Effect of Sucrose on the Perceived Flavor Intensity of Chewing Gum

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The release of sucrose and menthone from chewing gum was measured in-mouth and in-nose, respectively, during eating. Swabs of saliva were taken from the tongue and analyzed using a rapid, direct liquid−mass spectrometry procedure. Menthone concentration in-nose was monitored on a breath-by-breath basis using direct gas phase atmospheric pressure chemical ionization−mass spectrometry. Simultaneously with the volatile release, trained panelists followed the change in mint flavor by time−intensity (TI) analysis. Two types of commercial chewing gum were analyzed. Both showed that the panelists perception of mint flavor followed sucrose release rather than menthone release. The temporal analysis of the chemical stimuli, with simultaneous TI analysis, provided unequivocal evidence of the perceptual interaction between nonvolatile and volatile flavor compounds from chewing gum.

Keywords: *Sucrose; in mouth; chewing gum; menthone; nonvolatile*

INTRODUCTION

The perceived flavor of a food is derived from signals sensed by a variety of receptors located in the mouth and nose, which are then processed in the neural system. For convenience, the sensations of taste and aroma have often been investigated separately, but the potential interaction of volatiles and nonvolatiles should be considered. Besides invoking the sensation of taste, nonvolatiles can also enhance the perception of aroma compounds (Noble et al., 1993; Noble, 1996).

Both taste and aroma change over the period food is eaten, and the temporal changes of volatile flavors have been monitored in vivo in our laboratory using atmospheric pressure chemical ionization−mass spectrometry (APCI−MS) (Linforth et al., 1996). Changes in nonvolatile concentrations in-mouth have received less attention although chew and spit methods and in-mouth sensors (Davidson et al., 1998; Jack et al., 1995) have been used with some success in food systems. Both systems have limitations. Chew and spit experiments may be very time-consuming, while the response of sensors depends on the presence of sufficient saliva to carry the nonvolatiles to the sensors. In low moisture foods, in particular, lack of saliva can cause a slow response (Davidson et al., 1998).

Dawes and Macpherson (1993) sampled saliva using small, preweighed filter paper strips from different tooth surfaces at specific time points during the eating of chewing gum. From the strips they determined the distribution of sucrose around the mouth and the implications for the site-specificity of caries and calculus deposition. To sample saliva from the tongue, we used cotton buds which were weighed and extracted with solvent, and the sucrose concentration was determined by direct liquid phase API−MS. No chromatography was involved and resolution was entirely on the basis of *m*/*z* values. Recent developments in liquid chromatography−mass spectrometry (LC−MS) have allowed the quantitative detection of compounds in solution (Jáuregui et al., 1997; Kato and Numajiri, 1991), and we have adapted the technique for the analysis of nonvolatile flavor compounds in saliva. Since nonvolatiles are present at relatively high levels (g/kg) compared to volatile flavor compounds (mg/kg or μg/kg), sensitivity was not an issue.

The purpose of this paper was to monitor the temporal release of sucrose and menthone from chewing gum during eating and to relate the time-release curves to the sensory time−intensity (TI) curves. Chewing gum was chosen as the model food, as it is available in a variety of forms that exhibit different rates of sugar release. In chewing gum, volatile release is fairly rapid, but then the amount in-nose remains fairly constant over long periods of time (Harvey, 1997; Linforth and Taylor, 1998). Flavor perception however, decreases with time. Adaptation is one of the mechanisms that has been proposed to explain the phenomenon (Overbosch et al., 1991; Linforth and Taylor, 1998; Taylor et al., 1999), but the interaction of nonvolatile and volatile compounds may provide an alternative and/or additional explanation.

Duizer et al. (1996) used dual-attribute time−intensity to simultaneously measure the perceived sweetness and peppermint flavor of chewing gum and found that a gum with a faster release of sweetness enhanced the duration and intensity of sweet perception, as well as the duration of the peppermint flavor. Sensory work carried out by Valdés et al. (1956), using simple solutions containing sucrose, organic acids, and raspberry flavoring, also found that there was a tendency for the panel to ascribe more flavor to the sweeter samples. However, the ability to monitor the temporal stimuli close to the olfactory receptors, while simultaneously analyzing the signal in the higher brain (through TI analysis), provides additional information to investigate the phenomena.

