Real Time Volatile Flavor Release Monitoring and its Flavor/Food Application Using Proton Transfer Reaction Mass Spectrometry

Understanding how aroma compounds interact with and are released from simple and complex foods

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Flavors and fragrances usually are complex mixtures of molecules with different physical properties, including volatility, fat solubility and sensorial characteristics, covering a wide spectrum of threshold values. They are usually present in natural extracts or final products at levels in the order of ppb to ppm. Classical methods of flavor and fragrance analysis employ a range of techniques for the isolation, concentration and identification of aroma compounds. More recently, interest has grown in the understanding of how aroma compounds interact with and are released from simple and complex foods and cosmetic matrices. It is from these bases that aroma compounds are released in the mouth, for foods, or on the skin or hair for fragrances. Release of volatile components by definition is a kinetic process and has the dimensions of concentration and time. These dynamic aspects may be exemplified by:

- the general process of food consumption and aroma transfer and release during eating and drinking
- the release of aroma compounds during the consumption of natural foods, such as fruit and vegetables, a process often associated with *de novo* aroma generation
- the release of aroma molecules from fabricated foods, such as beverages and confectionery products
- the evaporation of perfume from real and simulated skin surfaces
- the evolution of fragrance compounds from flowers

Flavors and fragrances therefore fit significantly into this temporal environment. How, then, do we manage and monitor the concentration/time dynamics of these complex mixtures of small aroma molecules in the ppbv-ppmv in the gas phase in multifunctional matrices and in timescales from parts of a second to minutes and hours? Additionally, can we measure these flavor dynamics during the complicated eating process? Classical methods of aroma analysis are limited since they normally require a separation step, usually by gas chromatography, that interrupts the dynamic monitoring process.

The development of chemical ionization techniques, such as proton transfer reaction/mass spectrometry (PTR-MS), has made the continual monitoring of fast formation and release of volatile organic compounds possible.[1]

The purpose of this review is to describe the PTR-MS instrument and its operation, as well as to exemplify its potential in the areas of flavors/foods and, by extrapolation, its utilization for fragrance application.

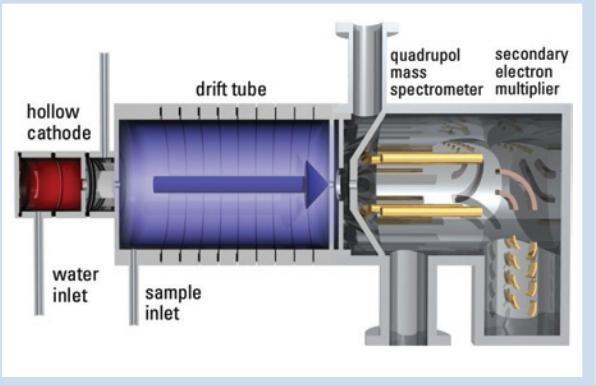
Methodology

The PTR-MS instrument and its operational details: The PTR-MS technique for volatiles measurement is rather easy to operate and requires a limited work-up procedure. Quantification of organic volatiles in the gas phase in the range pptv-ppmv also is possible without the addition of internal standards, and complex mixtures of aroma compounds, including flavors, can be monitored *in vitro* and in-nose

for selected ions in view of the relative simplicity of the spectra usually associated with the soft ionization procedure employed.[2] A constraint of PTR-MS is that specific unambiguous identification of compounds is not possible because PTR-MS is a uni-dimensional technique in which the primary information available is the protonated molecular mass or limited fragmentation of the same. Thus, the technique is unable to distinguish isobaric compounds, i.e. compounds of the same nominal but different accurate masses, and it relies on classical gas chromatography/electron impact mass spectrometry for chemical identification.

A schematic representation of the PTR-MS instrument is shown in F-1, along with an indication of the functional parts.

Cutaway sectional representation of the PTR/MS **F-1**



A more detailed description of the operation of the instrument can be found elsewhere, but, for clarity, some aspects of the processes involved in the real-time detection and quantification of aroma compounds are described.[3,4] The fundamental reaction utilizes proton transfer employing the hydroxonium ion H_3O^+ as the primary ion, this latter being produced at high intensity and purity in a hollow-cathode ion source. Proton transfer occurs with volatile species with proton affinities greater than that of water, 165.2 kcal/mol, namely, most organic species.[5] The primary ion does not react with the natural components of air, CO_2, N_2, O_2, due to their lower proton affinities; thus, they do not interfere in the aroma analysis procedure.

