

# Multimodal Sensory Integration during Sequential Eating—Linking Chewing Activity, Aroma Release, and Aroma Perception over Time

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Accepted March 2, 2012

## Abstract

The respective effects of chewing activity, aroma release from a gelled candy, and aroma perception were investigated. Specifically, the study aimed at 1) comparing an imposed chewing and swallowing pattern (IP) and free protocol (FP) on panelists for in vivo measurements, 2) investigating carryover effects in sequential eating, and 3) studying the link between instrumental data and their perception counterpart. Chewing activity, in-nose aroma concentration, and aroma perception over time were measured by electromyography, proton transfer reaction–mass spectrometry, and time intensity, respectively. Model gel candies were flavored at 2 intensity levels (low—L and high—H). The panelists evaluated 3 sequences (H then H, H then L, and L then H) in duplicates with both IP and FP. They scored aroma intensity over time while their in-nose aroma concentrations and their chewing activity were measured. Overall, only limited advantages were found in imposing a chewing and swallowing pattern for instrumental and sensory data. In addition, the study highlighted the role of brain integration on perceived intensity and dynamics of perception, in the framework of sequential eating without rinsing. Because of the presence of adaptation phenomena, contrast effect, and potential taste and texture cross-modal interaction with aroma perception, it was concluded that dynamic in-nose concentration data provide only one part of the perception picture and therefore cannot be used alone in prediction models.

**Key words:** adaptation, aroma perception, cross-modal interaction, PTR-MS, sequential eating, time-intensity

## Introduction

It is accepted that aroma and its perception play a critical role in food acceptance and liking by consumers. However, when developing a new product, the measurement of aroma perception requires time resource-consuming sensory tests. For this reason, there exists a desire to understand aroma perception mechanisms so as to develop methodologies to measure it, be it directly or indirectly by instrumental means to minimize the extensive sensory involvement.

Retronasal aroma perception is the result of a chain of events starting with the ingestion and breakdown of a food product which results in the release of aroma molecules that access the olfactory epithelium from the back of the mouth via the pharynx (Buettner and Beauchamp 2010). There, after binding to receptors, the odorant molecule is transduced into an electrical signal that is conveyed to the brain, where it ultimately converges with information from other senses including taste, vision, audition, and somatosensation to result in a sensation having characteristic intensity and temporal properties (Baek et al. 1999; Delwiche 2004; Frasnelli et al. 2005; Small and Prescott 2005; Prazeller et al. 2006).

In recent years, a lot of published research has focused on aroma release from food products in the context of mouth-space and nose-space aroma dynamics, establishing relative effects of food composition and texture (Cook et al. 2005; Linforth et al. 2005; Mestres et al. 2005; Bayarri et al. 2006; Gierczynski et al. 2007; González-Tomás et al. 2007; Burseg et al. 2009; Délérís et al. 2011), and aroma compounds characteristics (Linforth et al. 2010). But aroma release is not only influenced by the food matrix. Aroma release and therefore aroma perception are dynamic processes, influenced by constant changes in chewing, swallowing, salivation, and breathing patterns, as has been shown in recent published works (Guinard et al. 1997; Buettner 2002; Buettner et al. 2002; Blissett et al. 2006; Buettner et al. 2008). Moreover, food is rarely consumed as a succession of samples with interstimulus water rinses as is usually the case during the course of well-controlled psychophysics experiments. Indeed, food is consumed as a succession of bites, usually without significant delay or rinsing. Under these conditions, aroma perception over time can be influenced by carry over

effects, namely the influence of a previously evaluated sample can have on the perception of a subsequent sample. The effects of carryover on panelists' taste discrimination performances using various types of difference tests were well documented and summarized in the Sequential Sensitivity Analysis theory (O'Mahony and Odert 1985; Lee and O'Mahony 2007). To the authors' knowledge, no comparable studies have been carried out to examine the impact of carryover effects on aroma perception and in-nose concentration over time, in the case of solid foods.

The detailed investigation of the dynamics of in-nose aroma concentration or sensory aroma intensity perception was made possible by the use of dedicated instrumental devices of sensory methods. Two analytical techniques are available for the determination of the dynamics of aroma in-nose concentration: chemical ionization mass spectrometry (APCI-MS) and proton transfer-reaction MS (PTR-MS). The description, advantages, and limitations of these 2 techniques have been detailed many times (Hansel et al. 1995; Piggott 2000; Taylor et al. 2000). While the instrumental methodology is rather straightforward, the sensory protocols need to be adapted depending on the focus of the study. Two sensory methodologies are frequently used to capture the temporal aspects of aroma perception. Both methods rely on the use of a trained panel, using a predefined set of sensory attributes to describe their sensations. With time intensity (TI; Larson-Powers and Pangborn 1978), panelists score the perceived intensity over time for each sensory attribute on an intensity scale, one at a time. With Temporal Dominance of Sensations (TDS; Pineau et al. 2003, 2004), panelists are required to report in real time which sensory attribute is dominant, and additionally to report its intensity, in some forms of TDS (Labbe et al. 2009). The TI and TDS method without intensity scoring correspond to 2 distinct objectives: either the collection of the individual evolution of the perceived intensity of each sensory attribute or the assessment of the evolution of perceived dominant sensations over time. Differences can arise between the 2 methods because the dominant sensory attribute at a given time point is not necessarily the most intense. Each method has specific advantages and limitations (Le Révérend et al. 2008; Pineau et al. 2009). The advantage of TDS is that it captures well the sequence of perception of multiple sensory attributes over time, whereas TI is more suitable when one is only interested in the kinetic of one specific sensory attribute.

There can be different motives behind the study of aroma release. One motive can be to better understand the influence of physiological factors on aroma release and on the resulting sensory perception, as described earlier. In this case, the focus is on the human subjects, and it is of interest to understand and characterize their natural variability to potentially link it to other parameters such as preference (Roberts et al. 2004) or the regulation of food intake (Ruijschop et al. 2009). Another motive can be to assess the impact of formulation parameters of the food products on the aroma release and on

the resulting sensory perception. This situation corresponds to what O'Mahony and Goldstein (1987) described as "Sensory Evaluation I," where panelists are used as analytical tools, under controlled conditions and minimal "noise." In this situation, the experimenter chooses the most sensitive protocols available for sensory testing, since the goal is to detect differences between food samples in the most precise possible way. Typically, the training of sensory panelists aims at increasing their accuracy and reproducibility (Chambers et al. 2004) and also the consensus between the panelists (Labbe et al. 2004). In a similar way, the experimenter may choose to standardize the evaluation procedure of the samples for the instrumental measurement of aroma release. A frequent assumption is that standardizing the chewing and swallowing rhythm across panelists will increase the homogeneity of the data and the discrimination of the products (Frank et al. 2011). However, to the authors' knowledge, no published study focused on validating this assumption or comparing data obtained with "imposed" versus "free" protocols.

