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Flavor Release and Perception of Flavored Whey Protein Gels: Perception Is Determined by Texture Rather than by Release

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Five whey protein gels, with different gel hardnesses and waterholding capacities, were flavored with ethylbutyrate or diacetyl and evaluated by a 10-person panel to study the relation between the gel structure and the sensory perception, as well as the nosespace flavor concentration during eating. The sensory perception of the flavor compounds was measured by the time−intensity method, while simultaneously the nosespace flavor concentration was monitored by the MS−Nose. The nosespace flavor concentration was found to be independent of the gel hardness or waterholding capacity. However, significant changes in flavor intensity between the gels were perceived by the majority of the panelists, despite the fact that the panelists were instructed to focus only on flavor perception and to not take texture into account. From these observations it is concluded that the texture of gels determines perception of flavor intensity rather than the in-nose flavor concentration.

KEYWORDS: **MS−Nose; APCI; API; nosespace; time−intensity; flavor release; texture; whey protein gel; waterholding capacity; gel hardness**

INTRODUCTION

The influence of product structure and composition on the temporal aspects of flavor release has been the subject of many studies. The reason for this interest is that understanding of the principles governing flavor release and perception during eating might help to formulate food products with improved flavor characteristics. This is especially important for low-calorie products with, for instance, reduced levels of fat, sugar, or alcohol. The structure of a product will influence the transport of volatiles into the oral and nasal cavities, while the composition of a product will influence the interactions between flavor and non-flavor ingredients. Various authors have reviewed these flavor−matrix interactions (*1*−*4*).

Studies with liquid systems containing hydrocolloids (e.g., xanthan, hydroxypropylcellulose, sodium alginate, carboxymethylcellulose, and guar gum) have revealed that an increased viscosity due to an increase in hydrocolloid concentration results in a reduction of the flavor perception (*5*−*7*).

Protein and carbohydrate gels have been used to study the effect of matrix properties, such as gel hardness, waterholding capacity, and microstructure, on the perception of flavor. In general, the same trend was found for the gel systems as for liquid systems, i.e., an increase in gelling agent concentration causes a decrease in the sensory rating of the flavor perception (*8*−*10*).

In addition to these studies, which used sensory rating, the flavor perception of flavored protein and carbohydrate gels has been monitored by time−intensity (TI) methodology (*11*−*13*). It was shown that the maximum perceived flavor intensity (I_{max}) decreased with increasing gel hardness. An increase in gel hardness was achieved by an increase in gelling agent concentration. A similar TI study has been done with gels with equal gelling agent concentration (*14*). In this study gels were prepared with equal protein concentrations but with variation in salt type and ionic strength during preparation, which resulted in different rheological, microstructural, and waterholding properties. However, ionic strength and salt type might influence the interactions between flavor compounds and proteins. No relationship between gel hardness (fracture stress) and perceived intensity of flavor was found. However, the gel structure (i.e., stranded or particulate structure) seemed to have an effect. Gels with a particulate structure and low water holding capacity had a lower maximum perceived intensity than that of the gels with a stranded structure (*14*). Release of flavors was not measured in these studies.

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Several methods have been developed to measure flavor concentrations in real time in the nosespace of test-persons during eating (*15*−*17*). In the present study, we used the MS−Nose, developed by Taylor and co-workers (*17*). The system consists of a mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source, which is modified to allow analysis of the human breath. A small part of the test-person's breath is continuously sampled into the mass spectrometer. The system allows sensitive and fast monitoring of the in vivo flavor release. The release of various aroma compounds from gelatine gels has been measured with this instrument (*18, 19*). In both studies nosespace concentration measurements and time−intensity recordings were performed simultaneously. Baek et al. (*19*), who worked with furfuryl acetate, found a significant decrease in I_{max} and an increase in T_{max} (time to maximum intensity) for TI as the gelatine concentration increased. No correlation between I_{max} and the maximum nosespace concentration was found. However, the rate of volatile release seemed to correlate well with the sensory data (*19*). Linforth et al. (*18*) compared T_{max} values of nosespace concentration and sensory TI for menthol and dimethylpyrazine. When the maximum was reached fast, the sensory perception was found to lag behind the flavor release. When the maximum was reached after a longer period of time, the T_{max} of perception preceded the T_{max} of flavor release, due to a sensory adaptation effect (*18*).

