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Sensory Perception is Related to the Rate of Change of Volatile Concentration In-nose during Eating of Model Gels

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Abstract

The relationship between perceived aroma and the volatile concentration measured in-nose was investigated during eating of a model food. Sensory ranking and time–intensity analysis (TI) were used to measure perceived aroma, while in-nose volatile concentration was monitored by atmospheric pressure ionization mass spectrometry, which produced time release data. A gelatine–sucrose gel with a range of gelatine concentrations (2–8% w/w) and flavoured with furfuryl acetate was used as the model food. Sensory scaling showed decreased flavour intensities and TI showed a decrease in the flavour perceived over time, as the gelatine concentration increased. Studies in model systems and in people demonstrated that the different rates of release observed for different gelatine concentrations were not due to binding of volatile to protein in the gel, nor to mucous membranes, but were due to different rates of gel breakdown in-mouth. There were no significant differences in the maximum in-nose volatile concentrations for the different gelatine concentrations, so the amount of volatile present did not correlate well with the sensory analysis. However, the rates of volatile release were different for the different gels and showed a good correlation with sensory data.

Introduction

The link between the chemical signals (the stimuli) that cause flavour and the sensory perception (the response) they evoke has been the subject of much research. The concept of psychophysics was proposed 150 years ago to explain the observed relationship between the sweet response noted after stimulation of the taste buds with various sugars (for review see Hoppe, 1995). Stevens (1957) developed and elaborated methods for determining stimulus magnitudes and response sensations (often called direct scaling methods). The Power Law derived by Stevens has been widely applied to correlate taste and odour sensations with the concentrations of flavour chemicals in foods.

However, time–intensity analysis (TI) shows that there is a temporal dimension to flavour perception. Overbosch (1986) proposed that the temporal dimension to aroma perception was due to adaptation. If the receptors were subject to a constant level of stimulation by a volatile aroma compound, then the response should decrease with time and, eventually, no response would be observed. The rate of adaptation depends on the aroma molecule but, using data from the literature, Overbosch (1986) proposed a mathematical model that calculated the adaptive process and subtracted it from the stimulus applied, to obtain the actual signal that triggered the receptor. He predicted that significant adaptation could occur within the time taken to chew and swallow food. A refinement of the model was published in 1989 (Overbosch and de Jong, 1989). Overbosch (1986) acknowledged that the situation that occurs with a dynamic stimulus was more complex but showed an idealized plot of the relationship between stimulus and response. In his model, the time to maximum intensity (T_{max}) for both stimulus and response was identical but subsequent work (Linforth *et al.*, 1998) has suggested that another mechanism besides adaptation may occur in the early stages of eating. Furthermore, the averaging methods of Overbosch may have obscured subtle differences in T_{max} between stimulus and response curves. It should also be recognized that the Overbosch models consider the aroma of single volatiles and take no account of potential interactions between volatiles, which may change the relationship between stimulus and response, as has been proposed in other models (see for example Ennis, 1996).

The advent of methodology to follow volatile release as people eat foods [and at concentrations that relate to the odour thresholds of many aroma compounds (Linforth *et al.*, 1996)] has renewed interest in the relationship between aroma stimuli and perceived responses. There is evidence that flavour compounds are delivered at different rates to the

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receptors in a wide range of real foods, e.g. sugar confectionery (Ingham *et al.*, 1995), cheese (Delahunty and Piggott, 1995), chocolate (Roozen and Legger-Huysman, 1995) and tomatoes (Linforth *et al.*, 1994). Using a direct inlet system for atmospheric pressure ionization-mass spectrometry (API-MS) (Linforth and Taylor, 1998), the release of volatiles from foods can be measured in real time by sampling air from the nose or mouth of people eating foods, with detection at the 10 to100 ppbv level in the gas phase. The data obtained can be considered analogous to sensory TI data and, for convenience, have been termed time release (TR) curves.