The study presented here was designed with the following objectives. The first goal was to assess the impact of the evaluation protocol (free versus imposed chewing and swallowing rhythm) on the discrimination power of the measurement techniques (sensory and PTR-MS) and on the quality of the data (homogeneity between the panelists). It was hypothesized that imposing a common chewing and swallowing rhythm to the panelists should homogenize aroma release and aroma perception over time. Second, data were utilized for the determination and quantification of the impact of carryover effects (i.e., "the sequence effect") on perceived aroma intensity and on in-nose concentration under both protocols. Last, the experimental setup allowed the investigation of the links between chewing activity, dynamic aroma release, and sensory perception over time, under both protocols.

## Materials and methods

### Stimuli

Flavored gelled candies were prepared with the composition as shown in Table 1. Sucrose (Domino, Domino Foods, Inc.), gelatin (Gelatin 250 bloom type A, Gelita), and spring water (Alpine Valley Water) were mixed, heated (100 °C), and brought to full gelatin dissolution. Separately sucrose, corn syrup (Corn syrup 85 Brix, Cargill), and spring water were mixed and heated to 116 °C. The 2 warm solutions were mixed in a weight ratio of 1.06 gelatin solution to 1 sugar solution and stirred well. Food coloring (yellow 5, internal supply) and the desired amount of flavor (isoamyl acetate, internal supply) dissolved in triacetin were added in glass beakers, and the gelatin-sugar mix was added on top. Coloring agent was added so as to provide a more acceptable looking product. It was verified that the coloring in the gelled

candies did not interfere with measurements. Isoamyl acetate was chosen because of its recognizable aroma (“banana”). This study focused on a product containing a single aroma chemical rather than a mixture of chemicals as will be the case in future research.

Mixing was done manually, until the color looked uniform. Two concentrations of isoamyl acetate were prepared: high and low as detailed in Table 1. The concentrations were determined so as to be substantially different without being overwhelming.

The colored and flavored mix was then poured onto chocolate molds (Sugar Craft) to allow for consistent shape and weight (average weight  $5 \pm 0.15$  g) of the samples. The gels were covered with wax paper to prevent extensive drying and retain their soft chewy texture. Samples were prepared fresh before each evaluation day and kept in a refrigerator in sealed containers until the morning of evaluation.

### Panelists' recruitment and training

Twenty-nine panelists were recruited internally (11 men, 18 women) for their willingness to participate in an 8-week study. All panelists signed an informed consent document approved through the internal (Givaudan) Internal Review Board, and all studies being conducted were found to have the appropriate safeguard implements to ensure the rights and welfare of research participants in compliance with DHHS Regulations for the Protection of Human Subjects (45 CFR 46).

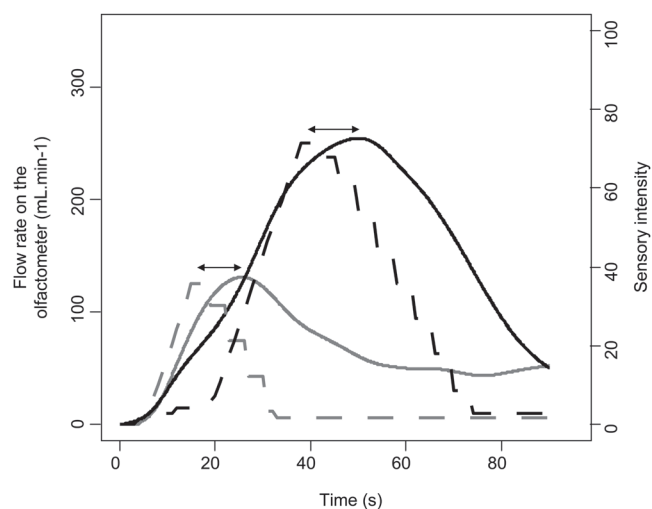
The goals of the training were the following. The first goal was to teach the panelists how to score the perceived aroma intensity on an intensity scale (without the time dimension). The second was to teach them to pay attention to variations of perceived aroma intensity over time. The third was to teach them to score perceived aroma intensity over time on an intensity scale, fourth to discriminate between sensory signals having different properties (different time at maximum intensity and different maximum intensities). The fifth goal was to align the panelists on their scoring of perceived intensity on the intensity scale by repeatedly showing them reference stimuli. Finally, the last goal was to get the panelists comfortable with the evaluation environment (PTR-MS,

electromyography [EMG] electrodes) before proceeding to the test.

The aforementioned goals were reached through 6 sessions of training. During the first 3 sessions, the stimuli presented to the panelists (isoamyl acetate aromas) were generated by an olfactometer (Virtual Aroma Synthesizer VAS technology), and during the last three sessions, the panelists were presented with similar stimuli as for the final evaluation (isoamyl acetate flavored candies). The VAS is a Givaudan-owned olfactometer to deliver controlled quantities of volatiles over time into a gas phase. In the first training session, the panelists were presented with 2 banana flavor intensity references (a low and a high reference) and learned to use an intensity scale to score the perceived intensity. In the second session, the panelists smelled an aroma stimulus in which intensity gradually varied over a 90-s time period: a slowly increasing and then decreasing signal. The panelists' task was to report when the intensity was at its maximum. In the third session (2 repetitions), the panelists were presented 2 signals, first signal 1 (overall low intensity and reaching the maximum at 17 s) and after a short break, signal 2 (overall high intensity and reaching the maximum at 40 s) as illustrated in Figure 1. The panelists' task consisted of scoring the perceived intensity over time for 2 different stimuli. Session 4 had 2 parts. First, the panelists were presented with 2 flavored gel candies representing 2 anchor points on the intensity scale (“low” at 20 and “high” at 80), then were asked to taste them and remember their intensities. Second, panelists were given a new unmarked sample (the “high” one) and scored its perceived intensity for 90 s. In session 5, the panelists were given first the anchor samples and were then required to score the respective intensity of a “low” and then a “high” for 90 s, without rinsing in-between. The last