Recently, we have developed a gel system based on whey protein, which allowed different rheological properties at equal protein concentrations (*20*). The rheological variation between the gels was obtained by variation in protein concentration during heating. In contrast to the gels of Gwartney (*14*), no salts or sugars, which could influence the flavor perception (taste−aroma interaction) were necessary for this purpose. Another advantage of the gelation system used in this study is that the used flavors are not exposed to heat, as gelation was performed at ambient temperature by mild acidification. Furthermore, the level of protein-related off-flavors is minimized by mild heating conditions.

The aim of the present study was to determine whether flavor release and perception would be affected by gel hardness and waterholding capacity and whether changes in flavor release correlate with changes in flavor perception. Diacetyl and ethylbutyrate were chosen as flavor compounds, because they represent a typical hydrophilic and hydrophobic compound, respectively. Release and perception of these flavor compounds from five different whey protein gels were studied by sensory TI and nosespace flavor concentration (NSC) using the MS−Nose.

MATERIALS AND METHODS

Materials. Whey protein isolate (Bipro, JE 153-9-420) was obtained from Davisco Foods International Inc., Le Sueur, MN. Specifications were as follows: pH 7.23; degree of methylation 94.75%; non-protein nitrogen 0.17%; ash 1.8%; lactose 0.34%; calcium 0.13%; total protein 93.39% (N × 6.38); α-lactalbumin 12.6%; bovine serum albumin 3.2%; immunoglobulin G 5.2%; *β*-lactoglobulin A 33.2% and *β*-lactoglobulin B 37.1%. Quest International (Naarden, The Netherlands) provided diacetyl and ethylbutyrate. Glucono-*δ*-lactone (GDL) was supplied by Sigma Chemical Co. (St. Louis, MO), and ethanol (>99.9%) was from J. T. Baker (Deventer, The Netherlands).

Preparation of the Gels. Whey protein isolate was solubilized in demineralized water at initial concentrations of 4%, 7.5%, and 11% (w/w) by gentle stirring for at least 2 h at ambient temperature. To obtain gels with different physical parameters (i.e., gel hardness and waterholding capacity) at equal protein concentrations, a two-step gelation procedure was followed (**Table 1**). First, solutions were heated at 68.5 °C in a waterbath for 3 h. During this heating step, the protein molecules formed aggregates of different sizes, depending on the initial protein concentration. After cooling to ambient temperature, parts of the protein solutions of 7.5% and 11% were diluted to final concentrations of 4% by addition of demineralized water. At this point, ethylbutyrate or diacetyl was added to all solutions to final concentrations of 150 ppm. Ethylbutyrate was diluted in ethanol before addition to the protein solution. This introduced 0.14% (w/w) ethanol into the ethylbutyrate gels. Diacetyl was diluted in water before addition to the protein solution. GDL was added to the solutions with final protein concentrations of 4, 7.5, and 11% (w/w), to a final concentration of 0.32, 0.52, and 0.86% (w/w), respectively. After addition of GDL the pH decreased slowly to a final value of 5.0, toward the pI of the protein (pH 5.1). During a period of 15 h at ambient temperature, the gels were formed. Subsequently, the gels were stored at 4 °C for a maximum period of 2 days.

Table 1. Physical Properties of the Whey Protein Gels[a]

gel no.	initial protein conc. (%)	final protein conc. (%)	hardness (kPa)	WHC (%)
1	4.0	4.0	13.4	71
2	7.5	4.0	41.6	74
3	11	4.0	35.0	76
4	7.5	7.5	114.8	84
5	11	11	211.5	86

[a] The initial and final protein concentrations during the preparation of the gels are also indicated.