The models of Overbosch (Overbosch, 1986; Overbosch and De Jong, 1989) provide one explanation for linking volatile concentration with aroma perception and are applicable to the TR and TI data now available with the real time assays. TI, however, is a scientific tool for following perceptual changes in trained panellists and it is unlikely that the typical consumer is consciously aware of the TI profile of foods when they judge flavour quality and/or intensity. The question remains, therefore, whether these time-related volatile profile changes during eating have any relevance to the consumer and their perception of food flavour and whether the measurement of TR data is useful in developing food products for the general public or just a scientific curiosity.

The purpose of this paper was to investigate how the release profiles of volatiles were related to aroma intensity as perceived by sensory scaling methods. Some TI was carried out as a comparison. Gelatine–sucrose gels were chosen as a model food as they are known to deliver flavour at different rates when the gel formulation is changed (Guinard and Marty, 1995; Wilson and Brown, 1997). To avoid any problems of interaction between volatiles, a single volatile (furfuryl acetate, FFA) was used which had an easily recognizable, characteristic flavour so that sensory panellists could readily recognize the compound. Another advantage of FFA was that it shows relatively low persistence in-mouth and therefore minimizes problems of carry over from one sample to another.

Materials and methods

Gelatine gel system

Gelatine (250 bloom strength; DGF STOESS, Germany) gels were prepared containing 20.5% w/w sucrose and 22.5% w/w glucose syrup (Cerestar, UK). The gels were prepared by dissolving the gelatine at 60°C, prior to mixing with the sucrose–glucose mix (which had been boiled and allowed to cool). The gels were flavoured by adding FFA (Firmenich SA, Switzerland), dispersed in propylene glycol, to the cooled gelatine–sugar mixture before gelation occurred. The gelatine gels contained different volatile and gelatine concentrations, as shown in Table 1. The gels were cut into cubes (6.0 ± 0.1 g) and stored overnight at 4°C. The following day, the gels were equilibrated at room temperature before being presented to the panellists.

Table 1 Concentrations of gelatine and FFA in the gels

Gelatine concentration (% w/w)	FFA concentration in gel (mg/kg)
2.0	100, 300, 600, 1000, 1500
3.5	300
5.0	0, 100, 300, 600, 1000, 1500
6.5	300
8.0	100, 300, 600, 1000, 1500

Sensory evaluation of gels: scaling

Sixteen trained panellists were asked to rate the relative maximum intensity of FFA perceived whilst eating gelatine gels. The samples were randomly coded with three-digit numbers and presented in random order. Panellists were asked to rate the samples relative to two reference samples: an unflavoured 5% w/w gelatine gel, representing average textural properties, and a flavoured 2% w/w gelatine gel, which caused the highest sensory perception. The two gels represented 0 and 10 on the intensity scale respectively. The panellists were trained to focus on the flavour and ignore differences in sweetness and texture. Each panellist assessed one set of gel samples and waited at least 2 min between samples. Odourless water and dry crackers were available to remove traces of gels between samples. The mean intensity values from the 16 panellists were calculated for each gel.

Sensory evaluation of gels: time intensity

Eleven trained panellists were asked to rate the perceived flavour intensity with time while eating the gels. TI was performed simultaneously with TR data collection and the TI signal was combined with the MS data using an analogue channel of the mass spectrometer (one data point collected every second). The resulting traces were processed to yield T_{max}, I_{max} and gradient data. Each panellist was given one set of gels and had 4 min between samples during which they could use water or crackers to remove traces of gel from the mouth. The breath of each panellist was monitored by API-MS before the next gel was consumed and no significant carry over between samples was observed.

Instrumental analysis: model system

The release of FFA was measured from a cube of gel as it dissolved in 100 ml of distilled water (37°C) in a 125 ml flask with stirring. Samples of the headspace (HS) were drawn into the API-MS at 12 ml/min. Release profiles for FFA were monitored in duplicate for up to 45 min.