**Table 1** Composition of the flavored candies (w/w%)

Ingredients	Low level	High level
Sucrose	31.99	31.93
43 DE corn syrup	19.59	19.56
Water	38.93	38.86
Gelatin (250 bloom)	9.45	9.44
Triacetin	0.02	0.16
Isoamyl acetate	$7.50 \times 10^{-3}$	$5.3 \times 10^{-2}$
Total	100	100



**Figure 1** Olfactometer flow rate over time (stimulus intensity; dotted lines, left vertical axis) and resulting perceived sensory intensity over time (solid lines, right vertical axis) for stimulus 1 (gray line, right vertical axis) and then stimulus 2 (black lines, right vertical axis).

session was organized for the panelists to get comfortable with performing the same sensory scoring task as in session 5, this time breathing into the PTR-MS inlet.

### Chewing and swallowing protocol

Two different types of panelist training protocols are found in the literature for this type of study: either an “imposed” protocol, where panelists are instructed when to chew the sample and/or when to inhale–exhale or a “free” protocol, where panelists chew, swallow, and breathe normally (Davidson et al. 1999; Buettner et al. 2008; Gierczynski et al. 2008; Ruijschop et al. 2009). In the study presented here, the 2 possibilities were tested and compared. The first protocol, called “free,” consisted of letting the panelists chew the sample at their own pace and swallow the pieces or saliva whenever needed. They were instructed to “breathe normally and regularly into the nose piece while evaluating the sample.” The second protocol, called “imposed,” consisted of standardizing the chewing and swallowing pattern of the panelists, with the hypothesis that it would also standardize their breathing and thus their release pattern. The pattern imposed was 1 chew/s and swallowing allowed every 20 s, as many times as needed in the 90-s period. These rhythms were based on preliminary tests and seemed reasonable for the panelists. The chewing rhythm was provided by a metronome beating in the background, and the swallowing times were indicated by the word “swallow” appearing on the computer screen. The panelists were asked to refrain from swallowing in-between specified swallowing times.

### Presentation design of the samples

Samples were presented in a sequence of 2 per sitting without rinsing or break in-between, following the 3 sequences: “high” followed by a “high,” “high” followed by a “low,” or “low” followed by a “high.” The “low” followed by “low” sequence was not evaluated in this particular study. Each sequence was duplicated for each panelist, and the duplicate sequences were done both with the “free” and “imposed” protocol (FP and IP, respectively). The presentation order of the 3 sequences was balanced using a latin square over the 29 panelists. All panelists started with the “free” protocol and then followed with the “imposed” protocol. Overall, each panelist tested 12 sequences, namely 2 replicates of high–high, high–low, low–high each with FP and then IP.

All measurements, EMG, PTR-MS, and sensory evaluation, were performed in parallel on each panelist.

### EMG measurements

The objective was to measure jaw muscles’ activation over time. The surface EMG signals were measured with a BioNex 4-channel Bio-Potential amplifier (MindWare Technologies LTD) with a total amplifier gain of 1000. A 16-bit A/D resolution converter digitalized the analog signals with a sam-

pling frequency of 1000 Hz for each channel and an input range of 20 V. Bipolar surface electrodes consisting of 3 circular Ag/AgCl disks (4 mm diameter) were used. The subjects’ skin areas were cleaned with a mild prepping lotion, and the determination of the best location for electrode placement above the masseter muscle was done via palpation. Two electrodes were attached using conductive gel and adhesive collars, spaced approximately 15 mm apart, and a third electrode (ground) placed on the wrist of the idle hand of the panelist. Data were collected as text files at a frequency of 1000 Hz.

### Instrumental setup (PTR-MS)

The objective was to measure breath volatile intensity and changes over time. The breath volatiles were monitored using a PTR-MS (Ionicon Analytick GmbH) as found in previous studies (Roberts et al. 2004; Mestres et al. 2005; Buettner et al. 2008; Gierczynski et al. 2008). The instrument parameters were setup as following: drift tube pressure: 2.05 mbar, drift tube voltage: 600 V, drift tube temperature: 80 °C, detector pressure:  $2.2 \times 10^{-5}$  mbar, pressure controller: 600 mbar, flow controller: 6.0 sccm, inlet temperature: 100 °C, voltages for  $U_{SO}$  and  $U_S$  were adjusted to keep a ratio of water clusters to primary ions ( $m/z$  21) of less than 3%. The instrument was set in selected ion monitoring mode (MID). The ions specific for isoamyl acetate were preliminary determined ( $m/z$  61 and 71, dwell time 100 ms each). In addition to monitoring the primary ions ( $m/z$  21, dwell 50 ms), the mass 59 (dwell 50 ms) was also monitored, signaling acetone as breathing indicator. Overall, one data point was acquired every 0.31 s. The data reported represent ion  $m/z$  71, only as both signals were similar and  $m/z$  71 was within linear range in all cases.

Panelists sat in front of the instrument and were instructed to breathe directly into a heated nosepiece (40 °C) attached to the instrument inlet. An initial baseline was first monitored to ensure quality of the breathing signal and correct position of the panelist. Data were later cut to fit the 90-s recording for each of the sample, based on the visible breathing pattern as recorded by acetone.

### Sensory evaluation

For each sample evaluation, panelists were instructed to score the perceived “banana” intensity while eating the sample for 90 s. Specific evaluation protocols and instructions to the panelists are detailed hereafter.

The data acquisition system was built in-house under the R environment version 2.12.0 (R Development Core Team 2009) using the packages *tecltk* and *gWidgets*. The panelists had to hit a “start” button when they first started chewing the sample, and then used the time-intensity scale with a slider to score intensity in real time. The intensity scale had 2 anchors, a “low” and “high,” corresponding to the anchor products presented to the panelists during the training



phase. Data were collected as text files at a frequency of 10 Hz.

