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# Aroma Release and Retronasal Perception during and after Consumption of Flavored Whey Protein Gels with Different Textures. 1. in Vivo Release Analysis

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The influence of gel texture on retronasal aroma release during mastication was followed by means of real-time proton-transfer reaction mass spectrometry and compared to sensory perception of overall aroma intensity. A clear correlation was found between individual-specific consumption patterns and the respective physicochemical release patterns in vivo. A modified data analysis approach was used to monitor the aroma changes during the mastication process. It was found that the temporal resolution of the release profile played an important role in adequate description of the release processes. On the basis of this observation, a hypothesis is presented for the observed differences in intensity rating.

**KEYWORDS: Time−intensity; proton-transfer reaction mass spectrometry; PTR-MS; mastication; aroma release**

## INTRODUCTION

Aroma release and perception during mastication and food consumption is influenced by physicochemical and also physiological parameters, which are on the verge of being understood. It is obvious that release patterns of odorants change significantly, depending on the food matrix composition, for example, fat content. A series of publications has, up to now, dealt with this physicochemical release aspect. For viscous or gel systems, it has been often found that aroma and/or taste sensory intensity decreases with increase in viscosity or gel hardness (*1*−*4*). From a physicochemical point of view, some investigations showed that the viscosity of solutions or gel hardness has no considerable effect on aroma release, whereas others have reported reduced release of volatiles, mainly under dynamic conditions, when viscosity or hardness of gels, respectively, was increased (*5*−*12*). The release of odorants was also not only reported to be influenced by gel composition but to be strongly dependent on the chemical structure of the volatile (*13*). On the other hand, it was found that solutions with similar viscosity but different thickener systems do not necessarily induce the same flavor perception, so that, apart from viscosity, other effects such as aroma-matrix binding or other types of interactions were discussed (*14*). As a recent example, Boland et al. investigated the physicochemical release of volatiles from pectin, gelatin, and starch gels, differing in rigidity. The gelatin gel was much more rigid than the other two gel systems. Under static conditions, significantly higher partition coefficients were found in pectin gel, whereas the gelatin and starch gels did not differ significantly from each other. Under dynamic conditions, the "maximum intensity" ($I_{max}$) values, the slopes of release curves and cumulative release values were significantly lower for the gelatin gel, whereas the other two gels did not significantly differ. Also, a number of the investigated compounds had the highest "time until $I_{max}$ is reached" ($t_{max}$) values in gelatin gel. This different behavior under dynamic conditions was assumed to be related to the significantly higher rigidity of the gelatin gel. However, also opposite reports can be found, such as salting-out effects upon thickener addition. Therefore, flavor release patterns as a function of texture modification are still difficult to predict. The complexity of the task becomes evident when the literature on models, theories, and predictors targeting at aroma release as function of structure and matrix composition is surveyed (*15*, *16*).

On the other hand, when food systems are subjected to oral treatment, it quickly becomes evident that several events take place, making prediction of aroma release in vivo a challenging task (*17*, *18*). From in vivo studies we know that, first of all, there is obviously a considerable range of consumption patterns among humans as a high variability of aroma release during chewing is observed, for example, during nose space analysis (*12*). This variance between panelists has often been reported. However, when aroma release in vivo has been observed, chewing and swallowing patterns have usually not been regarded

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in detail and/or not taken into account with regard to data analysis. To deal with this problem and to accomplish tendencies from the raw data, generally a wide panel with a broad number of replicates has been used. In other studies, even precisely defined eating protocols did not overcome this problem, so that complex statistical calculations are generally employed in data analysis (*11*, *19*). Despite these attempts to solve the problem, generally, the physiological reasons for the observed interindividual differences remained more or less unclear.

