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Release and Perception of Ethyl Butanoate during and after Consumption of Whey Protein Gels: Relation between Textural and Physiological Parameters

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The influence of gel texture on parameters such as positioning of food material in the oral cavity during mastication, and salivation, and their influence on aroma release in vivo was studied. Retronasal perception was followed by means of time-resolved sensory evaluation, while volatile release patterns were observed by means of PTR-MS. A clear correlation was found between individual-specific consumption patterns and the respective sensory perception. Also, gel texture could be clearly correlated with respective physicochemical release patterns in vivo and to the corresponding retronasal aroma perception.

KEYWORDS: Time-intensity; chewing position; saliva; tooth; PTR-MS; mastication; TA; gel-texture

INTRODUCTION

A vast number of studies have dealt up to now with aroma release phenomena in vivo and their relation to sensory perception. Real food systems were investigated as well as model systems such as gels or dairy products, applying an array of different techniques such as atmospheric pressure chemical ionization (APCI-MS), proton-transfer reaction mass spectrometry (PTR-MS), exhaled odorant measurement (EXOM), and many more (*1*−*6*). However, the findings obtained often seem not fully consistent or even contradictory. Reasons for this might be, especially for in vivo analysis of aroma release, different sample consumption protocols for panelists. For example, some studies involved highly standardized guidelines for consumption but still obtained considerable variation in volatile release profiles (*3*, *7*, *8*). Completely free eating modes led to even higher variance between panelists which could not be fully explained on a physiological basis and required extensive panelist numbers with subsequent complex statistical data treatment (*9*). Concerning data analysis for real-time aroma release studies in vivo, recent studies furthermore showed that precise recording of events such as sample introduction or swallowing and incorporation of these parameters into final data analysis is a crucial factor for interpretation of the obtained release curves (*7*, *8*). Subject-specific consumption patterns were also observed in a recent study on model dairy desserts, where panelists could be, consistently with their chewing patterns, grouped according to the perceived aroma intensities (*3*).

In other gel studies, not only the influence of texture but also of artificial saliva addition and simulated mastication was followed. Techniques involved a model mouth system with on-line monitoring of volatile release by PTR-MS analysis, while static headspace analysis was used to determine partition coefficients of compounds depending on the gel textural properties (*1*, *10*). When observing the dynamic release from pectin gels differing in hardness in the model mouth system, reduced release of volatiles was reported when viscosity or hardness of the gels was increased (*1*). Also, it was found that of all parameters studied (mastication, saliva, gel hardness), mastication rate was the parameter with the largest influence on aroma release. Increase in mastication rate increased overall aroma release. This was attributed to the increase in surface area due to sample breakdown. Compared to a nonchewing condition, the maximum aroma intensity was much higher in the chewing condition and was reached later and aroma decline was much slower. On the other hand, a decrease of aroma release during mastication was found in the presence of saliva. In this context, it is interesting to note that an opposite effect was observed when the influence of artificial saliva addition on dynamic aroma release from different gels (pectin, gelatin, starch) was studied (*10*). Here, high aroma liberation was observed for the comparatively rigid gelatin gel, while the other softer gels remained unaffected. Generally, the effects of gel type were much more pronounced in this study as completely different gel systems were investigated than in the pectin study.

The topic of the first part of this investigation (*7*) on model gel systems was a detailed in vivo release analysis in real time with special focus on the impact of the chewing period, termed oral or preswallow phase, the swallowing event itself, and the subsequent release after the gel bolus has been swallowed. Therefore, detailed data analysis has been developed showing

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Table 1. Rheological Properties of Gels with Different Protein Concentrations at Two Different Deformations

protein content (%)	R_{max} (mN)	reversible deformation (mm)	reversible deformation (%)
		50% compression	
4	39	2.70	54
6	120	2.95	59
7	149	3.10	62
8	195	3.23	65
10	281	3.50	70
		80% compression	
4	84	0.30	4
6	560	2.50	31
7	834	3.30	41
8	1000	3.45	44
10	1429	3.50	47

that averaging of overall release profiles for different panelists and calculation of the "classical" parameters I_{max}, T_{max}, and area under the curve (AUC) was unsatisfactory. The present study will now focus on physiological parameters in relation to gel texture, such as salivary flow and chewing patterns, and their possible relevance for the respective odorant release patterns observed in vivo. Thereby, not only factors depending on sample differences, such as texture, but also those leading to variations between panelists were studied.

MATERIALS AND METHODS

Chemicals. Ethyl butanoate was obtained from Aldrich (Steinheim, Germany). The odorant was freshly distilled prior to analysis. Chemical and sensory purity was checked by gas chromatography−olfactometry (GC/O) as well as gas chromatography−mass spectrometry (GC−MS).

Whey protein isolate (Bipro, JE 153-9-420) was obtained from Davisco Foods International, Inc., Le Sueur, MN, and glucono-δ-lactone (GDL) from Aldrich (Steinheim, Germany).