### Data analysis

The data were analyzed using R version 2.12.0 (R Development Core Team 2009). For both sensory and PTR-MS data, each individual time-intensity curve (for each sample and each panelist) was smoothed using the `smooth.spline` function from the `spline` package (`spar = 0.75`) to remove noise in a similar manner as Dijksterhuis and Eilers (1997), using cubic smoothing splines. Such curves can be described with various parameters, depending on the focus of the study. Several parameters were extracted for each of these individual curves, such as maximum intensity ( $I_{\max}$ ), time to maximum intensity ( $T_{\max}$ , in seconds), and area under the curve (AUC). Time “0” in all cases represented the time at which the sample was put in the mouth and the panelist started chewing. Because the PTR-MS and sensory recordings were done in parallel, all files had the same duration. The subsequent sensory TI and PTR-MS individual smoothed curves were then averaged for each time point across panelists.

For subsequent data analyses, each stimulus was considered differently depending on the preceding stimulus, as described by Lee and O'Mahony (2007). Five samples were thus defined: L (low in first position), H (high in first position), hL (low preceded by high), hH (high preceded by high), and IH (high preceded by low). Two analyses of variance (ANOVAs) were conducted on each of the data sets and each protocol for each TI parameter, with the main effects panelist, sample, repetition, and their interactions. In the first ANOVA, the “sample” effect was composed of 2 stimuli: high versus low level. In the second ANOVA, the “sample” effect was composed of 5 stimuli: L, H, hL, hH, and IH. For the second ANOVA, significant differences between the stimuli were determined by the Tukey's test ( $\alpha = 0.05$ ). In addition, ANOVAs were conducted to test for protocol effect within the sensory or PTR-MS measurements, as well as for position effect (samples shown in first or second position). Additionally, average curves were created by calculating the average for each time point across panelists and repetitions on the smoothed data. As noted by Dijksterhuis and Piggott (2001), TI parameters should not be extracted from the average curves. Therefore, the average curves were only displayed for illustrative purposes, and ANOVAs were performed on the TI parameters extracted from individual TI curves.

The EMG data analysis was inspired by Neto et al. (2007), using the R packages `mFilter`, and `dplR`. First, a high-pass filter with a cutoff at 110 Hz was applied, in order to remove the 60 Hz electrical noise and concentrate on the power spectrum of higher energy. The signal was then full-wave rectified, and then a Morlet wavelet analysis was applied. For each time point, the sum of power was calculated for periods that comprised between 0.002 and 0.016 s. The resulting signal was smoothed using the same spline function and setting

as for the sensory and PTR-MS signals. Finally, average curves were created by calculating the average for each time point using the smoothed data. Because no significant sample effect was identified, curves were averaged across panelists, repetitions, and samples for each protocol.

## Results

### Olfactometer and sensory TI

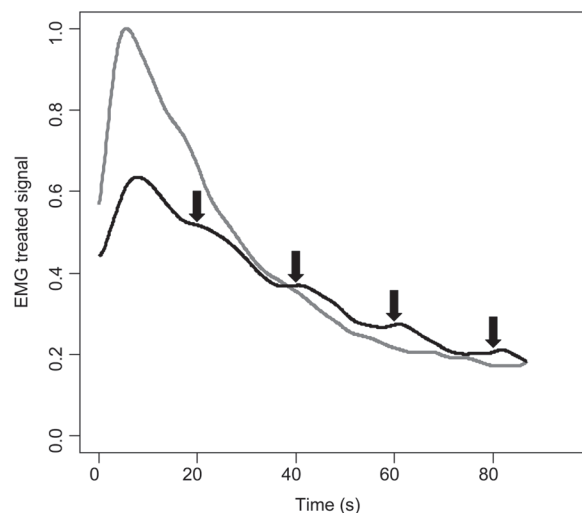
During the training phase, the panelists scored the perceived aroma intensity over time of 2 stimuli delivered by the VAS olfactometer (Figure 1). The concentration changes of the odorant over time induced by the flow rate changes for these 2 stimuli had a near bell shape, as depicted in Figure 1. Stimulus 1 had a lower maximum intensity than stimulus 2. Overall, the shapes of the resulting perceived intensity curves over time for the 2 stimuli were very similar to the shapes of the curves of the concentration changes of the odorant over time. As expected, stimulus 2 was perceived as more intense than stimulus 1. Interestingly, a consistent 9–10 s delay in  $T_{\max}$  could be observed between the stimulus signal and its corresponding perceived intensity signal, regardless of the type of stimulus. In addition, the delay was maintained in the decreasing slope, as is most visible with the highest stimulus level (black line). This suggested a constant and consistent delay between the occurrence of a stimulus change of intensity and the panelist's answer.

### The EMG

The EMG signal was recorded to monitor chewing activity over time (Figure 2). Expectedly, there were no substantial effects of aroma concentration (“high” vs. “low” samples) on the chewing activity curve shape (data not shown). There were notable differences between the chewing activities in FP and IP. The chewing activity in FP was early and intense from the beginning to 5.5 s and steadily declined until 90 s (end of the evaluation period). More precisely, a change of declining slope could be observed around 25 s (first slope from maximum to 25 s and second slope from 25 to 90 s), and the baseline activity was reached around 60 s. In IP, however, the chewing activity appeared as less intense in the beginning but lasted longer than in FP. The change of slope only appeared at around 40 s (compared with 25 s in FP). Interestingly, some local changes in slopes were visible on the IP curve at time around 20, 40, 60, and 80 s, which were consistent with the swallowing events required in this protocol. Such local changes were not visible in the FP, where the swallowing events were not synchronized across panelists.

### In-nose aroma concentration—FP

The dynamic in-nose aroma concentration was measured with a PTR-MS (Figure 3a). Each of the average curves presented the typical dynamic aroma release profile as has been published many times in the literature (Linthorpe et al. 1999; Harvey



