In-Mouth Measurement of pH and Conductivity during Eating

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A dental brace containing two sensors was constructed to allow the in-mouth monitoring of conductivity and pH during eating. The salt release from peanuts, salt and vinegar crisps, cheese, and mashed potato was measured during eating. There was a lag of 22 s after placing the crisps in the mouth before there was a significant increase in conductivity. This highlighted the problems of measuring salt release from dry foodstuffs. The conductivity electrodes were not ion specific, and consequently, when testing processed cheese, the high ion content (salt cations and anions) contributed to the signal, producing a complex release curve. The acid release from a blackcurrant gelatine gel and an orange was successfully measured. The average minimum pH reached was 3.8 with the gel, and 4.5 with the orange. It was found that the buffering capacity of saliva affected the pH of successive replicates.

Keywords: *Nonvolatile, in-mouth; pH; conductivity; salt*

INTRODUCTION

Although food flavor is usually perceived by humans as a single, overall effect, it is actually composed of signals from a variety of sensors located chiefly in the mouth and nose. The sensations of taste and aroma occur when the sensors are stimulated by chemical compounds released from the food during eating. Sensory studies have shown that the perceived flavor intensity (and sometimes the flavor quality) changes with time during eating, and measurement of the volatile components during eating has established that the volatile concentration in-nose also changes with time [see Taylor (1996) for review]. When trying to relate the perceived flavor of a food with its flavor composition, the potential interaction of volatiles and nonvolatiles should be considered, since besides invoking the sensation of taste, nonvolatiles can also enhance the perception of aroma compounds (Noble et al., 1993; Noble, 1996).

To measure the concentration changes of nonvolatiles in-mouth during eating, sensors are required that are robust and have a rapid response time because food is eaten over short time periods (typically 30−60 s). Placing of the sensors is also important so that nonvolatile compounds released from the food into the saliva phase can be detected and a representative signal obtained. Not all parts of the mouth are in intimate contact with the food and mixing will not be homogeneous. A further consideration is that the sensors should not interfere with the person's ability to chew and swallow food.

Jack et al. (1995) described preliminary experiments to measure conductivity in-mouth during eating of cheese, using a dental plate fitted with electrodes. In this paper, a similar approach has been taken with a range of foods, but the dental plate has been fitted with a pH electrode as well as a conductivity sensor.

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The choice of sensors was determined partly by the availability of suitable devices and partly by the potential for measuring parameters that affect the release and/or perception of food flavor. Acid is one of the basic tastes recognized in humans, and measuring pH in-mouth indicates the changes that occur. Acidity can also affect the ionization of other molecules which may affect their volatility. Salt is another basic taste perceived in mouth but is also thought to act as a flavor enhancer for other flavor molecules. Conductivity was used to estimate sodium concentration in-mouth during eating, but it is a nonspecific test for sodium because other ions could also affect conductivity (e.g., Ca^{2+}) and results need to be interpreted with caution. For both sensors, the performance with different food samples was tested to determine whether it was possible to obtain real-time measurements during eating and to determine the variability in one person when samples of the same food were eaten on different occasions.

MATERIALS AND METHODS

Apparatus. A dental plate containing two sensors was constructed to allow the in-mouth monitoring of conductivity and pH. Impressions of the subject's upper dentitions were taken, and the dental appliance was fabricated from a clear acrylic dental plastic, with stainless steel retention clasps. This fitted closely to the subject's top palate and did not interfere with a normal eating pattern.

Conductivity Measurements. Two stainless steel electrodes (2 mm in diameter, 1 mm apart) were embedded in the roof of the appliance at the point where the tongue contacts the palate during eating (Jack et al., 1995). The electrodes were connected to insulated conducting wires (twin screened cable, stock number 367189, RS, U.K.) which passed behind the wisdom teeth of the subject, around the outside of the teeth and out through the side of the mouth. This allowed normal chewing, with minimal interference from the wires. The wires were connected to an analogue conductivity meter (CM35, Walden Precision Apparatus Limited, Cambridge, U.K.) with the output digitized using an 8 channel, A/D, 16 bit converter (ADC-16 High-Resolution Data Logger, Pico Technology Limited, Cambridge, U.K.).

