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Simultaneous instrumental and sensory analysis of volatile release from gelatine and pectin/gelatine gels

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Abstract

Release of volatiles from gels during eating was measured by monitoring the volatile composition of breath, using on-line atmospheric pressure chemical ionisation–mass spectroscopy (APCI–MS), and by simultaneous sensory time–intensity (TI) evaluation. The time–release (TR) and TI curves were very similar, suggesting a simple relationship between changes in volatile concentration over the eating time course and the intensity of volatile perception. Analysis of the T_{max} values (time to maximum intensity) showed that the ratio between the T_{max} for the sensory and instrumental data (sensory T_{max}/instrumental T_{max}) varied with the instrumental T_{max}, such that at low T_{max} values the ratio was >1.0 and, as T_{max} increased, the ratio decreased to below 1.0. This trend suggested that with short eating events there may be a lag in perception after the maximum breath stimulus concentration. Conversely if the eating process took longer, sensory adaptation might occur.

Keywords: APCI; TI; Gelatine volatile release

1. Introduction

Aroma is acknowledged to play a major role in determining flavour quality and there has been a long standing interest in relating the flavour perceived to the mixture of chemicals that are responsible for that perception. Most people are familiar with the concept of an initial food flavour that develops as the food is chewed, and may then be followed by an aftertaste. Sensory analysis using the time–intensity (TI) technique can measure the flavour changes that occur over the eating time course, and has been used to study the effects of the food matrix on flavour release and perception. Wilson and Brown (1997) have shown that increasing the gelatine content of gelatine gels modifies both the break strength and the melting point. This in turn affects the eating pattern of individuals and also has significant effects on the perceived flavour intensity of the gels (Guinard & Marty, 1995; Wilson & Brown, 1997).

Techniques to measure volatiles in the nose or mouth during eating using Tenax traps to collect samples of breath (Delahunty, Piggott, Connor, & Paterson, 1994; Ingham, Linforth, & Taylor, 1995; Linforth, Ingham, & Taylor, 1996; Roozen & Legger, 1995), have demonstrated that the actual concentrations of flavour compounds change with time and this has been termed the time release (TR) profile. However, analysing the TR profile by such methods is very time-consuming due to the number of separate chromatograms that have to be run to produce just one time course.

Alternative methods were developed using membrane separators to allow the introduction of volatiles into the electron impact source of a mass spectrometer whilst excluding air and water (Soeting & Heidema, 1988). These enabled the analysis of TR profiles in real time (although the system does have limitations because of the selective permeability of the membrane for different compounds). Overbosch used this system to produce TR curves which could then be compared with TI data (Overbosch, 1987). The TR and TI profiles were found to exhibit differences consistent with those predicted by a theoretical model, on the effect of adaptation on the relationship between TI and TR curves (Overbosch, 1986).

Alternative methods have subsequently been developed which do not utilise membranes to separate the gas phase from the ionisation region of the mass

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spectrometer. The technique of atmospheric pressure chemical ionisation–mass spectrometry (APCI–MS) has been widely used in our laboratory to analyse gas phase samples in real time. The method has some limitations because it can only discriminate compounds on the basis of mass; however, it can provide rapid and detailed information on the breath-by-breath release of volatiles in the expired air from the nose (Linforth et al., 1996). This breath-by-breath profile is thought to be very similar to that sensed by the olfactory epithelium and may be different from the volatile composition of the food and the headspace profile, because of the changes that occur to the physical state of foods during eating (Overbosch, Afterof, & Haring, 1991; Taylor & Linforth, 1996).

Preliminary experiments (Linforth, Taylor, & Brown, 1998; Taylor & Linforth, 1997) studied the simultaneous TI and TR profiles of gelatine gels containing benzaldehyde or isoamylacetate. The traces obtained showed that the sensory perception lagged behind the in-nose concentration, suggesting a delay between the presentation of the stimulus and the sensory response. However, with limited time and just two panellists, the data set was not sufficient for rigorous data analysis. TI and TR traces were also obtained from chewing gum using a wider range of people (Linforth & Taylor, 1998). In this case, although the concentration of menthol and menthone increased and then remained at a constant level, perception of mint flavour decreased substantially within 5 min. This is presumably the result of olfactory adaptation (Köster & Wijk, 1991).