Instrumental analysis: TR during eating

Panellists were given samples of the gels to eat while the

FFA content in-nose was monitored by sampling the air flow from one nostril over a 2 min period (no specific eating instructions were given). The breath was introduced (25–30 ml/min) into a modified API source (Linforth and Taylor, 1998) fitted on a Platform II Quadrupole mass spectrometer (Micromass, Altrincham, UK), where the FFA was ionized by a 4 kV corona discharge. The mass spectrometer was used in positive ion mode, with a cone voltage of 20 V, and set to monitor *m/z* 80.8 (a fragment ion of FFA). The raw breath by breath traces were converted into TR curves by smoothing the peak height data and converting peak height into actual concentration in air (nanolitres of volatile per litre of air; ppbv) after calibration of the API-MS interface with a series of FFA standards. From the TR curves, the parameters maximum intensity (I_{max}) and time to maximum intensity (T_{max}) were calculated along with the rate of volatile release, which was defined as the gradient on the up-slope t_{25} (time to 25% of I_{max}) and t_{75} (time to 75% of I_{max}).

Results

Sensory evaluation of gelatine gels containing FFA

Initially, the gel samples containing FFA were evaluated sensorially to ensure that this volatile showed release characteristics from the gelatine–sucrose system similar to the volatiles used previously (benzaldehyde, D-limonene, ethyl butyrate: Guinard and Marty, 1995; commercial banana flavour: Wilson and Brown, 1997). Sixteen panellists each ate a gel and recorded their relative overall flavour intensities using two reference gels. Figure 1 shows the mean flavour intensities (sixteen panellists) from five gel samples containing 2–8% w/w gelatine but with FFA at the same concentration (300 mg/kg). There was a clear trend of decreasing sensory intensity with increasing gelatine concentration and the sensory values for the 2% w/w and 8% w/w gels were significantly different ($P < 0.001$, ANOVA), which agrees with previous work (Guinard and Marty, 1995; Wilson and Brown, 1997).

Sensory evaluation: TI

TI was carried out using gels of different gelatine concentrations (2–8% w/w), all of which contained the same amount of FFA (300 mg/kg). The TI data were averaged using a method similar to that described by Overbosch *et al.* (1986). The TI data were first normalized in the intensity direction to the numeric average of I_{max}, followed by averaging in the time dimension (Figure 2). The TI profiles showed a significant difference between the different gels, with the I_{max} decreasing ($P < 0.001$, ANOVA) and the T_{max} increasing ($P < 0.001$, ANOVA) as gelatine concentration increased. Again, this confirmed the results obtained with banana flavour by Wilson and Brown (1997) and, despite the different volatiles, the increase in gelatine concentration in both studies resulted in a decrease in sensory perception.

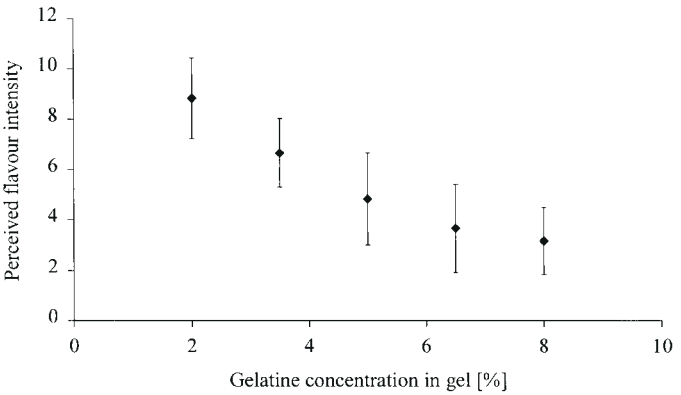

Figure 1 Average sensory score (± SE) of perceived flavour intensity for sixteen panellists eating gelatine gels of different gelatine concentrations flavoured with 300ppm FFA. Each panellist ate one set of gels.

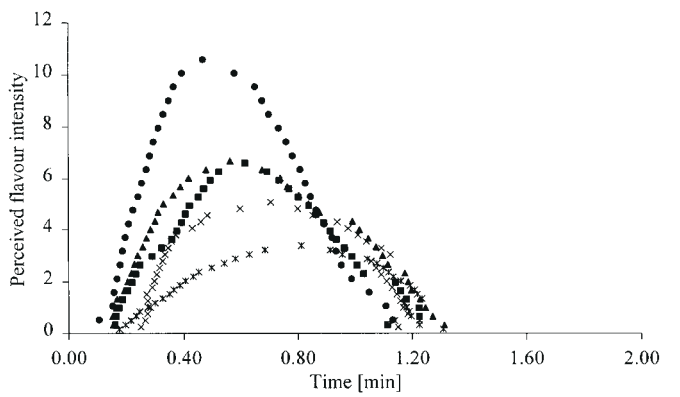

Figure 2 TI profiles of perceived flavour intensity of FFA (300 mg/kg). ●, 2%, ■, 3.5%, ▲, 5%, ×, 6.5% and ✻, 8% w/w from gelatine gels. Each curve is the mean result for eleven panellists each eating one sample of each gel.