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Table 1. Average I_{max} and T_{max} Values for Salt Release from Various Foods, Together with the Standard Deviation (SD) and Coefficient of Variation (CV)[a]

food stuff	replicate 1	replicate 2	replicate 3	avg	SD	CV (%)
salt and vinegar crisps, I_{max}	0.64	0.59	0.45	0.56	0.1	17
salt and vinegar crisps, T_{max}	90	90	81	87	5.2	6
peanut, I_{max}	0.21	0.31	0.34	0.29	0.07	24
peanut, T_{max}	97	94	86	92	5.7	6.2
Cheddar, I_{max}	0.35	0.50	0.44	0.43	0.07	17
Cheddar, T_{max}	74	89	96	86	11	13
Dairylea, I_{max}	2	2.3	2.3	2.2	0.18	8.1
Dairylea, T_{max}	86	87	86	86	0.58	0.67
mash (unsalted), I_{max}	0.54	0.47	0.40	0.47	0.07	15
mash (unsalted), T_{max}	69	64	76	70	6	8.7
mash (salted), I_{max}	1.6	1.8	2.1	1.9	0.26	14
mash (salted), T_{max}	73	69	65	69	4	5.8

[a] I_{max} was determined in sodium equivalents (NaE; g/100 g), while T_{max} was measured in seconds (s).

The apparatus was calibrated using sodium chloride solutions (0, 0.5, 1, 2, 3, and 4%) outside of the mouth. Conductivity was measured in arbitrary digitized units and, by plotting the square of the conductivity against sodium chloride concentration, a straight line calibration was generated.

pH Measurements were performed using a Beetrode micro pH electrode (diameter 0.1 mm, Beetrode NMPH2B, World Precision Instruments, Herts, U.K.), which was also embedded in the roof of the appliance. The electrode was connected to an analogue pH meter (C18, Walden Precision Apparatus). The pH electrode was calibrated by placing the dental appliance into a beaker containing standard pH 4 and 7 buffer solution. During the calibration and in-mouth monitoring, a reference salt bridge was created by having the subject dip one finger into a 3 M KCl solution containing the reference electrode (DRIREF-2, World Precision Instruments) (Fejerskov et al., 1992).

Time−intensity (TI) measurements were obtained using a custom-built potentiometer fitted with a lever, which could be moved along a marked scale to indicate perceived flavor intensity rating. The panelist was trained prior to the experiments and was consistent in detecting differences in the salt content of the foods. The digitized outputs from the pH, conductivity, and TI apparatus were captured by a data acquisition software package (Picolog, Pico Technology) running on a PC.

Materials. The chemicals used in this study were all analytical reagent grade and obtained from Fisher Scientific (Leics. U.K.). Food samples were obtained from a local supermarket and included salt and vinegar crisps (Walkers, Leics, U.K.); original salted peanuts (KP, Leics, U.K.); Dairylea chunky spreadable cheese (Kraft Jacobs Suchard, Belgium); Cheddar cheese (Sainbury's own English Mature Cheddar, U.K.); instant mashed potato mix (Sainsbury's own label), an orange (Moroccan Shamouti variety), and blackcurrant jelly concentrate (Sainsbury's own label). All products were eaten as purchased except for the instant mashed potato, which was hydrated [83:17 water:mash (w/w)]. The hydrated mash was eaten as prepared and with 2% sodium chloride added.

The eating protocol was similar for all measurements of salt and pH. Readings were taken every second, and with each sample, the subject was asked to make chewing movements before the food was introduced, to obtain a baseline recording for each sensor. The subject introduced the food into the mouth after 50 s of baseline signal had been obtained. All experiments used one person with sound dentition, who was told to chew using the same rate and force for every sample. There was no visual feedback to the person eating the food samples as the Picolog program only allowed the subject to see the time of chewing, and not the sensor traces. All samples were analyzed in triplicate. Water was used to rinse the mouth between samples, and 10 min was allowed between each sample to clear the palate and minimize the effects of fatigue.

Sample size depended on the product; 5 g of crisps and peanuts, 9.8 g of Cheddar, 11.6 g of Dairylea, and a 10 g sample of mashed potato were used for conductivity measurements. An 11.5 g block of undiluted blackcurrant table jelly (gelatine) concentrate and two segments (12.5 g) of a fresh orange were monitored for acid release.