The primary proton transfer reaction occurs as follows:

$$R + H_30^+ \xrightarrow{k} RH^+ + H_20$$

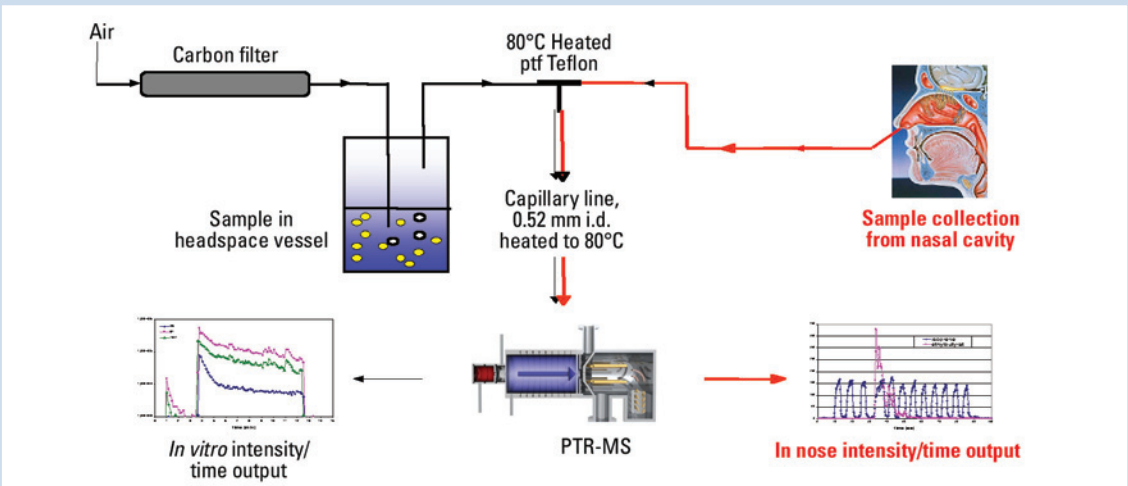
where R is the reactive volatile organic compound, and k is the reaction rate constant; with reaction rates for nearly all volatile organics equal to the respective gas kinetic collision frequencies.[3] The main advantage of the proton transfer reaction is that, in most cases, although there are some exceptions, there is limited fragmentation of the product ion, the RH^+ species. This factor permits the analysis of complex mixtures of volatile compounds, provided they have different molecular masses.

A small, defined amount of sample gas, ca.15 cm^3 min^{-1}, passes through a flow controller and enters the drift tube for analysis. Product ions are monitored in the downstream quadrupole mass selector. Under typical operating conditions, only a small fraction of the primary ions reacts with the R species so that the density of the product ions $[RH^+]$ is given by the relationship:

$$[RH^+] = [H_3O^+] (1-e^{-k/[R]t}) \sim [H_3O^+] [R] \cdot kt$$

$$\text{or } [R] = 1/kt \cdot [RH^+]/[H_3O^+]$$

Monitoring modes for *in vitro* and in-nose aroma analysis **F-2**



where k is the reaction rate constant (ca. 2×10^{-9} cm^3 sec^{-1}) for proton transfer from H_3O^+ to R, and t is the transit time (ca.105 x 10^{-6} sec) of H_3O^+ in the drift tube.[4, 6]

The relative constancy of the values of k and t in the drift tube permits calculation of gas phase concentrations. This ability to measure aroma compounds quantitatively in the gas phase is very important because with this knowledge, it may be possible to relate individual aroma compound concentrations, as measured in the nose during consumption of a food, to the perceived sensory response.[7]

Experimental setup for sampling: Gas samples may be analyzed directly from *in vitro* or from in-nose/in-mouth environments. Volatiles in the gas phase are conducted via a heated, inert transfer line directly into the drift tube of the PTR-MS at a flow rate, depending on the application, of 50-150 mL min^{-1}, of which 15 mL min^{-1} was sampled to the PTR-MS for analysis (F-2).

