Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 108 (2008) 1234–1246
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical Methods

Dynamics of retronasal aroma perception during consumption: Cross-linking on-line breath analysis with medico-analytical tools to elucidate a complex process

Andrea Buettner [a,b], Sabine Otto [a], Ambros Beer [c], Montse Mestres [d], Peter Schieberle [a], Thomas Hummel [e,*]

[a] Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, 85748 Garching, Germany
[b] Fraunhofer-Institute, Process Engineering and Packaging, Product Safety and Analysis, Giggenhauser Strasse 35, 85354 Freising, Germany
[c] Institut für Röntgendiagnostik, Technische Universität München, Ismaninger Strasse 22, 81675 München, Germany
[d] Facultat d'Enologia, Dept Química Analítica i Orgànica, Universitat Rovira i Virgili, C/Ramon y Cajal 70, 43005 Tarragona, Spain
[e] Smell and Taste Clinic, Department of Otorhinolaryngology, University of Dresden Medical School, Fetscherstrasse 74, 01307 Dresden, Germany

Received 17 November 2006; received in revised form 12 March 2007; accepted 15 March 2007

Abstract

An analytical concept is developed for the on-line investigation of the temporal and spatial dynamics in ortho- and retronasal odor perception. It aims at the elucidation of the relationship between the molecular level and perception during and after administration of a chemical stimulus. One basic principle is to apply precisely defined odorous stimuli, and to measure their temporal characteristics using an on-line analytical technique, proton-transfer reaction mass spectrometry (PTR-MS). In parallel, the subjects' response to the stimulus can be investigated with electrophysiological and psychophysical techniques. This approach can be modified to elucidate the processes involved in the perception of food and drinks. Panellists may take an active role as they modulate chemosensations with certain patterns of mastication/swallowing. Using videofluoroscopy or real-time magnetic resonance imaging these mechanical oropharyngeal processes may be visualized.

Keywords: PTR-MS; Olfactometer; Sensory analysis; Videofluoroscopy; Magnetic resonance imaging; Olfactory event-related potentials

1. Introduction

During the last years research on retronasal aroma perception has been dominated by "nosespace" and "mouthspace" analyses involving different trapping or mass spectrometric techniques (Hansson, Giannouli, & van Ruth, 2003; Taylor, 1996). Often, the focus was placed on physical release phenomena such as partitioning of odorants between polar and non-polar phases and air, and the influence of dynamic physiological processes such as salivation and breathing. This is mirrored by the development of a series of mathematical approaches which model in vivo retronasal release phenomena and their cross-linking to sensory perception (Harrison, 1998; Janestad, Wendin, Ruhe, & Hall, 2000). Generally, the time course of retronasal odor perception during and after food consumption is influenced not only by food matrix composition but also by physiological factors. Apart from dilution effects due to salivation and aroma transfer by breathing, other factors include oropharyngeal performance during mastication and swallowing (Burdach & Doty, 1987), differences in the topographical adsorption of odorants to the oral and pharyngeal mucosa and the olfactory epithelium (Buettner & Schieberle, 2000), interaction between odorants and salivary constituents (Friel &

* Corresponding author.
E-mail address: thummel@mail.zih.tu-dresden.de (T. Hummel).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.03.042

Taylor, 2001; Hussein, Kachikian, & Pidel, 1983; Taylor, 1996), and the formation of adhesive coatings by food matrix constituents on oral and pharyngeal mucosa.

Differences between ortho- and retronasal perception of odors have attracted much attention (Burdach, Kroeze, & Koster, 1984; Diaz, 2004). However, as retronasal stimuli were presented orally most investigations were biased by factors such as gustatory/trigeminal chemosensory/somatosensory co-activation. In this respect it is especially important to note that the transfer of odorants from the oral to the nasal cavity can be mechanically blocked by the soft palate which is under the subjects' control. In fact, subjects can be trained with regard to their velopharyngeal movements in order to produce maximum odorant transfer during retronasal evaluation. This situation also introduces additional variation to the results obtained from panellists (Buettner, Beer, Hannig, & Settles, 2001).

To elucidate the dynamics of ortho- and retronasal aroma transfer, intranasal airflow patterns have been previously studied and models have been developed to mimic in vivo physiological conditions (Hornung, Youngentob, & Mozell, 1987; Keyhani, Scherer, & Mozell, 1997). Generally, results obtained and conclusions drawn by different authors were not fully consistent and do not allow a simple answer to the question why ortho- and retronasal perceptions can differ. Generally, it has been assumed that odor concentrations measured at the nostrils in the exhalation breath during mastication would resemble those being effective at the receptor site (Taylor, 1996). However, recent findings indicate that an intranasal gradient pattern develops, with spatial and temporal variations in odorant concentration, depending on the compound's respective chemical structures (Frasnelli, van Ruth, Kriukova, & Hummel, 2005).

When summarizing available literature on this topic, one realizes that on-line cross-linking of the different distinct analytical aspects might be key to answer questions on discrepancies between ortho- and retronasal sensory perception. Therefore, the goal of the present work was to develop a concept that combines precisely controlled odor stimulation at specific sites within the naso-pharyngeal space in vivo, with the on-line monitoring of the spatial and temporal distribution of the stimulus in vivo at defined intranasal locations using an adequate analytical tool offering sufficient sensitivity and selectivity, as well as the required temporal resolution (compare Frasnelli et al., 2005). Furthermore, this concept potentially also involves the on-line assessment of the chemical stimulus and its neuronal processing, both at a peripheral (olfactory epithelium) and a central-nervous level (brain). Last but not least, it comprises the qualitative and quantitative sensory evaluation by the panellist.

A key component of the model is that parameters are controlled which might interfere via retronasal odor presentation, e.g., salivation, swallowing, or breathing. As already performed in previous studies (Heilmann & Hummel, 2004; Small, Gerber, Mak, & Hummel, 2005), odors can be introduced to the nasal cavity through cannulas such that precisely the same stimulus is presented in the front or the back of the nasal cavity.

For a complete picture of the retronasal processes, subsequently the dynamic processes of food consumption have to be taken into account. To follow their influence on the dynamics of retronasal aroma perception, the approach combines on-line breath analysis via proton-transfer reaction mass-spectrometry (PTR-MS), together with sensory analysis and medical monitoring techniques (radiological and nuclear imaging techniques, event-related potentials, electro-olfactograms) (Buettner & Welle, 2004). PTR-MS analysis is characterized by short response times (generally 200 ms or below) and relatively high sensitivity with detection thresholds in the range of approximately 20 ppt_v (Hansel, Jordan, Warneke, Holzinger, & Lindinger, 1998). In the following, this concept will be illustrated using examples related to wine and gel consumption.

2. Materials and methods

2.1. Chemicals

Ethyl butanoate was obtained from Aldrich (Steinheim, Germany). The odorant was freshly distilled prior to analysis. Chemical and sensory purity was ascertained by gaschromatography–olfactometry (GC/O) as well as gaschromatography–mass spectrometry (GC–MS). Phenyl ethanol for olfactometer studies was from Sigma (Deisenhofen, Germany; order number P6134). Whey protein isolate (Bipro, JE 153-9-420) was from Davisco Foods International Inc., Le Sueur, MN, and glucono-δ-lactone (GDL) from Aldrich (Steinheim, Germany).

2.2. Wine samples

The following Chardonnay wine was selected for investigation: 1999 Merryvale Reserve Chardonnay, 14.5% by volume, Napa Valley, Merryvale Vineyards (St. Helena, California, USA). Prior to this study the composition of the wine in terms of odorants and volatiles has been studied extensively (Buettner, 2004a).

2.3. Preparation of gels

Gels with 4% ("soft" gel), and 10% ("hard" gel) protein concentration, respectively, were prepared and flavoured with ethyl butanoate according to the procedure described in Weel et al. (2002). Gels were freshly prepared, kept at 4 °C between sessions and applied immediately for analysis.