Preparation of Gels. Gels with 4%, 6%, 7%, 8%, and 10% whey protein in distilled water were prepared and flavored with ethyl butanoate exactly according to the procedure described in (*2*). Gelling was allowed for 15 h at room temperature in open-ended syringes with an inner diameter of 9 mm for texture analysis (TA) and of 18 mm for sensory tests. To avoid contamination or aroma losses during the gelling process, the ends of the syringes were protected with Parafilm. Ethyl butanoate content in the samples was checked by means of stable isotope dilution assays according to (*11*). Gels were kept at 4 °C between sessions and stored at this temperature for a maximum period of 48 h. Prior to analysis, they were pulled out of the syringe by means of the piston and freshly cut into cylinder-shaped samples of 0.65 mL for TA-testing and 2 mL for determination of chewing position, for determination of salivation and also for sensory evaluation and PTR-MS analyses.

Texture Analysis. TA was performed by means of a texture analyzer (Stable Microsystems, U.K.) with the following parameters: temperature 22°C; test-speed for compression 0.5 and 2 mm/s for the upstroke the higher speed giving a better impression of the elastic (solid) character. The reversible deformation during upstroke was monitored by the distance until the relaxation stress of the sample was zero again and was expressed as % of the total compression (Table 1). The maximum force (R_{max} (mN)) as a function of the deformation monitored the hardness of the cylindric sample of 10 mm height compressed by 50% and 80% of its original height. To get an idea of the force the tip of the tongue exerts on a piece of gel freshly introduced to the mouth, a cylindric hard piece of gel was fixed onto the hook of the SMS/Kieffer-Rig, placed just behind the incisors and touched by the tongue as was the case during the sensory evaluations.

Panelists. Seven panelists (two male, five female, age 22−40, nonsmokers) were recruited from the Technical University of Munich. They exhibited no known illnesses at the time of examination and normal olfactory and gustatory function. In regular weekly training sessions, panelists were tested for their sensory performance with selected suprathreshold aroma solutions prior to participation in the experiments, while subjective aroma perception was tested with a defined set of aroma substances and an internally developed "flavour language" (*12*). The panelists had a normal salivary flow, tested in model chewing experiments as described in (*7*). Intraoral analyses were performed 2 h after breakfast and thorough cleaning of the teeth and oral cavity with a commercial toothpaste (5 min).

Determination of Chewing Position. Gels with 4%, 6%, 7%, 8%, and 10% protein concentration, respectively, were singly presented to the panelists in random order for uninstructed consumption. After chewing and swallowing, panelists were asked about the main chewing positions for each gel. They had not only to tell the preferred side of chewing (if any), but also to locate the area of chewing (incisors, eyeteeth, premolars, molars). Experiments were performed three times for each panelist and gel on one day and were repeated on two following days.

After this, panelists were asked to chew the softest and the hardest gel, thereby deliberately changing their preferred chewing positions to the opposite. Panelists had to rate the degree of convenience/familiarity of this changed chewing pattern on a seven-point scale from 0 (unfamiliar/inconvenient) to 3 (highly familiar/convenient). Furthermore, they evaluated the overall aroma perception of gel samples on a seven-point scale (steps of 0.5 for rating) from 0.0 (not perceivable) to 3.0 (very intense) while chewing normally and also when chewing with the changed pattern.

Determination of Salivation. Weighted gel samples (3 replicates each) with 4% and 10% protein concentration with and without aromatization with ethyl butanoate, respectively, were singly presented to seven panelists in random order with a 30 min break between samples. After each sample evaluation, panelists rinsed their mouths with water. Panelists were asked to chew each sample singly for 1 min according to their normal chewing behavior and to avoid swallowing during the chewing procedure. After 1 min, panelists spat out the total amount of sample and saliva present in their oral cavities and the spit-off samples were weighed again. The degree of salivation was calculated from the weight difference compared to the sample before chewing.

Sensory Evaluation and PTR-MS Analysis. Sensory analyses were performed in a sensory panel room at 21 ± 1 °C at three different sessions. The samples with 4 and 10% protein content, respectively, were taken into the oral cavity and chewed for 30 s with closed lips and without swallowing. Then, panelists were instructed to swallow the entire bolus and, after that, to continue chewing for 60 s. The different gels were presented in triplicate to the panelists. The order of the gels was randomized with a 15 min break between samples, and after each evaluation, the panelists rinsed the oral cavity with tap water. No information about the purpose of the experiment or the exact composition of the samples was given to the panelists.

Panelists were *not* specifically trained to produce TI curves but should indicate during the whole chewing procedure the moments of intense aroma perception by raising their thumbs. End of subjective aroma perception should be indicated by raising the whole hand.

In parallels to sensory evaluation, nosespace air was sampled with two glass tubes fitted into the nostrils (*7*). 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.