Things seem to become even more complicated when psychophysical interaction phenomena are studied. Mainly in the past decades, interaction phenomena between different sensory modalities are increasingly discussed, leading either to enhancement or reduction of aroma sensory impressions when "congruent" or "incongruent" sensations are perceived at the same time (*20*−*23*). This has been reported for the degree of pleasantness, congruency, and enhancement of, for example, fruity or savory aroma in the presence of sweet or salty taste, and vice versa, and also for sensations such as texture (*12*). It has been shown that the sensory perception of aromas changed due to changes in the concentration of matrix constituents (tastants), whereas the actual in-nose odorant concentration or the odorant application to the panelist remained the same (*19*, *24*). Even when tastants were reduced in only their sensory perceptability, for example, due to thickener addition above the point of random coil overlap ($c^*$), but not in their real sample concentration, the same effect occurred (*5*). In the case of texture, Weel et al. reported that the texture of gels, rather than the in-nose concentration, determines the perception of flavor intensity (*12*). Similarly, mouthfeel sensory signals were discussed in terms of perception interaction with taste and aroma (*1*). Taking into account psychophysical phenomena like these, prediction of aroma sensations in complex food systems seems to be close to impossible.

To reduce the complexity, most studies focused on food model systems, mainly gel systems, spiked with relatively simple aroma compositions, some utilizing precisely defined chewing protocols, whereas others preferred uninfluenced natural consumption (*4*, *12*, *19*). Model systems were varied in texture, type, and amount of gelling agent, addition of tastants, or variation in odorant composition. In agreement with the findings of Carr et al. (*10*) and Guinard and Marty (*9*), Baek et al. reported higher $I_{max}$ and lower $t_{max}$ values for softer gels than for harder ones, indicating a quicker liberation from the softer material (*4*). This seems to be contradicted by the recent studies of Weel et al. on nose space analysis using APCI-MS, by which no differences in physicochemical release characteristics $I_{max}$, $t_{max}$, and total odorant release were observed (*12*). However, also in that study, the sensory perceptions of the soft and hard gels differed significantly. In agreement with previous texture-related studies, as discussed above, the softer gels were described as significantly more aroma-intense. As a consequence, aroma intensity perception was discussed to be influenced by texture sensation rather than aroma release during eating. It has to be stated that for both studies different chewing protocols were used; Baek et al. used a completely free mode, whereas Weel et al. gave precisely defined instructions. Therefore, results and conclusions might be difficult to compare.

The present work, which is performed with gel model systems, also focuses on the texture aspect. To allow better comparison with previous findings, gel systems and aromatization as used by Weel et al. were studied (*12*). The aim is to find a connective link between pure physicochemical release patterns and perceived sensory impressions. Time-resolved analysis of the eating process should be performed with special emphasis on individual patterns of consumption, which means it depends on the typical behavioral modes of single panelist. If sensory differences between aromatized gels of different hardness would be observed, the reasons for these differences should be elucidated. Part 1 of the work mainly deals with the comparison of odorant release patterns in relation to the textural properties of the gels and the respective sensory perception, whereas the second part (*25*) will focus on the characterization of human physiological parameters and the influence of textural gel properties on mastication.

## 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 from Davisco Foods International Inc., Le Sueur, MN, and glucono-$\delta$-lactone (GDL) was from Aldrich.

**Preparation of Gels.** Gels with 4 and 10% protein concentrations, respectively, were prepared and flavored with ethyl butanoate according to the procedure described in ref *12*. Gels were freshly prepared, kept at 4 °C between sessions, and stored also at this temperature for a maximum period of 48 h.

**Panelists.** Seven panelists (two males, five females, ages 22−40 years, 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, whereas subjective aroma perception was tested with a defined set of aroma substances and an internally developed "flavor language" (*26*). The panelists had normal salivary flow, tested in model chewing experiments as described in ref *25*. Intraoral analyses were performed 2 h after breakfast and thorough cleaning of the teeth and oral cavity with a commercial toothpaste (5 min).

**Sensory Evaluation.** Assessors were trained in preceding weekly training sessions in recognizing orthonasally and retronasally ∼150 selected odorants at different odorant concentrations according to their odor qualities. Participation in these sessions was at least for one year prior to participation in the actual sensory experiments. Panelists were always asked to score odor intensities on a seven-point scale (steps of 0.5 for rating) from 0.0 (not perceivable) to 3.0 (very intense). Sensory analyses were performed in a sensory panel room at 21 ± 1 °C 2 h after breakfast at three different sessions (different days). Sessions for one panelist did not last longer than 1 h each.

