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Flavor Release Measurement by Atmospheric Pressure Chemical Ionization Ion Trap Mass Spectrometry, Construction of Interface and Mathematical Modeling of Release Profiles

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An instrumental on-line retronasal flavor analysis was developed to obtain information about the release of flavor compounds in expired air from humans during eating. The volatile flavor compounds were measured by ion trap mass spectrometry with an atmospheric pressure chemical ionization source (APCI). An interface was designed to sample the breath directly from the nose. The repeatability in vitro for seven different flavor compounds came out with relative standard derivation less than 10% in most cases, which is acceptable. In vitro quantification was carried out by a determination of the concentration in the gas phase over a flavor solution by GC/MS, followed by measurements of intensities by the APCI ion trap. Ion suppression by acetone in the breath was negligible at concentration levels relevant in these experiments. The instrumental limits of detection for menthone and menthol coincide with that of the flavor detection threshold. An application study on the release of menthone and menthol from chewing gum by a group of six test persons was performed. Flavored chewing gum was used as a model matrix because of the long chewing periods and the simplicity of the system. It is concluded that the interface and the method can be used to measure breath from the nose. A mathematical model of the data was developed to give a quantitative method for description and characterization of the release of flavor compounds. The release profiles consisted of two sequences, one for a chewing period, and one for a phasing out process. The proposed method for modeling provided a reasonable description of the release process. In addition to flavor compounds, this new interface and mathematical application could provide information on chemicals in the human breath, which could be interesting, for example, within medical diagnosis.

Mass spectrometric techniques for breath-by-breath (BBB) analysis of expired air and the application in medicine was first described in 1983.[1] Adaptation of the methodology to measure the concentration of flavor compounds released from food during mastication in nasally expired air was demonstrated by Soeting and Heidema.[2] Correlation of BBB analyses of food with simultaneous assessment of sensory perception is an imperative technique in exploring sensory responses. Thus, the technique may be used to gain basic knowledge on parameters affecting flavor perception.

BBB analysis is complicated because of the high background signal from the presence of air, carbon dioxide, and other human metabolites, primarily acetone. Acetone is produced by fatty acid catabolism, transported by the blood to the lungs and released via the blood−air interface. Thus, acetone can be used as marker for the respiration cycle. The acetone level in expired air of normal subjects ranges from 0.6 to 2 ppm.[3] The concentration of flavor compounds released from food into the breath will often be much lower, requiring very low instrumental limits of detection.[4]

Taylor and Linforth described in a patent[5,6] an apparatus and methods for determination of volatile flavor compounds on-line in human breath by mass spectrometry. The methodology is based on direct introduction of expired breath into an atmospheric pressure chemical ionization (APCI) mass spectrometer. In the APCI source, protonated water clusters of varying size are formed from the moisture in the expired air.[1] Proton-transfer reaction occurs between the analyte and the protonated water cluster to form protonated analyte−water clusters of varying sizes. The water

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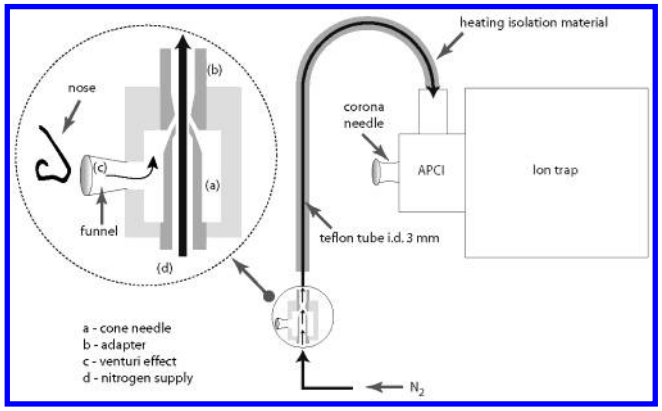

Figure 1. Schematic representation of the APCI-MS ion trap coupled up with a retronasal interface as inlet. External Teflon funnels were available to adjust for nose size. a, cone needle; b, adapter; c, venturi effect; d, nitrogen supply.

is stripped off by evaporation and induced collisions with heated nitrogen molecules and the protonated analyte is transmitted to the analyzer.

APCI ionization is a relatively gentle method of ionization, which implies that the reagent ions contain sufficient energy to ionize the molecule, but not enough energy to cause extensive fragmentation. The most common product is the protonated molecular ion $[M + H]^+$, but alcohols and most aldehydes dehydrate to $[M - H_2O + H]^+$. Quantification by APCI-MS is a challenge, partly because the ionization is competitive and incomplete. Furthermore, if one particular volatile component is present at much higher level than the other volatiles, the major component might suppress ionization of the minor component as a result of what is known as the matrix effect.[7]

The aim of this study was to develop an instrumental on-line retronasal flavor measurement with high stability, low limit of detection, and reliable quantification and to evaluate the method in an application study. The main focus was to construct an APCI-MS interface to perform on-line measurement of expired air from the human nose during eating and to establish a quantitative analysis for describing and characterizing the outcome of the instrumental analyses, namely the release of flavor compounds. The application study aimed to evaluate if the time of the day the measurements took place affected the release of flavor and the breath. The requirement for the instrumental setup was flexibility and that only minor instrumental changes on the APCI source were needed. Furthermore, it was important that the test persons were affected as little as possible to avoid unpleasant feelings that could influence flavor release. Additionally, the direct inlet should be easy to handle and clean after contact with the human nose.