The increase in T_{max} might be explained by an increase in melting point (Wilson and Brown, 1997), a different rate of dissolution and/or a different rate of breakdown due to chewing efficiency (Brown *et al.*, 1995).

The differences in I_{max}, however, might be due to binding of the volatiles to gelatine, which reduced the amount of volatile available for release—a situation that has been reported for various volatiles and proteins (see e.g. Nawar, 1971; Solms *et al.*, 1973; Landy *et al.*, 1995). Alternatively, the decrease in I_{max} with increasing gelatine concentration could be due to a slower rate of release in mouth, with the high gelatine gels releasing the same amount of volatile as a low gelatine gel, but over a longer time period. Since the time in-mouth is limited, the slower release rate might produce a lower I_{max} value for high gelatine gels.

Instrumental analysis: model system

To investigate which of these mechanisms was valid, a model system was set up. Gels containing the same concentration

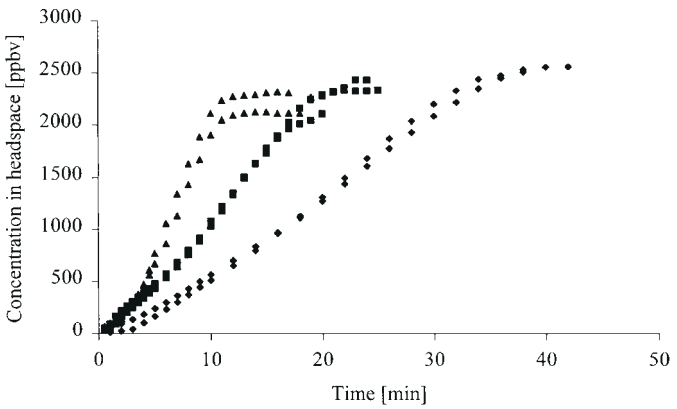

Figure 3 Release of FFA from gelatine gels in model systems. ▲, 2%, ■, 5% and ◆, 8% w/w. Duplicate release curves are shown for each gel concentration.

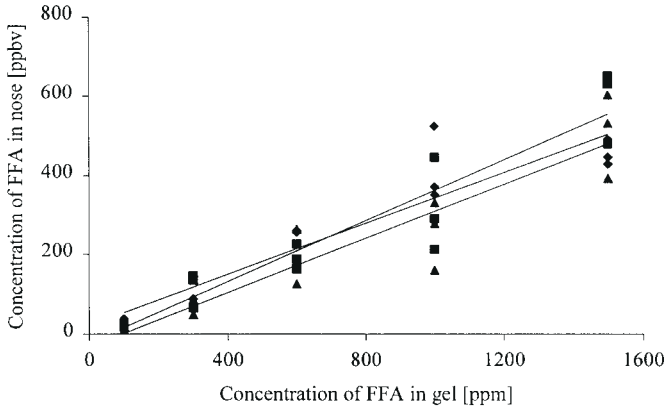

Figure 4 Effect of FFA concentration on TR I_{max} for one panellist, consuming gels with different gelatine concentrations: ◆, 2%, ■, 5% and ▲, 8%.

of FFA (300 mg/kg) but different gelatine concentrations (2, 5 and 8% w/w) were placed in water and stirred, and the FFA release was measured by monitoring the HS until the gel had completely dissolved. The results (Figure 3) demonstrated that the three gels released FFA at different rates, with the softest gel (2% w/w gelatine) becoming fully dissolved after 10 min, whereas it took 40 min for the harder gel (8% w/w gelatine) to dissolve completely. However, irrespective of gelatine concentration, all gels reached the same concentration of FFA in the HS when fully dissolved, showing there was no binding of FFA to gelatine under these conditions and that slower release was the most likely explanation for the difference in TI performance of the gels. This agrees with work by Harrison and Hills (1996), who found that increasing the gelatine and sucrose concentrations of a gel system resulted in reduced release rate for a water-soluble dye which they used as a marker for gel dissolution.