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 was 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 (three samples of each type of gel). The order of the gels was randomized. No information about the purpose of the experiment or the exact composition of the samples was given to the panelists.

For comparative evaluation of the hard and 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. Panelists were *not* asked to produce "time intensity" curves but to score the overall fruity odor quality (maximum intensity) of the samples on a seven-point scale from 0.0 to 3.0.

**Breath Sampling.** Nose space air 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 proton-transfer reaction mass

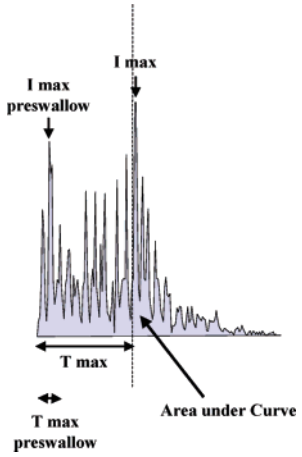


**Figure 1.** Parameters for the analysis of PTR-MS data.

spectrometer (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 nose space 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.** The PTR-MS technique has been extensively discussed in a series of review papers (*27*−*29*). Briefly, it combines a soft, sensitive, and efficient mode of chemical ionization (CI), adapted to the analysis of trace volatile organic compounds (VOCs), with a mass filter. In this study, 15 sccm of 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 that of 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%), which 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).

**PTR-MS Data Analysis.** General analysis of the raw PTR-MS data has been performed in a comparable way as it has been done before in nose space analysis. Parameters calculated involved the areas under the curves (AUC), the maximum intensity of the release profile ($I_{max}$), and the time necessary to reach the maximum intensity ($t_{max}$) (cf. **Figure 1**). However, in the present study and unlike in most previous studies, the mean of the single determinations was not calculated first, extracting the mentioned parameters therefrom, but the single raw data was analyzed for AUC, $I_{max}$, and $t_{max}$ and later on averaged, according to the needs of the analysis (mean values for single panelists, mean values for all panelists combined, etc.).

The same was done for the "preswallowing curves" by using only the raw data obtained during chewing of the gels until (and exclusively) the swallowing event itself. This phase can also be termed as the "oral" phase of consumption. The maximum intensity of this time interval represents "$I_{max}$ preswallow", and the time necessary to reach $I_{max}$ preswallow is termed "$t_{max}$ preswallow".

## RESULTS

**Influence of Gel Texture on Sensory Perception.** Sensory analysis has been performed as follows: the maximum overall retronasal aroma intensities of the softest and hardest gels (4 and 10% protein contents, respectively) were evaluated with chewing and swallowing according to the protocol given above. Intensity rating resulted in a significantly higher mean intensity for the soft gel (2.7, standard deviation of ±0.3) than for the hard one (1.7, standard deviation of ±0.3).