To determine the sensitivity of the nose-space flavor concentration measurements, gels with increasing concentrations of diacetyl and ethylbutyrate were prepared, according to the procedure described above. The protein concentrations of the solutions used for these gels during heating and gelation were 11% and 4%, respectively. Diacetyl and ethylbutyrate were both present in each gel at concentrations of 100, 125, 150, 175, and 200 ppm.

Gel Hardness. Gel hardness was determined by a texture analyzer (type TA-XT2, Stable Micro Systems Ltd., Godalming, U.K.). Approximately 24 h after the addition of GDL, a grid was pressed into the gels. The device consisted of four blades (45 × 1.5 × 2 mm) of stainless steel arranged in a double cross. A force-time curve was obtained at a constant rate of 0.3 mm/sec for a 10-mm displacement. Gel hardness was expressed as the stress (Pa) at the maximum peak of the force−time curve (*21*, *22*).

Water Holding Capacity (WHC). A 40-g portion of gel was minced with a plunger in 60 crushing movements and put into a centrifuge tube. The gel was centrifuged for 30 min at 160*g*. After centrifugation the serum was removed and weighed. The WHC (%) of the gel was defined as the mass fraction of the retained water from the total amount of water present in the gel (*23*, *24*).

General Setup of Flavor Release Measurements and Time−Intensity Recordings. Ten panelists were familiarized to the aroma of diacetyl and ethylbutyrate and trained to produce TI curves, while their nosespace volatile concentration was measured simultaneously by the MS Nose, during five training sessions in which three samples were judged by each panelist. The panelists were instructed to chew regularly (independent of the gel hardness) for 30 s without swallowing, then to swallow the entire bolus, and, after that, to continue chewing for 60 s.

The different gels flavored with either diacetyl or ethylbutyrate were presented in triplicate to each panelist as cylinder-shaped samples of 2 mL (3 samples of each type of gel). The order of the samples was randomized, and to prevent sensory fatigue the samples were presented in five sessions of three gels. Before every session a non-flavored gel with medium hardness (gel no. 3) was presented as a blank, followed by a flavored gel with medium hardness (gel no. 3) as reference. TI recordings and NSC measurements were conducted simultaneously.

No information about the purpose of the experiment or the kind of presented sample was given to the panelists.

Nosespace Flavor Concentration Measurements. While panelists were eating the gels, the nosespace concentration of diacetyl or ethylbutyrate was monitored by sampling the airflow from one nostril over a 1.5-min period.

By resting one nostril at a plastic tip attached to a pipe, the tidal flow of air from the nostril was allowed to pass back and forth through this pipe. In this way, the normal breathing pattern was not disturbed. A small part of the breath in the pipe was sampled through a capillary tube (0.53 mm i.d., heated to 100 °C) positioned at a right angle with the pipe, within the flow of breath. Because only a small part of the breath was sampled, the measurement was independent of the nostril used.

The sampled part of the breath was introduced (75 mL/min) into the source of a MS−Nose atmospheric pressure chemical ionization gas phase analyzer (APCI-GPA) attached to a VG Quattro II mass spectrometer (Micromass UK Ltd., Manchester, U.K.).

The compounds were ionized by a 3.0 kV discharge. Source and probe temperatures were 80 °C.

Diacetyl and ethylbutyrate were analyzed in selected ion mode (0.2 s dwell on each ion), at cone voltages of 19 and 20 V, respectively. Acetone, always present in the human breath as a result of the fatty acid metabolism, was measured as an indicator of the panelists breathing pattern. Breath volatile concentrations were expressed as peak height in arbitrary units and divided by a factor 5×10^6.

Time−Intensity Recordings. TI curves were recorded by FIZZ software (Biosystemes, Couternon, France) over a 1.5-min period. Panelists were instructed to rate the perceived diacetyl or ethylbutyrate flavor on a scale from 0 to 10. The maximum intensity of the reference gel was agreed by the panelists to have a flavor intensity of 5 on the 0−10 scale. The panelists were instructed not to let textural effects influence their perception of the flavor intensity.