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10.1021/jf9901082 CCC: $18.00
Published on Web 09/15/1999

Table 1. Actual and Measured Concentrations of Standard Solutions of Sucrose Using the Swabbing Technique Followed by API−MS Analysis Where Each Value Is the Mean of Three Determinations

actual sucrose concentration (g/100 g)	average measured sucrose concentration (g/100 g)	standard deviation
2.0	2.4	0.1
4.0	4.4	0.5
8.0	7.9	0.6

MATERIALS AND METHODS

Materials. Two commercial chewing gums were tested: a "stick" type gum and a "tablet" type gum. The main sweetener used in both gums was sucrose. The cotton buds used were the standard cosmetic type. Firmenich (Geneva, Switzerland) supplied the menthone used. Fisher Scientific Ltd. (Leicestershire, England) supplied the sucrose, methanol, and hexane.

Saliva Sampling. Three panelists placed the gum samples in their mouths and chewed at a specified location in the mouth at a rate of 80 chews/min using a metronome. They were instructed to take a swab from a specific location on the tongue using a cotton bud.

Saliva was sampled from the mouth of panelists at 0, 10, 20, 30, 40, 50, 60, 120, 180, 240, and 300 s. Not all time points were obtained from a single gum sample (this would be physically difficult), instead the time points were split into three subsets. Four replicates of saliva samples per time point were taken for each of the two gums; hence, the in-mouth sucrose concentration data for each panelist were obtained from 12 samples of gum. Panelists rinsed the mouth with water to clear the palate, and 15 min breaks were taken between samples to minimize the effects of fatigue.

Each cotton bud was weighed before and after swabbing, to determine the weight of saliva swabbed, and then the sucrose was extracted in 3 mL solutions of methanol:water (50:50 (v/v)).

Sucrose Analysis. A mass spectrometer equipped with a liquid chromatography interface (Platform LCZ, Micromass, Manchester, England) was used for the analysis of sucrose. The sucrose was monitored in the negative ionization selected ion mode (m/z 340.9) using L−APCI−MS. A mobile phase of methanol−water (50:50 (v/v)) was continuously pumped into the interface, at a rate of 0.4 mL/min using a source block temperature of 150 °C and a desolvation temperature of 400 °C. A 10 μL aliquot of saliva samples or standards was injected via a Rheodyne injection loop (Rheodyne). The sucrose entering the source was then ionized by a 3 kV corona discharge; and the signal for sucrose was optimized by using a cone voltage of 18 V. The concentration of sucrose in the saliva was estimated by comparing the peak areas obtained for sample injection with those obtained for a set of seven standards (0.1−0.00156 g sucrose/100 mL methanol:water) and then correcting for salivary weight differences. To assess the efficiency of the extraction process, swabs were taken from standard sucrose solutions outside of the mouth (Table 1).

Measurement of Breath Menthone Concentration. The breath volatile analysis was conducted simultaneously with the sensory TI analysis. Previous work has shown that the menthol and menthone are released similarly in chewing gum (Linforth and Taylor, 1998). Therefore, only the release of menthone was followed in this experiment. Eleven panelists (different from those used for the sucrose analysis) trained in TI analysis placed the samples in their mouth and were told to chew at a rate of 80 chews/min using a metronome. While they ate, an open-ended plastic tube placed in one nostril guided the breath over the sampling port of the interface. The plastic tube did not interrupt the normal breathing pattern, and during exhalation breath entered the plastic tube allowing breath to be sampled into the mass spectrometer.

The gas phase in the tube was sampled continuously at 30 mL/min through the heated (60 °C) interface into the APCI source of the mass spectrometer (Platform II, Micromass, Manchester, England). There, the menthone (m/z 155.0) was ionized by a 4 kV corona discharge (cone voltage 20 V) before being sampled into the high vacuum region of the mass spectrometer.

