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Measurement of volatile release in the mouth

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Using extra strong mints as a simple food system, which contains high levels of volatiles and a simple aroma profile, different methods of sampling the volatiles in the mouth during eating have been investigated. Release of volatiles in the mouth is important in determining the profile perceived by the receptors in the nose and thus relates directly to our perception of aroma when food is eaten. Direct introduction of volatiles from the mouth and nose into a mass spectrometer did not provide information on the volatile profile, as the air introduced greatly reduced the sensitivity of the machine, and volatiles could not be reliably detected above the background noise. Cryogenic trapping on fused silica capillaries followed by gas chromatography and mass spectrometry did give profiles which were different for headspace, mouthspace and nosespace. Problems with loss of volatiles after trapping and the presence of water on the traps limited the usefulness of this method. Trapping on Tenax traps overcame some of these problems, and similar profiles to those obtained with cryogenic trapping were obtained. The amounts of two major volatiles in the headspace and nosespace were estimated and found to be menthone (8·32 and 24·3 mg m^{-3} air) and menthol (2·59 and 4·3 mg m^{-3} air), respectively. The concentrations of menthone in both headspace and nosespace were below the reported odour threshold value, but menthol was present in concentrations above the odour threshold. The method shows that volatile profiles during eating can be measured, but further development is required to improve sensitivity if the technique is to be applied to other foods.

INTRODUCTION

Most methods for determining volatile profiles in food are based on the analysis of *whole foods*, whether by solvent extraction or by headspace sampling. While this indicates the profile when we smell food prior to consumption, the physicochemical environment changes markedly when we eat food, and this affects the volatile profile and our perception of aroma. Mastication involves the addition of saliva (and amylase) to food, the comminution of food into smaller particles with a greater surface area and, sometimes, a change in temperature, which also plays a role. With emulsified products like butter, the initial phase (water-in-oil) is broken down in the mouth and this will inevitably affect the partition of volatiles between the oil and water phases with a concomitant change in the volatile profile.

The profile of volatiles in the mouth is transferred to the nasal membranes and it is presumably this profile which the brain recognises as characteristic of a particular food. The ability to measure this profile in the mouth/nose is, therefore, of interest as it relates more directly to the human perception of aroma when food is consumed. There is little information on flavour release in the mouth, mainly due to the lack of suitable methods, although some pioneering work has been published (Haring, 1990). Apart from the interest in understanding how aromas are released in the mouth, there are potential applications in product development. For instance, food products are often reformulated, to reduce cost, to reduce fat content or to replace a hydrocolloid. These changes will also affect the flavour of the food as the flavour compounds will partition in a different ratio between the phases or adsorb specifically to macromolecular components. If technologists could measure the volatile profile released in the mouth by the original product, new formulations could be checked against this profile to ensure that flavour release was still the same. The information provided by the method would be specific and demonstrate which compounds were over- or under-expressed. With this knowledge, flavours for the food could be reformulated. Currently, the major method of assessing the effect of different formulations on flavour release is sensory analysis. While this will always be the ultimate test for a food product, analysis of flavour release would provide a useful tool to assist in flavour reformation.

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This paper investigates three methods for analysing the volatile profile released in the mouth from foods. The terms mouthspace and nosespace have been used to describe the volatile profiles that exist in the mouth and nose during eating. Because eating is a fairly rapid process, sampling is required over a period of about 1 min. The amounts of volatiles released from food can be extremely small, so, to overcome potential problems of sensitivity, extra strong mints were used as they represent a simple system.

MATERIALS AND METHODS

Direct introduction of volatiles into mass spectrometric detector (MSD)

A Hewlett-Packard 5970 MSD (Manchester, UK) was connected to deactivated fused silica restriction tubing (1 m; 0·1 mm i.d.; Chrompack, London, UK) to restrict the flow of air to 1 ml min^{-1}. The free end of this tubing was held near to the nose or mouth (about 5 mm distance) to sample volatiles in the nosespace or mouthspace. The samples were drawn into the MSD by the vacuum present at the source. Samples were collected from both the mouth and the nose during the course of eating extra strong mints (Trebor, Rowntrees, UK). The MSD was operated in the selected-ion monitoring (SIM) mode, and the ions monitored were at *m/z* 44 (for carbon dioxide) and *m/z* 138 and 139 for the mint volatiles (menthol and menthone, respectively).

Cryogenic trapping of mint volatiles

The trap consisted of a length of column (1 m; 0·22 mm i.d. BP-20 column, 0·25 μm film thickness; SGE, Milton Keynes, UK) which was placed in a flask containing liquid nitrogen, such that the central 450 mm of the trap was cooled. A vacuum pump was connected to one end of the trap, which allowed air to be drawn through the trap (flow rate 20 ml min^{-1}; duration of trapping 10 s). For mouthspace and nosespace samples, air was sampled from one breath (via either the mouth or the nose, respectively), 30 s after the introduction of a mint into the mouth and subsequent mastication. Headspace samples were collected from the headspace above one mint in a 250 ml flask after equilibration for 1 h.