EXPERIMENTAL SECTION

Design of Interface. The volatile flavor compounds were measured using an ion trap mass spectrometer (Agilent 1100 LC/MSD Trap 4.0, Frankfurt, Germany) with an atmospheric pressure chemical ionization source (APCI). However, a modified spray probe was used. The developed nose−instrument interface and the overall instrumental arrangement is shown in Figure 1. A funnel of Teflon was designed as the inlet for the human breath from the nose. The funnel can easily be changed and cleaned. Teflon was chosen as the material because of the low carry-over effect.

The normal APCI probe was replaced by a modified ESI probe in which the normal ESI needle top was removed to facilitate the higher flow of air, because only gaseous samples were to be analyzed; i.e., no solvent had to be vaporized in the source. The modified ESI probe was coupled by a Teflon tube (i.d. 3 mm, length 80 cm) to a custom-designed connecting link. In the opposite end of the Teflon tube, a Swagelock T-fitting (SS-400-3, Swagelock, U.S.A.) was attached. Inside the T-piece a cone-shaped needle (a in Figure 1) was placed close to an adapter (b in Figure 1), machined to fit over the needle cone. All parts were of stainless steel. By adjusting the position between these parts, a venturi effect was obtained by a high inlet nitrogen flow from the needle (d in Figure 1). This resulted in a small vacuum in the sampling funnel (c in Figure 1), drawing nasally expired air into the system. The nitrogen flow was supplied through the nebulizer gas line, giving the instrumental software control of the flow. Optimizing experiments revealed a nebulizer gas pressure of 30 psi and a drying gas flow of 1 mL/min yielded the most sensitive results (data not shown). A source temperature of 200 °C was used. The Teflon tube was wrapped in electrically heated tape and coupled to a temperature controller. The temperature was maintained at 45 °C to avoid condensation of water vapor.

In Vivo Measurement (Expired Air from Humans). The nose was placed in the funnel so that breaths from the nose could be sampled into the APCI source and at the same time allow the subject to breathe laboratory air. Thereby it was possible to register breathing pattern and the release of flavors. The breathing pattern of acetone (m/z 59 $[M + H]^+$) was routinely monitored to ensure a correct position of the nose in all in vivo measurements. The volatile flavor compounds were ionized by a corona discharge of 4000 nA. The ion trap scanned from 15 to 350 m/z with an accumulation time of 300 ms. Chewing gum was used as the model matrix because of the long chewing periods and the simplicity of the system. All chewing gums were without sugar coating but with added sweetener. Peppermint oil and menthol were used as flavors. The chewing gums were obtained from Dandy A/S (Vejle, Denmark).

In Vitro Measurement (Headspace Measurement). Headspace measurement was performed by substituting a Teflon tube of a length of 20 cm and i.d. 3 mm for the funnel. The instrument settings were as described in the previous section. Flavor solution, 25 μL, was added to 10 mL distilled water in a 100-mL screw-cap bottle. The concentration and composition of the flavor solutions are given in the following sections. The bottles were placed in a water bath at 37 °C for at least 30 min to obtain water/gas phase equilibrium. Pilot experiments have showed that this was sufficient to reach equilibrium for the volatile compounds in these tests (data not shown). The water bath was placed on a magnetic stirrer to allow stirring of the solution while measuring. Prior to measurement, the screw cap was changed to a modified screw cap with a hole for the Teflon tube (i.d. 3 mm). The release profiles were followed over time, typically for 1 min (triplicate analysis). Raw data for mass/charge ratios were exported to the MatLab software (MatLab version 6.0 Release 12, MathsWork, U.S.A.). The differ-

(7) Taylor, A. J.; Linforth, R. S. T.; Harvey, B. A.; Blake, B. *Food Chem.* **2000**, *71*, 327−38.

ences between minimum and maximum intensities were calculated as δ values. The minimum signal represented the background and was the average intensity obtained during a suitable period prior to the experiment.

Repeatability in Vitro. A stock solution of seven volatile compounds, (−)-menthone (≥99%) and (−)-menthol (≥99%) (Fluka, Buchs, Switzerland), 2-pentanone (92%), 2-hexanone (98%), 2-heptanone (98%), ethyl hexanoate (99%), and ethyl octanoate (99.5%) (Aldrich-Chemie, Steinheim, Germany), was prepared in 96% ethanol. The compounds were selected on the basis of their molecular weight, volatility, and functional group. Menthone and menthol were chosen because of their importance as flavor compounds in many sweets and medical products. The repeatabilities of the method were checked by headspace measurement during a 4-week period with one to four weekly determinations and in a day with four measurements the same day. Two concentration levels were used (2.5 μg flavor compound/g ethanol and 25 μg flavor compound/g ethanol). The release of the compounds 2-pentanone (m/z 87, $[M + H]^+$), 2-hexanone (m/z 101, $[M + H]^+$), 2-heptanone (m/z 115, $[M + H]^+$), menthol (m/z 139, $[M - H_2O + H]^+$), ethyl hexanoate (m/z 145, $[M + H]^+$), menthone (m/z 155, $[M + H]^+$), and ethyl octanoate (m/z 172, $[M + H]^+$) were followed for 1 min. The δ intensities of each of the seven compounds were determined.