**Influence of Gel Texture on Volatile Release in Vivo: Real-Time PTR-MS.** *Analysis of Overall Release Profile.*

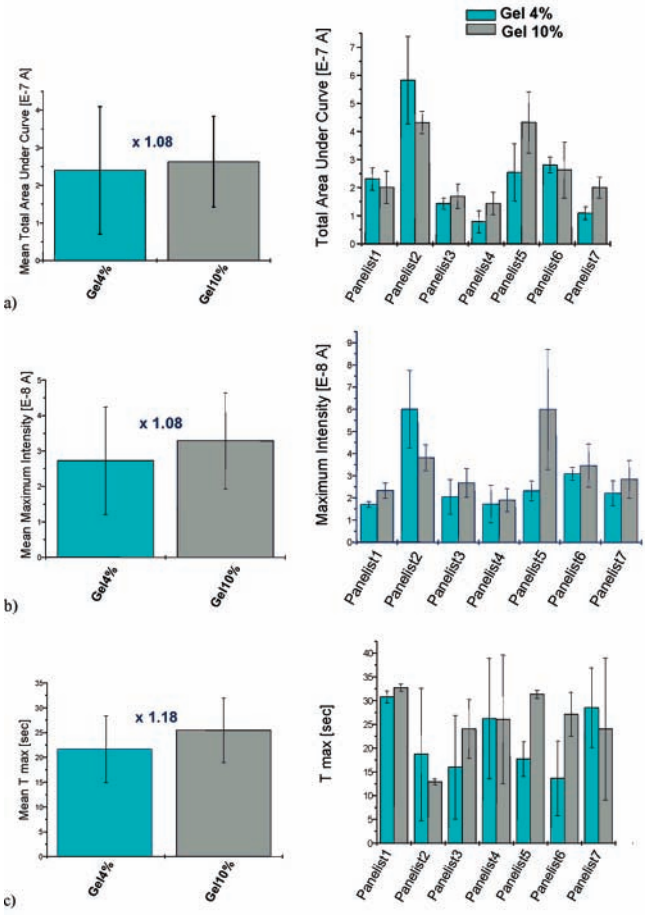


**Figure 2.** Analysis of total PTR-release profiles from consumption of soft and hard gels (4 and 10% protein contents, respectively): (**a**) area under curve; (**b**) maximum intensity; (**c**) time until maximum intensity is reached (left side, mean of seven panelists; right side, data of single panelists, average of three determinations; standard deviations are given as error bars).

During consumption of the soft and hard gels (4 and 10% protein contents, respectively) according to the instructions described above, ethyl butanoate exhaled from the nose was measured by means of PTR-MS (raw data). Each determination was performed three times for each gel and each panelist. The averaged data of all replicates are displayed in **Figure 2**, both for mean values obtained for all panelists (left side) and for the averaged values for each single panelist (right side). When the overall mean values (left side) for the total areas under the release curves (**Figure 2a**) and the maximum intensity ($I_{max}$; **Figure 2b**), as well as the time until $I_{max}$ is reached ($t_{max}$, **Figure 2c**), are considered, no significant difference between the release of ethyl butanoate from the soft and hard gels was found. The broad range of the error bars for standard deviation indicates high variability among the panelists. This is in agreement with the plots showing the single panelists' data (right side) with high interindividual variation and also high standard deviations for each single panelist. This is valid for all three parameters. It has to be stated that the standard deviation found for $t_{max}$ was (with the exception of panelist 1) particularly high. Single panelists' data did not allow a clear interpretation, whereas the mean data of all panelists would lead to the conclusion that no difference was observable for the release of ethyl butanoate from soft and hard gels, respectively.

*Raw Data.* The high variations observed led to a closer look at the raw data. Examples of characteristic release profiles for

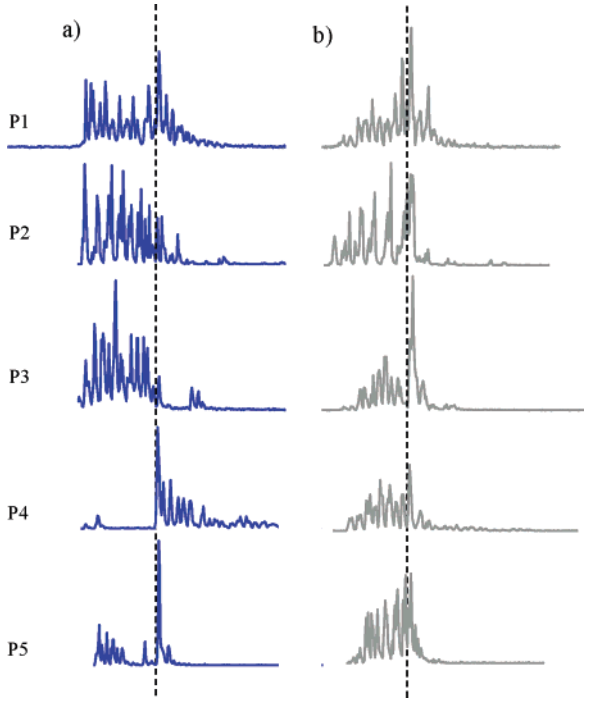


**Figure 3.** Selected ion trace (*m*/*z* 117) from PTR nose space analysis of the consumption of (**a**) soft gel (4% protein content) and (**b**) hard gel (10% protein content) for five different panelists (P1–5). The dashed line indicates the swallowing event.