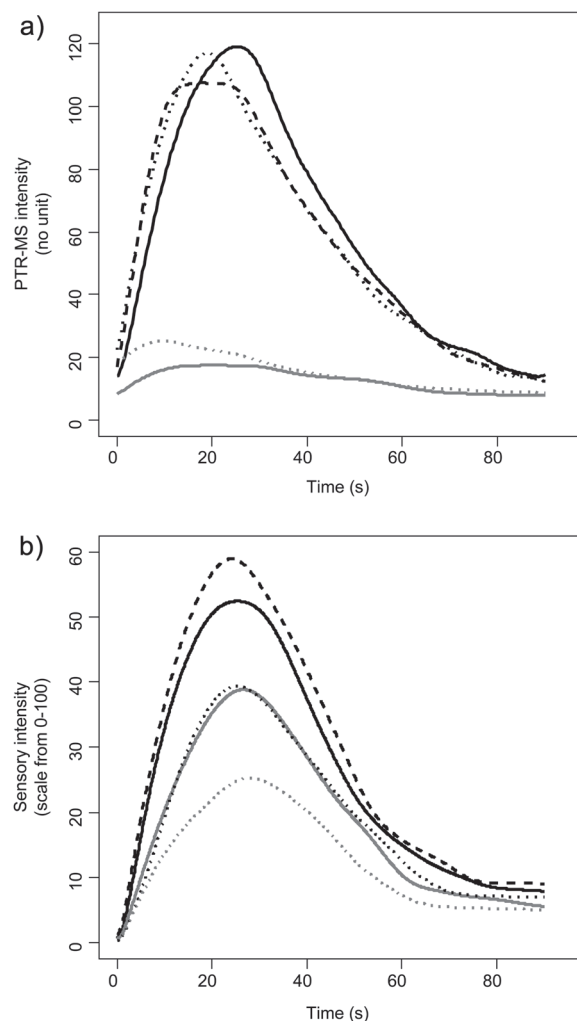
**Figure 2** EMG treated signal (normalized intensity over time) for the FP (gray) and IP (black). For IP, the black arrows indicate the times when the panelists were asked to swallow.

and Barra 2003; Krause et al. 2011). As anticipated, the candies with a low aroma concentration were overall significantly ( $P < 0.05$ ) less intense than the ones with a high concentration, considering both  $I_{max}$  and AUC (Table 2, Figure 3a). There was no significant ( $P > 0.05$ ) carryover effect for  $I_{max}$  or AUC. On the time axis, however, some notable differences could be observed. Overall,  $T_{max}$  for “high” occurred significantly ( $P < 0.05$ ) later than  $T_{max}$  for “low” (32.9 s for H vs. 25.4 s for L) (Table 2, Figure 3a). More specifically, high in second position reached  $T_{max}$  slightly but significantly earlier than high in first position (32.1 s in first position vs. 28.6 s in second position) and the low samples followed a similar pattern (24.4 s in first position vs. 18.5 s in second position).

Under this protocol, specific swallowing events cannot be singled out in the average curves and are therefore not represented in Figure 3a.

#### In-nose aroma concentration—IP

The dynamic in-nose aroma concentration for the various stimuli under IP (Figure 5a) presented some common points to the FP condition (Figure 3a): overall the “high” level was significantly more intense than the “low” level (Table 2). However, IP curves also presented noticeable differences compared with FP curves. There was a significant ( $P < 0.05$ ) carryover effect for AUC (Figure 4f). There was also a trend ( $P < 0.10$ ) for the samples in second position (hH, IH, and hL) to have a higher AUC than the samples in first position (L and H). The dynamic aspect of the curves was also different between FP and IP. In FP, the 3 average curves for the “high” level stimuli had a similar  $T_{max}$  and so did the 2 average curves for “low” level stimuli. This was not observed with IP, where significant ( $P < 0.05$ ) carryover effects were noted for  $T_{max}$  (Figures 4e and 5a):  $T_{max}$  came significantly earlier



**Figure 3** Average time-intensity signals for (a) PTR-MS and (b) sensory measurements under FP. The stimuli are marked as follows: L, low in first position (gray solid lines); hL, low preceded by high (gray dotted lines); H, high in first position (black solid lines); IH, high preceded by low (black dashed lines), and hH, high preceded by high (black dotted lines).

for hL than for L (14.5 and 43.7 s, respectively;  $P < 0.05$ ). hH came significantly ( $P < 0.05$ ) earlier than H, and IH was intermediate. Finally, the average  $T_{max}$  was significantly earlier with FP than with IP (27.4 and 33.5 s, respectively;  $P < 0.05$ ).

In addition, similar patterns as those seen in the EMG signals under IP (Figure 2) could be noticed on the PTR-MS IP curves. Some local changes in slopes were visible at times around 20, 40, 60, and 80 s, concurrent with the swallowing events (as indicated by the arrows).

#### Sensory intensity—FP

Overall, very satisfactory panel performances were obtained under the FP condition, as denoted by a significant sample effect for  $I_{max}$  and AUC, a relatively small panelist effect and the absence of a significant panelist  $\times$  sample interaction (Table 2). The panelists' effects on  $I_{max}$  and AUC denoted

**Table 2** Results of the ANOVAs for the time intensity parameters extracted from PTR-MS and sensory data

		Main effects and interactions	Time intensity parameters		
			lmax	Tmax	AUC
PTR-MS	FP	Panelist	2.18 (***)	5.38 (***)	3.51 (***)
		Sample	110.66 (***)	38.11 (***)	143.11 (***)
		Repetition	1.01	6.41 (*)	0.04
		Panelist × sample	0.94	2.77 (***)	1.09
		Panelist × repetition	0.76	1.06	0.76
		Sample × repetition	0.62	0.64	0.81
	Imposed protocol	Panelist	1.71 (*)	1.05	1.98 (**)
		Sample	53.21 (***)	6.31 (*)	99.64 (***)
		Repetition	1.63	9.77 (**)	0.49
		Panelist × sample	0.76	1.02	0.75
		Panelist × repetition	1.13	0.66	1.37
		Sample × repetition	1.23	12.57 (***)	0.01
Sensory	FP	Panelist	5.2 (***)	31.65 (***)	11.81 (***)
		Sample	73.94 (***)	0.93	58.87 (***)
		Repetition	1.89	2.57	0.64
		Panelist × sample	1.23	0.71	0.82
		Panelist × repetition	0.65	0.69	0.66
		Sample × repetition	0.34	0.22	1.64
	Imposed protocol	Panelist	6.92 (***)	24.02 (***)	6.92 (***)
		Sample	77.74 (***)	14.36 (***)	75.9 (***)
		Repetition	2.7	1.74	3.67
		Panelist × sample	0.96	1.41	0.96
		Panelist × repetition	0.59	1.35	0.8
		Sample × repetition	1.73	0.35	1.3

*F* values and corresponding significance levels under parenthesis. The sample effect corresponds to high or low level. The “×” symbol denotes interactions between 2 factors. For example, “panelist × sample” denotes the interaction between panelist and sample. Significance codes for the *P* values: 0 < “\*\*\*” < 0.001 < “\*\*” < 0.01 < “\*” < 0.05.