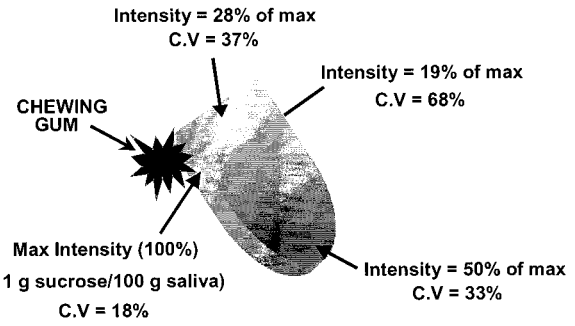

Figure 1. Comparison of the maximum intensity of sucrose and the average coefficient of variation (CV) on the right side of the tongue, with the maximum intensity and CV measured at three other locations (left side, back, and tip) from eating a commercial spearmint chewing gum over a 5 min period. The intensity is the mean of three replicates.

A hexane solution of menthone (concentration 35 μg/mL) was used for the calibration of the APCI−MS. The standard was introduced and volatilized, into the nitrogen make up gas entering the source (10 L/min), using a syringe pump at flow rate of 1.5 μL/min (Harvard Apparatus, Cambridge, MA). This showed the signal intensity (peak height) produced when 52.5 ng/min of menthone enters the source. Comparison of the peak heights for the standard with those for the menthone in each breath allowed an estimation of the maximum amount of menthone (ng/min) entering the source over each exhalation. Since the breath sampling rate was known (20 mL/min), we were able to estimate the breath menthone concentration that would result in the observed rate (ng/min) entering the source. These values were determined and expressed as parts per billion in the gas phase (ppbv). Three replicates were taken for each gum, panelists rinsed the mouth with water to clear the palate, and 15 min breaks were taken between samples to minimize the effects of fatigue.

Sensory Analysis. Panelists trained in TI analysis were instructed to rate the intensity of the overall mint flavor of the gums by moving a lever with a marked scale to indicate perceived flavor intensity. Prior to the main experiment, the panelists were given samples of both gums to provide them with an example of the maximum intensity of the mint flavor they would be likely to experience. Panelists were not comparing the intensity of one chewing gum against the other, they were instructed to rate the intensity of each gum relative to the maximum for that gum. Moving the lever generated a linear analogue signal, which was fed directly into one of the analogue channels of the mass spectrometer. The resulting TI curves were processed with the software provided with the mass spectrometer.

RESULTS AND DISCUSSION

Analysis of Sucrose. In preliminary experiments, four different locations on the tongue of one panelist were examined to investigate sucrose distribution and establish suitable locations for swabbing (Figure 1). The work carried out by Dawes and Macpherson (1993) found an uneven distribution of sucrose in saliva on different tooth surfaces. Our results also revealed an uneven but fairly consistent distribution of sucrose on the tongue. Gum was chewed on the right side of the mouth and swabs were taken (at 0, 0.5, 1, 2, 3, 4, and 5 min) from each location. The maximum concentration was obviously found at the point of chewing (11 g/100 g of saliva) with lower concentrations at the left-hand side of the tongue (19% of max), the back of the tongue (28% of max) and the tip of the tongue (50% of max). Given

Table 2. Mean Sucrose Concentration (g/100 g of Saliva) from Four Replicates, on the Right Side of the Tongue of Three Panelists, at Specific Times during the Eating Process, from Two Types of Commercial Chewing Gum, together with the Standard Deviation (SD) and Percentage Coefficient of Variation (% CV)

	panelist 1			panelist 2			panelist 3		
time (s)	mean	SD	% CV	mean	SD	% CV	mean	SD	% CV
				Stick Chewing Gum					
10	5.1	2.5	48.5	8.8	5.3	60.0	4.3	2.0	47.4
20	5.7	2.3	40.3	12.6	3.7	29.3	5.1	1.1	21.9
30	8.0	3.9	48.1	12.5	5.0	39.9	9.7	4.4	45.1
40	5.1	1.4	26.7	17.8	4.9	27.4	9.7	4.9	50.1
50	6.1	1.9	31.6	14.4	2.5	17.1	16.6	3.2	19.5
60	8.2	2.6	31.8	12.2	3.8	31.3	9.1	2.0	22.5
120	8.5	1.3	14.8	12.4	3.6	28.9	11.0	4.5	41.5
180	4.7	0.6	13.7	8.1	3.1	38.4	6.2	1.2	19.3
240	4.3	1.1	25.3	3.8	2.5	64.7	3.1	1.2	38.6
300	2.9	0.7	24.4	2.3	1.1	46.5	1.8	0.8	44.4
mean % CV			**30.5**			**38.4**			**35.0**
				Tablet Chewing Gum					
10	18.0	2.7	14.8	30.5	8.5	27.9	15.3	10.4	67.5
20	16.3	3.0	18.7	23.2	4.9	21.4	13.8	3.1	22.9
30	12.3	3.2	26.0	22.2	5.0	22.4	15.2	4.6	30.0
40	10.3	4.1	39.4	21.4	4.4	20.6	14.5	11.0	76.2
50	9.3	2.8	30.2	20.2	8.3	41.0	13.2	4.7	35.8
60	9.2	4.9	53.3	20.2	5.0	24.9	14.0	4.4	31.8
120	2.9	0.9	32.7	13.3	3.1	23.3	7.8	2.6	33.7
180	1.9	0.4	18.5	7.7	2.6	33.5	4.4	1.5	34.1
240	1.1	0.2	19.1	4.4	1.9	44.1	2.1	0.7	33.3
300	0.5	0.1	18.5	2.6	1.2	46.2	0.8	0.6	76.2
mean % CV			**27.1**			**30.5**			**44.1**