During the whole gel chewing sequence, as described above, the nosespace volatile concentration was measured simultaneously by using real-time PTR-MS. By resting the nostrils at the glass tubes, the tidal breath flow from the nostril was directly sampled without disturbance of the normal breathing or gel consumption pattern. PTR-MS data was always recorded together with the sensory evaluation by the panelists and their respective manual aroma indications as described above. Panelists were not allowed to look at any time at the data recording system and had no visual, acoustical, or other indication on when odor signals were detected by the MS system.

The PTR-MS technique has been extensively discussed in a series of review papers (*13*−*15*). Briefly, it combines a soft, sensitive, and

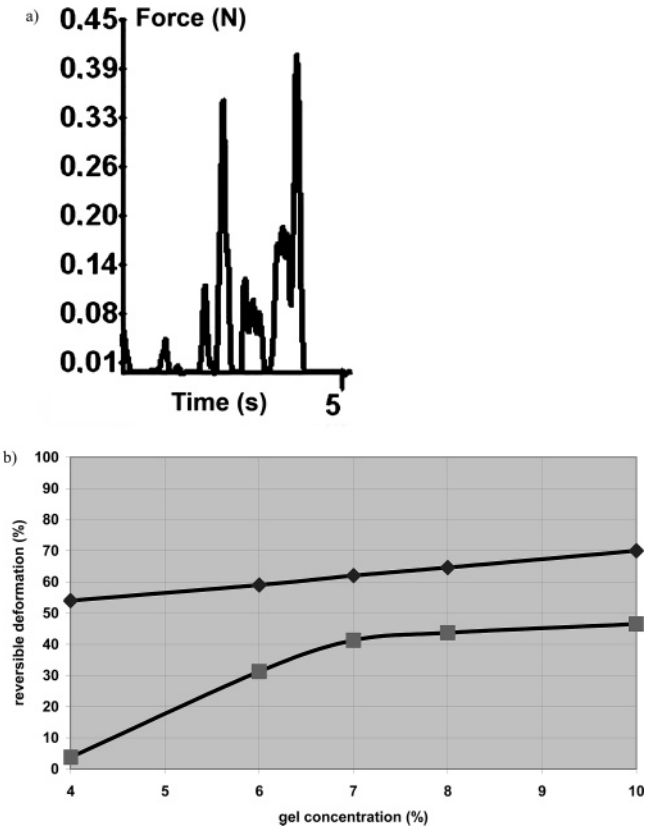

Figure 1. (**a**) Force the tip of a tongue exerts on a hard gel during the first sensory evaluations. (**b**) Reversible deformation of gels during upstroke as a function of the gel–protein concentration. Diamonds = 50% of compression, squares = 80%. Each determination was performed in duplicate; maximum relative deviation ±4%.

efficient mode of chemical ionization (CI), adapted to the analysis of trace 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 that have proton affinities larger than water (proton affinity of H_2O: 166.5 kcal/mol) are ionized by proton transfer from H_3O^+, and the protonated VOCs are mass analyzed. 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 panelists' breathing patterns), and ethyl butanoate were analyzed in the selected ion mode (masses 49, 69, and 117, respectively).

RESULTS

Gel Texture Determination. Cylindric gels of standardized size and weight were analyzed by compression with a texture analyzer to monitor the influence of gel texture on chewing. The maximum peak force after compression was defined as hardness of the gel. The upstroke-speed was set to a higher value of 2 mm/s to imitate the rapid withdrawal of the teeth or of the tongue after its first more tentative approach. To find conditions similar to which the gels were subjected within the mouth, the intensity of compression was varied. With 50% of compression forces between 39 and 281 mN were monitored with similarly high reversible deformations for all of the gel-concentrations. With 80% of compression, 4% and 6% gels were largely broken as shown by the poor reversible deformation (**Figure 1**). The more concentrated gels showed good recovery. Comparing the maximum peak force values and the respective reversible deformabilities of Table 1 to the force the tip of the tongue can exert on the gels (**Figure 1**), it is obvious that the 4% and 6% gels will be perceived as being more fluid or papescent. On the basis of the good correlation between the hardness or the reversible deformability of gels and the chewing position the compression to 80% was considered to be the best method for the characterization of the gels.

Influence of Gel Texture on Chewing Position. Unin-structed chewing of the gels with protein contents of 4, 6, 7, 8, and 10% protein concentration, respectively, and interrogation of the panelists of their preferred chewing positions for each gel revealed a very clear local differentiation between the gels according to the gel textures. All panelists reported unanimously that intra-oral positioning for the softest gel (4%) was always in the frontal part of the oral cavity, in the area of the incisors and eyeteeth. This gel had a predominantly fluid character at small forces (**Table 1** and **Figure 1b** lower curve) with the reversible deformability being only 4%. Most panelists (five) reported that slight chewing occurred involving the incisors, while two panelists reported that they just pressed the gel against the hard palate by using their tongue.