Quantification in Vitro. The headspace (10 mL) was drawn from a 100 mL bottle containing a menthone and menthol solution into a gastight syringe (see in Vitro Measurement section for sampling). The gas was applied to an adsorbent trap (Perkin-Elmer, U.S.A.) containing 225 mg Tenax GR (Chrompack, Bergen op Zoom, The Netherlands). Five different concentration levels were used, and measurements were performed in triplicate. The flavor compounds were analyzed and quantified by GC/MS as described in Haahr et al.,[8] except that the detector operated in selected ion monitoring mode (SIM) at m/z 112 for menthone and m/z 71 for menthol.

Quantification of menthone and menthol was performed by determining the concentration of the compounds in the gas phase both by GC/MS and by APCI-MS as described above. This procedure allowed a calibration curve between APCI-MS-intensities and concentration in the gas phase in ng/mL according to GC/MS.

To assess the ion suppression by acetone in the APCI source, the above experiment was repeated with addition of different levels of acetone (0, 0.1, 0.3, and 0.6 ppm).

In Vitro Limit of Detection for Menthone and Menthol. The limit of detection was calculated as three times the noise[9] and was converted to concentration by the calibration curves. The noise was determined from six blank samples, that is, the headspace of 10 mL distilled water.

In Vivo Limit of Detection for Menthone and Menthol. Three female subjects determined the in vivo limit of detection by testing three blank samples (12.5 μL of pure ethanol 96% in 5 mL distilled water). The analytical procedure was as follows: (1) The subject breathed 20 s without sample in the mouth, (2) 5 mL of solution was administrated into the mouth, (3) the solution was swirled in the mouth for a further 20 s to obtain mouth temperature, (4) the sample was swallowed, and (5) the subject breathed again for 20 s. To test the flavor-detection threshold parallel, the subjects tested four concentration levels of menthone (0.12−0.73 μg/mL) and menthol (1.06−18.7 μg/mL) in triplicate. The breathing pattern and the release of menthone and menthol were followed continuously. After each sample, the subject was asked to note the perceived flavor.

Application Study. "Time of Day" Effect on in Vivo Measurements. The study consisted of four identical series of two chewing sessions (i.e., eight measurements in total). The series were carried out at four different time points in the course of 1 day with ∼2 h between them. Six female subjects were each served one chewing gum at each session. The experimental procedure involved (1) 2 min of breathing, (2) chewing gum was administrated into the mouth, (3) 2 min of chewing (with the molars only), (4) the chewing gum was spit out, and (5) 3 min of breathing. In the chewing period, the subjects chewed 20 s on one side of the mouth, swallowed the saliva, chewed for 20 s in the other side of the mouth and so forth. No instructions were given concerning chewing rate. The subjects were told to keep the mouth closed. The release profiles of acetone, menthol and menthone were followed over time. To compare the level of acetone within the day for each subject, a one-way ANOVA and a multiple range test were performed (Statgraphics plus 4.1 Manugistics, U.S.A.). Periodic breathing time and average amplitude were calculated in MatLab software.

MATHEMATICAL DATA MODELING OF RELEASE PROFILES

A model was established to give a quantitative description and characterization of the release of flavor compounds. During the chewing period, the release of flavor compounds depended on several factors, such as the size and weight of the chewing gum, the chemical composition of the chewing gum, and the test person. After the chewing period, the chewing gum was spit out, and the concentration of the flavor decreased first rapidly and then more slowly. This period is termed the phasing out process. During this period, the concentration decay depended on other factors than those for the chewing period. It was therefore expected that two different characterizations were to be applied, one for the chewing period and one for the phasing out process.

The release and decay of the flavor in the expired air acts according to different complex dynamic processes.[10] A detailed and accurate description of those processes will most likely never be established, and therefore, we are looking for some operational approximative models. The dynamic behavior of the signals calls for a description in terms of a dynamic model. In the following so-called intervention, modeling will be suggested and used for a description of the characteristics.

Overview of Time Series Intervention Analysis. Intervention analysis may be viewed as a type of regression analysis in which one or more predictor variables observed at equally spaced time points are postulated to have an impact on a response variable. The analysis has a close link to system modeling seeking in building-transfer functions for describing the relationship between one or more input variables and a response variable. The

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(9) Knoll, J. E. *J. Chromatogr. Sci.* **2001**, *23*, 422−25.