five different panelists during consumption of the soft and hard gels, respectively, are given in panels **a** and **b** of **Figure 3**, with mainly two different patterns. Although most panelists exhibited release profiles as shown for panelists 1−3, for consumption of the soft gel there are also a few panelists who showed a different profile (panelists 4 and 5).

Related to the first pattern, it can be seen that higher amounts of ethyl butanoate are released from the soft gel right after introduction into the oral cavity. Obviously, the most important differences occur already during chewing, in the time period termed by us as "preswallow" or "oral" phase. Generally, a relatively high initial quantity of ethyl butanoate is quickly liberated from the soft gel, often followed by a subsequent slow decrease, whereas for the hard gel a reduced initial intensity, sometimes with delayed onset, and a steady increase can be observed with a considerable aroma pulse when it is swallowed. To be exact, generally for the soft gel there were two main events where $I_{max}$ values occurred: the first relatively soon after introduction of the sample into the oral cavity and some initial chewing actions in the first half of the chewing period and the second associated with the swallowing event at ∼35 s. For the hard gel, $I_{max}$ was usually found only after swallowing associated with the swallow breath at ∼35 s, but in some cases a higher intensity was already reached a bit prior to swallowing during chewing (cf. **Figure 3**, panelist 2). It has to be stated that the localization of $I_{max}$ either at the first or second position for the soft gel, or either right before or after swallowing for the hard gel, not only varied between panelists but could also be found within replicates of one panelist. That means that, for example, panelist 1 exhibited in two replicates the highest intensity right after introduction of the soft gel, whereas for the third sample, the highest intensity was found after swallowing. However, localization of the two $I_{max}$ values was generally very reproducible.

In agreement with this it has to be mentioned that the general release profiles were very reproducible in their shape, but did

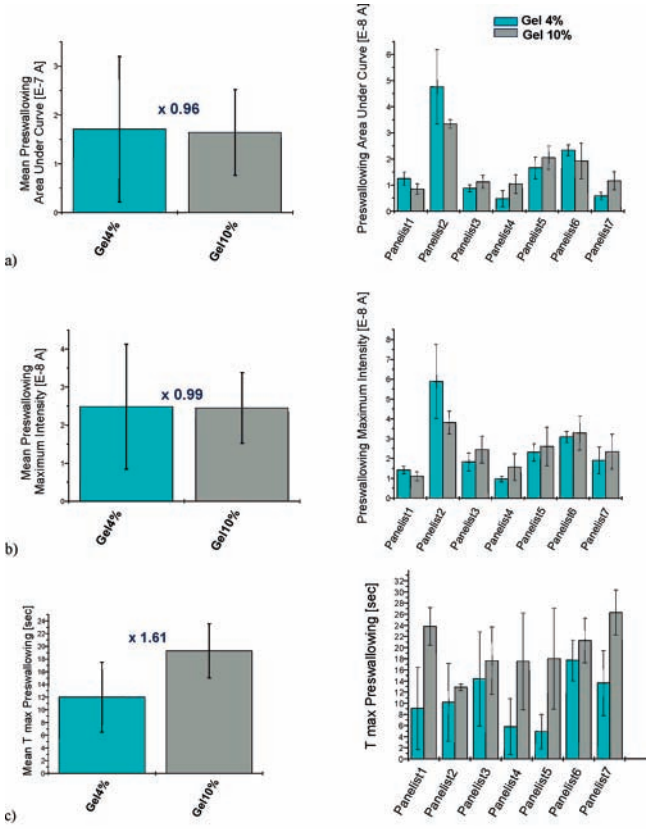


**Figure 4.** Analysis of preswallow phase PTR-release profiles from consumption of soft and hard gels (4 and 10% protein contents, respectively): (**a**) area under curve; (**b**) maximum intensity; (**c**) time until maximum intensity is reached (left side, mean of seven panelists; right side, data of single panelists, average of three determinations; standard deviations are given as error bars).