2.4. Panellists

Panellists were non-pregnant, non-smoking volunteers of the Technical University of Munich, exhibiting no

known illnesses at the time of examination and with normal olfactory and gustatory function. Subjective aroma perception was normal in the past and at the time of examination. The panellists reported a normal salivary flow and were selected for their excellent oral hygiene. Ten assessors (five male, five female; age range 24–53 years, mean age 34) were recruited and trained in preceding training sessions.

2.5. Sensory evaluation

Panellists were always asked to score odor intensities on a seven-point scale from 0.0 (not perceivable) to 3.0 (very intense) in steps of 0.5 units. Sensory analyses were performed in a dedicated quiet room at 21 ± 1 °C during three different sessions on separate days.

2.6. Wine samples

Samples (4 °C) were freshly opened and immediately used for sensory evaluation. The wine (25 mL each), was singly presented to the sensory panel for retronasal evaluation in a covered glass (capacity 45 mL). A sip of the sample was taken, and kept in the mouth for a defined time interval (20 s) with closed lips and closed velum while it was swished in the oral cavity. At defined time intervals the velum was opened deliberately by performing velum pumping actions as described previously (Buettner, 2003). After approximately 10 s of pumping, the sample was again kept within the oral cavity without swallowing. This pumping and non-pumping action was repeated once. Then, the sample was swallowed, and after certain time intervals, the pumping actions were repeated several times. Panellists

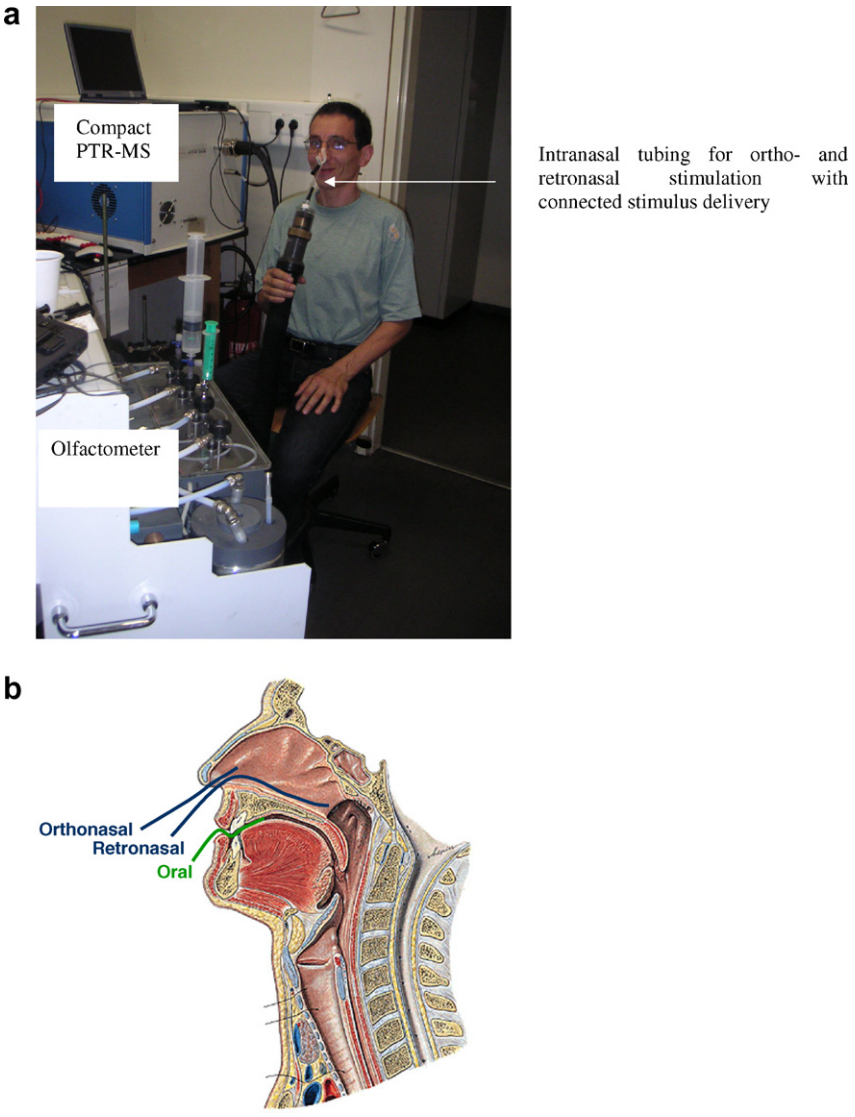

Fig. 1. (a) Picture of the experimental setup for the coupling of intranasal or intraoral stimulation by means of an olfactometer (OM2S; Burghart instruments, Wedel, Germany) together with on-line PTR-MS analysis. The temporal and spatial spreading of volatiles within the nasal cavity, or of the temporal and spatial progress of volatiles from the oral to the nasal cavity, respectively, is monitored. Discrete locations of in vivo stimulation are schematically represented in (b). Intranasal (ipsi- or contralateral) stimulation is performed either ortho- or retronasally.

were asked to indicate the moment of intense aroma perception by raising their thumbs during the tasting procedure.

2.7. Gels

Gels were freshly prepared and immediately applied to sensory evaluation. The samples were singly presented to the sensory panel for retronasal evaluation. Two milliliters of the respective sample were taken into the oral cavity and chewed for 30 s with closed lips and without swallowing. Then, panellists were instructed to swallow the entire bolus and, after that, to continue chewing for 60 s. The different gels were presented in triplicates to the panellists (3 samples of each type of gel). The order of the gels was randomized. Subjects were not informed about the composition of the samples.

For comparative evaluation of the hard and the soft gels, respectively, one sample was first evaluated in randomized order, then, after a 15 min break and rinsing of the oral cavity with tap water, evaluation of the second sample was performed.