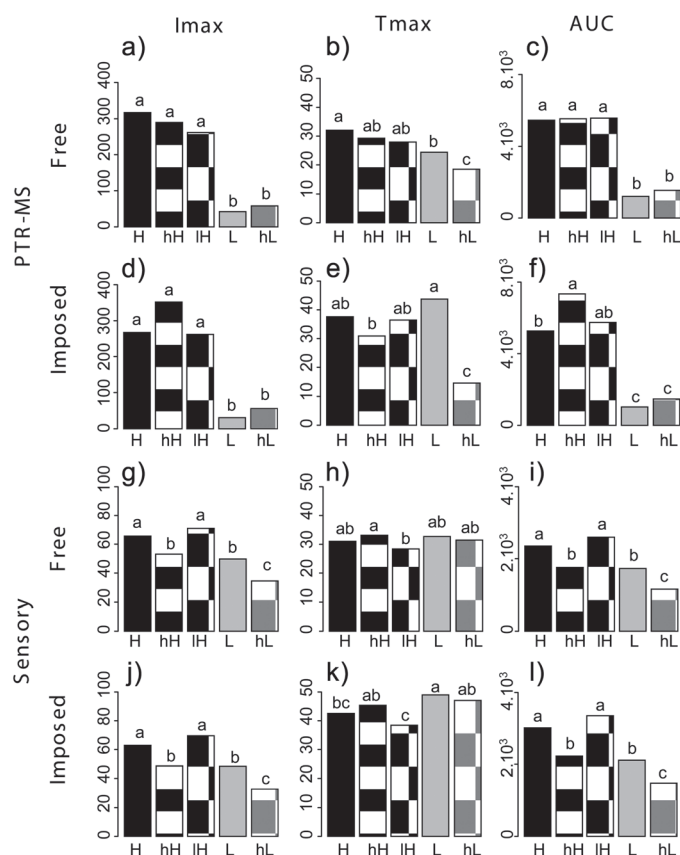
a different usage of the scale, despite training, but this is very common in sensory time-intensity studies (Guinard et al. 2002). The average sensory curves (Figure 3b) presented a typical shape of sensory TI curves as previously reported in the literature (Dijksterhuis and Piggott 2001). As anticipated, and similar to in-nose concentration curves, “low” samples had a lower intensity than “high” samples (Table 2). However, contrary to instrumental curves, there were significant ( $P < 0.05$ ) carryover effects for both Imax and AUC (Figure 4g,i). Overall, there was a (nonsignificant) trend for LH to be scored as more intense than H (Imax are 71.1 and 65.6, respectively, on average), in turn significantly ( $P < 0.05$ ) more intense than hH (Imax is 53.1, on average) (Figure 4g). Similarly, L was scored as significantly ( $P < 0.05$ ) more intense

than hL (Imax are 50.0 and 34.6, respectively). Interestingly, Imax and AUC were not significantly different ( $P > 0.05$ ) for L and hH, meaning that under certain experimental conditions, the low and the high levels were perceived as isointense.

Contrary to the instrumental curves, there was no significant difference ( $P > 0.05$ ) between the “low” and “high” level for Tmax (Table 2). Tmax was similar for all 5 stimuli (31.3 s, on average), and the time of return to baseline level was around 60s for all types of samples (Figure 3b).

#### Sensory intensity—IP

Similar to the results with FP, very satisfactory panel performances were obtained with the IP (Table 2). As



**Figure 4** Values of the TI parameters under the 2 conditions, FP and IP, for PTR-MS and sensory measurements. Within a plot, stimuli that share a common letter are not significantly different ( $P = 0.05$ ).

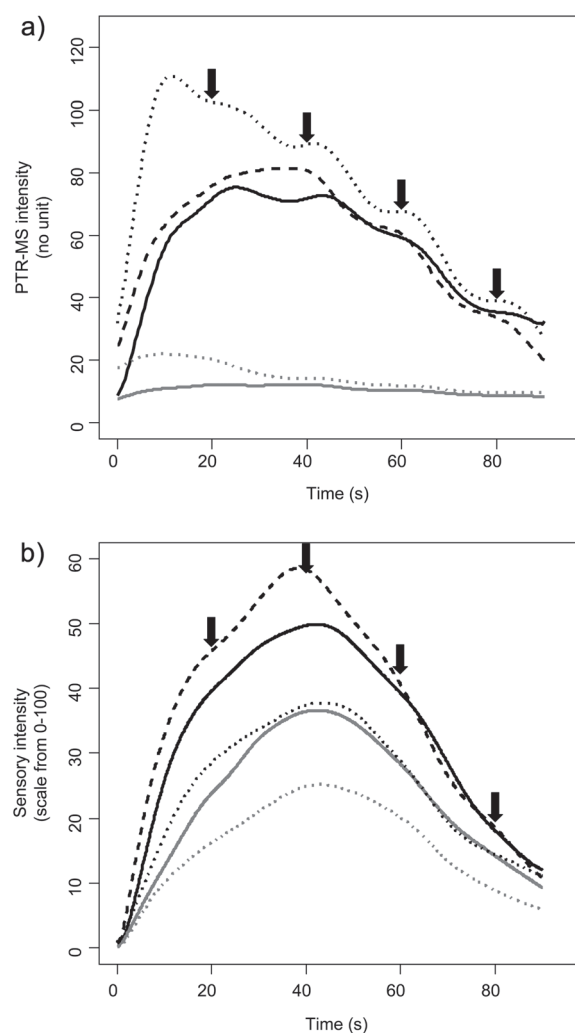
previously, overall, the panelists perceived the “high” as more intense than the “low” stimulus, as denoted by significant ( $P < 0.05$ ) sample effects for I<sub>max</sub> and T<sub>max</sub> (Table 2). Also, with IP, the same type of carryover effect as with FP was found for both I<sub>max</sub> and AUC, in the same direction (Figure 4j,l).

Beyond these similarities, there were also substantial differences between the sensory intensity curves with FP (Figure 3b) and with IP (Figure 5b). The relative T<sub>max</sub> for each of the average curves were significantly later with IP than with FP (44.1 and 31.3 s, respectively;  $P < 0.05$ ). In addition, the “return to baseline level” observed with FP at around 60 s did not occur with IP, at least not within the 90 s experimental framework.