In contrast to this, all panelists reported that the hardest gel (10%) was always treated by chewing actions of the molars in the back part of the oral cavities and that the bolus breakdown products were always kept in the cheeks until further disruption and/or prior to swallowing. For the gels with medium gel hardness (6, 7, and 8% protein content), panelists reported a transitional state.

These results led to the idea of manipulating the chewing position of the panelists deliberately by asking them to change their behavior. That means, they were told to "chew" the soft gel in the back part, and to treat the hardest gel with their frontal teeth such as incisors and/or eyeteeth. All panelists reported that this change in chewing position did not display any normal or natural chewing behavior to them. In both cases (soft gel in the back part, hard gel in the front), the artificially changed chewing behavior was rated on a seven-point scale from 0 (highly unfamiliar/inconvenient) to 3 (highly familiar/convenient) as very unfamiliar and inconvenient (score between 0 and 0.5 for all panelists).

Also, a sensory evaluation of the overall aroma intensity of the gel samples with changed chewing patterns was performed during the mastication phase (15 s after bolus intake). In that way, and on a seven point scale from 0 to 3, all the panelists found that when the soft gels were placed in the "wrong" position compared to the normal one, the fruity aroma impression decreased, at least, by 0.5 points. To be exact, the intensity mean value was 2.0 for the normal chewing and 1.5 for chewing in back position. On the other hand, the rating of the hard gel remained almost constant as showed the intensity mean value of 1.3 for the normal chewing versus 1.2 for modified chewing position. In all cases, the relative standard deviation (rsd) between the different panelists evaluation was less than 12%.

Influence of Gel Texture on Salivation during Mastication. Based on the above observations, salivation during chewing of the two most extreme gels, the 4 and the 10% gel, was studied in the following:

As discussed above, the chewing location differed considerably depending on the hardness of the gel applied. According to the distribution of the major salivary glands predominantly in the rear part of the oral cavity (*16*), and since chewing was generally more intense for the harder gels, it was assumed that natural chewing of the hard gel in this area might stimulate saliva production more than chewing in the frontal part.

To proove this, salivation of 4% and 10% gels, during a time interval of 1 min of chewing (without swallowing and without

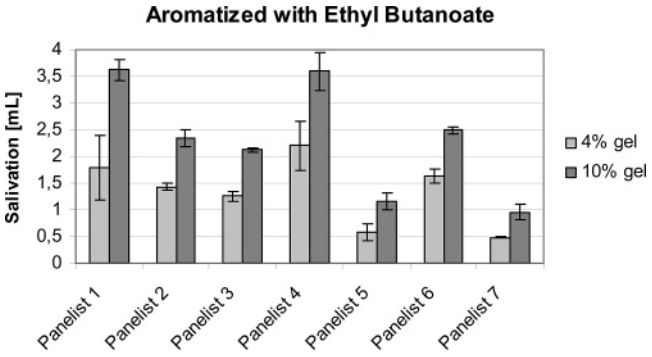

Figure 2. Determination of salivation during chewing of soft and hard gels (4 and 10% protein content, respectively), with aromatization, for seven different panelists. Data for each panelist are the mean of three replicates; standard deviations are given as error bars.

instructions concerning chewing positions), was determined by spitting out the bolus together with the produced saliva. To observe the influence of texture exclusively, the gels were also administered without aroma addition to the panelists.

It was found that the saliva production was indeed considerably increased by a factor of about 1.8 on average for all panelists, while the increase factor for the single panelists varied between 1.5 and 2.0 (cf. **Figure 2**). Apart from this, considerable inter-individual variation in saliva production was observed, not only between the soft and the hard gels, but also within one gel system. On the other hand, the coefficients of variance found for each single panelist for different replicates of one sample were generally low (in most cases less than 10%).

When applying a statistical t-test (α 0.05) to compare the saliva production during chewing of aromatized gels with that of nonaromatized gels, it was found that the values obtained were comparable for each panelist (data not shown). This means that there was no significant aromatization effect on salivation. The variations found were within the error of the single determinations (see coefficients of variance for single panelists data).

Influence of Gel Texture on Volatile Release in Vivo: Sensory Perception and Real-Time PTR-MS Analysis. In part one of this investigation (*7*), a sensory evaluation of the overall aroma intensity of the soft and the hard gels was performed.

In the present study, the temporal resolution of retronasal aroma perception should be elucidated in more detail. This goal was also based on the above-mentioned observations from gel texture analysis and of the chewing experiments. For this reason, again the two extremes, the 4 and the 10% gels, were selected for investigation. Therefore, one of the key questions was whether panelists were able to separate between single aroma release events as they are, for example, observed for some distinct chewing actions or the swallowing event itself (*5*), or whether their aroma perception followed a kind of "time-intensity-pattern" as described in the literature numerous times. To obtain the panelists' response as unbiased as possible, they were not instructed in any type of temporal rating according to the time−intensity methodology, but the only instruction for them was to raise their thumbs for aroma perception, indicating a rough estimation of the perceived intensity by the height at a certain time (differentiation between medium- and high-intensity only).