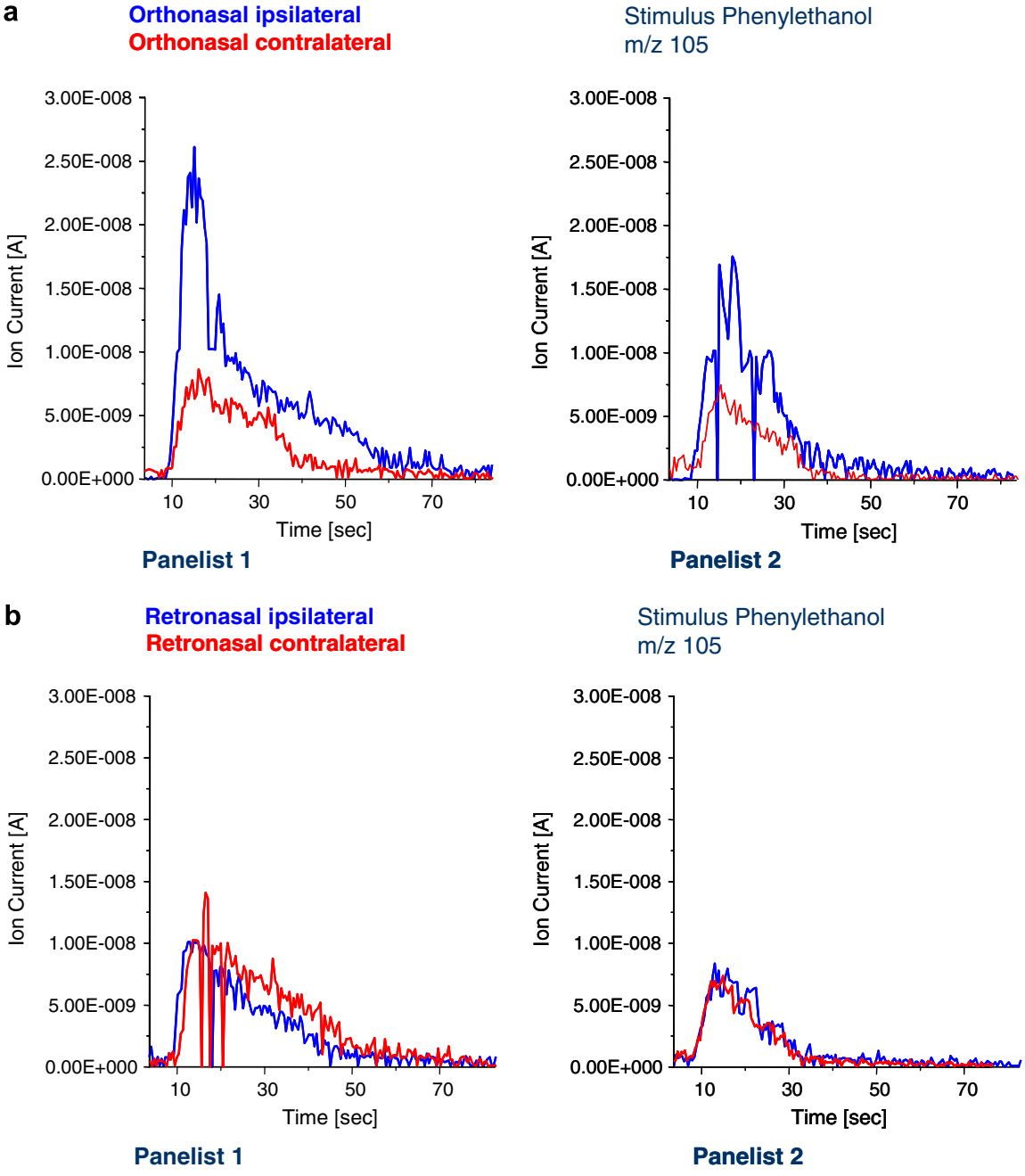

Fig. 2. Observation of the mass trace *m*/*z* 105 (from stimulus phenyl ethanol) by means of PTR-MS during in vivo ortho- and retronasal stimulation (ipsi- and contralateral) with defined phenyl ethanol-pulses from an olfactometer. Pulse intensity: 0.3 μg/ml, stimulus duration 5 s. Comparison of characteristic spreading profiles between two panellists.

2.8. Olfactometer

Stimuli were presented either ortho- or retronasally by means of a computer-controlled air-dilution olfactometer (OM2s; Burghart instruments, Wedel, Germany). This stimulator allows the presentation of odor concentrations with rectangular-shaped time intensity functions. Mechanical stimulation is avoided by embedding stimuli into a constant flow of odorless, humidified air of controlled temperature (80% relative humidity, total flow 8 l/min, 36 °C) (Kobal, 1981).

2.9. Intranasal tubes for ortho- and retronasal stimulation

For retronasal stimulation odors were released into the epipharynx cranially of the soft palate through tubing which was positioned under endoscopic control. For orthonasal stimulation an identical tube was placed in the anterior portion of the nasal cavity. Specifically, two plastic tubes of 3.3 mm outer diameter and 15 cm length were attached to each other, so that the opening of the two tubes were 6.5 cm apart. Tubes were cut from a sterile suction catheter made from soft polyvinyl chloride available

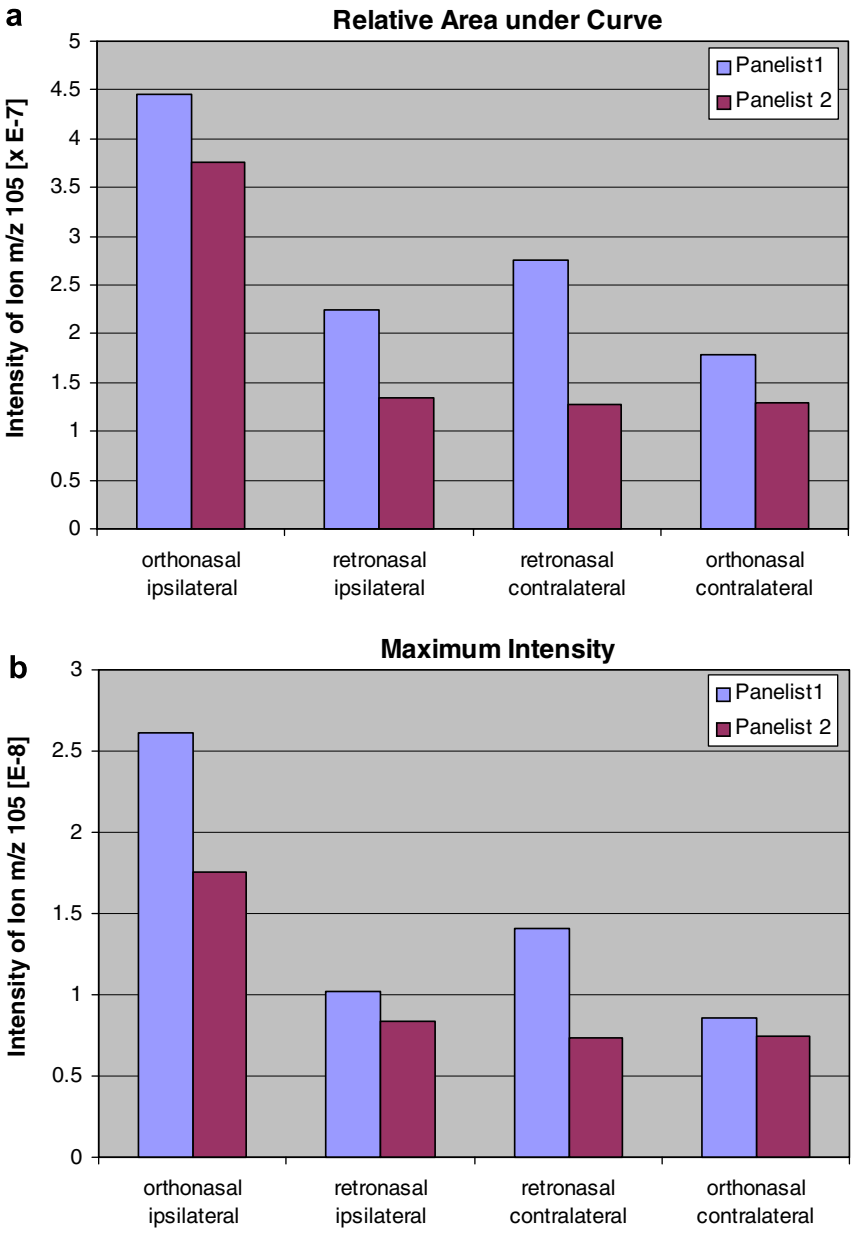

Fig. 3. (a) Total areas under the curve and (b) I_{max}'s from PTR-MS raw data obtained after in vivo ortho- and retronasal stimulation (ipsi- and contralateral) with defined phenyl ethanol-pulses from an olfactometer. Pulse intensity: 0.3 μg/ml, stimulus duration 5 s. Comparisons of single determinations between two panellists are shown.

through medical supply (Braun Melsungen AG, Melsungen, Germany). The ends of the two tubes were bent in an angle of 45° (Fig. 1). This was achieved by warming the end of the plastic tubes above a commercially available heater (Mirror Heater, Haeberle, Stuttgart, Germany). The tubes were placed inside the nose under endoscopic control (3 mm diameter, 30° vision angle rigid endoscope, Karl Storz, Tuttlingen, Germany) such that the opening of one of the tubes was just beyond the nasal valve and the opening of the other tube was in the epipharynx (Fig. 1). The tubes were attached to the nose by adhesive tape, so that the "retronasal" tube, ending in the epipharynx, was placed beneath the "orthonasal" tube, ending in the nasal vestibulum. For stimulus presentation the tubes were connected to the outlet of the stimulator.