Similarly to EMG curves and instrumental curves, the swallowing events appeared visibly and some local (though small) changes of slope were notable on the sensory intensity curves (Figure 5b).

## Discussion

The present study had 3 goals. First, a comparison of the choice of method and protocol (namely IP vs. FP) was



**Figure 5** Average time-intensity signals for (a) PTR-MS and (b) sensory measurements under the IP. The stimuli are marked as follows: L, low in first position (gray solid lines); hL, low preceded by high (gray dotted lines); H, high in first position (black solid lines); IH, high preceded by low (black dashed lines); and hH, high preceded by high (black dotted lines). The black arrows symbolize the times panelists were required to swallow.

carried on. Second, the impact of carryover effects on perceived intensity and in-nose concentration was investigated. Last, the links between chewing activity, in-nose aroma concentration, and sensory perception over time were evaluated. The following discussion addresses the 3 objectives.

## Comparison of protocols

The first objective of the study was to determine if the use of a standardized evaluation protocol for the panelists with defined chewing and swallowing rhythms (IP) would provide substantial benefits over an FP, both for the measurement of the in-nose aroma concentration (PTR-MS) and of perceived aroma intensity over time (sensory TI). Areas of interest include interpersonal variability, agreement between



the panelists, ability to discriminate between the high and low aroma intensity levels, and practical ease of use of the protocols.

As described earlier and quite unsurprisingly, there is an important interpersonal variability both in the global amount of volatile exhaled by the panelists (PTR-MS) and in the usage of the sensory scale, as denoted by the significant panelist effect on the curve parameters (Imax and AUC). Considering AUC, this variability is slightly reduced with IP compared with FP, both for instrumental and sensory measurements, but the improvement is limited. Moreover, the agreement between the panelists to detect “high” as being more intense than “low” was very satisfactory with both protocols (IP and FP) and both types of measurements (in-nose concentration and sensory perception), as reflected by the absence of significance of the panelist  $\times$  sample interaction. In this regard again, IP does not provide any improvement over FP. On another level, it is interesting to notice that the interpersonal differences noted in instrumental data are similar to or lower than the variability in sensory data, with both protocols. While the existence of substantial interpersonal differences in *in vivo* PTR-MS data is recognized (Roberts et al. 2004), to the authors’ knowledge, these differences have not been previously reported and compared with the magnitude of interpersonal differences commonly found in sensory studies. With this in mind, it was investigated if panelists’ ability to discriminate between the low and the high levels was related to instrumental measurements of in-nose aroma concentration. No such link was found: the  $R^2$  between PTR-MS and sensory individual panelists’ sample  $F$  values for Imax and AUC are 0.0234 and 0.0133, respectively, and no differences were observed between the 2 protocols. For this present study, it can be hypothesized that differences between the panelists both at the peripheral (at the receptor level) or central level (different scale usage by the panelists) superseded differences between the panelists in terms of in-nose aroma concentration. To summarize, using IP instead of FP did not reduce interpersonal variability nor increase agreement between panelists, neither for PTR-MS nor sensory data.

Another important aspect is the comparison of the ability to discriminate between the “high” and “low” levels with both approaches (in-nose concentration and sensory perception). The discrimination power of the PTR-MS measurement appears to be very satisfactory under both protocols, even larger with FP compared with IP, as indicated earlier. The discrimination power in sensory measurements is also very satisfactory under both protocols, with no strong evidence of one protocol being superior to the other. The absence of obvious benefits of using IP versus FP is contrary to the study’s initial hypothesis, stating that standardized chewing and swallowing rhythms across panelists would limit interpersonal variability and maximize the discrimination power for both instrumental and sensory measurements. One explanation could be that the intensity differences between the samples were rather

straightforward, leaving not much room for improvement under any protocol. Finally, from a pragmatic standpoint, the FP is simpler to use than IP and requires less training of the panelists. Moreover, the presence of a beating sound and signal to swallow was mentioned by some panelists as being “distracting” from the task (scoring intensity). Overall, the present study showed no obvious benefits (enhanced data homogeneity or discrimination power) of imposing a strict protocol.

### Sequence effect, in-nose concentration, and perceived intensity

The second objective of the study was to assess the impact of carryover effects on perceived intensity and in-nose concentration. The impact of carryover effects on perceived intensity were analyzed taking into account the concept of conditional stimuli (Lee and O’Mahony 2007). For both FP and IP, significant and consistent carryover effects were observed for sensory data. The fact that hH and hL are respectively perceived as less intense than H and L is most likely due to adaptation. In this case, the first sample—or “adapting stimulus”—is “high” and tends to induce adaptation at this level. Therefore, when the second sample is evaluated, either a “high” or a “low” level (that is at the same level or below the adapting stimulus), it tends to be perceived as less intense than if it had been in first position (H or L). Under these circumstances, one could have expected IH to be perceived as less intense than H. Indeed, adaptation to the first sample (low) should have decreased the perceived intensity of the second sample (high). However, IH is perceived as about as intense as H. Here, a contrast effect may have overruled the adaptation effect induced by the “low” sample and enhanced the perceived intensity of the second sample “high.” In this case, adaptation and contrast effects seem to have canceled each other out. All together, the results revealed significant carryover effects on perceived intensity, which can be explained by adaptation and contrast effects.

Carryover effects of one sample to the next on in-nose aroma concentration can be expected to originate from physicochemical phenomena happening in the oral cavity. Indeed, after swallowing food, particles remaining in the mouth may participate to extend the duration of aroma release. Aroma compounds adsorbed to the oral mucosa can also subsequently be released from it (Buettner and Welle 2004). Both factors are expected to increase the overall amount of aroma measured in the oral cavity for the samples evaluated in second position compared with those evaluated in first position. Consistent with this hypothesis, an ANOVA revealed that samples in second position have a significantly ( $P < 0.05$ ) higher AUC than the samples in first position, with IP. Also, still with IP, hH has a significantly higher AUC than H, and IH is intermediate. This further supports the aforementioned hypothesis of isoamyl acetate being released into the mouth and nose cavities after swallowing,

both from the oral mucosa and food remains in the mouth. However, contrary to IP, with FP, the AUC of the samples evaluated in second position is not significantly higher ( $P > 0.05$ ) than the AUC of the samples in first position. This difference between the two protocols can be attributed to the shorter residence time of the samples in the mouth, and subsequently to a longer natural cleansing period before the evaluation of the second sample. Indeed, under IP, the chewing time was longer than under FP, therefore the residence time of the samples in the mouth was increased, aroma release in the nose was delayed, and the resulting sensory perception lasted longer, overall.