Gel Preparation for in Vitro Measurement of Flavor Release. Each gel was directly prepared in a 10-mL headspace vial (3 mL of gel per vial) and flavored with 5, 10, or 25 ppm of diacetyl and ethylbutyrate, according to the procedure described above.

Another series of gels was prepared according to the procedure, except for the protein concentration during gelation, which was 10 times diluted for each sample to avoid gel formation. The solutions were centrifuged (30 min, 9600*g*), and the resulting pellet was freeze-dried (72 h). The dry protein was suspended in demineralized water to the original concentrations and amounts of 3 mL were put in 10-mL headspace vials. Diacetyl and ethylbutyrate were added to final concentrations of 5, 10, and 25 ppm.

Headspace Gas Chromatography (HSGC). The headspace flavor concentrations were allowed to reach equilibrium during overnight storage at ambient temperature. The headspace flavor concentration was analyzed by gas chromatography. To this end 1000 μL of headspace was injected splitless on the column after 20 min incubation at 35 °C. A GC-8000top gas chromatograph (CE Instruments, Milan, Italy) was equipped with a CP-SIL 5 CB Low bleed column (30 m × 0.25 mm; film thickness 1.0 μm; Chrompack, Middelburg, The Netherlands) and a flame ionization detector (FID). The oven temperature was initially 40 °C for 5 min, then raised at 15 °C/min to 150 °C and was kept at 150 °C for 5 min. Inlet and detector temperatures were 250 and 225 °C, respectively. Gas flow rates were as follows: hydrogen, 35 mL/min; air, 350 mL/min; makeup nitrogen, 30 mL/min. The headspace concentrations were expressed as peak areas in arbitrary units.

Confocal Scanning Laser Microscopy (CSLM). Imaging was performed using a Leica confocal scanning laser microscope, type TCS-SP, configured with an inverted microscope, and an ArKr laser for single-photon excitation. The protein gels were stained by applying 2 μL of an aqueous solution of 0.05% (w/w) Rhodamine B to 200 μL of gel. The 568-nm laser line was used for excitation inducing a fluorescent emission of Rhodamine B, detected between 600 and 700 nm.

RESULTS AND DISCUSSION

Preparation and Physical Properties of the Gels. The hardness and WHC of the five different gels produced are shown in **Table 1**. Gels 1, 2, and 3 have the same final protein concentration (4%) and they can be compared without taking into account compositional effects. To obtain a set of gels with a wider range of physical parameters, two gels with a higher final protein concentration (gels 4 and 5, 7.5 and 11% protein, respectively) were included.

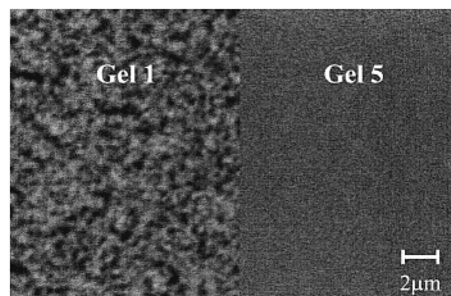

Figure 1. CSLM images of the structures of gels nos. 1 and 5 (**Table 1**).

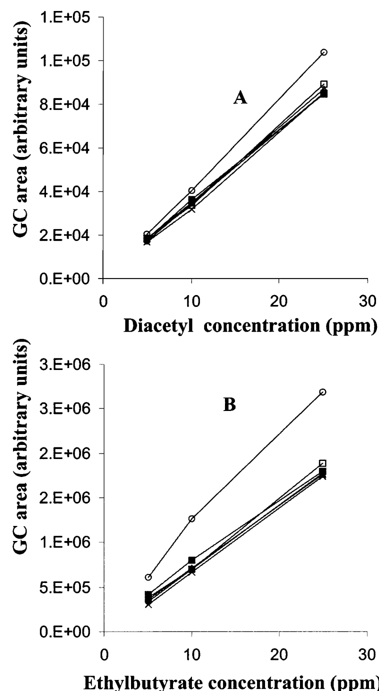

Figure 2. Equilibrium HS concentration of diacetyl (A) and ethylbutyrate (B) above whey protein gels 1 (●), 2 (□), 3 (△), 4 (×), and 5 (■) (**Table 1**). The open circles (○) represent water samples.