2.10. PTR-MS

The PTR-MS technique has been extensively discussed in a series of review papers (Hansel et al., 1998). Briefly, it combines a sensitive and efficient mode of chemical ionisation (CI), adapted to the analysis of trace volatile organic compounds (VOCs), with a mass filter. In this study, 15 sccm gas was continuously introduced into the drift tube (CI cell). The drift tube contained besides the buffer gas, a controlled ion density of H_3O^+. VOCs with proton affinities larger than water (proton affinity of H_2O: 166.5 kcal/mol) are ionised by proton transfer from H_3O^+, and the protonated VOCs are mass analysed. The ion source produces nearly exclusively H_3O^+ ions (>98%), that are extracted and transferred into the drift tube. Acetone, isoprene (both as indicators for the panellists' breathing patterns), phenyl ethanol, ethyl acetate and ethyl butanoate were analysed in the selected ion mode (masses 59, 69, 105, 89 and 117, respectively). The PTR-MS system used in the present study was a Compact PTR-MS (Ionicon, Innsbruck, Austria).

2.11. Breath sampling

Nose space air during wine tasting and gel eating was sampled with two glass tubes fitted into the nostrils. The transfer line was a heated silo steel capillary with an inner diameter of 0.5 mm. A small fraction of 15 sccm was introduced into the drift tube of the PTR-MS. The tubes were heated at 50 °C, to prevent condensation along the sampling line. For the air sampling from a certain nostril during olfactometer stimulation, the volatiles were directly withdrawn with the silo steel capillary from the respective position. For the wine drinking and gel chewing experiments, nosespace volatile concentration was measured simultaneously to consumption by using real-time PTR-MS, as described above. By resting the nostrils at the glass tubes, the tidal breath flow from the nostril was directly sampled without interference with breathing or of wine or gel consumption.

2.12. PTR-MS data analysis

Analysis of the raw PTR-MS data has been performed as described previously (Mestres, Moran, Jordan, & Buettner, 2004). Parameters calculated involved the total amounts of odorants detected, given as areas under the curves (AUC), the maximum intensity of the release profile (I_{max}) and the time necessary to reach the maximum intensity (T_{max}). Unlike most previous studies, the mean of the single determinations was not calculated first, extracting the mentioned parameters therefore, but the single raw data was analysed for AUC, I_{max} and T_{max}, and later on averaged, according to the needs of the analysis (mean

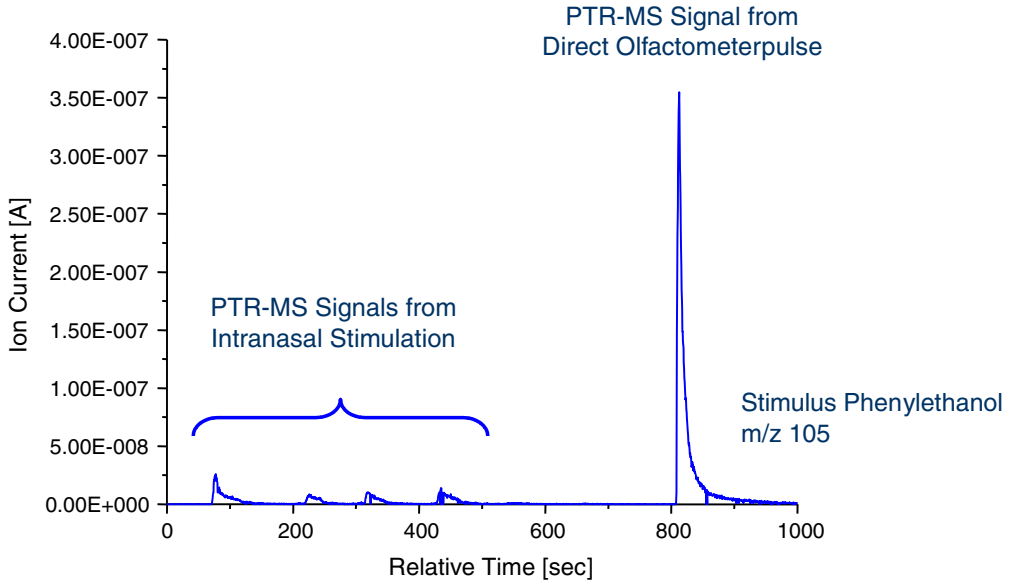

Fig. 4. Comparison between total intensities of ion m/z 105 obtained from in vivo and ex vivo phenyl ethanol-pulses from an olfactometer. Pulse intensity was always constant (pulse intensity: 0.3 μg/ml, stimulus duration 5 s.).

values for single panellists, mean values for all panellists combined).

2.13. Videofluoroscopy

Videofluoroscopy was performed using a conventional fluoroscopy unit (Philips Diagnost 76) connected to a S-VHS video recorder and monitoring was taped on a conventional S-VHS videotape. Temporal resolution was 25 images/s. Iotrolan (Isovist®, Schering, Berlin, Germany) served as liquid contrast agent. Images were acquired in the sagittal plane during swallowing as described previously (Buettner et al., 2001).

2.14. Nasal stimulation

Panellists were instructed in oral breathing using velopharyngeal closure (Kobal, 1981). Thereby, ortho- and