Generally, high concurrence between aroma detection via PTR-MS and sensory aroma detection was observed for all panelists.

Five representative breath profiles for the consumption of the soft gel are displayed in **Figure 3a**−**e**. Panelists were highly effective in indicating not only most of the single peak events with precise timing but also by rating those of highest intensity by raising their thumbs accordingly. The same was observed for the consumption of the hard gels.

Also, some panelists indicated aroma perception after swallowing even when no more distinct signal was obtained by PTR-MS.

Based on this observation, the correct peak detection via hand indication versus the total number of PTR signals, as obtained during the chewing and swallowing period (including the exhalation breath following swallowing = "swallow breath, cf. (*5*)), was analyzed. Therefore, all PTR peaks exceeding 10% of the maximum intensity of the respective chewing sequence were counted and compared with the total number of sensorically correct identified peaks by means of hand identification. The data obtained for both the soft as well as the hard gel for all seven panelists are displayed in **Figure 4**. It can be seen that for both gels, panelists were able to separately perceive at least 50% of the recorded PTR peaks as single peak events and that in some cases correct identification even went up to 80% values. On average, panelists performed about 65% correct identification for both gel systems. There was no significant preference for one gel system with regard to correct sensory peak identification, indicating that performance was independent of the respective gel hardness. Only panelist 4 seemed to perform significantly better for the hard gel, while the opposite was true for panelist 2.

Nosespace analysis during consumption of gels showed temporal release differences with an high initial onset of aroma release during chewing of the soft gel, while the aroma release of the hard gel slowly increased with the maximum being reached about at the time of swallowing. Specific data on this observation is not presented here as this finding is fully consistent with data published in part 1 of this investigation (*7*).

Apart from these textural differences, high interpersonal variability was observed, predominantly for the chewing of the soft gel (e.g., P1,2,3 and P4,5 in (*7*), **Figure 3**). Generally, two different modes of release could be distinguished for the soft gel. It was found that five panelists exhibited a release profile with high initial aroma liberation during chewing, and a subsequent decline, sometimes with a second "I_{max}" at the time when the gel is swallowed (three characteristic examples in **Figure 3a**−**c**). However, two panelists only showed some initial peaks, followed by some tiny peaks or even an aroma-transfer-free time interval during the oral phase (**Figure 3d**,**e**). Then, with the first exhalation right after swallowing, a major aroma pulse occurred.

Accordingly, these panelists reported only some initial aroma perception followed by no or only minor perception during "chewing" of the soft gel prior to swallowing (**Figure 3d**,**e**).

DISCUSSION

Influence of Gel Texture on Chewing Position. The chewing experiment revealed a very clear sensory separation of chewing locations by the panelists, predominantly for the soft and the hard gel, while the medium soft gel required a transitional position. Due to the unanimity of the result, it can be assumed that the observed "normal" oral positioning of the gels depending on their texture indeed represents the main chewing behavior. This behavior agrees with the results obtained by TA: gels with higher protein contents needed higher pressure to be broken. Accordingly, the "abnormal" chewing is refused. Previous studies have shown that the tactile rating by tongue,