(10) Harrison, M.; Hills, B. P.; Bakker, J.; Clothier, T. *J. Food Sci.* **1997**, *62*, 653−58.

most important difference is that in transfer-function modeling, the predictor variables are quantitative, whereas in intervention analysis, the predictor variables are only indicators (typically taking the value 1 or 0).

The Intervention Model. An intervention model is a model that can describe the changes in an output process that are due to external phenomena, which is then considered as input into the system. For intervention models, the input is a qualitative variable, whereas the input to a transfer function model is a quantitative variable. The qualitative input is described by the intervention function defined as

$$I_t = \begin{cases} 1 & t = t_0 \\ 0 & t \neq t_0 \end{cases} \quad (1)$$

where it is assumed that the intervention takes place at time point $t = t_0$.

In the literature, it is also seen that the intervention function is defined as a step function, that is, as $J_t = I_t + I_{t-1} + I_{t-2} + ...$ This alternative definition will be used in the modeling later on.

Box−Jenkins Form. The intervention model can be written on the so-called Box−Jenkins form

$$Y_t = \frac{\omega(B)B^b}{\delta(B)} I_t + \frac{\theta(B)}{\phi(B)} \epsilon_t \quad (2)$$

where I_t is the intervention function. Furthermore, $\phi(B)$, $\theta(B)$, $\delta(B)$, and $\omega(B)$ are polynomials in the backshift operator (defined as $BY_t = Y_{t-1}$)

$$\delta(B) = 1 + \delta_1 B + ... + \delta_r B^r \quad (3)$$

$$\omega(B) = \omega_0 + \omega_1 B + ... + \omega_s B^s \quad (4)$$

$$\varphi(B) = 1 + \varphi_1 B + ... + \varphi_p B^p \quad (5)$$

$$\theta(B) = 1 + \theta_1 B + ... + \theta_q B^q \quad (6)$$

Furthermore, b is an integer-valued time delay. Finally, ϵ_t is a white noise process, that is, independently and identically distributed random variables with mean 0 and variance σ^2.

Output Error Form. An output error model (OE model) is a model with the form

$$Y_t = \frac{\omega(B)}{\delta(B)} I_{t-b} + N_t \quad (7)$$

where there is no model for the noise term N_t. The parameters of the models are most often estimated by the output error method (OEM). This will be elaborated in the section on estimation.

Identification and Estimation. To determine the nature of the transfer function component $\omega(B)/\delta(B)$ and the time delay b, plots of the response variable and attempt to diagnose the form of the impact of the intervention were examined. At the same moment the chewing gum was spit out, the dynamic system changed, and therefore, two different time windows (modeling windows) were used. The first window covered the chewing period, and the second window covered the entire period after the chewing gum was spit out. The initial concentrations for each period was estimated as an integral part of the estimation method. Various estimation methods are suggested in the literature.[11,12] In the present study, most attention was paid to modeling the low-frequency behavior of the variations and, in particular, the stationary concentration and a characteristic time constant associated with the approach to the stationary concentration. In that case, the output error method was found to be most adequate for estimation. Least squares and prediction error methods were also considered, but we found that these methods put too much emphasis on the high-frequency behavior of the signals.

By applying the output error method, the parameter estimates were obtained as

$$\theta = \arg\min\{S(\theta) = \sum_{t=1}^{N} N_t^2(\theta)\} \quad (8)$$

where

$$N_t(\theta) = Y_t - \frac{\omega(B)B^b}{\delta(B)} X_t \quad (9)$$

$N_t(\theta)$ is the simulation error, since it represents the deviation between Y_t and the output from the model where X_t is input without considering the noise. For intervention models X_t is equal to either I_t or J_t. The covariance matrix for the parameter estimates are often very difficult to calculate.[12] Furthermore, the most common validation techniques cannot be applied, for example, test for white noise. Therefore, the method should be used only under special conditions. In the present case where most attention was put on estimating the low-frequency dynamics and, in particular, on finding a characteristic time constant associated with the stationary concentration, the output error method was the most reasonable method.

Time Constants and Stationarity. As mentioned previously, the model considered was of the form

$$Y_t = \frac{\omega(B)}{\delta(B)} J_{t-b} + N_t \quad (10)$$

where J_t is the step function.

Alternatively, the model is written

$$Y_t = h(B)J_{t-b} + N_t \quad (11)$$

where the transfer function $h(B) = \omega(B)/\delta(B)$ is introduced.

If all of the roots to $h(z^{-1}) = 0$ are inside the unit circle in the complex plane, then the transfer function is stable. Assuming stability and neglecting the noise term N_t, the concentration Y_t in the model (11) will approach a stationary concentration given by

(11) Brockwell, P. J.; Davis, R. A. *Introduction to Time Series and Forecasting*; Springer-Verlag: NewYork, 1996.

(12) Ljung, L. *System Identification: Theory for the User*; Prentice-Hall: New York 1987.

$$Y_\infty = h(1) = \frac{\omega(1)}{\delta(1)} \tag{12}$$

This equation is used for finding the stationary concentration. The rate of convergence toward the stationary concentration is given by the time constants of the transfer function, and ultimately, the largest time constant determines the speed. In the actual case, the smaller time constants turned out to describe the dynamics of the initial dynamics shortly after the chewing begins.