This paper presents simultaneous TI and TR data from gelatine/sucrose gels containing either menthol or dimethyl pyrazine and pectin/gelatine/sucrose gels containing ethyl butyrate. The composition of the gels was chosen to provide a range of release times that lay between those of the chewing gum and gelatine gels described above. The single volatiles were chosen as each has a characteristic, easily identifiable aroma. The TR and TI curves were obtained for each gel sample and the relationship between them examined.

2. Materials and methods

2.1. Gel systems

The composition of the gelatine gels is shown in Table 1. The gels were prepared by dissolving gelatine (250 Bloom) in water at 60°C in a water bath, before it was combined with the sucrose/glucose syrup solution (which had previously been boiled and cooled to below 100°C). The volatiles (dimethyl pyrazine and menthol) were dissolved in propylene glycol prior to their addition to the gelatine/sugar solutions, when they were at approximately 60°C.

Table 1
The composition of the gelatine gels

Gel	% Gelatine	% Sucrose	%Glucose syrup	Menthol (ppm)	Dimethyl pyrazine (ppm)
1	3	15	20	0	250
2	6	30	40	0	500
3	9	30	40	0	1000
4	3	15	20	150	0
5	6	30	40	300	0
6	9	30	40	450	0

The pectin/gelatine gels were prepared by adding a solution of glucose syrup, sugar and sorbitol to a sugar/pectin solution. A solution of gelatine and citric acid was then added to produce a gel with a final composition of: 0.75% pectin, 7.5% gelatine, 4% sorbitol, 30% sucrose, 30% glucose syrup, and 0.5% citric acid. This solution was then diluted to produce gels with pectin concentrations of 0.625, 0.5 and 0.375%. Ethyl butyrate (600 ppm) was added to the gels as described above.

The gels were cut into cubes (6 g) and stored at room temperature for 24 h before presentation to the panellists.

2.2. Instrumental analysis

The breath volatile composition was monitored using a mass spectrometer (Platform II, Micromass, Manchester, UK) fitted with a modified atmospheric pressure chemical ionisation source (Linforth & Taylor, 1998). As the individuals ate the gelatine gels, a small proportion of their breath was drawn into the source (25 ml min^{-1}) via a heated (60°C) deactivated fused silica transferline. Volatile compounds were ionised by a 4 kV corona discharge, then sampled into the high vacuum region of the mass spectrometer. Selected ion mode (0.05 s dwell on each ion) was used to monitor menthol, m/z 138.9 (MH^+-H_2O), dimethyl pyrazine, m/z 108.8 (MH^+) and ethyl butyrate, m/z 116.8 (MH^+). Calibration was achieved by comparison of the peak heights of the compounds present in breath, with the peak heights produced by the introduction and vaporisation of a hexane solution containing known amounts of each compound.

2.3. Sensory analysis

Six panellists trained in time intensity (TI) analysis were given two samples of each of the gels to familiarise themselves with the flavour characteristics and maximum flavour intensities they would experience. They were then instructed to move a lever to show their perception of the aroma compounds when eating the gels (one replicate of each gel per panellist). The panellists were asked to focus on the temporal changes that

occurred over the eating time course (onset of flavour, rate of increase, rate of decline) and move the lever accordingly. The output from the lever was fed directly into one of the analogue channels of the mass spectrometer (data points were collected once a second), allowing simultaneous collection of TI and instrumental data.

3. Results and discussion

3.1. TI and TR profiles of gelatine gels

The composition of the gelatine gels was varied (Table 1) to produce a series of samples that were broadly similar but which would be expected to have different release characteristics. The gels were presented to the panellists with no specific eating instructions such that the duration of the eating process was not regulated. The combination of different physical properties and variation in the eating pattern (of individuals) was intended to create a wide range of eating times and therefore provide TR and TI curves with differing values for parameters such as T_{max} (time to maximum volatile concentration or perceived intensity, respectively).

Average TI and TR curves were produced using two methods. The first calculated the numeric average of the intensity values for the six panellists at regular time intervals (a value at 0.5 min would represent the average of the intensity values for all six panellists at that time). However, this method has the disadvantage that the average value can be influenced if one panellist has a particularly high or low intensity value at that time value compared to the others (Liu & MacFie, 1990). The second approach was based on the method of Overbosch, Van den Enden, and Keur (1986) where the curves for all six panellists were first normalised to the numeric average of I_{max} and then the median time values were determined at regular (every 5%) intensity values. Thus a data point at 50% of I_{max} on the upslope of the TR curve would represent, the median of the time it took for all six panellists to achieve a breath concentration of 50% relative to the maximum.