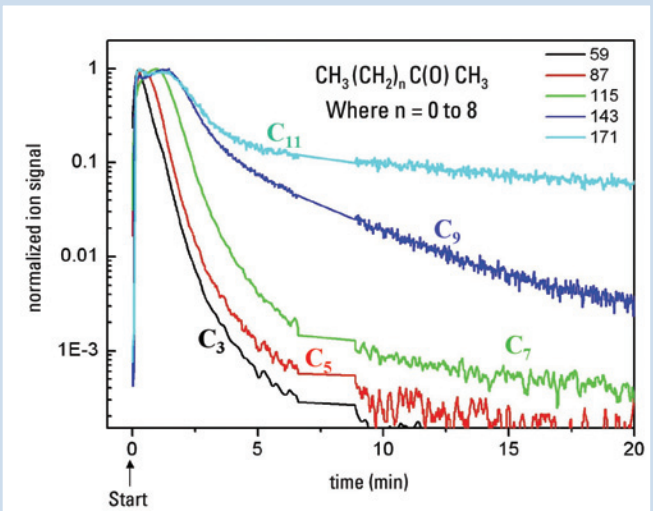
Connection from the *in vitro* vessel or nose is via an inert fused silica capillary line, 0.52 mm id. and length 100 cm. The transfer line can be heated from 50°-150°C. The scan speed for monitoring the primary ion $H_3{}^{18}O^+$(m/z 21) and the aroma compound(s) selected for tracking, e.g. isoprene, measured at m/z 69 ($C_5H_8 \cdot H^+$), is set, depending on the speed of analysis and the number of ions to be monitored, from 20 msec to ca. 1 sed/atomic mass unit.

In vitro protocol: Samples for analysis were placed in a suitable septum sealed vessel with an inert gas inlet tube made from PFA of id. 2 mm (Item # KT845, Cole-Palmer Instrument Co., Vernon Hills, IL, 60061), dipping into the liquid, if present, or in the gas phase above a solid. The incoming air was purged of volatile organics by passing the gas through a Supelpur HC filter (Item # 2-2445-U, Supelco, Supelco Park, Bellefonte, PA, 16823). The exit line from the sample vessel was connected via 2 mm id. PFA to the transfer line, as above. For large samples, such as a whole apple, a core was cut through the intact fruit with a # 5 cork borer. Then immediately into one opening, the entry point, in the fruit to a depth of 1 cm was inserted a close-fitting glass connector, the other end of which was joined to the outlet from the carbon filter. An identical glass device was connected to the other exit point in the fruit and coupled to the transfer line to the PTR-MS. The advantage of this system was that a fresh-cut surface, of both defined sweep volume and surface area, could be evaluated.

In-nose protocol: For liquids, 4 mL of sample was taken into the mouth, after a number of initial breathing cycles and preceding the next intake of breath. Experiments were conducted either in the normal "drinking" mode without any liquid mixing and followed immediately by swallowing or by holding the sample in the mouth prior to swallowing. A subsequent swallowing cycle was repeated without further sample ingestion. The volatiles transferred during the post-swallowing cycles were monitored quantitatively.

For structured solids, such as chocolate or a fruit chew, 4-6 g were taken into the mouth, chewed and then swallowed following a normal eating pattern. This involved chewing the sample to an initial state of subdivision followed by a swallowing cycle where saliva, but minimum solids, was swallowed. Further subdivision of the sample by tongue and teeth action with subsequent swallowing cycles was repeated. When the size of the sample was reduced sufficiently, the majority of the mass then was swallowed as a pliable bolus. Subsequent swallowing action cleared any residual solid/saliva mix. At this stage, normal tidal breathing was resumed.

***In vitro* release, from a smelling strip, of odd carbon numbered methyl ketones** **F-3**



Applications of PTR-MS in Flavors and Foods

The examples given below were taken from the flavor/foods area. Extrapolation of the same approach to fragrances realizes opportunities for monitoring the release of this family of compounds from perfumes and cosmetic preparations applied to the skin and from living flowers.[2]

In vitro Monitoring

Release of aroma compounds from a smelling strip—what the flavorist senses: Approximately 0.5 mg each of an homologous series of methyl ketones, odd carbon numbers 3-11, were coated onto a piece of smelling strip and placed in a glass vessel, as described above for the *in vitro* procedure. The release of the individual ketones, after data normalization, was monitored continually for a period of 20 min (F-3).

The release profiles for the homologous series showed a decline pattern anticipated with the C_3 ketone decaying very rapidly, while the rate decreased as the carbon number was increased, in keeping with their corresponding vapor pressures of 30.8, 4.93 and 0.49 kPa at 25°C, for the $C_{3,5,7}$ ketones. Expansion of the first minute of the release profile, data not shown, still exhibited differences in release between the ketones. It would be equally possible to monitor the decay of a complete flavor under the same conditions. The ability to monitor analytically the decay profile sensed by the flavorist now becomes a possibility.