The time constants are related to the roots of the transfer functions. All of the roots of the transfer function are found as the roots to

$$\delta(z^{-1}) = 0 \tag{13}$$

where the roots are either real or complex. For a real and positive root, p_i ($|p_i| < 1$), the time constant is found as

$$\tau_i = -\ln\frac{1}{p_i} \tag{14}$$

In this study, it turned out that all of the largest roots where real and positive.

RESULTS AND DISCUSSION

Operation of Interface. The interface design presented in this paper is based on very simple materials and only a few custom-made parts. The breath is drawn into the source by the well-known venturi effect. Compared to the Taylor and Linforth patent,[5] we have moved the venturi effect from the APCI source to the nose interface. Therefore, this interface can be installed on any mass spectrometer having an APCI source. A funnel was chosen as inlet for the breath instead of a plastic pipet tip inserted directly into a nostril as described.[7] The pipet tip felt uncomfortable to the test persons in the present experiments. Furthermore, the funnel can be made to fit different persons. According to our experience, the funnel affects the subject to a lesser extent during breathing, drinking, and eating than a plastic tip. The test persons reported that they could chew habitually. The rate of sampling the headspace and the breath was found to be 4−10 mL/min.

In contrast to the interface patented by Linforth and Taylor,[5] our interface has a large volume of the inlet tube; thus, there is no restriction in the flow of air. Therefore, together with the relatively high flow of nitrogen, the dwell time is only ∼0.03 s for in vivo measurements and 1 s for the headspace analysis. Dwell times were not reported by Taylor et al.,[7] but they stressed that the delay in response time is insignificant. Doyen et al.[13] reported dwell times for the headspace analysis to be 2 s and for the in vivo studies to be 0.01 s.

As described in the Introduction, water is an important part of the ionization process. However, controlling the amount of water in the breath and in the surrounding area is difficult. The large flow of nitrogen used, as compared to the sampling volume, eliminated this problem; thus, it was only important to control the water content of the nitrogen. Addition of different amounts of water directly into the APCI source showed no effect on sensitivity in our instrumental setup (data not shown), whereas other authors found that increasing relative humidity of the APCI-MS ionization gases either decreased or increased instrument sensitivity.[4,14]

Repeatability in Vitro. The repeatabilities of in vitro headspace measurements of seven different flavor compounds were evaluated within a day and over a period of 4 weeks. The relative standard deviations (RSD) from the measurements are shown in Figure 2. For the 2-ketones, the repeatability was fairly good in both cases, with RSD in the range of 1−8% at both concentration levels. The RSD for the other compounds was ∼10% or less in the majority of the measurements. This was satisfactory taking into account that the RSD covered the uncertainty of the concentration and temperature (equilibrium shifting) and of the instrument. It was observed for the headspace measurements that a change of 1° C displaced the water/gas-phase equilibrium in the headspace and caused differences in the APCI intensities. Previously, it has been discussed among others whether compounds in a solution will interact with each other. Chaintreau et al.[15] concluded from their experiments that the composition of the mixture had very little influence on the partition at low concentration as used in this study.

Quantification in Vitro. Quantification of menthone and menthol was carried out by determining the concentration of the compounds in the gas phase both by GC/MS and by APCI-MS. The calibration curves for menthone were linear (0−471 ppb v/v) for each of the levels of acetone. There was a significant relationship ($p < 0.1$) between the concentration measurement by GC/MS and the APCI intensities for menthone. Testing the slopes and standard deviation against each other revealed that the calibration curves were not significantly different ($p < 0.5$). For menthol (0−985 ppb v/v), a polynomial regression of second order was used for menthol because it gave the best description of the relationship between the concentration and the intensities. Nonlinear quantification is reported by others.[16] Regression coefficients for menthone were between 0.998 and 1.00 and for menthol, between 0.997 and 0.998. As described in the Introduction, acetone is always present in expired air. Therefore, ion suppression by acetone in the APCI source was evaluated using different levels of acetone. For menthone, it can be concluded that the suppression was negligible at the concentration levels relevant for our experiments. Similar results were found for menthol, except at the high level of acetone. The standard curves reveal a deflection, as compared to the other curves, which might be assigned to the ion suppression phenomenon.

Detection Limits of Menthone and Menthol in Vitro and in Vivo. The limit of detection for in vitro measurements was 7.6 ng/L of headspace gas in the bottle for menthone and 6.2 ng/L of headspace gas in the bottle for menthol. The limits of detection for the in vivo measurement were found to be 1.7 ng/L of breath for menthone and 3.8 ng/L of breath for menthol (see Figure 3). Furthermore, it was found that the limit of detection for the instrumental method coincides with that of the flavor detection threshold. Figure 3 shows that there were large differences among the flavor threshold levels of the two compounds for the three subjects. This variation is probably due to variation in personal anatomy and the manner of handling solutions in the mouth. In genera, the determination of flavor detection threshold is not very accurate, for example, due to the variation in subjects. No