The trap was prepared for gas chromatography (GC) by connecting one end to a 60 cm length of column mounted in the injector (head pressure 2·5 psi; helium). The trap was still immersed in liquid nitrogen and liquefied air was removed by purging with helium for 30 s. The free end of the trap was then connected to the column (22 m × 0·22 mm i.d. BP-20, 0·25 μm film thickness; SGE). The connections were achieved by inserting the lengths of column into PTFE sleeves (6 cm) which had been stretched to produce a tight fit. The trap was then removed from the liquid nitrogen, and chromatography was started.

The volatiles were chromatographed using a temperature program from 35 to 180°C and ramped at 10°C min^{-1} after 1 min delay. Detection was achieved with the MSD in the SIM mode (*m/z* 138 and 139) and the peak areas of menthol, methone and their isomers were obtained by integration of the *m/z* 138 and 139 ion chromatograms.

Tenax trapping of volatiles

Samples were collected on to a Tenax trap (Unijector, SGE) via a length of deactivated fused silica tubing (1 m length; 0·53 mm i.d.) connected to the base of the Tenax traps. Air was drawn through the traps, using a vacuum pump (flow rate 20 ml min^{-1}, duration of sampling 1 min) connected to the other end of the trap.

Headspace samples were collected from the headspace above a mint in a 250 ml flask. Nosespace samples were collected from air near to the nose during the course of eating a mint. The samples were desorbed off the traps at 240°C (Unijector, SGE; head pressure 10 psi; helium) and refocused on the column (25 m × 0·22 mm i.d. BP-1; 1·0 μm film thickness, SGE) by cooling a 40 cm region of the column with liquid nitrogen. The volatiles were chromatographed from 50 to 200°C and ramped at 10°C min^{-1} after a 1 min delay. The amounts of menthol and menthone were estimated by integration of the *m/z* 138 and 139 ion traces as before.

Calibration

Solutions of menthone (80 μg ml^{-1}) and menthol (46·5 μg ml^{-1}) in methanol were prepared and 1 μl was applied to a Tenax trap. The trap was desorbed and chromatographed as described above and the peaks were integrated, using the *m/z* 138 and 139 ion traces. From the amount of menthone or menthol added and from the peak areas obtained by integration, an estimate of the amounts of menthol and menthone in the nosespace, headspace and mouthspace samples was calculated. Authentic standards were obtained from Aldrich (Gillingham, Dorset, UK).

RESULTS AND DISCUSSION

Volatile composition of extra strong mints

The volatiles above extra strong mints were sampled by a conventional headspace technique and analysed by GC–MS with the MSD in scan mode. The trace is shown in Fig. 1. The aroma profile of extra strong mints was extremely simple compared to most other foods, which can contain several hundred volatile compounds. The four components with retention times between 10 and 11 min were identified from their mass spectra and by chromatography of authentic standards as menthone (A), carvomenthone (B), carvomenthol (C) and menthol (D). While the positional isomers of

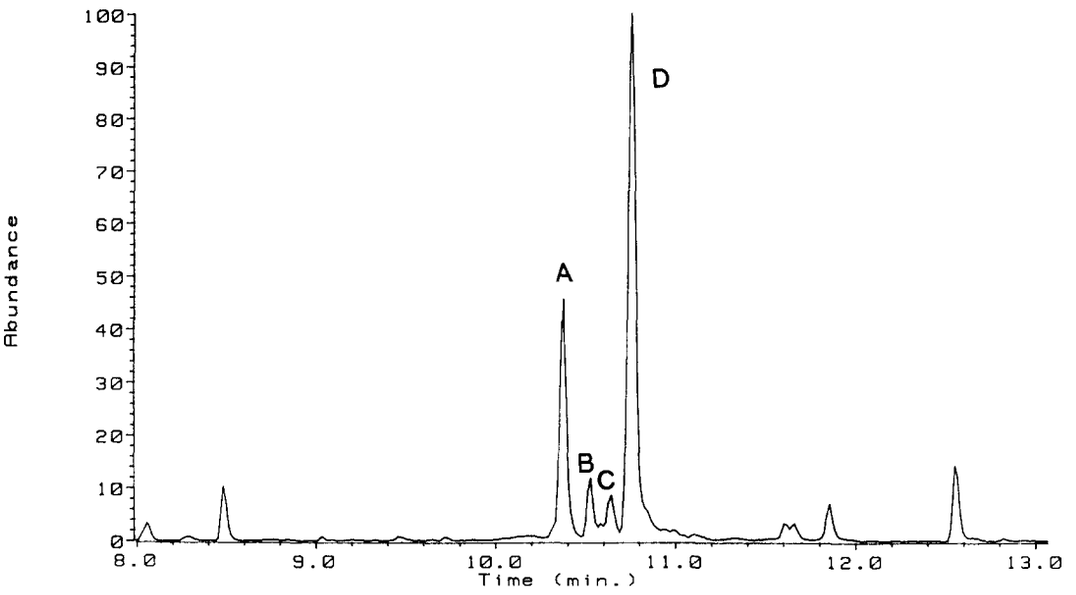

Fig. 1. Total ion chromatogram of a headspace sample of extra strong mints showing menthone (A), carvomenthone (B), carvo menthol (C), and menthol (D).