The curves produced by simple averaging or by normalising the data showed very similar patterns (Figs. 1 and 2, respectively) for both the TI and TR data, irrespective of the data analysis method used. This was true of the curves before and after T_{max} unlike the previous report on benzaldehyde and isoamylacetate release from gels (Taylor & Linforth, 1997) where the TI curve lagged behind the TR curve after I_{max} had been reached. The similarity between TI and TR curves suggested a simple linear correlation between the chemical stimulus and the perceived response. It was not necessary to use a logarithmic or power law function to adjust one set of data to fit the other as has been proposed in the Psychophysical Laws (see Hoppe, 1997, for review). Such a result was unexpected. The strong trigeminal cooling effect of menthol might have affected panellists' ability to follow the olfactory menthol signal and the 9% gels released volatiles over a long time (TR T_{max} up to 1.3 min) such that adaptation might have occurred.

While there was a good fit between the shape of the TI and TR curves, the panellists intensity scores did not correlate well with the in-nose concentrations of the

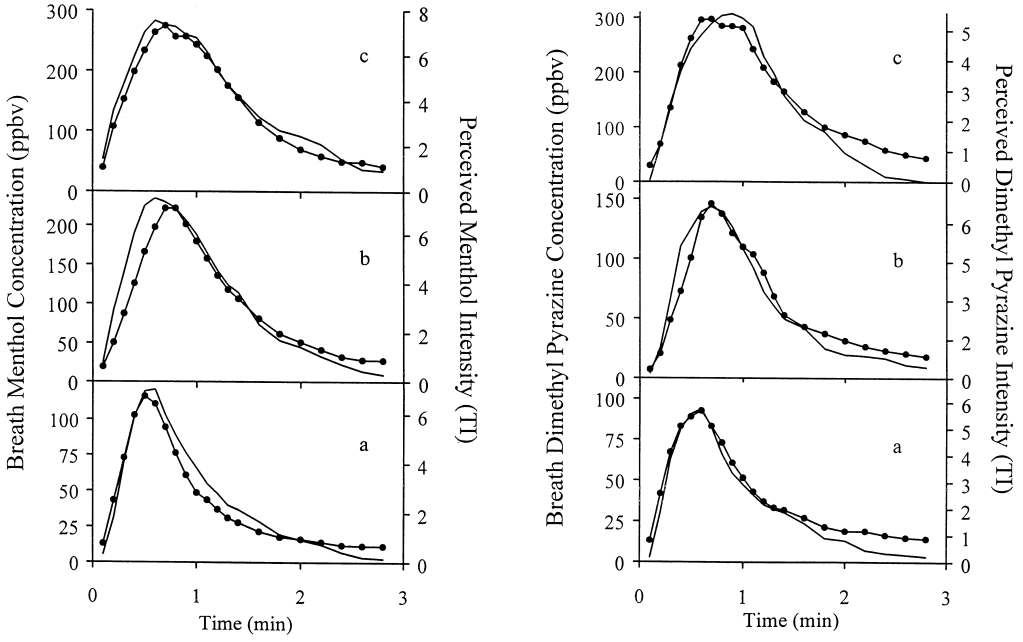

Fig. 1. Graphs of sensory (—) and instrumental (-●-) data for the 3 (a), 6 (b) and 9% (c) gelatine gels containing menthol or dimethyl pyrazine, produced by averaging the data points in the intensity dimension.