Release of aroma compounds from a cut apple: The setup used in this experiment was as described in the *in vitro* monitoring mode, as shown in F-2. Four ions corresponding to wound-generated C_6 aldehydes and acetaldehyde were monitored for a period of 22 min (see F-4).

The results demonstrated a rapid formation/release, then a decline of all of the indicated compounds except for acetaldehyde, which exhibits a buildup to a steady state flux, suggesting a different pathway of formation to the other components. The biochemistry behind the C_6 family of wound-induced aroma compound formation is well established and is part of the localized signaling pathway in plant defense response.[8]

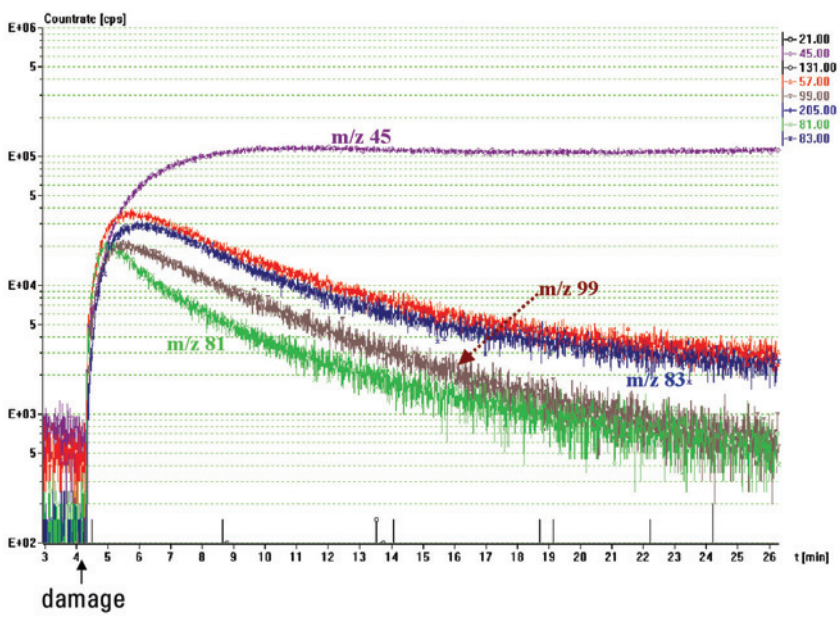
The relationship between the anatomy of the mouth and aroma release: The process of consumption and enjoyment of flavor in food is a complex phenomenon in which most of the signals for taste and aroma emanate from the mouth and, in the case of aroma, find their way to the olfactory epithelium, principally via the retro-nasal route. Retro-nasal perception is consequentially the result of the combined temporal olfactory outcome from aroma compounds released during mastication and breakdown of the food structure, including its mixing with saliva and swallowing of the saliva/food bolus. This perception process may be subdivided, based on knowledge of the processing of food in the mouth into two phases. Buccal, or pre-swallowing, perception is linked to the chewing of solid foodstuff and occurs when the soft palate closure opens only temporarily to enable vapors to pass in the direction of the nasal cavity while the food is being consumed. Full retro-nasal perception is associated with the post-swallowing process itself, the dominant phase in swallowing liquids or finely comminuted solid foods, when the soft palate closure opens fully following previous closing and opening of the epiglottis. The capability to monitor aroma release in the nasal cavity requires the rapid-response, high-sensitivity measurement available via PTR-MS. It is important, however, to note that, in the context of flavor perception, the process is multimodal and receives additional contributions principally from taste and texture.

A diagrammatic representation of the oral anatomy and its relationship to aroma release is shown in F-5.

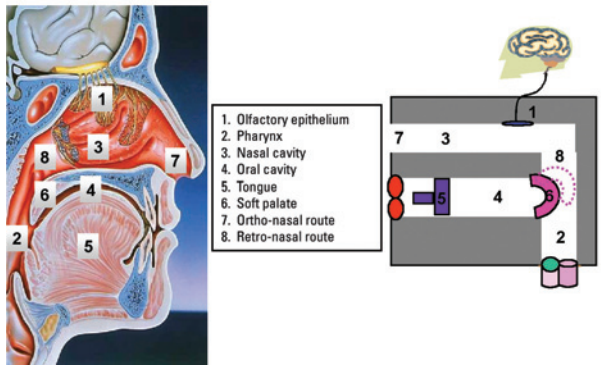
In-nose Monitoring

Consumption of a beverage: The sequence of events leading to the release of aroma compounds was demonstrated by consumption of 4 mL of a strawberry-