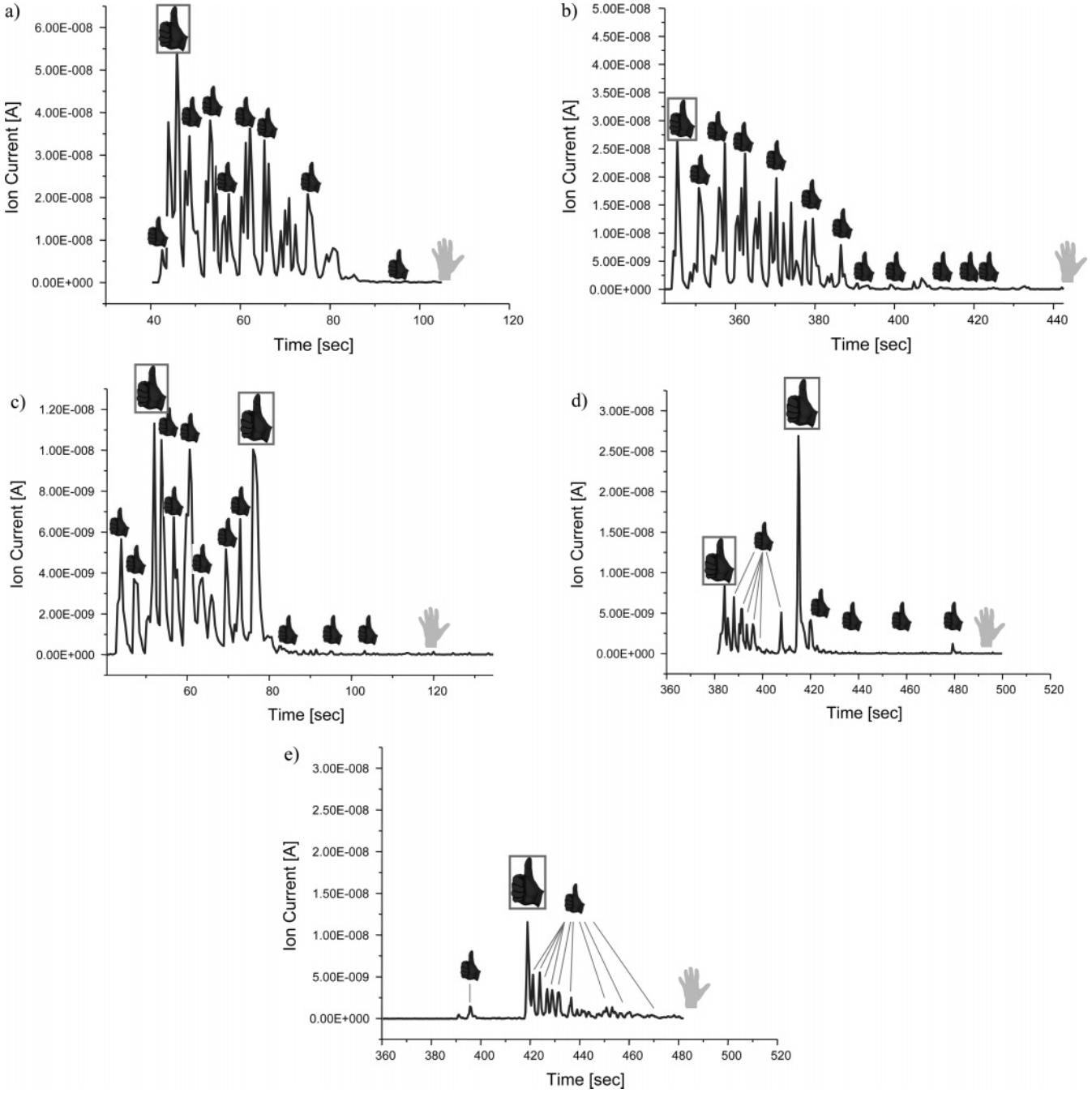

Figure 3. (**a**–**e**) Selected ion trace (*m*/*z* 117) from PTR nosespace analysis during the consumption of soft gel (4% protein content) for five different panelists. The symbol with the thumb up indicates medium (small symbol) and high (large symbol) aroma intensity, the raised hand indicates the end of sensory perception.

palate, and teeth is extremely sensitive, even to structural features of particles in the micrometer range (*17*). Therefore, it can be assumed that panelists were indeed able to clearly locate the predominant chewing locations.

For "abnormal" chewing, the reasons for the lower aroma intensity rating of the soft gel were not investigated. It might be due to "abnormal" salivation (dilution effect) or an "abnormal" closure of the velum (no retronasal perception) as result of the changed chewing pattern. As the soft gel turns relatively fast into a semiliquid state, the potential risk of gel material flowing into the pharynx is high for the back position so that the velum might be closed more readily than when consuming the soft gel according to the natural habit. This problem would not occur for the chewing of the hard gel in the frontal position so that no velum effect would occur there. On the other hand, the hard gel still requires intense chewing, also in the frontal position, so that intense up and down movement of the jaw induces intermittent opening of the velum−tongue barrier similar to the chewing actions in the back part. Based on these considerations, a velum effect would not be expected for the hard gel, which would be in agreement with the observed sensory rating. Also, the rating of the fruity impression for the hard gel is already relatively low so that a further decrease might not preponderate to the same extent as for the soft gel.

Influence of Gel Texture on Salivation during Mastication. Variations in salivary flow rates, as well as in saliva composition between humans are well-known phenomena (*16*).

The high differences observed in this study for the chewing of the soft and the hard gel are noteworthy, as despite the reported differences in hardness, both gels still have to be

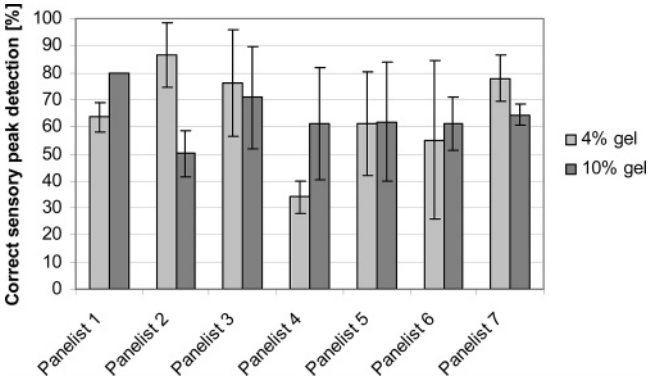

Figure 4. Correct sensory identification of PTR signals (≥10% of respective maximum intensity) during chewing and swallowing by means of hand sign indication, values are displayed as % correct hand indications related to total peak number. Data are the means of three replicates for each panelist and each gel.

