phase gels. *Biomol. Eng.* **2001**, *17* (4−5), 143–150.

(14) Taylor, A. J.; Linforth, R. S. T.; Harvey, B. A.; Blake, B. Atmospheric pressure chemical ionisation mass spectrometry for in vivo analysis of volatile flavour release. *Food Chem.* **2000**, *71* (3), 327–338.

(15) Buffo, R. A.; Zehentbauer, G.; Reineccius, G. A. Determination of linear response in the detection of aroma compounds by atmospheric pressure ionization-mass spectrometry (API-MS). *J. Agric. Food Chem.* **2005**, *53* (3), 702–707.

(16) Buffo, R. A.; Reineccius, G. A. Determination of linear response in the detection of mixtures of aroma compounds by atmospheric pressure ionization-mass spectrometry (API-MS). *Flavour Fragrance J.* **2008**, *23* (1), 16–22.

(17) Grab, W.; Gfeller, H., Flavorspace - A new technology for the measurement of fast dynamic changes of the flavour release during eating. In *Frontiers of Flavour Science*; Schiberle, P., Engel, K., Eds.; Deutsche Forschungsanstalt für Lebensmittel: Garching, Germany, 2000; pp 261−270.

(18) Inman, E. L.; Voigtman, E.; Winefordner, J. D. Calibration Curve Preparation of Analytes in Liquid Solutions by Means of an Exponential Dilution Flask. *Appl. Spectrosc.* **1982**, *36* (2), 99–102.

(19) Bylaite, E.; Adler-Nissen, J.; Meyer, A. S. Effect of xanthan on flavor release from thickened viscous food model systems. *J. Agric. Food Chem.* **2005**, *53* (9), 3577–3583.

(20) Yven, C.; Guichard, E.; Giboreau, A.; Roberts, D. D. Assessment of interactions between hydrocolloids and flavor compounds by sensory, headspace, and binding methodologies. *J. Agric. Food Chem.* **1998**, *46* (4), 1510–1514.

(21) Linforth, R. S. T.; Pearson, K. S. K.; Taylor, A. J. In vivo flavor release from gelatin-sucrose gels containing droplets of flavor compounds. *J. Agric. Food Chem.* **2007**, *55* (19), 7859–7863.

(22) Boutboul, A.; Giampaoli, P.; Feigenbaum, A.; Ducruet, V. Influence of the nature and treatment of starch on aroma retention. *Carbohyd. Polym.* **2002**, *47* (1), 73–82.

(23) Goubet, I.; Le Quere, J. L.; Voilley, A. J. Retention of aroma compounds by carbohydrates: Influence of their physicochemical characteristics and of their physical state. A review. *J. Agric. Food Chem.* **1998**, *46* (5), 1981–1990.

(24) Chana, A.; Tromelin, A.; Andriot, I.; Guichard, E. Flavor release from iota-carrageenan matrix: A quantitative structure-property relationships approach. *J. Agric. Food Chem.* **2006**, *54* (10), 3679–3685.

(25) Potineni, R. V.; Peterson, D. G. Mechanisms of flavor release in chewing gum: Cinnemaldehyde. *J. Agric. Food Chem.* **2008**, *56* (9), 3260–3267.

(26) Harrison, M.; Hills, B. P.; Bakker, J.; Clothier, T. Mathematical models of flavor release from liquid emulsions. *J. Food Sci.* **1997**, *62* (4), 653–664.