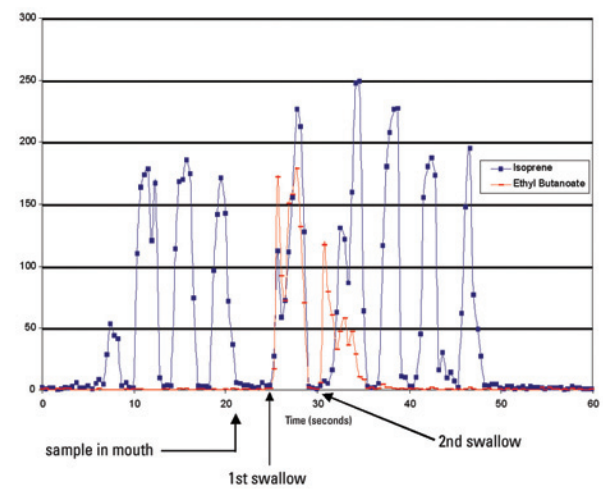
In vitro **release of wound-generated C_2 and C_6 aldehydes following mechanical damage to an apple** **F-4**



Sagital anatomical and functional section of the head and corresponding model of eating and related aroma transfer **F-5**



In-nose monitoring of the release of ethyl butanoate during consumption of a strawberry-flavored beverage (for experimental conditions, see text) **F-6**



flavored beverage, dosed at 0.1 percent v/v, and containing ethyl butanoate at a level of 1.5 percent m/m. The total content of the ester in the final product was 15 ppm, and the 4 mL consumed contained 60 µg of the ester. The pattern of release of the ion at m/z 117, corresponding to (ethyl butanoate)H^+, showed a simple pattern of aroma transfer from mouth to nasal cavity during the drinking mode. Isoprene in the breath, ion at m/z 69, was used as an index of breathing. The sample was taken into the mouth, held for 5 sec and then swallowed. In this mode, there is essentially one peak of aroma corresponding to the post-swallowing action (F-6). Up to the time of swallowing there was little or no transfer of aroma compounds from mouth to nasal cavity and, thus, no olfactory response. Additional lesser transfer of aroma occurs after a subsequent swallowing, this removing residual ethyl butanoate/saliva mix. The distribution of the ethyl butanoate signal transferred to the nose during the post-swallowing

phase between the first and second swallow, measured as the area under the appropriate peak, was 66 and 34 percent, respectively. Only traces of ethyl butanoate, <3 percent, were transferred to the nasal cavity following a subsequent third swallow. Other modes of swallowing liquids could yield different release profiles.

F-7 shows the sequential representation of events leading to the transfer of aroma compounds from the mouth to the olfactory area via the retro-nasal route for a simple beverage structure. This system transfer is controlled principally via the positioning of the soft palate, which, in order sequentially restricts liquid retention in the oral cavity, allows transfer of aroma compounds and liquid to the pharyngeal region but not the nasal cavity, and finally at the post-swallowing stage transfers aroma-laden gases to the olfactory region.

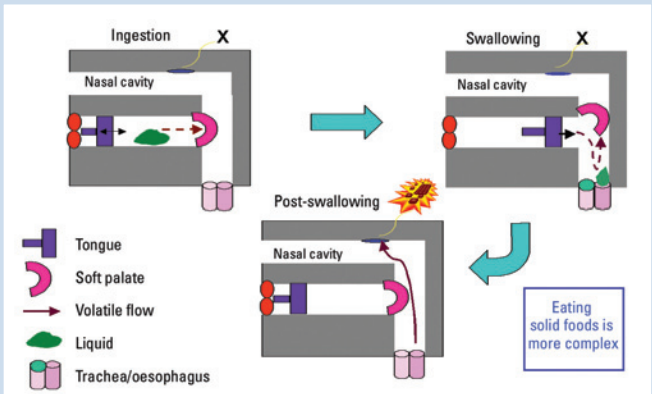
Consumption of a fabricated meltable product-chocolate: A single piece of dairy milk chocolate, 5.7 g, was placed in the mouth and chewed normally for ca. 19 sec corresponding to approximately 25 chews. Aroma release was monitored at m/z 73 and 87 corresponding to butanal and pentanal/isopentanal, respectively, released from the chocolate (F-8). Breathing pattern was monitored at m/z 69 corresponding to protonated isoprene. At this point the liquor was swallowed (swallow 1) and the residue mixed in the mouth for a further 15 sec, then swallowed again (swallow 2). Further mixing was carried out and the final swallow (swallow 3) made at ca. 40 sec after sample insertion in the mouth. The exercise was repeated and at the swallow 1 and 2 stages the sample was expectorated. On visual inspection at the first swallow stage the chocolate mass was not dispersed completely or melted, while at the second swallow the chocolate was melted completely and dispersed fully in the saliva. The extent of melting/dispersion, on inspection, appeared to be related to the amplitude of the aroma signals from the product. Additional work will need to be conducted to probe further these important food microstructure/flavor interrelationships.