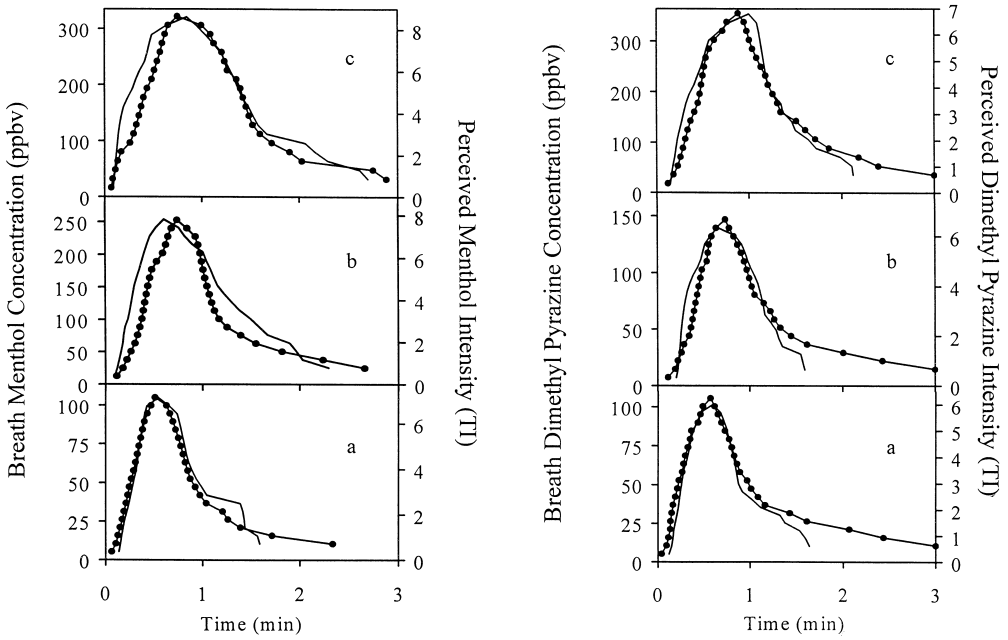

Fig. 2. Graphs of sensory (—) and instrumental (-●-) data for the 3 (a), 6 (b) and 9% (c) gelatine gels containing menthol or dimethyl pyrazine, produced by averaging the data points in the time dimension after normalisation to the average I_{max}.

volatiles showing that panellists could discriminate the qualitative aspects of the sensory release profiles but not the quantitative aspects. The panellists were given reference gels on the day before the TI analysis to calibrate their perceived intensity but none just prior to the TI analysis, as the high flavour intensity gels may have caused sensory adaptation and made the situation worse rather than better. Panellists were requested to focus on the rate of change of intensity and move the lever more quickly or more slowly according to their perceived rate of flavour change. Furthermore, to avoid sensory fatigue, samples were presented to the panellists in three consecutive half-day sessions with each panellist consuming one menthol and one dimethyl pyrazine flavoured gel in each session. Consequently, the intensity data from one panellist were obtained over a period of one and a half days from just three samples for each volatile. This factor may explain the poor correlation of perceived intensity and in-nose volatile concentration.

For the TR curves, there were effects of gelatine concentration on the shape of the curves which were paralleled by changes in the sensory data. The initial phase of the profiles (up to 75% of I_{max}) were very similar for all gels with only minor differences in the average T_{max} values (Table 2). The major effect of gelatine concentration was seen in the decline of TI and TR values after T_{max}. The 9% gelatine gels showed the slowest decline in volatile concentration after T_{max} while the 3% gels showed the most rapid decline. This may be attributed to differences in the persistence of gel fragments in-mouth after initial oral breakdown and maximum volatile release.

Table 2
Average T_{max} values and standard deviation SD for the gelatine gel TR curves

Compound	Gelatine %	Mean (min)	SD (min)
Menthol	3	0.52	0.07
Menthol	6	0.77	0.24
Menthol	9	0.79	0.29
Dimethyl pyrazine	3	0.52	0.13
Dimethyl pyrazine	6	0.79	0.16
Dimethyl pyrazine	9	0.87	0.29

Each result is based on the results from six panellists who each ate one sample of each gel.

3.2. TI and TR profiles of pectin/gelatine gels

Even the weakest of the gelatine gels produced average T_{max} values greater than 0.5 min (Figs. 1 and 2), which is much later than those observed in previous experiments (Linforth et al., 1998). This was thought to be primarily due to the behaviour of the compounds, rather than the gel system. Both dimethyl pyrazine and menthol show considerable persistence on the breath after the food bolus has been swallowed (Ingham, 1996; Linforth et al., 1996). This may be due to the persistence of the compounds in mouth, but, will also be influenced by their absorption to the nasal mucosa (Hornung et al., 1980). Persistent compounds will gradually build up on the nasal mucosa, and T_{max} will be dependent on the rate of release of the compound, its speed of migration to the nasal cavity and its elimination from the mouth and mucosal membranes.