In addition to measurement of the WHC and gel hardness, the microstructure of the five different gels was determined by CSLM. **Figure 1** shows the microstructures (length scale in μm) of gels 1 and 5. It is clearly seen that gel 1 has a coarser structure than gel 5, which is in good agreement with the results of the hardness and WHC measurements (**Table 1**). A gel with a more open structure will, in general, be softer than a gel with a more compact structure (*25*), and a relatively weak gel with an open structure will have a lower WHC (*26*).

The molecular interactions of diacetyl and ethylbutyrate with whey protein gels were studied by HSGC−FID measurements. The equilibrium headspace concentrations of diacetyl and ethylbutyrate above the gels were on average 15% and 40%, respectively, lower than those above water (**Figure 2**). However, there were no differences observed in equilibrium headspace flavor concentrations between the gels. If the molecular binding of diacetyl or ethylbutyrate with whey protein was important, one would expect a lower static headspace concentration for gels 4 and 5 compared to those of gels 1, 2, and 3. This is not

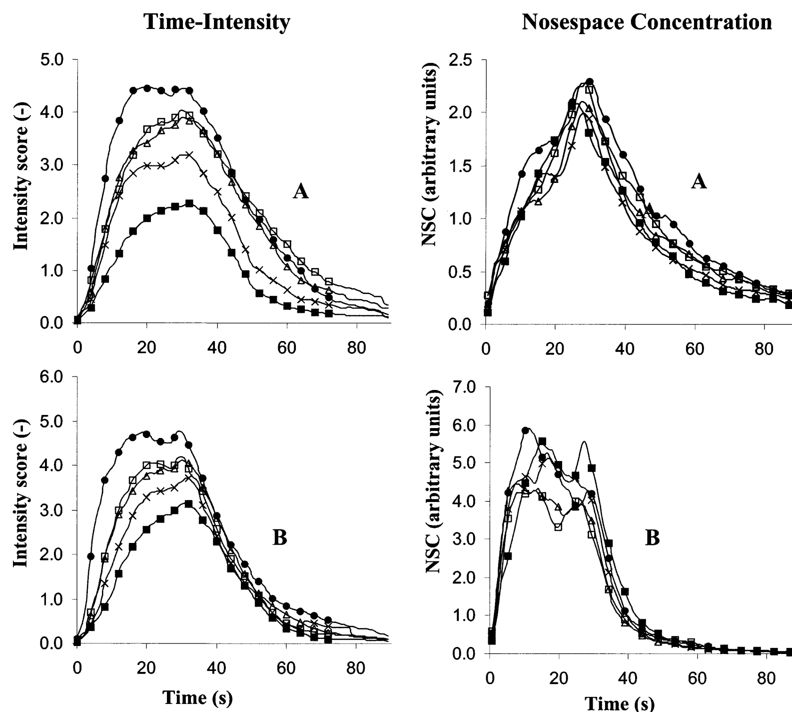

Figure 3. Averaged time–intensity recordings and relative averaged release profiles for gels 1 (●), 2 (□), 3 (△), 4 (×), and 5 (■) (**Table 1**), flavored with diacetyl (A) and ethylbutyrate (B).

observed in **Figure 2**. In addition to this, flavor binding was studied with nongelled samples with protein concentrations and applied heating conditions similar to those of the gelled samples. On rehydration the protein formed an insoluble dispersion. HSGC−FID analysis of the headspace of these samples did not reveal any differences in flavor binding for the different solutions (data not shown). Therefore, the differences in flavor release and perception of the studied gels can be directly explained by physical gel parameters, without the need to take final protein concentration and flavor−protein interactions into account.