Consumption of a dissolvable solid-fruit chew: A fruit chew containing a strawberry flavor, dosed at 0.3 percent v/v, was monitored in-nose during consumption. The initial hardness of the fruit chew was measured as the average force of compression recorded on the Stable Microsystems TA-XT2 Texture Analyser, application measurement BSW2/P6 for hardness and stickiness of chewy confectionery. Using a 6-mm cylinder probe and a 25-kg load cell the mean positive force value "hardness" was 6293 g. The progress of the release of ethyl butanoate, dosed in the flavor cocktail at 1.2 percent m/m, was monitored as before at m/z 117 (F-9).

A much more complex aroma release profile throughout a 50-sec time span was observed relative to the pattern for the simpler beverage system of F-6.

In addition to the aroma pulses due to the five post-swallowing cycles there were additional

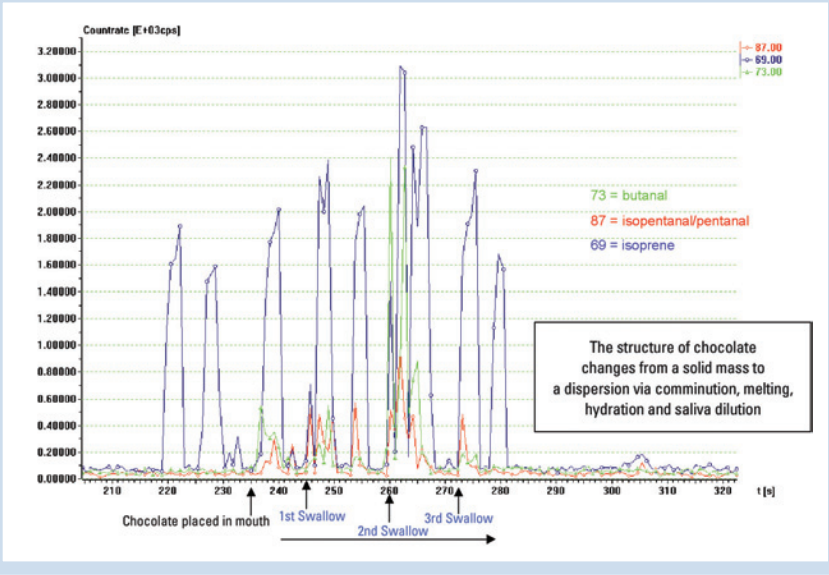
The flavor dynamics of consumption of a beverage **F-7**