RESULTS AND DISCUSSION

In-Mouth Salt Measurements. Food samples (salt and vinegar crisps, salted peanuts, Cheddar cheese, Dairylea cheese, and mashed potato with and without salt) were consumed by one person to avoid person-to-person variation. Plots of salt concentration (expressed as sodium equivalents) against time were constructed for each eating event and parameters such as the maximum salt intensity (I_{max}) and the time to maximum intensity; (T_{max}), were calculated from the curves. The reproducibility of the data (I_{max}, T_{max}) was assessed using the percentage coefficient of variation (%CV; standard deviation × 100/mean).

Table 1 shows the values obtained for the different foods with %CV values ranging 0.6−24%. Salted peanuts gave the highest variation, which was attributed to the fact that the salt was loosely dusted onto the surface of the nuts and, therefore, easily lost in the packet or on contact with the fingers. The salt content of each sample may therefore vary depending on the handling of the sample. Overall, the variation lay within the limits previously observed for in-mouth flavor release (Linforth et al., 1996).

Since food is not evenly mixed in mouth, there was some doubt whether the food bolus would make sufficient contact with the electrodes to produce a continuous signal. Although Jack et al. (1995) had produced traces from cheddar cheese, highly water-absorbent foods such as crisps present different problems, as they may absorb much of the saliva needed for the conductivity electrodes to function effectively.

Figure 1 shows the mean values obtained from eating three samples of salt and vinegar crisps.

Closer inspection of the trace in Figure 1 yields further information. The conductivity in-mouth was obtained for a period of 50 s, after which the crisps were introduced. However, there was a lag of 22 s after placing the crisps in the mouth before there was a significant increase in conductivity. Only after 72 s can an increase in conductivity be noted, suggesting that, before this time, the saliva containing salt does not reach the conductivity electrodes. Presumably, the introduction of crisps (typically 3−5% water) into the mouth absorbs much of the saliva from the oral cavity as the crisp matrix hydrates.

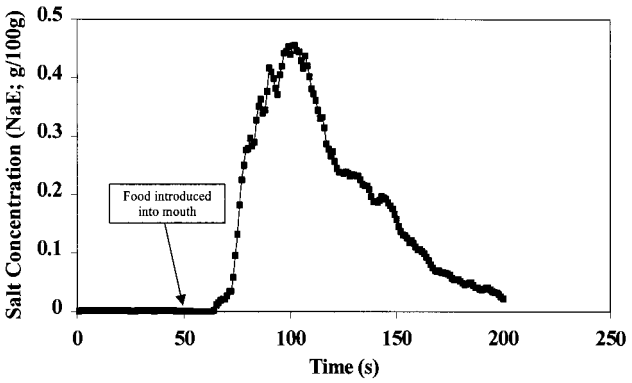

Figure 1. In-mouth salt concentration (■) [measured as sodium equivalents (NaE; g/100 g)] with time. The dental plate was fitted and the signal in the mouth was monitored (0−50 s) before the crisps were introduced at 50 s. Each point is the mean of three determinations.

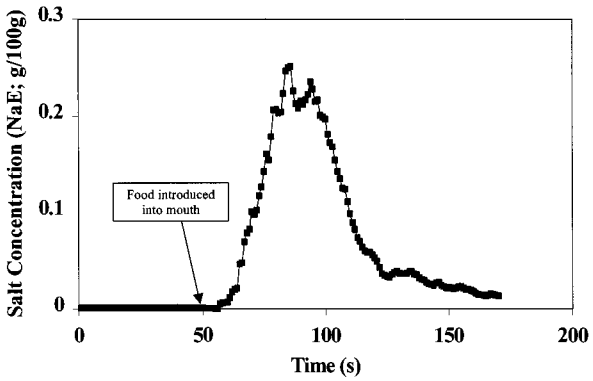

Figure 2. In-mouth salt concentration (■) [measured as sodium equivalents (NaE; g/100 g)] with time. The dental plate was fitted and the signal in the mouth was monitored (0−50 s) before the peanuts were introduced at 50 s. Each point is the mean of three determinations.