Effect of Gel Properties on Flavor Release and Perception. **Figure 3** shows the averaged time−intensity profiles and the corresponding averaged nosespace concentration curves of the five gels, eaten by 10 panelists in triplicate for diacetyl (A) and ethylbutyrate (B). Each curve in both figures represents the average of thirty single curves.

It can be seen in **Figure 3** that the perceived intensity of diacetyl and ethylbutyrate generally decreases with an increase in gel hardness (going from gel 1 to 5), averaged over the 10-person panel. However, no clear differences between the gels were found in the averaged nosespace flavor concentrations.

The results for both flavors are summarized in **Figure 4**. Averaging across the whole panel of I_{max} values of the TI curves and the nosespace concentration profiles shows a decrease of TI−I_{max} (maximum intensity of time intensity), while NSC−I_{max} (maximum intensity of nosespace flavor concentration) remains constant.

An explanation for the difference in release and perception might be that the current setup of the nosespace concentration measurements, using 10 panelists who assessed each gel in three replicates, was not sensitive enough to show subtle differences, which can be made visible by TI. Such an effect in nosespace concentration might be hidden because of a large person-to-person variation. To determine the sensitivity of the nosespace concentration method, physically identical gels (gel no. 3) with increasing concentrations of diacetyl and ethylbutyrate were

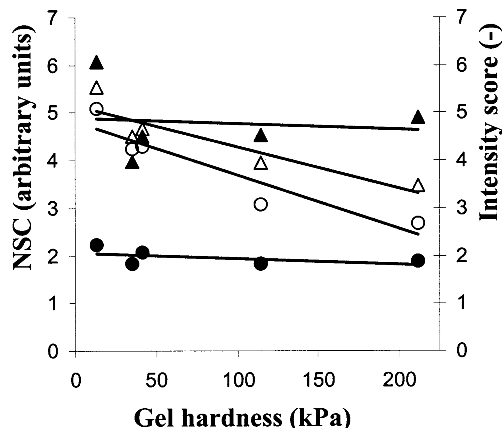

Figure 4. Relation between gel hardness and overall averaged values of I_{max} of NSC (closed symbols ● and ▲) and time intensity (open symbols ○ and △) for ethylbutyrate (triangles ▲ and △) and diacetyl (circles ● and ○). The lines reflect a linear regression estimate of the effect.

eaten by the panel. A higher concentration of the flavor compounds resulted in a higher averaged nosespace concentration curve (results not shown). The regression coefficients of the linear regression between flavor concentration and I_{max} of all individual curves were 0.99 and 0.97 for diacetyl and ethylbutyrate, respectively. This experiment clearly shows that a linear relationship exists between flavor concentration and NSC−I_{max} and that the method used to measure nosespace concentration is sufficiently sensitive to detect differences in flavor release. Therefore, the fact that no differences in nosespace concentrations are found between the five structurally different gels cannot be explained by a lack of sensitivity. The person-to-person variation does not hide the nosespace flavor concentration effect.

Clear differences in flavor release were found, but interestingly, the panelists indicated afterward that differences in flavor perception between the gels with different concentrations of diacetyl and ethylbutyrate were small. So, in this experiment

Table 2. Number of Panelists with a Significant ($p \leq 0.05$) Slope for the Linear Regression of WHC and Gel Hardness against I_{max} and T_{max} Values of the NSC and TI Signals for Gels Flavored with Diacetyl and Ethylbutyrate

predictor	dependent	NSC–I_{max}	NSC–T_{max}	TI–I_{max}	TI–T_{max}
diacetyl	WHC	2	0	6	1
	gel hardness	0	0	6	2
ethylbutyrate	WHC	2	2	6	4
	gel hardness	1	3	6	2

an increase in gel flavor concentration caused an increase in the nosespace concentration, due to flavor release but does not seem to increase the flavor perception. The opposite was the case in the study with gels with increasing gel hardness: the flavor release did not change, while the flavor perception decreased (**Figure 4**).