The pectin/gelatine gels were designed to produce T_{max} values earlier than those observed for the gelatine gels. This relied on two factors: first the gels were easily fractured and broke down quickly when eaten; second, ethyl butyrate exhibits very little persistence such that breath volatile concentrations should more closely reflect volatile release, when compared with either menthol or dimethyl pyrazine. All of the average TR curves exhibited earlier T_{max} values relative to the corresponding TI profiles (Fig. 3). These differences are similar to those observed in the preliminary experiments on benzaldehyde and isoamylacetate (Linforth et al., 1998).

It is also evident that there were substantial differences after T_{max} between the TI and TR curves (Fig. 3). The average TI curves lagged behind the TR profiles, taking up to twice as long to decline to 50% of maximum. Indeed the panellists were still recording substantial perceived intensities, when the average volatile concentration had declined to only 10% of the maximum intensity.

3.3. Correlation between the instrumental and sensory T_{max} results

The wide range of gel composition and the lack of eating instructions for the panellists were designed to give a range of different release times, for the study of the temporal aspects of the relationship between the stimulus (TR) and its perception (TI). However, averaging the TI and TR results, effectively reduced the range and number of values compared. Plotting the individual TI T_{max} against the TR T_{max} values (Fig. 4) showed the full range of values. There were clear differences between panellists (Fig. 4). Some of the panellists consumed the 9% gels as quickly as the 3% gels, resulting in early T_{max} values for the 9% gels.

Despite this variation, there was a clear correlation ($p < 0.001$, regression analysis) between the TI and TR values for individual samples (Fig. 4). This was an objective demonstration that the TI and TR curves were similar rather than by the visual comparisons noted in Figs. 1 and 2. Typically, the strong gels exhibited high values for T_{max} while the weak gels had low values. The linear regression trendline fitted through all sets of data irrespective of the gel system or volatile, suggesting a common trend.

Overbosch's theoretical model of the relationship between the stimulus and the perception over time (Overbosch, 1986) took into account the possible effects of adaptation, which could cause deviations between TI and TR curves late in the eating event. The model predicted that the TR and TI T_{max} values would be identical, such that maximum stimulus intensity would be perceived at the point of maximum stimulus delivery. Consequently the trendline for the data in Fig. 4 would

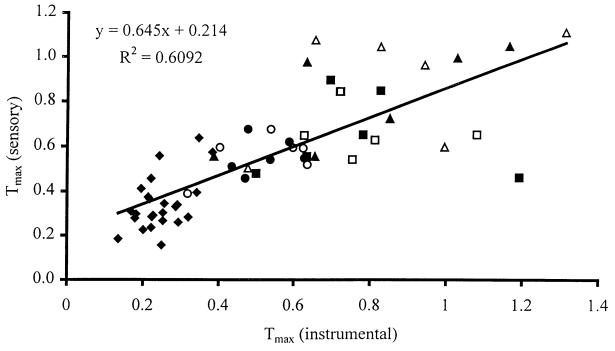

Fig. 4. Comparison of the instrumental and sensory T_{max} values for menthol-flavoured gelatine gels containing 3 (●), 6 (■) or 9% (▲) gelatine, dimethyl pyrazine-flavoured gelatine gels containing 3 (○), 6 (□) or 9% (△) gelatine or ethyl butyrate flavoured pectin/gelatine gels (◆).