the distribution of the sweet taste buds (located principally on the tip of the tongue (Guyton and Hall, 1996; Mackenna and Callander, 1997), this suggested that the maximum sucrose concentration experienced by the sweet taste receptors was about 5 g/100 g of saliva. The pattern of sucrose concentration across the tongue seemed fairly consistent as the variation (measured over all samples during the 5 min period) was relatively small (side of eating, 18%; left side, 68%; tip, 33%; and back, 37%). For the following experiments, panelists were instructed to chew on the right side of the mouth and swab the right side of the tongue.

A comparison of sucrose release from stick and tablet chewing gum was then carried out. Each panelist consumed 12 samples of gum to build the full time–release profile, and the mean sucrose concentrations are shown in Table 2. For the stick gum there were differences between panelists in the maximum concentration of sucrose in-mouth (8.5–17.8 g/100 g of saliva) and in the time taken to achieve maximum concentration despite the attempts to standardize chewing by using a metronome set at 80 chews/min. For the tablet gum, concentration differences between panelists were observed (15.3–30.5 g/100 g of saliva), but all three panelists achieved maximum concentration at 10 s. Table 2 also shows the variation in sucrose concentration with time, both between replicates for one panelist (intrapanelist variation) and between the three panelists (interpanelist variation). Variation between the replicates of one panelist was estimated by calculating the % CV value (SD × 100/mean) for each time point and then taking the mean of all the values. The mean values ranged from 27.1 to 44.1% for the three panelists and the two gums. The type of gum did not appear to affect the variation.

Comparing the maximum sucrose concentration of each panelist provided some measure of person to person variation. Panelists 2 and 3 showed similar

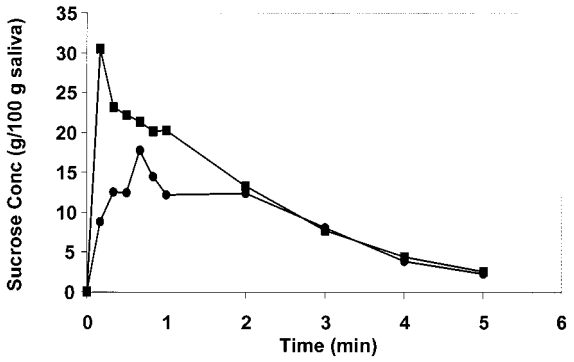

Figure 2. Comparison of the in-mouth sucrose concentration (g/100 g of saliva) between a stick (●) and tablet (■) type commercial chewing gum when consumed by one panelist. Gums were chewed on the right-hand side of the mouth and saliva samples were taken from the right side of the tongue. Each point is the mean of four values.

sucrose maxima for stick gum but panelist 1 showed only half the sucrose concentration (at maximum value). For tablet gum, panelists 1 and 3 were similar but panelist 2 showed higher concentrations (nearly double). The broad range of concentrations observed are most likely due to physiological differences between panelists, such as salivary flow rate, surface area of the teeth and tongue, and the chewing pattern adopted by each panelist. Each individual panelist seemed relatively consistent in their release of sucrose but the differences between panelists were greater.