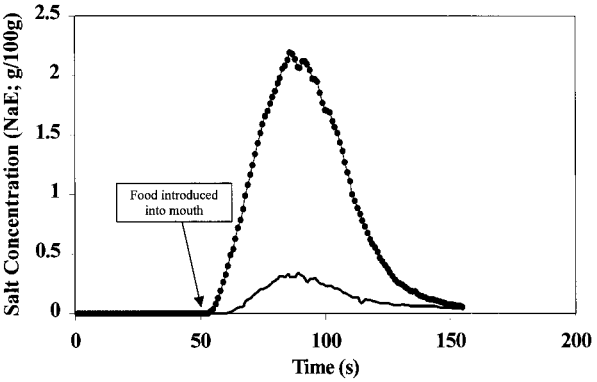

Figure 3. Comparison of the salt release [measured as sodium equivalents (NaE; g/100 g)] between Cheddar (—) and Dairylea (●) cheeses. The dental plate was fitted and the signal in the mouth was monitored (0−50 s) before the cheeses were introduced at 50 s. Each point is the mean of three determinations.

In contrast, the salt release curve from peanuts (Figure 2), had a lag phase 8 s shorter than the crisps, which was most probably due to the peanut absorbing less saliva.

Figure 3 shows the salt release curves of the Cheddar and Dairylea cheeses. The pack declarations list sodium concentrations of 1% for Dairylea and 0.7% for Cheddar. However, Dairylea recorded an I_{max} of 2.2% sodium equivalents while Cheddar showed an I_{max} of 0.42%. The discrepancies probably lie both in the properties of the

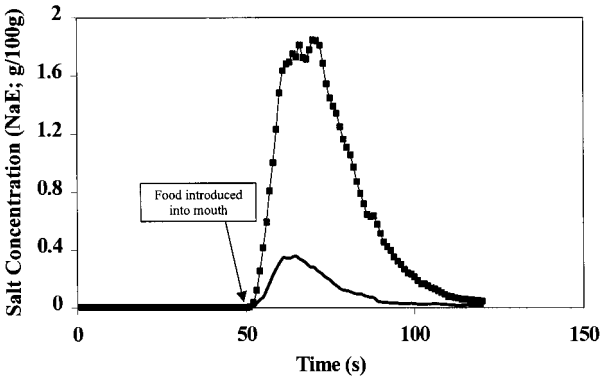

Figure 4. Comparison of the salt release [measured as sodium equivalents (NaE; g/100 g)] between standard mashed potato (—) and mashed potato with an additional 2% salt (■). The dental plate was fitted and the signal in the mouth was monitored (0−50 s) before the mashed potato was introduced at 50 s. Each point is the mean of three determinations.

cheese matrixes and in the chemical composition of the cheeses. Dairylea is a soft spreadable type cheese, and so its texture differs from the hard Cheddar. Dairylea therefore requires little effort to chew, and dissolves readily in the mouth. However, the Cheddar cheese structure requires more mechanical energy to breakdown, therefore lumps may remain in the mouth, which are swallowed whole. This means some of the salt may not be released in the oral cavity, which could explain the low I_{max} of the Cheddar.

However, the I_{max} of Dairylea was greater than the declared sodium content of the cheese. Further inspection of the declared additives shows that other salts are present as emulsifiers (E339, E341, and E452; the sodium, potassium, and calcium salts of various phosphate derivatives). The Codex Alimentarius recommends a maximum total level of emulsifying salts of 4%, but restricts phosphates to 3% (Kirk and Sawyer, 1991). These emulsifying salts almost certainly contribute to the conductivity measurements along with calcium naturally present in dairy products. Dairylea has a calcium content of 0.52%. However, up to two-thirds of the calcium was bound (Fox, 1997), and it is not known how much of the calcium is released upon chewing, if any. This demonstrates the limitation of conductivity measurements in foods where several types of ions are present.

Figure 4 shows the release curves for the mashed potato with and without added salt. Mashed potato was used as a model food because it has a consistent composition and the salt content can be manipulated easily. The unsalted mash has a low conductivity reading due to the small quantity of salt present in the mash powder. The average I_{max} measured for the salted mash was 1.85%, compared to an actual content of 2.4% which suggests that the majority of salt was released from this system and was available for in-mouth stimulation of the taste sensors.