Several other authors who studied various gel systems have previously reported a decrease in flavor perception with increasing gel hardness (*9*−*13*). However, in these studies the actual flavor release was not measured by in vivo nosespace measurements. On the basis of the results of our study we would suggest that the release of flavor from gels might not decrease with firmer gels, but that the flavor perception by panelists could be influenced by the textural properties of the gels in a psychophysical way. Psychophysics is the study of the relationship of what exists in the real world (stimulus or stimuli) and the human experience of these events (*27*). The influence of texture on flavor perception could be explained by the integration of the signals from various senses that reach the brain simultaneously during eating of a gel.

Statistical Analysis of Curve Parameters. In addition to graphical analysis of the data by averaging the NSC and TI curves for all panelists, statistical analysis has been applied. WHC and gel hardness are correlated (**Table 1**). Because of this, WHC and gel hardness have not been used in multivariate statistical analysis of the data, but strictly as univariate predictors. To facilitate the statistical analysis, each individual TI and nosespace concentration curve was summarized by a T_{max} and an I_{max} value. ANOVA with gel hardness and panelist as predictors, and T_{max} and I_{max} of both TI and nosespace concentration curves as dependent variables, showed that the factor "panelist" was highly significant ($p < 10^{-8}$). This means that the average values of the TI and NSC are different for each panelist. The Levene's test for heterogeneity in variance was significant ($p < 0.001$), indicating that variance also differs between panelists. Hence, the effect of gel hardness and WHC on the nosespace concentration and TI data was not analyzed across the whole panel. These data have been separately analyzed for each panelist. Linear regression has been applied to the dependent variables TI−I_{max}, TI−T_{max} (time of maximum intensity of Time−Intensity), NSC−I_{max} and NSC−T_{max} (time of maximum intensity of nosespace concentration), against gel hardness and WHC as predictors for each panelist separately, for both ethylbutyrate and diacetyl. **Table 2** summarizes for each combination of predictor and dependent variable the number of panelists showing a significant slope at a significance level of 0.05.

TI−I_{max} is the only dependent variable for which a considerable number (six out of ten) of significant slopes was found, for both ethylbutyrate and diacetyl. For NSC−I_{max} and T_{max}, and TI−T_{max}, only a few significant slopes were observed. This confirms the conclusions drawn from the graphical presentation in **Figure 4**, namely that only the TI−I_{max} is significantly correlated with the gel hardness. The actual p-values of the TI−I_{max} data have been summarized in **Table 3**. For each flavor compound the panelists can be divided into two groups: group I, consisting of six panelists who show a significant effect of gel hardness on TI−I_{max}, and group II with the four other panelists who do not exhibit this effect. Except for panelists 2 and 6, all panelists fall in the same group for each flavor. Panelist 2 belongs to group I for diacetyl and to group II for ethylbutyrate. For panelist 6 the opposite situation is observed.

Table 3. *P* values of the Linear Regression Per Panelist of Gel Hardness against I_{max} Values of the NSC and TI Signals for Gels Flavored with Diacetyl and Ethylbutyrate

panelist	diacetyl gel hardness	WHC	ethylbutyrate gel hardness	WHC
1	0.02a	0.03a	0.00a	0.00a
2	0.01a	0.01a	0.15	0.15
3	0.00a	0.00a	0.00a	0.00a
4	0.02a	0.02a	0.01a	0.02a
5	0.18	0.18	0.89	0.83
6	0.19	0.19	0.01a	0.00a
7	0.59	0.59	0.72	0.88
8	0.66	0.66	0.23	0.45
9	0.00a	0.00a	0.00a	0.03a
10	0.00a	0.00a	0.00a	0.00a

[a] a, Indicates significant at $\alpha = 0.05$ level.