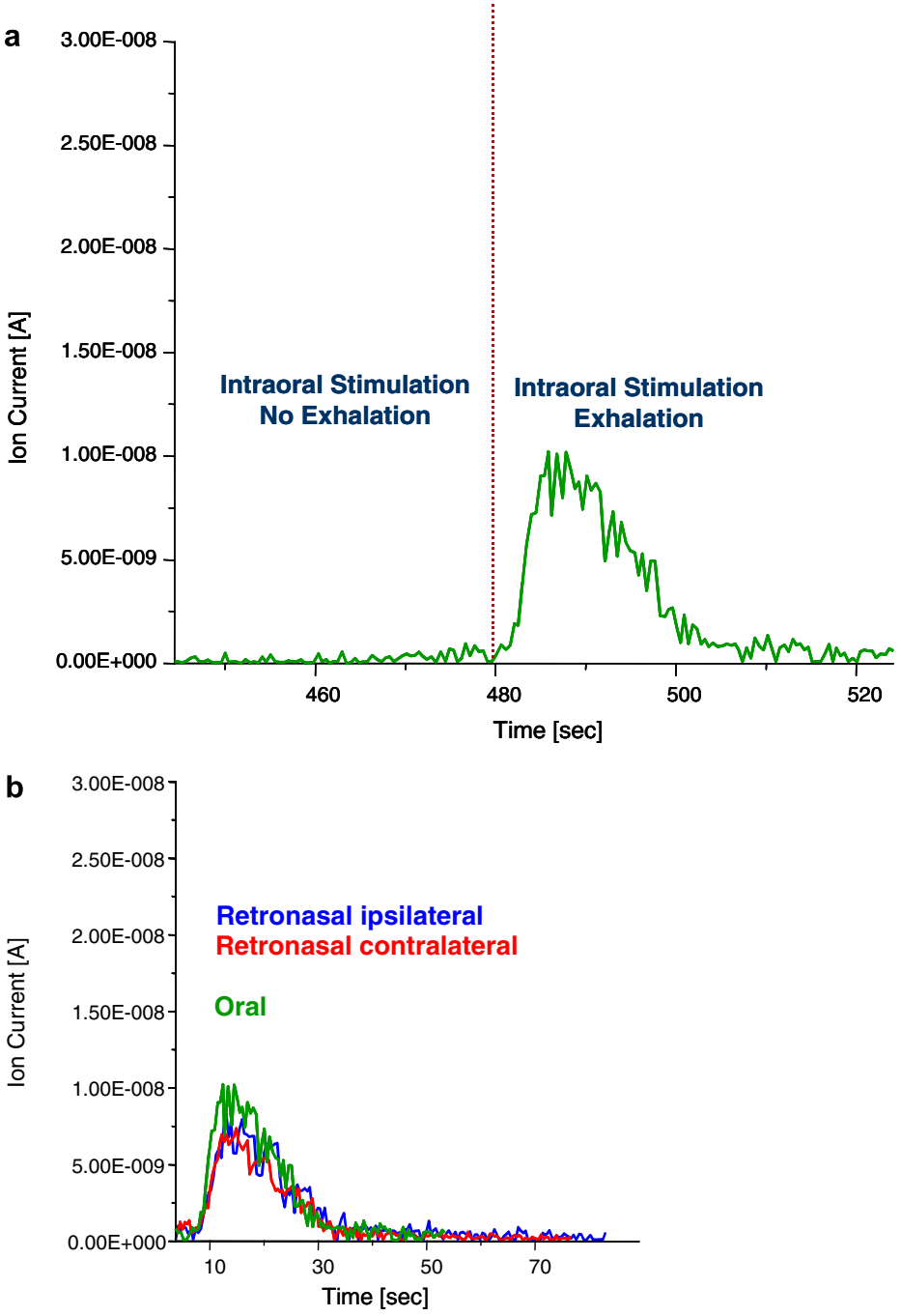

Fig. 5. (a) Observation of the mass trace *m*/*z* 105 by means of PTR-MS during in vivo oral stimulation (with and without exhalation) with defined phenyl ethanol-pulses from an olfactometer. Pulse intensity: 0.3 μg/ml, stimulus duration 5 s. Shown is a characteristic spreading profile of one panellist. (b) Comparison of in vivo spreading profiles obtained from oral stimulation (with exhalation) and from ipsi- and contralateral retronasal stimulation.

retronasal stimulation were simulated by use of an intranasal tubing system as described above (Heilmann & Hummel, 2004). Controlled stimulation with 200 ms pulses of 0.3 μg/ml phenyl ethanol (determined by gaschromatography) was either performed in the orthonasal or retronasal mode while the spreading of the stimulus to the ipsi- or contralateral orthonasal recording site was monitored by means of PTR-MS (Fig. 1).

3. Results

3.1. Coupling of in vivo olfactometer stimulation and PTR-MS analysis

A comparison of characteristic spreading profiles for two panellists are displayed in Fig. 2. Stimulation started 8 s after the onset of recordings, so that the delay time between stimulus application and signal recording was approximately 1–2 s, depending on the recording site.

Regarding the general shapes of the profiles and their temporal development, a high degree of similarity was seen for orthonasal contralateral, retronasal ipsilateral, and retronasal contralateral stimulation while the only profile exhibiting some deviation was that following orthonasal ipsilateral stimulation. That means, the most proximal stimulation varied considerably while the three distal modes resembled each other to a relatively high degree. The latter three showed a relatively fast increase in concentration at the recording site with approximately 5–8 s from the onset of stimulus increase until maximum concentration, while the decay was significantly slower taking with 35–45 s until baseline levels were reached. It has to be emphasized that these profiles represent the spreading of the stimulus without any interference with breathing. Therefore, relatively long intranasal lingering time intervals are observed. Additionally, it was carefully checked that the observed delay times measured in-nose were not due to adsorptive and carry-over effects of the applied cannula systems. This was ensured by measuring the odorant pulse directly at the olfactometer nosepiece, and on the end of the interconnected cannula. No differences were observed for onset, maximum intensity, and decline of the odorant pulse in the used setup.

In contrast to these profiles, the profile obtained after ipsilateral orthonasal stimulation exhibited a relatively sharp initial increase in concentration, followed by a quick drop to about half of the maximum concentration. Then, the shape changed and decreased relatively slowly, similar to what had been observed for the three other profiles. It became also evident that the highest concentration of the stimulus was obtained closest to the site of stimulus release and that the concentration was lowest at the site most distal from the point of release. This observation is mirrored when analysing the data with regard to pre-defined analytical parameters which were adapted from the interpretation of time-intensity data during consumption of foods (Birch & Munton, 1981): the analysis of the profiles in terms of maximum intensity (I_{max}), and the total stimulation seized by the area under the curve (AUC) (cf. Fig. 3). When comparing the profiles of the two panellists, it became evident that the general shapes were very similar but that, throughout all profiles, quantitative differences between panellists were visible. Generally, the concentrations obtained from the

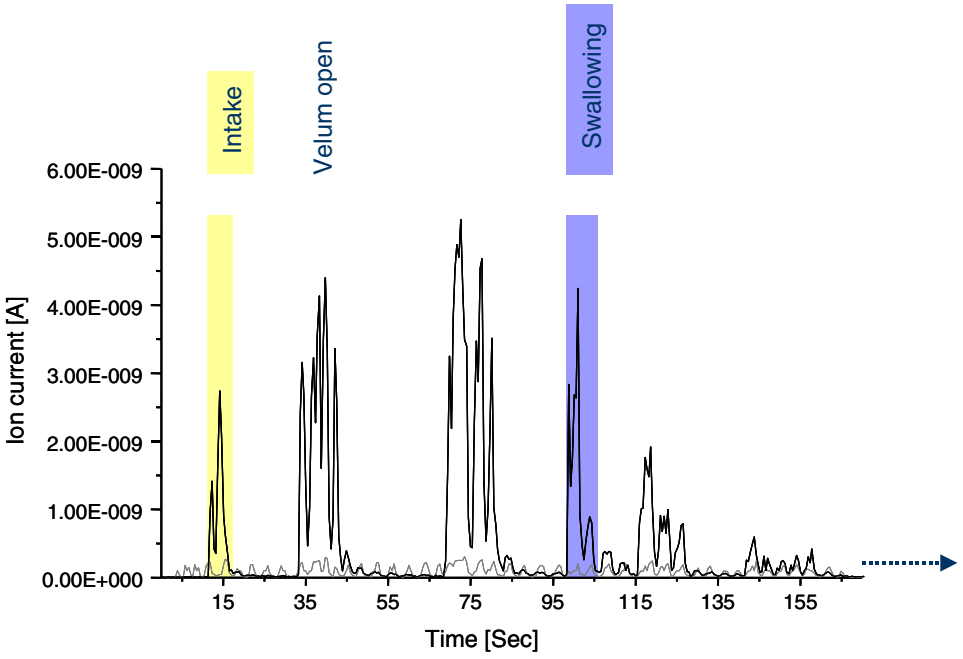

Fig. 6. Influence of velopharyngeal performance on retronasal ethyl acetate release during and after wine consumption, visualized by real-time PTR-MS breath analysis.

direct olfactometer pulse were higher than those obtained from intranasal stimulation (Fig. 4).

3.2. Oral stimulation

Olfactometer pulses were also applied intra-orally, first without exhalation, meaning without breathing during stimulation. In a second experiment, the stimulus was applied while the panellists exhaled through the nose once, then continued breathing through the oral cavity with velopharyngeal closure as described above. The stimulus profile obtained by PTR-MS analysis of the gas phase exiting the nostrils is shown in Fig. 5. During stimulation without exhalation no signal was obtained. On the other hand, with exhalation right after stimulation, a distinct aroma pulse was recorded at the naris.