Figure 5 shows the comparison of the measured salt release from mashed potato and the perceived salt concentration determined by TI analysis. Calculation of the %CV for the three replicate TI curves was 5.5% for I_{max} and 4.9% for T_{max}, showing that the results were very reproducible. This TI experiment was relatively simple using only one subject, with one level of stimulus. However, the results have highlighted some interesting points. A lag period can be seen between the concentration measured by conductivity and the perceived inten-

Table 2. Acid Release Measurements of Blackcurrant Jelly and Orange Segments Using the Beetrode Micro Electrode[a]

food stuff	replicate 1	replicate 2	replicate 3	avg	SD	CV (%)
blackcurrant jelly, pH_{min}	3.5	3.8	4	3.8	0.28	7.4
blackcurrant jelly, T_{min}	102	103	112	106	6	5
orange, pH_{min}	4.1	4.4	4.9	4.5	0.38	8.5
orange, T_{min}	81	69	83	78	8	10

[a] The minimum pH reached (pH_{min}) and the time (s) to the minimum pH (T_{min}) are shown, together with the standard deviation (SD) and the coefficient of variation (CV).

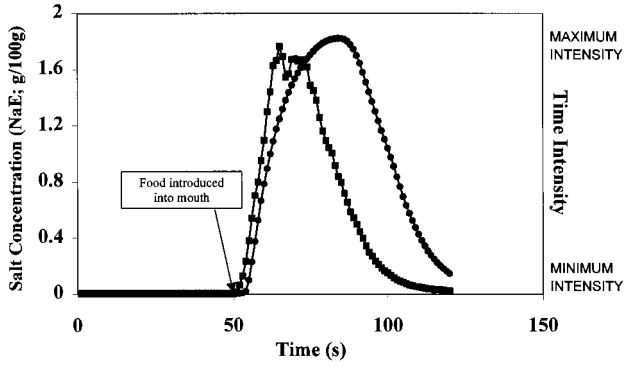

Figure 5. Comparison of the measured salt release (■) [measured as sodium equivalents (NaE; g/100 g)] and time−intensity values (●), for the mashed potato with an additional 2% salt. The dental plate was fitted and the signal in the mouth was monitored (0−50 s) before the mashed potato was introduced at 50 s. Each point is the mean of three determinations.

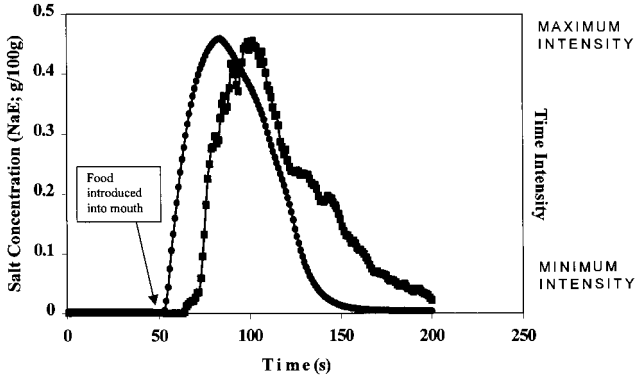

Figure 6. Comparison of the measured salt release (■) [measured as sodium equivalents (NaE; g/100 g)] and time−intensity values (●), for the salt and vinegar crisps. The dental plate was fitted and the signal in the mouth was monitored (0−50 s) before the crisps were introduced at 50 s. Each point is the mean of three determinations

sity. Overbosch et al. (1991) made similar observations on TI studies with aroma compounds and reported that "a subject's intensity estimate is the result of a signal integration over a period of several seconds". In this case, the subject was constantly comparing the present salt intensity with the previous intensity, and the outcome of that comparison determined whether the lever was moved, in which direction, and to what extent. This means that when the maximum level had been reached, the subject did not know that it was the maximum level until a certain time later, when they compared the intensities of following salt signals. In this way, a lag phase can be produced between measured and perceived intensity.

The reverse occurred when dry foodstuffs were tested. Figure 6 shows the comparison of the measured salt release from salt and vinegar crisps and the perceived salt concentration from average TI curves. The %CV for

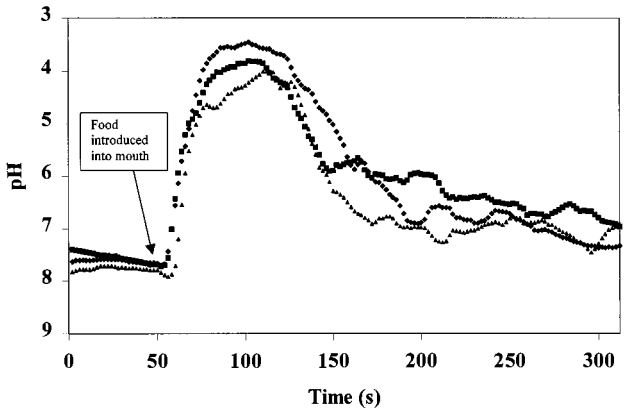

Figure 7. Change in mouth pH with time. The dental plate was fitted and the signal in the mouth was monitored (0−50 s) before the blackcurrant jelly was introduced at 50 s. Three replicates are shown [replicate 1 (◆); replicate 2 (■); replicate 3 (▲)].