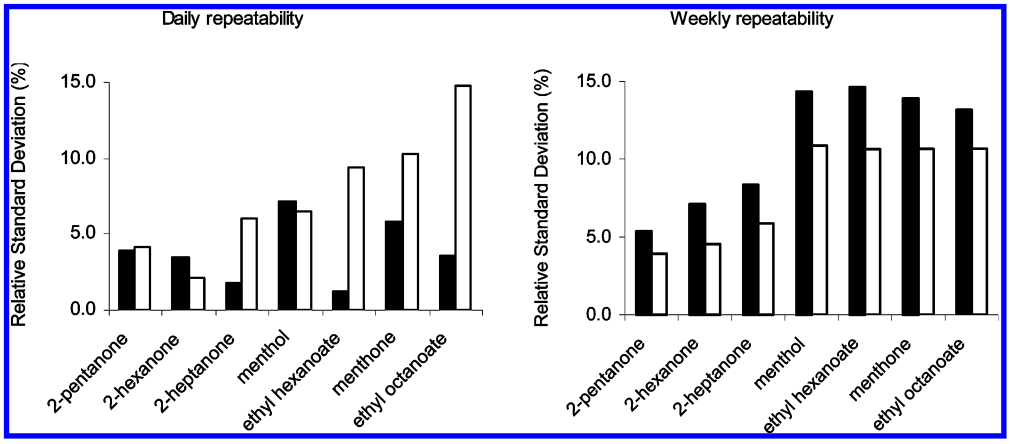

Figure 2. Repeatability of the retronasal APCI-MS measurement at two concentration levels within 1 day and in a period of four weeks (n = 3). (■) Flavor concentration of each compound 2.5 μg flavor/g ethanol (□) Flavor concentration of each compound 25 μg flavor/g ethanol.

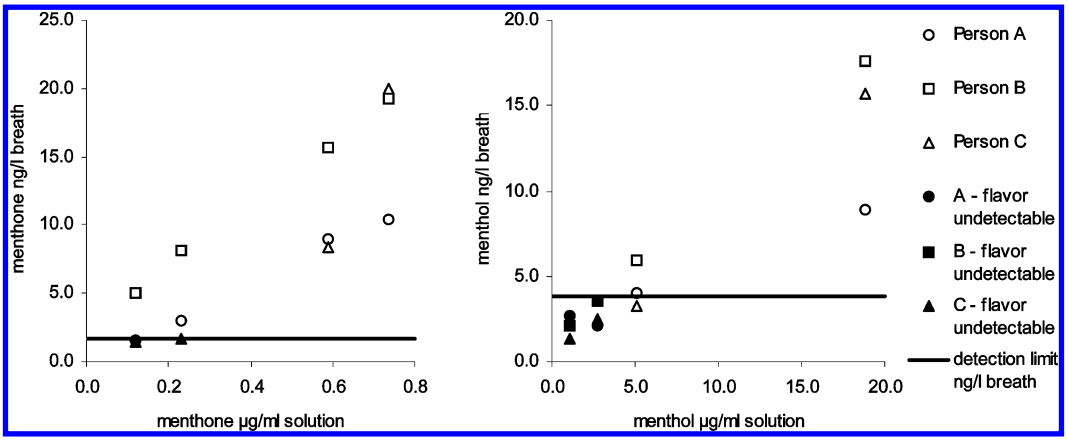

Figure 3. Experimental test of the in vivo limit of detection of menthone and menthol. The limit of detection is marked with a line.

Table 1. Parameters Covering the Breath[a]

subject	periodic time min	acetone amplitude intensity × 10^5	acetone level intensity × 10^5
1	3.84 ± 0.16^{w}	12.96 ± 3.11^{w}	33.7 ± 3.32^{w}
2	3.17 ± 0.14^{x}	4.78 ± 1.64^{xy}	23.5 ± 3.13^{x}
3	3.59 ± 0.24^{y}	3.43 ± 1.62^{x}	20.5 ± 1.42^{x}
4	3.27 ± 0.16^{x}	7.53 ± 4.42^{xy}	32.9 ± 4.62^{w}
5	2.84 ± 0.10^{z}	7.80 ± 3.38^{y}	42.5 ± 3.90^{y}
6	3.13 ± 0.24^{x}	7.96 ± 1.47^{y}	21.2 ± 3.35^{x}

[a] Means with the same letter in the respective columns are not significantly different for $p < 0.05$; $n = 4$.

information about detection limits by a retronasal method for menthone and menthol is available in the literature.

Application Study. Evaluation of the Breath. The level of acetone in the expired air for each person did not differ significantly ($p < 0.05$) among the series (data not shown) or among the measuring times. The first observation was expected, because acetone is a metabolic product. This could reflect that all subjects were in a normal state of nutrition and that there was less than 2 h since consumption of food. By contrast, it was found that there were differences among the subjects in the level of acetone, the acetone amplitude, and the periodic time (see Table 1). No correlation among the above-mentioned parameters was found. This is probably due to the limited number of participants and the biological variation.