3.3. Coupling of visualization of real masticatory processes/PTR-MS analysis/sensory analysis

3.3.1. Liquid foods

For real-time visualization of masticatory and swallowing processes, oropharyngeal performance was monitored by means of real-time videofluoroscopy, while observing selected marker volatiles (e.g., ethyl acetate) exhaled through the nose by means of PTR-MS. Using this approach, the impact of velo- and oropharyngeal performances on aroma transfer to the nose during tasting of wine was followed. A characteristic release profile obtained from PTR-MS analysis of ethyl acetate during wine tasting is shown in Fig. 6. It became evident that during a small sip of wine (intake), the velum was opened for a very short period of time as observed through videofluoroscopy. In agreement with this, an initial pulse of ethyl acetate was detectable in the expired air from the nose. When the sip was taken and the lips were closed, also the velum formed a border with the basis of the tongue as described in Buettner et al. (2001). Consequently, ethyl acetate was no longer detected in the breath. However, when the wine taster was instructed to open the velum deliberately, by performing distinct pumping actions (for details see Buettner, 2004b), simultaneous ethyl acetate detection by PTR-MS analysis took place. Also, the impact of swallowing was monitored using this setup. The moment of swallowing, as observed via videofluoroscopy, was directly followed by the ethyl acetate

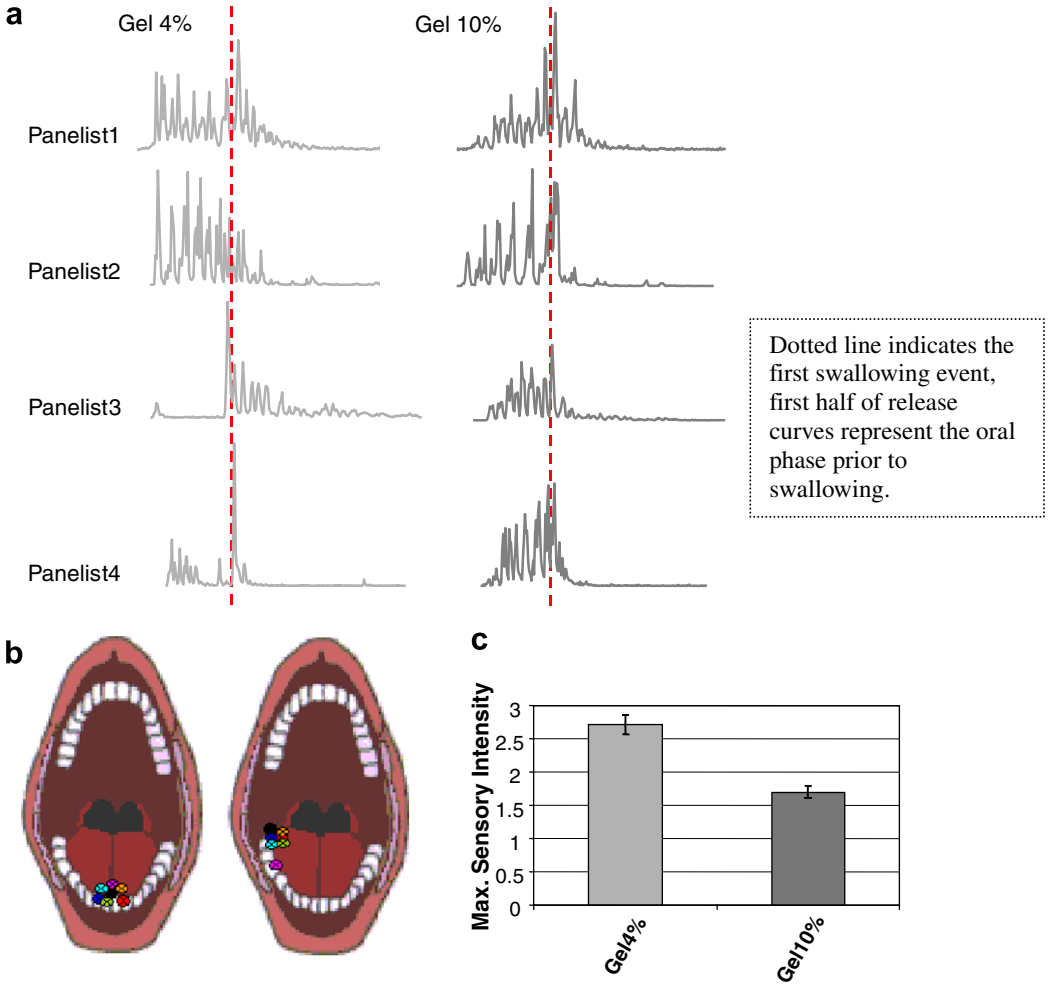

Fig. 7. Impact of gel texture on (a) ethyl butanoate release profiles during mastication, separately for individual panellists and (b) maximum retronasal aroma perception during consumption.

pulse right after swallowing during the so-called "swallow breath" (Buettner et al., 2001). When the wine was completely swallowed, subsequent velum pumping actions were observed to be accompanied by further VOC detection via PTR-MS analysis.

3.3.2. Solid foods

The same approach was used to elucidate the impact of chewing and swallowing of gel systems on retronasal aroma transfer. The consumption of gels with different protein content, and therefore different texture, was investigated. It was shown that for most panellists eating of the gels according to a defined eating protocol led to significantly different release profiles. During the oral phase, where no swallowing actions took place, a fast and intense onset of aroma transfer to the nose for the soft gel (4% protein content) was observed, while the mastication of the harder gel (10% protein content) led to a slow initial increase in intensity (cf. Fig. 7a, panellists 1 and 2 are shown as characteristic representatives). In both cases it was shown by means of videofluoroscopy that mastication actions with up and down movement of the jaw took place, which in turn led to intermittent opening of the velum-tongue border (Buettner et al., 2001). During those actions, volatiles were transferred to the nasal cavity. Panellists reported that "chewing" of the soft gel mainly took place in the frontal part of the oral cavity, close to the incisors, while the hard gel was chewed in the rear with the molars. Breakdown of the soft gel and spreading throughout the tongue and oral cavity occurred much faster (as reported by the panellists). The overall aroma intensity of the soft gel was rated as significantly higher than that of the harder gel (Fig. 7b). This effect on odor intensity might be due to sensorineural interactions between texture and odor, as indicated by recent studies (e.g., Bult, de Wijk, & Hummel, 2007; Hollowood, Linforth, & Taylor, 2002; Weel et al., 2002).

Nevertheless, the total amount of odorant released, as well as the maximum intensities obtained by PTR-MS analysis were more or less the same (Mestres et al., 2004, data not shown) while analysis of the data revealed the initial onset of VOC release to be much faster during the oral phase.

When investigating the PTR-MS release profiles, for the consumption of the soft gel, a second release pattern was observed for some panellists where almost no aroma

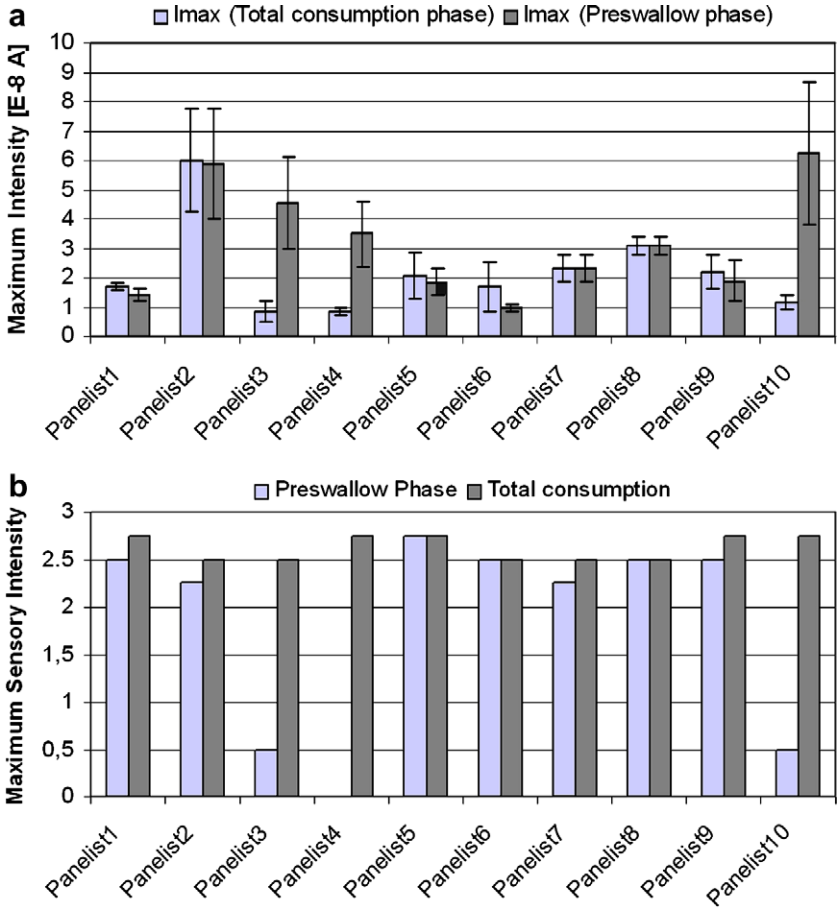

Fig. 8. (a) Analysis of pre-swallow phase (first 30 s of chewing prior to swallowing) and of total consumption phase PTR-MS release profiles from the consumption of soft gels (4% protein content); and (b) sensory evaluation of overall maximum sensory aroma intensity of the whole consumption sequence versus maximum sensory aroma intensity of the pre-swallow phase.