A hypothesis to explain the fact that the correlation between gel hardness and TI−I_{max} group II is not significant for group II, is that the panelists in this group produce nonconsistent results, and, therefore, do not show a significant correlation with gel properties. In that case, group II will have a higher standard error of their estimated slope. This has been tested for diacetyl and ethylbutyrate, and gel hardness and WHC, using ANOVA. There was no significant difference between groups I and II in their standard error values. This shows that the nonsignificance of group II is not caused by nonconsistent panelist performance. The panelists in group II produce results different from those of group I, but still in a consistent and reproducible way. The groups could not be characterized by any differences in age, experience, or gender.

A second hypothesis is that the panelists in group I allow that their flavor perception is influenced by gel texture, whereas the panelists in group II are able to separate flavor perception from texture perception. Interestingly, the panelists were explicitly instructed not to let textural effects influence their perception of the flavor intensity. Despite this, an effect of texture on flavor perception was found for a considerable number of panelists. Moreover, for the general consumer, perception occurs spontaneously, and psychophysical interactions between the senses are likely to occur, which emphasizes the potential importance of texture for flavor perception.

Influence of the Eating Protocol for in Vivo Measurement on the T_{max} Value. The studies of Baek et al. (*19*) and Linforth et al. (*18*) are comparable to our work, because they have also measured TI and NSC simultaneously, using gelatine gels. In contrast to our study, in which the panelists followed a strict protocol, the panelists in these two studies were allowed to chew and swallow whenever they wished. Similar to our results, Baek et al. (*19*) found no significant difference in the NSC−I_{max} values, but a significant difference in TI−I_{max} and TI−T_{max} values ($p < 0.001$, ANOVA).

In addition to this, a statistically significant ($p < 0.001$) decrease in the rate of release with increasing gelatine concentration was found. The rate of release was defined as the

nosespace concentration I_{max} divided by the timespan between the timepoints of the increasing phase corresponding with 25% and 75% of the T_{max} value. For our dataset a significant correlation (p value of 0.037) was found between gel hardness and rate of release for ethylbutyrate. For diacetyl a nonsignificant p value of 0.24 was obtained. The fact that we find a much lower significance for ethylbutyrate than Baek et al. (*19*) and no significance at all for diacetyl, can be explained by comparing the different eating protocols of both studies. Because the T_{max} is usually determined by the moment of swallowing (*28*), the effects in T_{max} measured by Baek et al. (*19*) are primarily caused by the fact that people chew longer on harder gels. In our eating protocol, the panelists had to swallow the entire bolus after thirty seconds of chewing, irrespective of the gel hardness. In this way, the chewing and swallowing time becomes independent of the gel hardness, which should result in a more objective monitoring of the effect of gel structure on the rate of release. To prove this, our set of gels with increasing gel hardness were eaten in triplicate by three panelists without the use of the eating protocol, while their nosespace concentration was measured simultaneously. In contrast to the experiments in which a protocol with a fixed swallowing time was used, a much higher significant positive correlation between gel hardness and NSC−T_{max} was found for both flavor compounds. P values of 6.8×10^{-7} and 0.01 were obtained for ethylbutyrate and diacetyl, respectively (no further results shown).

Linforth et al. (*18*) have found differences in the ratio between sensory and instrumental T_{max} values. These effects are not reproduced in our study, due to the fixation of T_{max} at 30 s by use of a protocol, as discussed above.

CONCLUSION

The main conclusion is that for the used whey protein gel system a change in texture determines the perception of flavor intensity in a psychophysical way, through a change in mouthfeel, and not through a change in the nosespace concentration of flavor compounds. Apparently, flavor nosespace concentration does not always determine flavor perception, suggesting that for some food applications changing product structure might be an effective tool to adjust flavor perception.

ABBREVIATIONS USED

CSLM, confocal scanning laser microscopy; FID, flame ionization detector; GDL, gluconodeltalacton; HSGC, headspace gas chromatography; I_{max}, maximum intensity; NSC, nosespace concentration; TI, time intensity; T_{max}, maximum intensity; WHC, waterholding capacity.

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