menthone and menthol were separated under the chromatographic conditions used, the optical isomers of menthol were not resolved. These compounds have significant odour properties in extra strong mints and were used as markers for further experiments. This was done so that the sampling methods described below could be investigated without the complication of many peaks.

Direct sampling of mint volatiles

Classical volatile analysis involves separation of the volatiles on a GC column, followed by identification by MS. However, with the simple mint system characterised by menthol and menthone (plus the isomers), GC separation is not entirely necessary. Instead, the compounds can be detected in the MSD, using SIM of the characteristic ions of these compounds. From the mass spectra, the significant ions for menthone (relative abundance in parentheses) were 41(100), 55(66), 69(73), 97(31), 139(39) and 154(26) and for menthol 41(98), 55(60), 71(100), 81(80), 95(74), 123(27) and 138(14). Similar mass spectra were obtained for carvomenthone and carvomenthol. The ions at *m/z* 138 and 139 were chosen for menthol/carvomenthol and menthone/carvomenthone, respectively. Operating the MSD in the SIM mode increased sensitivity, but the profiles obtained were different from those obtained in scan mode because the MSD is measuring only part of the ion current. Therefore, direct comparison of the profile in Fig. 1 (scan) and the data in Tables 1 and 2 (SIM) is inappropriate.

A system was set up where volatiles from the mouth or nose could be sampled through a piece of restriction tubing (to limit the flow to 1 ml min $^{-1}$) straight into the MSD with the vacuum in the MSD drawing samples through. The MSD was set to SIM (*m/z* 44 for carbon dioxide and *m/z* 138 and 139 for mint volatiles. Figure 2 shows a typical trace.

The upper trace shows the increase and decrease of carbon dioxide (*m/z* 44), which is related to the individual breaths of the subject. The lower trace shows the background signal (*m/z* 138–139) when air was introduced (0 to 0·8 min) and when air from the nose was sampled (0·8 to 1·3 min). At 1·3 min a mint was put in the mouth and chewed with the lips sealed and the restriction tubing held close to the nose. For the next minute, the nosespace was sampled and from 2·4 to 3·1 min the mouthspace was sampled by breathing in and out through the mouth with the tubing held close to the

Table 1. Relative amounts of the four major peaks (A, B, C, D) of extra strong mints (expressed as a percentage of the total area) obtained from methenol extracts (MeOH), cryogenic trapping of headspace (HS), nosespace (NS) and mouthspace (MS)

Sample	Peak A	Peak B	Peak C	Peak D
MeOH	41·8 ± 0·9	8·9 ± 0·9	8·0 ± 0·5	38·8 ± 2·9
HS	47·3 ± 0·9	9·6 ± 0·7	7·2 ± 1·7	32·6 ± 3·5
NS	67·4 ± 1·2	11·2 ± 1·4	4·2 ± 0·7	15·6 ± 0·9
MS	50·9 ± 1·4	11·4 ± 1·0	3·3 ± 0·2	30·8 ± 1·9

Values are the means of three determinations ± standard deviation.

Table 2. Main peak areas and percentage of total areas for the four major peaks (A, B, C, D) of extra strong mints obtained from Tenax trapping of headspace (HS) and nosespace (NS)

Sample	Peak A	Peak B	Peak C	Peak D
HS	4 841 ± 1329	1 503 ± 209	368 ± 96	3 600 ± 1 095
Area (%)	49·0 ± 4·4	10·8 ± 1·0	3·8 ± 0·5	36·5 ± 4·5
NS	14 170 ± 1471	2 499 ± 248	406 ± 30	1 997 ± 62
Area (%)	74·2 ± 1·0	13·1 ± 0·6	2·1 ± 0·1	10·5 ± 0·8

Values are the means of three determinations ± standard deviation. Units for peak area are arbitrary units.