Evaluation of Release Profiles. Two representative types of flavor release profiles for menthone were identified. One type had five very distinctive local maximum values (Figure 4A, marked with arrows) and the other one was relatively smooth (Figure 4B). The most plausible explanation for the differences in release pattern from the different subjects is that swallowing interacts differently with respiration. It was reported that swallowing, whether spontaneous or induced, interrupts respiration[17,18] and that it may result in a "burst" of air (in this study, menthone and menthol) from the nose.[19] It was also reported that the volume of post-swallowing breaths increased both in spontaneous swallows and in water-induced swallows, and that this increase was not associated with the timing of the swallowing with relation to the phase of the respiratory cycle.[17] In this study, the swallowing was standardized by time, but the act of swallowing was not forced. The influence of swallowing on the release profiles was revealed by the ion trace from acetone. This trace was affected by prolonging the duration of either the expiration or the inspiration according to when the swallowing took place; this corresponds with results obtained by others.[17,18,20] Furthermore, it has been shown that swallowing occurred during both inspiratory and expiratory phases of the respiratory cycle, with ~80% occurring during the expiratory phase.[17] This seemed also to be the case in this study, although another method and other compounds were measured.

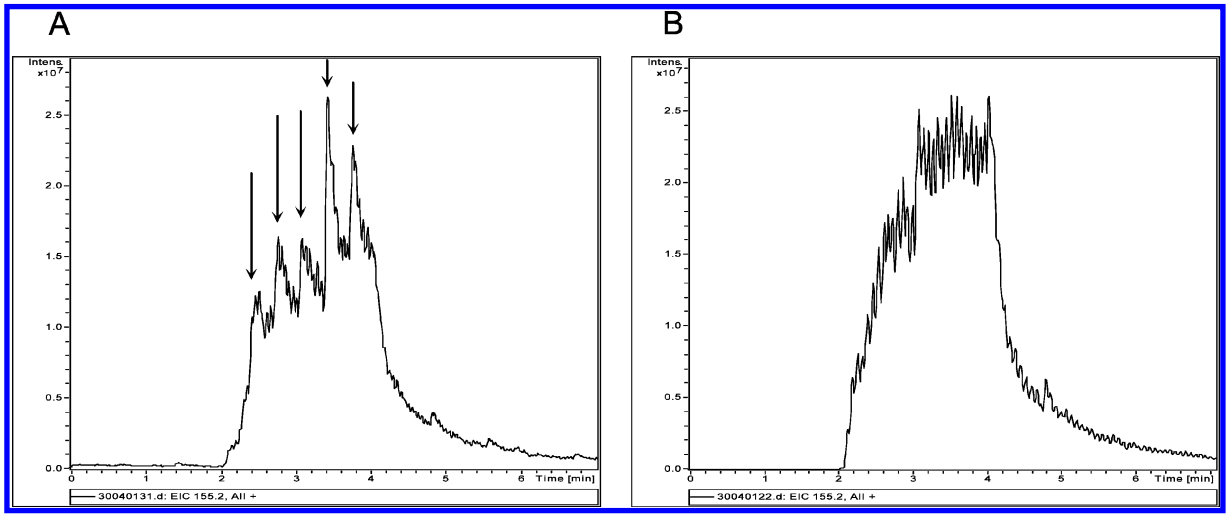

Figure 4. Flavor release profiles of menthone (*m*/*z* 155, $[M + H]^+$) from chewing gum chewed by two subjects. The arrows indicated the act of swallowing. The release profile under exhalation does not return to baseline as a result of the cloud of expired air around the sampling zone and the diffusion into the laboratory air.