differ in overall amount of release, mainly between panelists. Therefore, the relatively high standard deviations in $I_{max}$ and AUC are related to the absolute concentration differences between panelists and replicates, not to differences in the shape of the release curves. The high standard deviations for $t_{max}$ can be related to the fact that $t_{max}$ values were located at considerably different positions and then averaged.

With regard to the second release pattern for consumption of the soft gel, only some minor aroma pulses occurred upon introduction of the soft gel into the oral cavity, followed by chewing periods with very little or often even aroma-transfer-free periods. Then, right at the moment of swallowing, associated with the "swallow breath", a major aroma pulse occurred. It has to be stated that two panelists showed this pattern. The release profile obtained for the hard gel resembled those of the other panelists. The existence of this second pattern leads to further increase in variation of $I_{max}$, $t_{max}$, and AUC when these values are averaged (**Figure 2**), mainly for the soft gel. Therefore, taking into account the observed differences between the releases for the soft and hard gels, a more detailed approach of analyzing the raw data was chosen. Obviously, the most striking differences between the soft and hard gels occurred in the chewing phase before swallowing, so data analysis was focused on the "preswallow phase", as described above.

*Analysis of Preswallow Phase.* Analysis of the AUC, $I_{max}$, and $t_{max}$ of the preswallowing phase was performed as mean values for the single panelists, as well as the average of all determinations for all panelists analogous to the analysis performed before on the overall release profiles (cf. **Figure 4**).

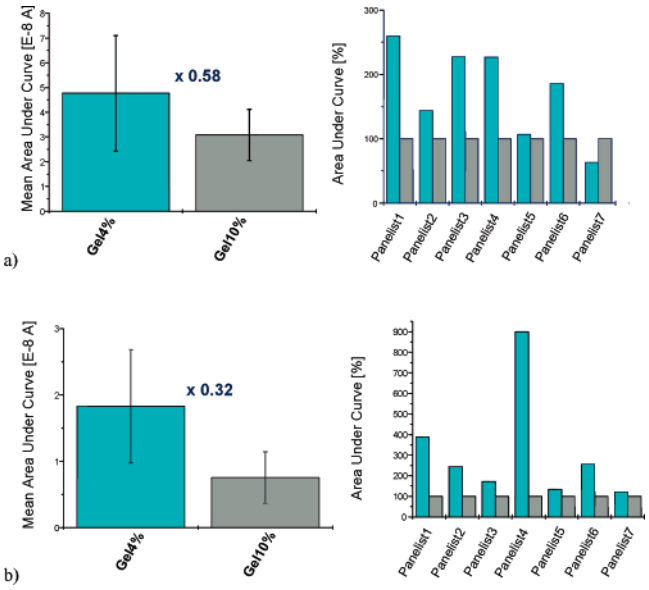


**Figure 5.** First impression of chewing: analysis of areas under curve of PTR-release profiles from consumption of soft and hard gels (4 and 10% protein contents, respectively) obtained for the first (**a**) 10 s and (**b**) 5 s of chewing, respectively (left side, mean of seven panelists; right side, data of single panelists, average of three determinations; standard deviations are given as error bars).

From a study of the combined data of the preswallowing phase for all panelists, again no differences were found between the soft and hard gels for AUC and $I_{max}$ (**Figure 4a**,**b**). Also, the single panelists' values showed again considerable variability. However, a significant difference was observed for $t_{max}$ (**Figure 4c**): in comparison to the cosumption of the hard gel, a considerably lower time interval was necessary to reach $I_{max}$ when the soft gel was consumed with ~7 s time difference. This observation was true not only for the mean value of all panelists but also for the single panelists' data, with all $I_{max}$ values of the hard gel being reached later than for the soft gel. The single time intervals varied from 3 to 15 s difference.