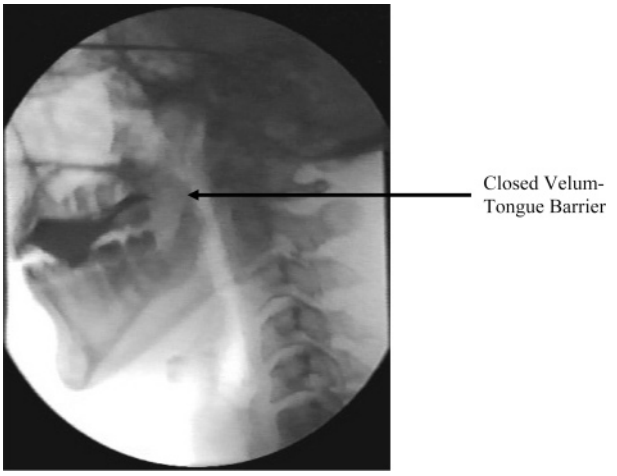

Figure 5. Observation of the oral and pharyngeal segments of panelist 4 by videofluoroscopy during mastication (preswallowing phase) of the soft gel (4% protein content).

regarded as very similar since both exhibit gel structure and have very similar water content. On the other hand, there was no distinct aromatization effect on salivation. This observation would be in agreement with other findings, where presentation of pleasant odors did not change resting and stimulated parotid salivary flow in humans (*18*). In another study, chewing was reported as a much stronger stimulus for salivation than aroma (*19*). According to this, it could be assumed that mastication just covers the impact of odor on salivation rate.

The possible influence of these differences on aroma liberation from the gels cannot be answered from these observations. Therefore, additional model studies on static and dynamic release patterns under natural salivation conditions need to be performed.

Influence of Gel Texture on Volatile Release in Vivo: Sensory Perception and Real-Time PTR-MS Analysis. In part 1 of this investigation (*7*), a sensory evaluation of the overall aroma intensity of the soft and the hard gels was performed. Generally, the soft gel was rated as much more aroma intense than the hard one despite the fact that the total released amount of odorant during the whole time course of eating did not differ. However, it was found that the shapes of the release profiles of the soft and the hard gel were significantly different with a very high initial liberation from the soft gel, while the hard one exhibited a slowly increasing release profile. According to these findings, the hypothesis of the relevance of the "first impression" on intensity rating has been proposed.

In the present study, the temporal resolution of retronasal aroma perception was elucidated in more detail. High concurrence between aroma detection via PTR-MS and sensory aroma detection was observed for all panelists.

Also, some panelists indicated aroma perception after swallowing even when no more distinct signal was obtained by PTR-MS. The reason could be that for these panelists the detection threshold of ethyl butanoate was below the detection limit of the PTR-MS system so that the panelists still perceived traces of the compound which were not visible any more. However, a comparison of sensory and analytical detection threshold has not been performed in this study.

In agreement with our previous study (*7*), nosespace analysis during consumption of gels showed temporal release differences with an high initial onset of aroma release during chewing of the soft gel, while the aroma release of the hard gel slowly increased with the maximum being reached about at the time of swallowing. Apart from this, two different modes of release for the soft gel were observed. From physiological studies on chewing patterns, the answer to this observation can be drawn as follows: Observation of the soft gel chewing process by means of videofluoroscopy showed that most panelists performed chewing actions where the connection between soft palate and tongue intermittently opened, thereby allowing an aroma transfer from the oral to the nasal cavity (online-observation of exhaled volatiles from the nose via PTR-MS, unpublished data). However, some panelists performed no real "chewing" but more precisely shearing actions with mainly back and forth or sideways movements of the jaw rather than an opening of the jaw and the teeths. For these panelists, the soft gel was mainly pressed with the tongue in the frontal part of the oral cavity against the hard palate. No opening of the velum−tongue border occurred, it was tightly kept closed (cf. **Figure 5**), allowing no or only minor transfer of volatiles from the oral to the nasal cavity. This goes along with the observation that these panelists also reported only some initial aroma perception followed by no or only minor perception during "chewing" prior to swallowing. On the other hand, when these panelists were instructed in chewing with jaw opening, thereby opening also the velum−tongue border, a significant increase in aroma transfer was observed, even exceeding the amounts detected during the preswallow phase from the hard gel (**Figure 6a−c**).