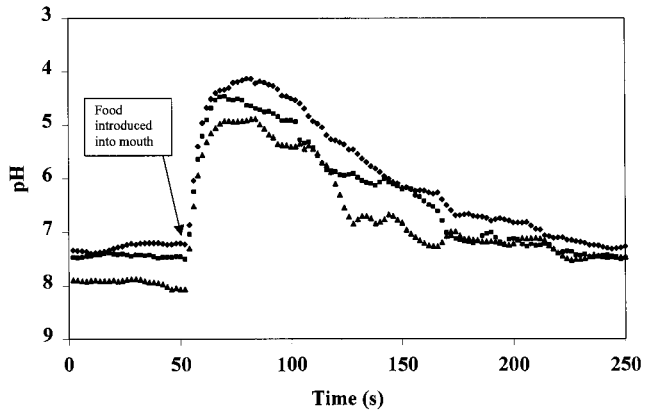

Figure 8. Change in mouth pH with time. The dental plate was fitted and the signal in the mouth was monitored (0−50 s) before the orange segments were introduced at 50 s. Three replicates are shown [replicate 1 (◆); replicate 2 (■); replicate 3 (▲)].

the TI curves was calculated as 2.3% for I_{max}, and 1.4% for T_{max}. This time the measured salt release curve lagged behind the TI curve. This may be explained by the low water content of the crisp. As discussed earlier, a low water content caused a delay in electrode response. However, the salt receptors of the subject may not exhibit such a delay, and consequently salt was perceived on the tongue before it was measured by the sensors on the dental plate.

In-Mouth pH Measurements. The acid release from blackcurrant jelly concentrate and orange segments was successfully measured. Along with the %CV (Table 2), visual inspection of the three replicates for both the jelly and orange (Figures 7 and 8) highlighted the reproducibility of the pH measurements.

When the minimum pH (pH_{min}) and the time to minimum pH (T_{min}) were determined (Table 2), it was

found that, for each successive replicate, the pH_{min} increased slightly even though the subject had a resting period of 10 min between samples and cleared the palate with water. This occurred when consuming both the jelly and orange segments. To investigate whether this was a drift problem with the Beetrode pH electrode, the subject was asked to expectorate into a beaker and the salivary pH was measured using a Jenway 3320 pH meter, using a general purpose combination electrode (BDH Gelplas, cat. number 309/1050/03) before and after eating a sample of jelly. An increase in the salivary pH was still observed.

When no food is being eaten, saliva has a pH between 6.0 and 7.4. Once chewing begins, more saliva is produced, which contains especially large quantities of potassium and bicarbonate ions. It is these latter ions that counteract the pH change due to acid from the foodstuff. However, even after the food is swallowed and the acid levels decline, the counterions are continually produced thereby causing the resting pH to be higher than normal. During active secretion, this buffering effect of the saliva can cause the in-mouth pH to approach 8.0 (Ganong, 1993). The acid release curves show that, with both the orange and jelly samples, the decrease in pH was rapid and initial mouth pH values were not restored for periods of around 250 s.

The microelectrode used produced adequate data but was prone to breakage, and ideally, an improved design is needed that is small, sturdy and flat. This would allow testing of harder foods, not just gelatine gels and soft fruits, which break down in the mouth relatively easily.

CONCLUSIONS

The experiments have shown that nonvolatile release can be measured in mouth, but there are certain considerations when carrying out such a task. The water content and textural properties of the food will affect the measurements of the changing salt and pH levels. This is because these sensors rely on the compounds to be dissolved in a certain amount of saliva, before they can be detected.

Future work will include the examination of the interactions between volatile and nonvolatile components of food by simultaneous measurements using the dental appliance and atmospheric pressure ionization-mass spectrometry (API-MS) (Linforth et al., 1996).

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