The relationship between perceived sweetness and sucrose concentration is $P = aS^b$ where P is sweetness perception, S is sucrose concentration, a is a constant, and b is 1.3 for sucrose (the power law relationship: Hoppe and Kroeze, 1995). However, the sensory effects of these variations in concentration are difficult to assess, and it is difficult to state categorically whether the increased sucrose concentration in one panelist can be translated into a different sweetness perception compared to another panelist without knowing the response of each individual to sucrose. Because of this, all the sucrose release data were pooled and the mean values for the three panelists and each of the two gums were calculated. Even though the swabbing method is fairly rapid, it was not feasible to collect data from the 11 trained panelists used for the volatile and TI analyses.

Although the actual in-mouth sucrose concentration varied between panelists, inspection of the sucrose release traces for the two gum types showed clear differences. Figure 2 shows the sucrose release for stick and tablet gum for one panelist. Normalizing the sucrose release data removed the concentration differences between panelists and showed that the overall trends of the release curves were similar for each panelist (data not shown). That is, the stick gum took longer to reach the maximum intensity and declined at a slower rate than the tablet gum. The different rates and patterns of release are due to the distribution of sugar in the two gums. Tablet gum is sugar coated allowing rapid release of sucrose, whereas the sucrose of the stick gum is embedded in the gum matrix, which results in slower release.

The stick gum trace in Figure 2 shows some spiking around 1 min of eating. Since sucrose concentration, at any particular time, is the net result of sucrose release and dilution due to salivary flow as well as the timing

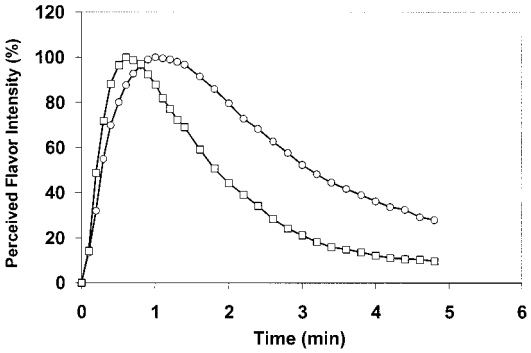

Figure 3. Perceived intensity of overall mint flavor with time from a stick (○) and tablet (□) type commercial chewing gum. Values have been normalized for the intensity and are the means of 11 panelists.

and frequency of sucrose removal by swallowing, it is not surprising that the release profile is not entirely smooth. A possible scenario for the stick gum trace in Figure 2 is that, just prior to sampling data for point 4, the panelist tended to swallow and decreased the sucrose concentration. Controlling swallowing during the eating process is difficult as panelists find it uncomfortable to eat if they cannot swallow at will.

Volatile and TI Analysis. The volatile and TI analysis were performed simultaneously with a trained TI panel. Panelists were instructed to follow the change in mint flavor of the gum with time. The TI curves for the stick and tablet gum were different, and Figure 3 shows the two traces where the maximum intensities have been normalized to 100 to allow easy comparison. There were differences between the two T_{max} (time to maximum) values (average of 0.6 min for tablet gum, 1 min for stick gum (*t*-test calculated difference; $P < 0.001$)) and the rate at which perceived mint flavor decreased. This can be illustrated by considering the flavor intensity at 5 min, which for stick gum was 28% of the maximum value, while for tablet gum the value was just 9.5% of the maximum. These differences were also statistically significant (*t*-test; $P < 0.004$). The variation in T_{max} between the replicates of each one of the 11 panelists was estimated by calculating the % CV value. These values ranged from 0 to 36.7% for the stick gum and from 5.6 to 41.8% for the tablet gum. The variation between the mean T_{max} values of all 11 panelists was 32% for both types of gum. When the menthone concentrations in-nose were plotted, it was found that the average breath concentration reached a steady state (after the increase in menthone concentration during the first minute) and remained relatively constant. The average menthone concentration at 5 min had increased 6.6% for the stick gum and decreased 25.3% for the tablet gum. This was consistent with results reported by other workers (Harvey, 1997; Linforth and Taylor, 1998). Figure 4 shows a plot of the menthone, sucrose, and TI parameters for stick gum, and Figure 5 shows the data for tablet gum. Again the TI and sucrose values in both figures have been normalized to allow for easier comparison, but the menthone values are absolute, in-nose concentrations.