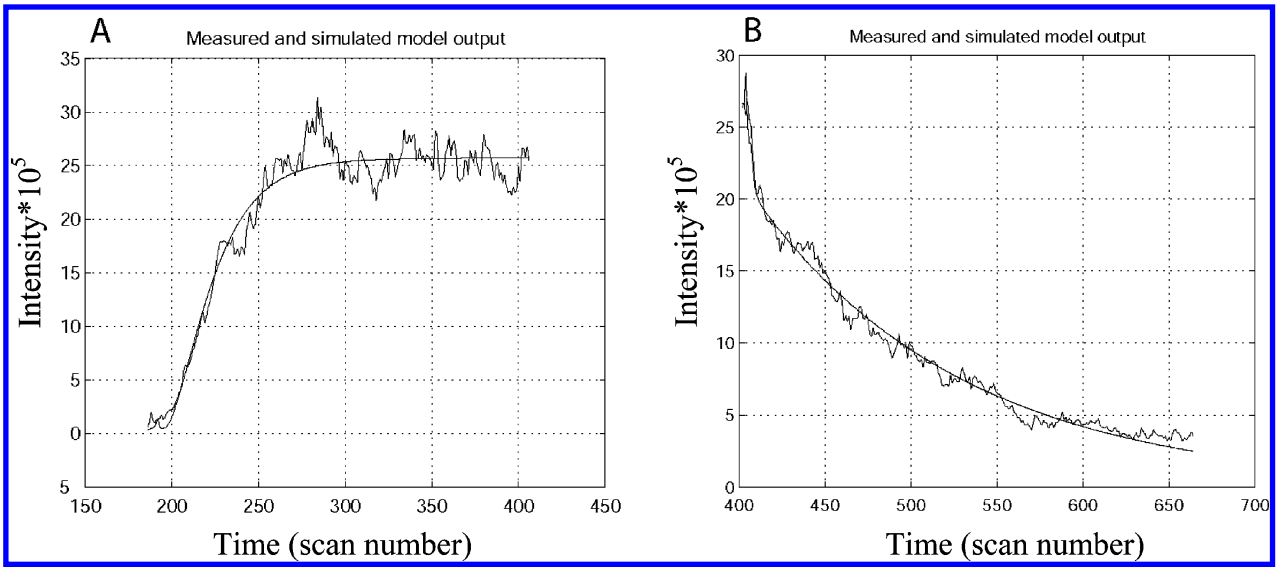

Figure 5. Release profiles for menthol (*m*/*z* 139, $[M - H_2O + H]^+$) for one subject. Part A shows measured and simulated model output for the chewing period, and part B shows the phasing out period.

The other type of release profiles (Figure 4B) showed a smooth progress, with high amplitudes in the respiration cycles. The effect of swallowing was less pronounced or not found in this profile. Looking into the ion trace of acetone, it could also be seen that a swallow did not show a prolonged duration of the next respiratory cycles. The differences between the two release profiles indicate that considerable differences exist among humans in the relationship of swallowing to the respiratory cycle.[17]

Evaluation of the Data Modeling on the Release Profiles. Harrison et al.[10] have developed theoretical models simulating the flavor release from liquid emulsion and in mouth[10,21] based on thermodynamically parameters, such as diffusion constants and partition coefficients. But their differential equations cannot be directly used in the handling of release profiles obtained by the BBB method. To the best of our knowledge, the only empirical data handling methods that have been implemented for BBB results are smoothing methods used by Taylor et al.[21] Smoothing, on the other hand, is quite a rough method, leaving out details that may be of importance. In this study, we focused and succeeded in developing a dynamic model based on intervention modeling. Release profiles for menthol were chosen to test the modeling process. Figure 5A and B shows the measured release profiles together with the profile simulated using the estimated

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Table 2. Estimated Parameters from the Data Modeling[a]

subject	chewing time constant intensity × 10^5/min	phasing out time constant intensity × 10^5/min	max intensity intensity × 10^5
1	69 ± 1^{w}	109 ± 10^{w}	36 ± 2^{w}
2	60 ± 4^{w}	132 ± 53^{w}	45 ± 1^{x}
3	60 ± 7^{w}	149 ± 27^{w}	28 ± 6^{wy}
4	51 ± 37^{w}	114 ± 19^{w}	31 ± 8^{wy}
5	46 ± 3^{w}	126 ± 9^{w}	31 ± 3^{wy}
6	173 ± 60^{x}	152 ± 32^{w}	26 ± 8^{y}

[a] Means with the same letter in the respective columns are not significantly different for $p < 0.05$; $n = 4$.

model. For both the chewing period and the phasing out period, a fourth order model (i.e., $\delta(B)$ is a fourth-order polynomial) showed the best fit. Parameters estimated from the model were (a) a time constant describing the ascending process for the chewing period, (b) a time constant describing the phasing out process and (c) a predicted maximum intensity (Table 2). The two processes must be different because of the changed dynamic (chewing as opposed to breathing). The estimated time constants for the chewing showed that subject 6 differed significantly ($p < 0.05$) from the others in having a much higher value, that is, having a slower release rate. This corresponds with the lowest maximum intensity for subject 6. Subject 2 had the highest value for estimated maximum intensity ($p < 0.05$). Looking into the breathing pattern, there was no clear answer as to why subject 6 had a slower release rate or why subject 2 had a higher maximum intensity. The parameters for the breathing and the three estimated parameters can together give an adequate description of the whole release process. Other parameters, such as chewing frequencies, saliva production or anatomical features, may have influence on the release rate, but were not investigated.

CONCLUSION

A method for measuring retronasal flavor release on-line and for analyzing the resulting data was developed and tested. The instrumental setup consists of a custom-designed interface combined with an APCI ion trap. The interface can be implemented on most mass spectrometers with an APCI source without rebuilding. The setup shows high repeatability in both in vitro and in vivo measurements and has an instrumental limit of detection for menthone and menthol that coincides with the flavor detection threshold. The ion suppression of the APCI source due to acetone in the breath seems to be negligible in the concentrations used. However, one should be aware that suppression of ions always will affect quantitative measurements. An output error method mathematical model for analyzing the release data was established. The model can be used to estimate the chewing and phasing-out-process-time constants and maximum intensity. From these three parameters, it is possible to assess the picture of the whole release process. These data allow for building a database system to describe the release characteristics of different flavor-matrix system averages of parameters.

The type of interface and the mathematical application could be used in other fields for research needing a real-time monitoring mass spectrometry, such as metabolic studies of malabsorption or studies of halitosis.

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