transfer occurred during the oral phase (cf. Fig. 7, panellists 3 and 4 are shown as characteristic representatives). On the other hand, the swallow breath peak obtained then was immediately relatively high. In Fig. 8a, the peaks with the highest maximum intensities (I_{max}) of the whole chewing sequence are shown in comparison to the maximum intensities reached during the pre-swallowing phase only (I_{max} peaks during chewing before swallowing). I_{max}'s for these panellists were low during the oral phase of consumption, but increased dramatically after swallowing which is mirrored by the high I_{max}'s for the overall consumption phase. This obviously limited or blocked transfer was observable for 3 out of 10 panellists (panellists 3, 4, and 10), and went along with a drastically reduced aroma perception during the pre-swallowing phase (Fig. 8b). The three panellists reported that they barely noticed any aroma while having the soft gel in the oral cavity, but perceived an intense aroma burst right after swallowing. This goes along with the high aroma intensity rating for the overall consumption sequence, and is substantiated by the PTR-MS data.

Visualization by means of videofluoroscopy of the oral and pharyngeal processes showed that these panellists pressed the material with the tongue against the hard palate in the frontal region of the oral cavity. This means, the gel structure was disrupted without real chewing actions. Accordingly, no jaw movement occurred and no velum opening took place.

4. Discussion

4.1. Coupling of in vivo olfactometer stimulation/PTR-MS analysis

4.1.1. Nasal and oral stimulation

The present results indicate that PTR-MS can be used not only to detect volatiles exhaled from the nose but also to monitor the temporal and spatial distribution of volatiles within the nasal cavity or their progress from the oral to the nasal cavity via the pharynx, respectively. To meet this demand, the PTR-MS technique was coupled with defined stimulation from an olfactometer, producing defined odor pulses at certain locations within the nasal, oral, and pharyngeal cavities. Using this approach, the intranasal spreading of the volatiles can be monitored directly. The presently observed profile from ipsilateral orthonasal stimulation exhibited a relatively sharp initial increase in concentration, followed by a quick drop to about half of the maximum concentration. Then, the shape changed and followed a slower decrease pattern, similar to that observed for the three other spreading patterns. This can be explained by the close proximity of the stimulation site to the recording site. It can be assumed that for the sharp initial peak those stimulus compounds are recorded which had not yet interacted with the nasal mucosa. On the other hand, the flatter profile should be due to those molecules which came already into contact with nasal mucosa, so that the shape of this part of the profile is flattened and broadened.

The slight differences in the panellists' profiles corroborates the idea that there are interindividual variations in stimulus spreading patterns. This is in agreement with reports on interindividual variations in the nasal anatomy such as differences in intranasal surface areas (Damm et al., 2002). To gain detailed insight into interindividual differences further investigations with a larger panel group and different stimuli are needed.

The direct comparison of the odor pulse at the olfactometer's outlet and the respective signals obtained from intranasal recordings indicated a huge discrepancy. As all experiments were performed under velopharyngeal closure, the only sources for odor "losses" have to be located within the nasal cavity. First of all, it has to be kept in mind that the administered odorants distribute throughout both nasal cavities. That means, detection at a certain location within the nasal cavity only represents a fraction of the original pulse. Nevertheless, one can assume that the stimulus adsorbs to the mucosal lining which was first described by Mozell as a chromatographic separation of odorants along the olfactory mucosa (Mozell, 1964). It has been reported that the spreading patterns along or within the mucosal tissue are influenced by the chemical structure of the odorant as it had been proposed more than 50 years ago (Adrian, 1950). Based on this observation, a "mass transport model of olfaction" has been proposed (Hahn, Scherer, & Mozell, 1994). It can be assumed that these processes differ, depending on the direction of airflow, meaning orthonasal or retronasal stimulus presentation. This would result in different activation patterns at the olfactory epithelium for the same odorant. This hypothesis could now be substantiated by further experiments using the approach proposed above.

For oral stimulation, the effect of exhaling during stimulus application on aroma transfer became evident. Obviously, the velum does not form any effective seal in this situation. Therefore, the aroma pulse during exhalation can be observed, while, without exhalation, no such transfer occurred via the pharynx to the nasal cavity. It is interesting to note that the profile obtained from oral stimulation very much resembled that from retronasal stimulation. This might indicate that, compared to the retronasal stimulation, during the passage from the oral cavity via the pharynx no significant changes occurred, and that the most drastic decrease of odor concentration would be due to adsorption within the nasal cavity. On the other hand, it has to be kept in mind that the initial transfer phase occurred under dynamic conditions, as an exhalation was needed to obtain any transfer at all. During retronasal stimulation, no breathing took place. While this represents a limitation for direct comparisons of the obtained data, the high similarity between the shapes from retronasal and oral stimulation is striking.

These results indicate that the temporal and spatial distribution of an odor can be monitored using the proposed experimental setup. It is also possible to investigate the

influence of the physicochemical properties of the stimulus on the spreading pattern.

4.2. Coupling of visualization of real masticatory processes/PTR-MS analysis/sensory analysis

4.2.1. Liquid foods

Apart from the passive stimulation with an olfactometer, the real food consumption with its dynamics adds a further dimension to the analytical task. First of all, the mechanical processes during chewing and swallowing need to be visualized as they can influence retronasal aroma transfer to a major extent (Buettner et al., 2001). This impact on odor transfer can be directly traced when coupling the real-time observation of velopharyngeal movements, e.g., by means of videofluoroscopy, or real-time magnetic resonance imaging with the on-line observation of the volatiles in the breath, e.g., by means of PTR-MS analysis. The recording of the volatiles can be either performed according to the conventional approach at the nostrils, but also directly at the olfactory epithelium using a cannula system to withdraw the volatiles directly from there. In agreement with previous findings on the impact of swallowing, sipping, and velum-pumping actions, the corresponding VOC signals were detectable by means of PTR-MS (Buettner, 2003; Burdach & Doty, 1987). After complete swallowing of the wine, further VOC signals were detectable during continued velum pumping actions. This finding corroborates the idea that traces of odorants can be present in the oral cavity for a long time after swallowing, so that they can be perceived even after food consumption and add to so-called "aftertaste" sensations.

Regarding the sensory impressions during the evaluation process it was found that the panellists always perceived retronasal aroma impressions at those times when the PTR-MS signals were also observed. This demonstrates that by cross-linking the mentioned techniques, the impact of certain physiological actions on retronasal aroma transfer and perception can be directly characterized.

4.2.2. Solid foods

By coupling the on-line visualization of the mastication process with breath-VOC monitoring via PTR-MS different factors were elucidated which influence retronasal aroma transfer. First of all, the differences in textural properties of the two investigated gel systems were found to be a main parameter. The faster and steeper increase of breath-VOCs observed for the soft gel was most likely due to the faster breakdown of the gel structure and, consequently the faster release of the volatiles.