Instrumental analysis: TR

The model system work suggested that the rate of release was responsible for the differences in TI. Binding of FFA to gelatine could not explain the TI differences but there was a possibility that FFA could be binding to membranes in the mouth and nose so that only a portion of the FFA release was actually transported to the olfactory receptors. To test this hypothesis, the in-nose concentration of FFA during eating was monitored for a series of gels with different volatile and gel concentrations using one panellist to minimize person-to-person variation. If no significant binding of the volatile was occurring, plotting the maximum concentration of FFA in-nose against the gel FFA concentration should produce a linear plot. Figure 4 shows the data obtained from three replicates of three different gel concentrations with five FFA concentrations. Despite the variable nature of the eating event, the lines are linear, suggesting that no significant binding of FFA occurs between the release event in-mouth and perception in-nose. The above experiment was repeated using three extra panellists. The relationships between the gel volatile concentration and breath volatile concentration were still linear for each panellist (data not shown), but there were substantial differences between panellists in the amount of FFA released from the same gels, presumably because of the different chewing patterns of the panellists (Brown *et al.*, 1995).

Comparison between instrumental and sensory data

Gels containing 300 mg/kg FFA were prepared with gelatine concentrations of 2, 5 and 8% w/w, and simultaneous TI and TR data were collected from 11 panellists who ate one sample of each gel. The mean values for the two sets of data are plotted in Figure 5 and show that there is a clear decrease in sensory perception with increasing gelatine concentration; however, the changes in volatile concentration in-nose are less clear. Statistical analysis of the the three gel concentrations showed no significant difference in the TR I_{max} values but a significant difference ($P < 0.001$, ANOVA) for the TI values. These findings suggest that, in this system, perception is not directly related to the maximum volatile concentration in-nose as might be predicted by the basic Power Law. This supports the necessity to modify the Power Law to take account of temporal changes. However, whereas Overbosch based his model on data obtained from a constant volatile stimulus, these data were obtained from a food during eating where the stimulus is dynamic and changes rapidly over a period of just over 1 min (see TR data in Figure 5).

To test whether the Overbosch (1986) adaptation concept was applicable to the data reported here, the ratios of the TI:TR T_{max} values were calculated from each of the individual curves that made up the mean values in Figure 5 and then plotted against the TR T_{max} values. If adaptation was occurring, then the receptors should become progressively less receptive to an increase in TR so that the TI peak

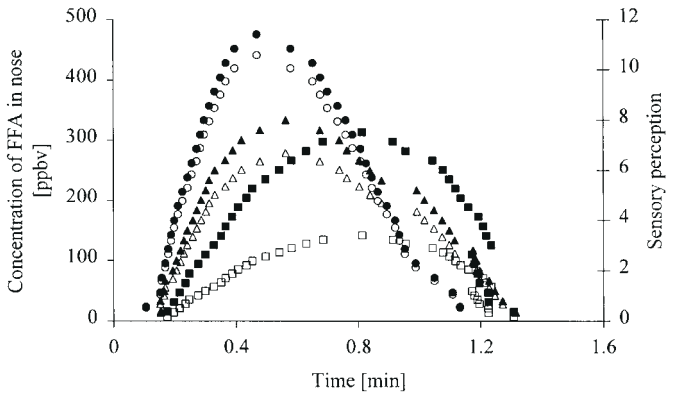

Figure 5 Volatile release profiles (TR; solid markers) and TI sensory data (open markers) from ●, 2%, ▲, 5% and ■, 8% w/w gelatine gels flavoured with 300 mg/kg FFA. Each curve is the mean value obtained from 11 panellists each eating one sample of each gel.