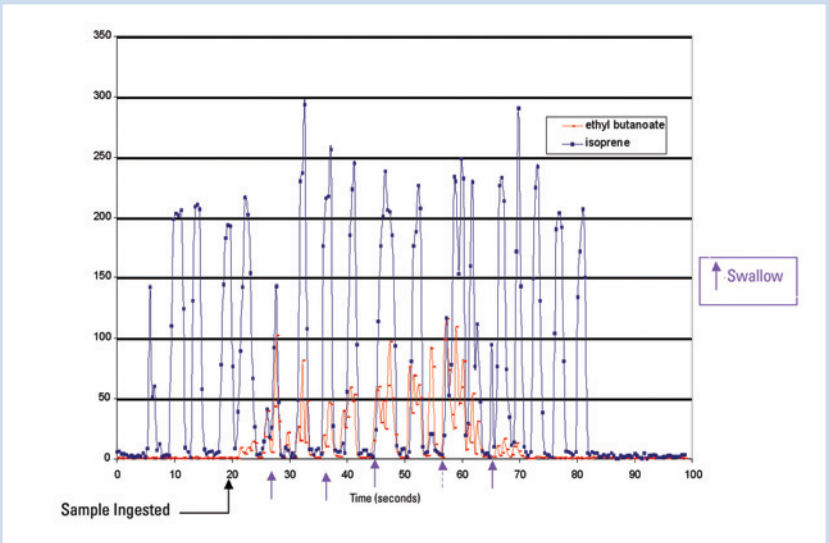
inter-swallowing buccal pulses brought about by the mouth chewing action. This meant that, although there was "chewable" material in the mouth, there was transfer of aroma-laden air to the nasal cavity. The distribution of the nasally transferred ester between the post-swallowing and buccal phases was 57.9 and 40.3 percent, respectively. It should be noted that in the case of the solid chew there was a gradual increase in the amount of ethyl butanoate transferred as the eating of the product progressed; this maximizing at 28.7 percent of the total signal at the stage when the bolus was swallowed; the dotted arrow in F-9. For the "buccal" phase there also was a buildup of ester transfer to the nasal cavity with a maximum, at 18.9 percent of the total signal, in the phase preceding the swallowing of the bolus. This was in contrast to the simple situation that existed for the beverage. These differences were rationalized in terms of the gradual erosion and solution of the solid fruit chew, whilst the water-based beverage was mixed immediately with saliva, and virtually all of the ester that was transferred to the gas phase in the nasal cavity was completed within a period of about 11 sec. The pulsatile aroma transfer observed during consumption of liquids and solids probably is in contrast to the more continual exposure of the taste receptors to sapid compounds in the mouth occurring at the same time. The nature of the aroma pattern may have repercussions on the process of adaptation.[9]

It is well established that the release of aroma compounds from food products is affected significantly by the ingredients' type and composition; fat level affect-

In-nose release of aroma compounds during the eating of chocolate (for experimental conditions, see text) **F-8**



In-nose aroma release during consumption of a strawberry-flavored fruit chew (for experimental conditions, see text) **F-9**



ing both the rate and extent of release of aroma compounds. These manifestations are more pronounced when the ingredients are assembled into fabricated structures, such as oil in water emulsions. The breakdown of these simple and more complex microstructures in the oral cavity dominates the final stage of flavor release.[10] It is clear from the consumption of chocolate and a fruit chew that a major component of the observed release profiles is dependent on the rate of breakdown of the product in the oral cavity. The establishment of guidelines for the factors controlling flavor release provides opportunities for optimization of ingredients, product microstructure and the breakdown of the microstructure in the mouth or in the particular application, such as flavor release during baking where the same general principles apply. Therefore, it is clear that, in order to control flavor delivery from foods, essential components are a knowledge of ingredients, structure assembly of fabricated products and an insight into the functional role of the oral cavity in product disassembly. This knowledge base is not generally within the traditional remit of flavor houses, except for those whose expertises include ingredients, emulsifiers and functional food systems. The marriage of the flavorist expertise and flavor science with food science is a powerful and essential requirement for progress in this area of flavor release and control.

Summary

New developments in measurement capability invariably provide an impetus to progress in understanding. The introduction of PTR-MS provided such a stimulus to the real-time measurement of aroma release under *in vitro* and in-nose conditions. The ability to monitor quantitatively levels of complex aroma mixtures applied in products and under normal eating conditions provides a reality and capability to link these analytical measurements to sensory observations under the same conditions. Many applications are possible, some of which are described above, providing a snapshot of what can be achieved in the food/flavor area with obvious extrapolation to fragrance systems.

The combination of this measurement capability in conjunction with the expertise of the flavorist, flavor scientist and physical food scientist provides a unique knowledge base from which to understand the complexity and control the release of flavor from food microstructures.

The above approach now is being applied within Danisco in its new range of Commonsense® flavors.[7]

Future Work

Further refinements in the measurement technique could include improvement in the ability to discriminate isobaric molecules by replacing the quadrupole

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mass filter by ion trap-based mass spectrometers with the additional capability for collision-induced dissociation or in-trap chemical reactions. Studies in this direction look promising.[11]

On the flavor front, food designs are anticipated in which the delivery of the flavor from the product is harmonized with the structure and microstructure breakdown of the food in the particular release environment, leading to potential one-stop-shop applications; this will require the flavorist and flavor scientists to integrate their activities with different science disciplines. The fusion of these multifunctional activities with linkages to the sensory perception of flavor will complete the circle.

Acknowledgments

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