*First Impression.* According to the observation on the initial release differences from soft and hard gels (cf. Raw Data), the AUC of the first 10 s of the chewing sequence (right after introduction of the gels into the oral cavity) was calculated (**Figure 5a**). In agreement with what has been seen from the raw data, twice as much ethyl butanoate was detectable when the soft gel was consumed (averaged data). When the single panelists' data were studied, this trend was confirmed for all except one (panelist 7). This focused data analysis showed that indeed the initial aroma release is significantly increased for the soft gel in comparison to the hard gel. The effect becomes even more pronounced when only the first 5 seconds of the chewing sequence is studied, with a 3-fold higher aroma quantity being liberated from the soft gel (**Figure 5b**).

## DISCUSSION

The lower sensory rating of aroma intensity for the harder gel in comparison to the soft gel is consistent with previous findings (*4*, *12*). Baek et al. observed, under unstandardized eating conditions, that the maximum sensory intensity was reached much earlier for softer gels. When the results of the sensory time intensity rating given by Weel et al. are carefully studied, it can be seen that $I_{max}$ is reached for the hard gel a bit later than 30 s (right after swallowing, Figure 3b in ref *12*). Furthermore, for the soft gels, panelists recorded a much earlier $I_{max}$ at ~20 s and also a kind of second $I_{max}$, at about the time when swallowing occurred. These results would be fully consistent with our analytical observation, with faster release of the odorant during eating of the soft gel, and a second event, when the swallowing occurs. Nevertheless, these differences are not as evident when the statistical data analysis is studied (Table 2 in ref *12*). Here, only for two panelists was gel hardness significantly correlated to the respective sensory $t_{max}$. A more detailed discussion on the exact timing of the retronasal sensory perception will be presented in ref *25*.

The "classical" parameters $I_{max}$, $t_{max}$, and AUC of the overall nose space release profiles seem to completely agree with the findings of Weel et al. (*12*), implying that there are no physicochemical release differences, whereas sensory perception of the hard and soft gels is not at all the same. This would, at first sight, support the idea that texture rather than aroma release determines aroma sensory perception.

However, this is contradicted by the more focused analysis of the preswallowing phase, by which the most significant differences were found in the progress of the release profiles. Despite the fact that the total amount released was still the same under the given eating conditions, it became evident that the timing is completely different, with $I_{max}$ being reached much earlier (~7 s on average) in the preswallowing phase of the consumption of soft gels. Especially when the relatively short chewing times during "normal mastication" are borne in mind, this can be regarded as a considerably long time interval.

This deviating observation might be explained as follows: averaging of data from different panelists with different chewing patterns, as it was, up to now, a general approach, resulting in the three parameters $I_{max}$, $t_{max}$, and amount of total odorant release, can result in not only a loss in singular information but also misleading interpretation of the data. For example, according to Gaussian distributions, averaging can lead to curve-like shapes for the averaged data, whereas the real release pattern for single panelists might have consisted just of one or a few peak events. This effect can be easily observed when $t_{max}$ values of the consumption of the soft gel, which are induced by two completely different release processes (~12 s for the first $I_{max}$ and ~35 s for the swallowing event related one), are averaged. One ends up with a newly created $I_{max}$ between both original ones at ~23 s.

Therefore, in agreement with the physicochemical findings of Baek et al., determined by means of APCI-MS with other gel systems in dynamic headspace model experiments as well as for in-nose determinations, the absolute release from all gels did not differ significantly, but the rate of physicochemical release differed with a delay in release from the harder gels. Possible explanations for the delay in $t_{max}$ are a quicker liberation from the softer material due to a faster breakdown of the matrix during mastication and higher surface formation. These effects have been discussed before (*30*, *31*). Probably, different breakdown patterns go along with different consumption patterns.This topic will be discussed in a further investigation dealing with the physiological effects of texture modifications (*25*).