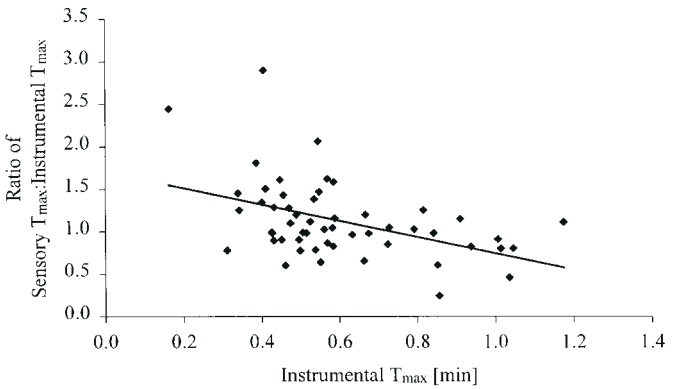

Figure 6 Relationship between the ratio of the sensory T_{max} and Instrumental T_{max} plotted against instrumental T_{max}.

should occur before the TR peak and the ratio of TI:TR should be <1. Figure 6 shows that the data are scattered but the line of best fit ($R = 0.421$) reveals that the TI:TR ratio is in fact >1 until the TR T_{max} reaches 0.6 min, after which TI:TR is <1. Although these results need to be treated with caution, due to the scatter and the fact that they represent only one volatile, they suggest that there are two processes taking place, an initial lag phase where the T_{max} for perception occurs later than the T_{max} of the stimulus and then an adaptive phase where the T_{max} for perception occurs earlier than the T_{max} of the stimulus. However, similar trends have been noted with other volatiles (Linforth *et al.*, 1998) and further modification to the models proposed by Overbosch (1986) and Overbosch and de Jong (1989) may be necessary to describe the full range of events that occur over the time course of eating.

Another parameter that can be extracted from the data is the rate of release, which expresses the FFA concentration in-nose as a function of time. The rate of release was defined as the gradient (1). The points t_{75} and t_{25} were used because,

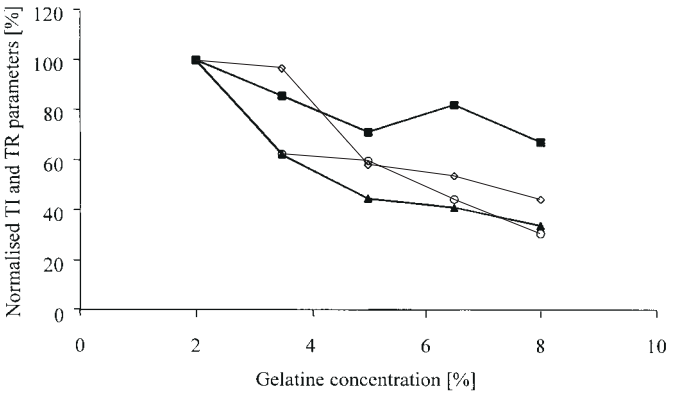

Figure 7 Relationship between sensory and instrumental data as gel concentration changes: ■, TR I_{max}, ◇, sensory score, ○, TI I_{max} and ▲, TR gradient. For a comparison, all data have been normalized so that the values for the 2% w/w gel are 100% w/w. Each point is the mean value from 11 panellist eating five different gels flavoured with 300 mg/kg FFA.

in most cases, this region of the TR curve was essentially linear whereas the situation was more complex above, and below, these values.

$$\text{Gradient} = I_{max}/2(t_{75} - t_{25}) \quad (1)$$

To compare the relationships between the TR I_{max}, the TI I_{max}, the sensory scaling score and the gradient, the values were all expressed on a relative scale (the value for the 2% w/w gel was taken as 100%). A plot of the relative values against gel concentration (Figure 7) suggested that the gradient correlated better with the sensory values than TR I_{max}. To confirm this trend, the actual values were analysed statistically. The changes in the actual TR I_{max} values were not statistically significant. There was, however, a significant decrease in the instrumental gradient ($P < 0.01$) and in the two sensory parameters TI I_{max} value ($P < 0.001$) and sensory scaling score ($P < 0.001$) with increasing gelatine concentration. These experiments demonstrate that the temporal aspects of volatile release are related to aroma perception and that the ability to measure volatile concentration in-nose simultaneously with aroma perception provides new opportunities for examining the relationship between the volatile stimulus and the aroma response.

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