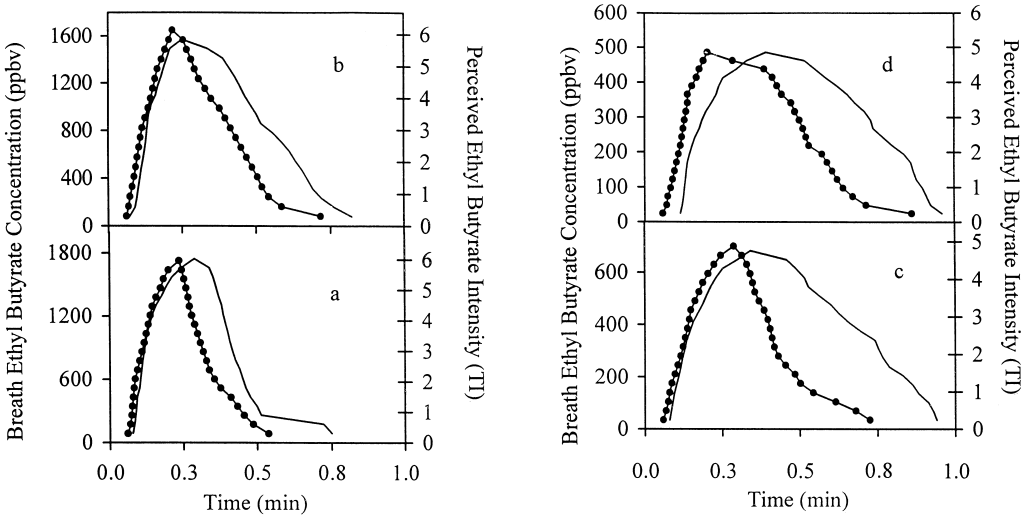

Fig. 3. Graphs of sensory (—) and instrumental (-●-) data for the 0.75 (a), 0.625 (b), 0.5 (c) and 0.375% (d) pectin/gelatine gels containing ethyl butyrate produced by averaging the data points in the intensity dimension.

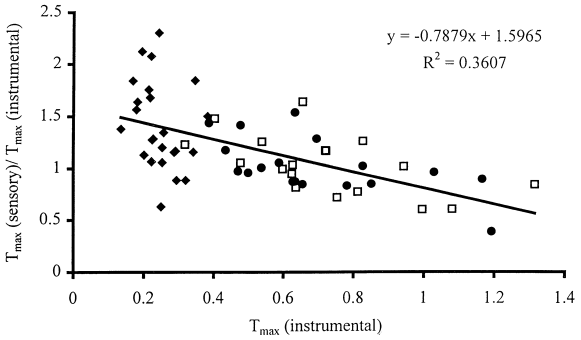

Fig. 5. Ratio of sensory to instrumental T_{max} data for menthol (●), dimethyl pyrazine (□) and ethyl butyrate (◆) gels at different instrumental T_{max} values.

be expected to be linear and to pass through the origin. However, the trendline did not pass through the origin, which suggests some deviation from the model.

To visualise the deviations within the data set more accurately, the ratio of the TI T_{max} and TR T_{max} values was calculated and each value plotted against the TR T_{max} to see if there was a shift in the TI/TR ratio with time of TR T_{max} (Fig. 5). If the TI and TR T_{max} values were identical, a ratio of 1.0 would be returned. However, when the samples were eaten quickly (low TR T_{max}) the sensory (TI) T_{max} tended to be later than the instrumental (TR) T_{max} yielding ratios >1. This implied some sort of lag between the volatile compound entering the nose and the panellist moving the TI lever in response to the stimulus. Similar observations were made by Overbosch and coworkers (1991), who reported that the T_{max} of TI curves "usually occurs a number of seconds later than in the MS breath measurements". Aroma sensations exhibit a delay in reaching maximum intensity, gradually increasing over the first few seconds of stimulus presentation. This has been attributed to the temporal integration of the stimulus (Berglund & Lindvall, 1982) and may account for the lag observed in the TI traces. Experiments demonstrating temporal integration (Overbosch, De Wijk, De Jonge, & Köster, 1988), studied the perception of constant stimuli delivered to the panellists for different periods of time, after a phase during which no stimulus was delivered. Typically the perceived intensity increases over the initial 5–10 s period of perception before declining. These observations, might be expected to relate more closely to the processes that occur at the onset of aroma detection rather than events at or around T_{max}. However, temporal integration could occur throughout the eating time course, resulting in substantial differences between TI and TR curves (particularly in the early phases of eating).

When the samples were eaten slowly, the ratio of the TI/TR T_{max} values was less than 1. This could be explained by the occurrence of adaptation to the aroma which causes a decrease in perception and therefore shifts the TI T_{max} value to an earlier time. This is in agreement with the chewing gum experiments (Linforth & Taylor, 1998).

4. Conclusions

These experiments suggest that the relationship between volatile concentration in-nose and sensory perception of that volatile is dependent on the physiological processes that cause lag and adaptation. These in turn are controlled by the eating process (rate of chewing, saliva flow, swallowing, etc.) and, to understand the process, will require an integration of science from several disciplines.

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