These results are in agreement with the findings of Hansson et al., where the influence of mastication rate and salivation on in vivo aroma release from different gel systems was investigated by PTR-MS (*4*). For strong gels, significant aroma release was observed when the gels were chewed, while the "chewing" of viscous solutions did not lead to exhalation of odorants from the nose, indicating that no aroma transfer from mouth to nose was possible. The explanation is that for viscous solutions the velum−tongue border was (unconsciously) kept closed during movement of the jaw to prevent choking. For rigid gels, this is not necessary, as the breakdown bits are no self-flowing system and need special swallowing actions to be transferred from the oral cavity to the pharynx. Therefore, opening of the velum during chewing of the hard gel occurs, together with aroma transfer to the nose. In the present study, the softest gel exhibits already more compactness than a viscous solution, so that both types of behavior for the panelists are found. Some panelists were still treating the soft gel similar to a viscous solution, while others already changed their chewing pattern according to that of a more rigid system. It is assumed that from the physiological

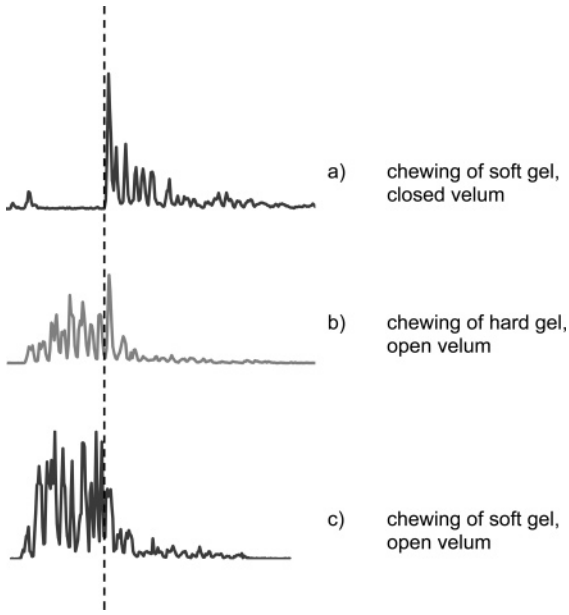

Figure 6. Selected ion trace (*m*/*z* 117) from PTR nosespace analysis of the consumption of (**a**) soft gel (4% protein content) with closed velum, (**b**) hard gel (10% protein content), and (**c**) soft gel (4% protein content) with open velum, representative data of one panelist (P4). The dashed line indicates the swallowing event.

point of view, the softest gel used in this study displays, in terms of mouthfeel and oral breakdown, the connective link between viscous and gel system. This is especially true when looking at the physical changes of the gel during oral treatment. When expectorating the soft gel after the 30 s time course of chewing, indeed a completely viscous solution was obtained with no more solid structures. However, when expectorating the solid gel after mastication, many small solid bits were found in a viscous salivary solution.

In conclusion, the high correlation between sensory and analytical aroma detection was surprising as such precise sensory location of aroma peaks during consumption has not been reported before. On the contrary, some authors previously reported some lag between the sensory perception of maximum intensity and the highest in-nose concentration with the sensory perception exhibiting a delay in reaching maximum intensity (*20*, *21*). This was observed mainly for very short eating intervals (*21*) and was explained by the fact that "intensity estimation is part of an integration process which may take up to 10 s" (*20*). Nevertheless, in the present study, there was surprising consistence in the timing and the estimation of intensity between sensory perception and in-nose odorant detection. However, it has to be stated that the eating intervals in our study were considerably longer, so that direct comparison might be not possible.

Apart from this, it has commonly been taken for granted that retronasal perception follows a curvelike release profile. At least in the present case, where no tastants are present, this seems not to be true. Interestingly, in another study the same experimental setup with sensory evaluation according to a time intensity approach resulted in a sensory rating according to a "classical" curvelike style (*2*). The same effect was observed in our group when panelists familiar to time−intensity rating perfomed the sensory evaluation. It can be speculated that the drawn sensory curves were just a result of panelists' training on time−intensity curves. On the other hand, tastants are often included in studies on model or food systems and their sensory rating, with the aim to be as close as possible to real foods. Recent studies indicate that our retronasal aroma intensity rating seems to be highly influenced by the presence of congruent or incongruent tastants (*22*−*24*). As a consequence, any shape of sensory time−intensity curve might be falsified in the presence of tastants and might only resemble the time course of tastant perception. This would, e.g., agree with the findings of Davidson et al. where retronasal aroma perception during chewing of chewing gum was reported to decrease in high correlation with the intraoral decrease of sweet tastants concentration during chewing, while the actual aroma concentration declined much slower (*24*). In conclusion, the coupling of on-line breath analysis, sensory evaluation, and physiological considerations specifically for single panelists showed that retronasal aroma perception exhibited very high temporal resolution which is not fully mirrored by application of for example "classical" time−intensity methodology. Furthermore, this sensory resolution was directly related to aroma transfer as observed by breath analysis.

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