*Hypothesis of the "First Impression".* As discussed above, the observed differences in physicochemical release rate from gels with different hardness agree with previous findings for gelatin gels of different gel strengths (*4*). In these studies, the authors aimed at explaining the discrepancy between perceived intensity and actual odorant concentration using the Overbosch adaptation concept. On the basis of this theory, it was discussed that the sensory maximum would deviate from the time when

the highest volatile concentration is reached. The authors reported both an initial lag phase, in which the sensory $t_{max}$ occurred later than the release $t_{max}$, and an adaptive phase with the opposite effect. This is, in our study, not the case. Neither for the soft nor for the hard gels did we observe any deviation of the physicochemical from the sensory timing. On the contrary, panelists were highly effective in indicating not only single peak events with precise timing but also rating those of highest intensity with exact time determination (*25*). In conclusion, only the absolute and overall rating of intensity differed between gel samples according to texture and was not consistent with the absolute aroma concentration released.

Our explanation for this phenomenon is that in the case of the soft gel, there is an immediate and very high release of odorant which is, from a psychophysical point of view, "compared" with the status prior to consumption when no aroma was present. As a consequence, this sudden increase should be perceived as a kind of aroma flash. However, for the hard gel there is a relatively slow increase in intensity. We assume that each step in increase is rated compared to the status before and therefore not rated as drastically in change as for the sudden flash. One might compare this phenomenon to other types of sensory perception. For example, when cold hands are introduced into warm water, this temperature change is sensed as very intense and dramatic, and therefore the water temperature is rated as very high. On the other hand, when hands are slowly warmed to the same temperature, the difference is not as drastic and the final temperature is sensed as much less severe. This means the temperature intensity is rated lower for the steady increase. Another example might be the switching on of light in a completely dark room in comparison to a slow dimming procedure to the same brightness. The flash is sensed as much more intense so that we even might close the eyes to protect ourselves.

Both examples reported here involve some response of the sensing systems to the sensation events, which can be achieved more efficiently when the change in sensation is slower. For the aroma-sensing system there might be a similar process involved that adjusts itself to the presence of a stimulus. From our everyday experience in the laboratory, we observed a comparable phenomenon when, for example, a solution containing an odorant is sniffed in a room that is already to some extent "perfumed" with the same odorant. If the concentration in the solution is relatively high, the odorant is still sensed, whereas for a lower concentration there might be no more detection possible. However, this can be quickly achieved by simply leaving the room and changing the environment for the olfactory comparison to background. Only a few seconds of "uncontaminated" background sniffing of environmental air is needed to regain the ability of perceiving also the low odorant concentration. We assume that the aroma-sensing system is continuously performing a kind of autozero. If the environmental or in- and exhaled concentration of an odorant exhibits a certain concentration, the olfactory system is adjusted appropriately.

In conclusion of the above-said, we propose the hypothesis of the "first impression": the total amount of an aroma stimulus released is not the only key factor determining the intensity rating of the stimulus, but the release rate of the compound also has to be taken into consideration. As a consequence, it can be effective to incorporate such quick-releasing systems (e.g., as particles) into relatively slow-releasing matrices to increase the overall aroma intensities of these systems by inducing a high initial sensation. Unlike Weel et al., we propose this rating of the first impression to be one of the driving forces of aroma intensity rating in vivo, not the perception of texture. This hypothesis needs to be further substantiated by specific model experiments.

This study shows that a detailed knowledge of the physiological processes is the first and unavoidable premise when in vivo release studies during consumption are performed and interpreted. From our point of view and on the basis of the present study, solving the problem of highly variable panelist data by increasing the number of panelists and replicates of experiments with subsequent averaging is not the best option, but needs to be replaced by precise experiments taking into account different stages of the mastication process and detailed physiological studies. Some aspects of this topic related to the explanation of chewing differences between panelists and their relationship with their patterns of release will be discussed in ref *25*.

## ABBREVIATIONS USED

PTR-MS, proton-transfer reaction mass spectrometry; AUC, area under curve; $I_{max}$, maximum intensity; $t_{max}$, time until $I_{max}$ is reached.

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