Individual consumption patterns were found to play a major role for odor release. For example, for the chewing of the soft gels, the panellists could be divided in two groups: Chewers with fast and intense initial onset of VOC release and non-chewers, who pressed the material against the palate. In the latter case, the velum-tongue border remained efficient, thereby blocking the retronasal aroma transfer until the sample was swallowed. On the other hand, the swallow breath peak obtained was much higher for the "non-chewers" compared to the "chewers". Obviously, all volatiles released during oral treatment cumulated within the oral cavity prior to swallowing. Interestingly, this analytical finding was confirmed by the time-resolved sensory evaluation from the respective panellists who reported no or only minor aroma perception during the oral phase of soft gel consumption but reported intense aroma perception right after swallowing.

5. Conclusion

The present examples show that the cross-linking of the visualization of the processes in vivo by medico-analytical tools, together with sensory analysis and real-time analysis of the aroma chemicals provide conclusive evidence of the direct relationships between certain anatomical processes and individual aroma perception. Future studies may also include the assessment of electrophysiological recordings from the olfactory epithelium or recordings of event-related potentials (e.g., Heilmann & Hummel, 2004; Hummel et al., 2006). The joint analysis of the electrophysiological and psychophysical responses in combination with the analysis of the intranasal distribution of odors at a high temporal resolution will provide the basis for the comprehensive description of individual responses to certain stimuli.

Acknowledgements

This work was supported by the Hochschulwissenschaftsprogramm HWP II, the Deutsche Forschungsgemeinschaft (grant Bu1351/1), the Deutsche Forschungsanstalt für Lebensmittelchemie, and a grant from the Deutsche Forschungsgemeinschaft to TH (HU441/2).

References

Adrian, E. D. (1950). Sensory discrimination with some recent evidence from the olfactory organ. *British Medical Bulletin, 6*, 330–333.

Birch, G. G., & Munton, S. L. (1981). Use of the "SMURF" in taste analysis. *Chemical Senses, 6*, 45–52.

Buettner, A. (2003). Physiology and chemistry behind retronasal aroma perception during wine tasting. In A. Lonvaud-Funel, G. de Revel, & P. Darriet (Eds.), *Proceedings of the VIIth International Symposium d'Oenology* (pp. 566–569). Paris: Editions Tec & Doc.

Buettner, A. (2004a). Investigation of potent odorants and afterodor development in two chardonnay wines using the Buccal Odor Screening System (BOSS). *Journal of Agricultural and Food Chemistry, 52*, 2339–2346.

Buettner, A. (2004b). Spaß an Essen und Trinken – Retronasale Geruchswahrnehmung. *Nachrichten aus der Chemie, 52*, 540–543.

Buettner, A., Beer, A., Hannig, C., & Settles, M. (2001). Observation of the swallowing process by application of videofluoroscopy and real-time magnetic resonance imaging-consequences for retronasal aroma stimulation. *Chemical Senses, 26*, 1211–1219.

Buettner, A., & Schieberle, P. (2000). Changes in the concentrations of key fruit odorants induced by mastication. In: D. D. Roberts, & A. J. Taylor (Eds.), *Flavor release. ACS Symposium Series 763* (pp. 87–98).

Buettner, A., & Welle, F. (2004). Intra-oral detection of potent odorants using a modified stir-bar sorptive extraction system in combination with HRGC/O called Buccal Odor Screening System (BOSS). *Flavour and Fragrance Journal, 19*, 505–514.

Bult, J. H. F., de Wijk, R. A., & Hummel, T. (2007). Investigations on multimodal sensory integration: texture, taste, and ortho- and retronasal olfactory stimuli in concert. *Neuroscience Letters, 411*, 6–10.

Burdach, K. J., & Doty, R. L. (1987). The effects of mouth movements, swallowing, and spitting on retronasal odor perception. *Physiology & Behavior, 41*, 353–356.

Burdach, K. J., Kroeze, J. H., & Koster, E. P. (1984). Nasal, retronasal, and gustatory perception: an experimental comparison. *Perception & Psychophysics, 36*, 205–208.

Damm, M., Vent, J., Schmidt, M., Theissen, P., Eckel, H. E., Lotsch, J., et al. (2002). Intranasal volume and olfactory function. *Chemical Senses, 27*, 831–839.

Diaz, M. E. (2004). Comparison between orthonasal and retronasal flavour perception at different concentrations. *Flavour and Fragrance Journal, 19*, 499–504.

Frasnelli, J., van Ruth, S., Kriukova, I., & Hummel, T. (2005). Intranasal concentrations of orally administered flavors. *Chemical Senses, 30*, 575–582.

Friel, E. N., & Taylor, A. J. (2001). Effect of salivary components on volatile partitioning from solutions. *Journal of Agricultural and Food Chemistry, 49*, 3898–3905.

Hahn, I., Scherer, P. W., & Mozell, M. M. (1994). A mass transport model of olfaction. *Journal of Theoretical Biology, 167*, 115–128.

Hansel, A., Jordan, A., Warneke, C., Holzinger, R., & Lindinger, W. (1998). Improved detection limit of the proton-transfer reaction mass spectrometer: on-line monitoring of volatile organic compounds at mixing ratios of a few pptv. *Rapid Communication in Mass Spectrometry, 12*, 871–875.

Hansson, A., Giannouli, P., & van Ruth, S. (2003). 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. *Journal of Agricultural and Food Chemistry, 51*, 4732–4740.

Harrison, M. (1998). Effect of breathing and saliva flow on flavor release from liquid foods. *Journal of Agricultural and Food Chemistry, 46*, 2727–2735.

Heilmann, S., & Hummel, T. (2004). A new method for comparing orthonasal and retronasal olfaction. *Behavioral Neuroscience, 118*, 412–419.

Hollowood, T. A., Linforth, R. S. T., & Taylor, A. J. (2002). The effect of viscosity on the perception of flavour. *Chemical Senses, 27*, 583–591.

Hornung, D. E., Youngentob, S. L., & Mozell, M. M. (1987). Olfactory mucosa/air partitioning of odorants. *Brain Research, 413*, 147–154.

Hummel, T., Heilmann, S., Landis, B. N., Reden, J., Frasnelli, J., Small, D. M., et al. (2006). Perceptual differences between chemical stimuli presented through the ortho- or retronasal route. *Flavor and Fragrance Journal, 21*, 42–47.

Hussein, M. M., Kachikian, R., & Pidel, A. R. (1983). Analysis for flavor residuals in the mouth by gaschromatography. *Journal of Food Science, 48*, 1884–1885.

Janestad, H., Wendin, K., Ruhe, A., & Hall, G. (2000). Modelling of dynamic flavor properties with ordinary differential equations. *Food Quality and Preference, 11*, 323–329.

Keyhani, K., Scherer, P. W., & Mozell, M. M. (1997). A numerical model of nasal odorant transport for the analysis of human olfaction. *Journal of Theoretical Biology, 186*, 279–301.

Kobal, G. (1981). *Elektrophysiologische Untersuchungen des menschlichen Geruchssinns*. Stuttgart: Thieme Verlag.

Mestres, M., Moran, N., Jordan, A., & Buettner, A. (2004). Aroma release and retronasal perception during and after consumption of flavored whey protein gels with different textures. Part I: in vivo release analysis. *Journal of Agricultural and Food Chemistry, 53*, 403–409.

Mozell, M. M. (1964). Evidence for sorption as a mechanism of the olfactory analysis of vapours. *Nature, 203*, 1181–1182.

Small, D. M., Gerber, J. C., Mak, Y. E., & Hummel, T. (2005). Differential neural responses evoked by orthonasal versus retronasal odorant perception in humans. *Neuron, 47*, 593–605.

Taylor, A. J. (1996). Volatile flavor release from foods during eating. *Critical Reviews in Food Science and Nutrition*, 765–784.

Weel, K. G. C., Boelrijk, A. E. M., Alting, A. C., van Mil, P. J. J. M., Burger, J. J., Gruppen, H., et al. (2002). Flavor release and perception of flavored whey protein gels: perception is determined by texture rather than by release. *Journal of Agricultural and Food Chemistry, 50*, 5149–5155.