Both Figures 4 and 5 clearly show that the perceived mint flavor does not follow the change in menthone concentration. Instead, both TI mint curves follow the sucrose release curves very closely. It is attractive to suggest that there is a relationship between the perception of mint flavor in chewing gum and the presence of

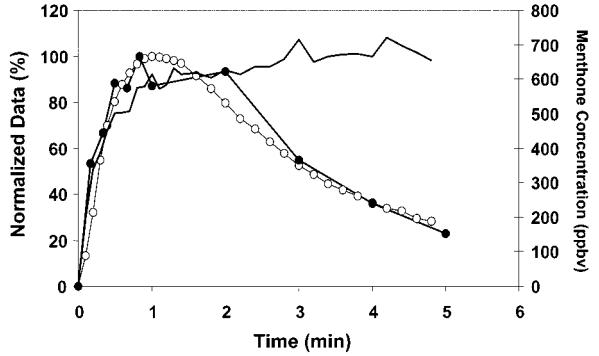

Figure 4. Sucrose release (●), menthone release (—), and perceived intensity of overall mint flavor (TI curve) (○), from a stick type commercial chewing gum. The sucrose release data are the mean values from three panelists, while the menthone release and perceived intensity values are the mean of 11 panelists. Sucrose and perceived intensity values have been normalized for easy comparison (maximum mean sucrose concentration was 12.4 g/100 g of saliva).

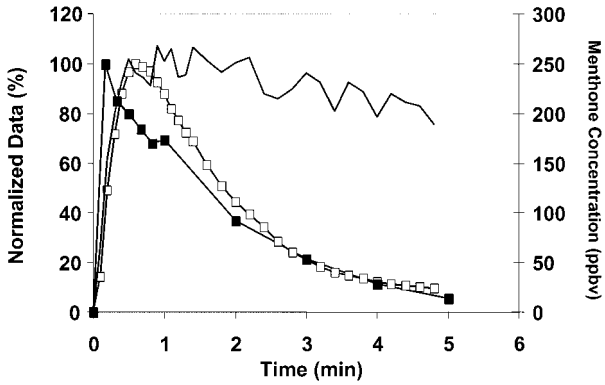

Figure 5. Sucrose release (■), menthone release (—), and perceived intensity of overall mint flavor (TI curve) (□), from a tablet type commercial chewing gum. The sucrose release data are the mean values from three panelists, while the menthone release and perceived intensity values are the mean of 11 panelists. Sucrose and perceived intensity values have been normalized for easy comparison (maximum mean sucrose concentration was 21.4 g/100 g of saliva).

sucrose in-mouth. These types of interaction have been reported previously and some examples are listed in the Introduction. However, there are other possible reasons for the results. Although the panel was instructed to follow mint flavor during their TI experiments, they may have confused loss of sweetness with mint flavor. The panel has been rigorously trained with a variety of food materials and has proved consistent in other experiments but may have become confused in this situation. Another reason is that the panelists became adapted to menthone flavor with time [see Overbosch et al. (1991) for discussion] and that the adaptation period coincided with the sucrose release timings.

CONCLUSIONS

Further experiments need to be devised to provide full answers to the above questions. Chewing gum is an unusual food that remains in the mouth for long periods. Patents have been issued for techniques that prolong sweetness (Cherukuri et al., 1992; Yatka et al., 1991), and studies using the techniques above may help explain the mechanisms involved in chewing gum flavor perception.

The results also demonstrate that the saliva sampling method using cotton bud swabs is effective and sufficiently rapid to be useful. The MS analysis is now being widened to include other nonvolatile flavor compounds so that the analysis can be applied to foods with mixtures of nonvolatiles (e.g., sugars and acids). Since the method can analyze for all these compounds simultaneously (providing they produce ions with different *m*/*z* values), it should be possible to analyze mono- and disaccharides and organic acids (e.g., malic, citric, and lactic) at the same time. This may shed more light on the nature of other taste−aroma interactions.

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Received for review February 11, 1999. Revised manuscript received July 10, 1999. Accepted July 19, 1999. J.M.D. is grateful to the Ministry of Agriculture Fisheries and Food for a studentship and to Firmenich (Geneva) for additional technical and financial support.

JF9901082