Finally, for both IP and FP, it can be noted that carryover effects observed for perceived intensity and for overall in-nose concentration can go in opposite directions. This indicates that the sole knowledge of the in-nose concentration at a given time point is not always enough to predict the perceived intensity of a given sample. This reinforces the need to consider past events to account for cognitive or physiological factors such as adaptation or contrast effects, as noted by Wright and Hills (2003). Although established under different experimental conditions, these recommendations are in-line with the results reported by Baek et al. (1999) and with the limitations of instrumental measurements of in-nose aroma concentration as highlighted in reviews on the topic (Piggott 2000; Buettner and Beauchamp 2010). To summarize, the panelists' perception of aroma intensity was strongly influenced by carryover effects, which could not be accounted for by in-nose aroma measurements because the latter revealed either no carryover effect (FP) or carryover effects going into different directions than for perception (IP).

### **Chewing activity, in-nose concentration, and perceived intensity**

The third objective of the study was to investigate the links between chewing activity, in-nose aroma concentration, and sensory perception over time. The examination of the times at maximum chewing activity, maximum in-nose concentration, and maximum perceived intensity under FP and for the high level suggest a clear "cause and effect" relationship. Indeed, under these conditions, the time at maximum chewing activity comes first (5.5 s), followed by the time at maximum in-nose concentration (between 20 and 30 s depending on the samples), followed by the time at maximum perceived intensity (around 30 s). This corresponds to the time needed for the gelled candy to dissolve, the aroma to migrate and reach the nose cavity in a breathing interval. Interestingly enough, the difference between the in-nose concentration  $T_{max}$  and perceived  $T_{max}$  is rather small (on average, 2–5 s). This tends to suggest that changes of aroma concentration in the nose would be perceived rather quickly, under normal eating conditions. The bottleneck stage therefore seems to be the time to reach the nose cavity. The existence of a time delay between the  $T_{max}$  of (retronasal) in-nose concentration

TI and the corresponding  $T_{max}$  of sensory TI curves was already reported before (Overbosch et al. 1991), and this phenomenon is usually attributed to the temporal integration of the stimulus (Berglund and Lindvall 1982; Linforth et al. 1999). The use of an olfactometer made it possible to investigate this effect further on orthonasal aroma perception by tightly controlling the variations over time of aroma concentration in the nasal cavity (orthonasal conditions), while asking the panelists to score perceived intensity. This revealed slightly larger differences between the times at maximum in-nose concentration and perceived intensity (about 9–10 s later). This suggests that when panelists only had the olfactory stimulus at their disposal (without textural and taste cues), it took them slightly longer to detect variations of aroma concentration in the nose. This also suggests that both texture and taste may have influenced aroma perception, in the present case. Another argument in favor of the influence of texture and sweet taste on aroma perception is the fact that bigger time discrepancies between in-nose aroma concentration and perception were observed for hL and hH, especially under IP where the residence time of the samples in the mouth was longer. For example, for hL under IP, the time at maximum intensity for in-nose concentration and sensory perception are 12 and 40 s, respectively. This represents a 28-s difference, which largely exceeds what was observed under orthonasal conditions (9–10 s). Another example of this phenomenon is the fact that under FP,  $T_{max}$  for H occurs significantly later than for L for the PTR-MS data but not for sensory data where both  $T_{max}$  occur at the same time. To summarize, a large part of these apparent discrepancies between  $T_{max}$  for in-nose concentration and sensory perception may be explained by the influence of sweet perception (Davidson et al. 1999) and potentially by the presence of food particles in the mouth and the texture perception through mastication activity (Weel et al. 2002). The present study focuses solely on gelled candy, but it could be expected that, for example, a hard brittle candy would provide different results. Similarly, a completely different matrix, such as a liquid food product, would behave differently in mouth and not provide a similar lasting perception as in-mouth particles do. Because of this, the present conclusions would have to be modulated for a product with a different texture and composition.

Finally, as could be expected, under IP, the influence of swallowing can clearly be seen on both the electromyographic measurements (transient increase in masseter activity right before swallowing) and on the in-nose concentration (transient increase right after swallowing), because all the panelists swallowed at the same times. The observed effect of swallowing on transient aroma in-nose concentration increase is consistent with previous studies (Buettner et al. 2001; Buettner et al. 2002; Hodgson et al. 2003). From the present study, however, it is not clear if this transient aroma in-nose concentration increase truly leads to an increase in sensory perception. It is possible that the panelists

consciously or unconsciously minimized the importance of this transient increase precisely because it came right after a swallowing event, the result being an integrated and smoothed signal over time (Mestres et al. 2006). This would be in accordance with Linforth et al. (1999), but not with Mestres et al. (2006), who reported a strong link between rapid aroma concentration changes in the nose and the resulting perception.

## Conclusions

The study aimed at assessing the benefits of imposing a strict chewing and swallowing pattern on panelists to carry out in vivo measurements, and about the quality of the link between instrumental data and their perception counterpart. Contrary to what was initially hypothesized, imposing a strict chewing and swallowing pattern on panelists did not provide substantial improvement of instrumental or sensory data in terms of reduction of interpersonal variability, increase of the agreement between the panelists, or ability to discriminate between the high and low aroma intensity levels. In addition, the study highlighted the role of brain integration on perceived intensity and dynamics of perception, in the framework of sequential eating without rinsing. Because of the presence of adaptation phenomena and contrast effects, dynamic in-nose concentration data provide only one part of the perception picture and therefore cannot be used alone in prediction models. The investigation of the time delays between the T<sub>max</sub> of chewing activity, in-nose concentration, and sensory perception signals strongly support the hypothesis that aroma perception was largely influenced by taste and texture.

## Acknowledgements

The authors wish to warmly thank Matt Spears for his precious support in organizing the panel and in carrying on the experiments so smoothly. Mark Yate's expertise in physiological measurements and help in setting up the EMG part is greatly appreciated. Last, the authors are thankful to all panelists for their patience and willingness to participate in the study.

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