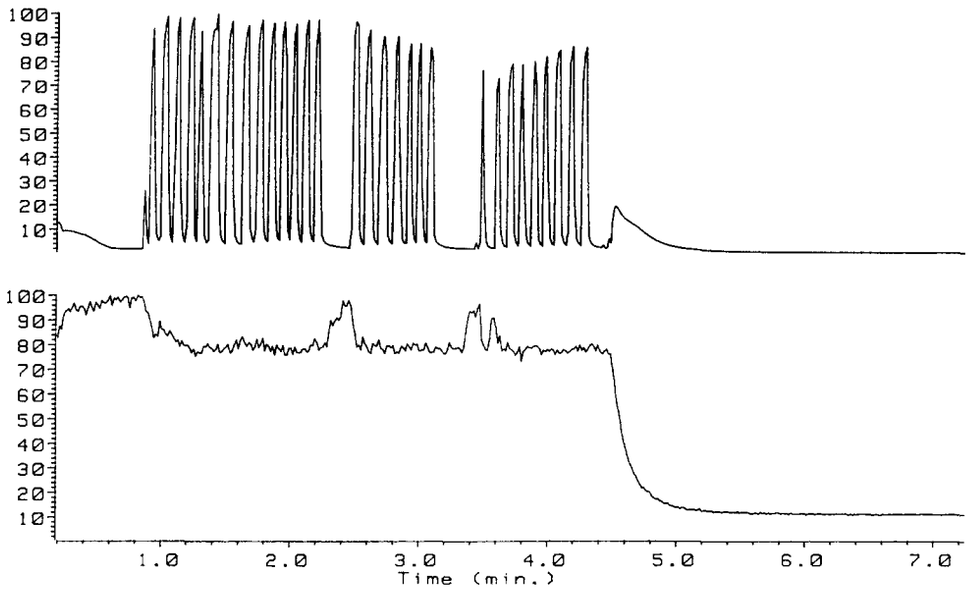

Fig. 2. Direct introduction of mouthspace and nosespace into the MSD. Upper trace (*m/z* 44, carbon dioxide) shows the breathing pattern of the subject. Lower trace (*m/z* 138 and 139) shows menthone and menthol. 0–0·8 min: air from laboratory sampled; 0·8–1·3 min: air from nose sampled; 1·3–2·4 min: mint chewed, nosespace sampled; 2·4–3·4 min: mint chewed, mouthspace sampled; 3·4–4·4 min: second mint introduced, mouthspace sampled; 4·4 min: sampling tube sealed.

lips. At 3·4 min, another mint was introduced and the mouthspace was sampled. Considering the high levels of volatiles in the mouth, the signals obtained are low and this is due to the low sensitivity of the MSD when air is the carrier gas. The background is much higher with air compared to the normal carrier gas, helium. An autotune of the MSD with air flowing showed a five-fold decrease in signal from the perfluorobutylamine standard. When the column was sealed (4·4 min) the background dropped rapidly. These experiments showed that direct analysis of mint volatiles was not possible with the MSD. A mass spectrometer with greater pumping capacity might have produced better results. It was obvious that air had to be separated from the volatiles prior to MSD analysis.

Cryogenic trapping of mint volatiles

Cryogenic trapping was performed on lengths of capillary column (1m length; 0·22 mm i.d.) of which the central 450 mm were immersed in liquid nitrogen. Air was drawn through the tubing by a vacuum pump, and the other end of the tubing was placed near to the nose or mouth or connected to a bottle containing mints for headspace collection. Although the lengths of capillary had a volume of 38 μl (by calculation), up to 3·3 ml of air could be drawn through the tubing and trapped without blocking the traps. Narrow bore fused silica capillary was used because it has excellent heat transfer properties during heating and cooling due to its low thermal mass. It was also easy to connect into the GC via the unions described in Materials and Methods.

Following removal of the trap from the liquid nitrogen, expansion of liquefied gases within the trap was substantial and caused the connections between the trap and the column to blow apart. This could be avoided by connecting one end of the trap to the helium supply for 30 s while keeping the trap in liquid nitrogen and purging out the liquefied gases. After purging, the other end of the trap was connected to the column and samples were chromatographed and detected by the MSD operating in the SIM mode.

Samples were collected from the mouth and nose of a subject chewing a mint or by collecting the headspace above a mint placed in a sealed bottle. In addition, a methanolic extract of mints was prepared and the results of these experiments are shown in Table 1. The profiles obtained from the four sampling methods show interesting differences. The methanolic extract profile showed peaks A and D dominant and roughly equal. In the nose, however, peak A represented 67·4% of the volatiles with peak D falling to 15·6%. The mouthspace and headspace profiles were similar with respect to peaks A and D. Table 1 shows that the values obtained for the three replicates were fairly consistent with a mean coefficient of variation (CV) (SD × 100/mean) of 8·12% and maximum and minimum values of 23·6 and 1·78%, respectively. These values are within the limits expected for headspace analysis (Larsen & Poll, 1990). Breakthrough was investigated by connecting two traps in series and chromatographing each in turn. No volatiles were detected in the second trap, showing that breakthrough was insignificant.

This system had its advantages and disadvantages. Trapping was complete as shown by the breakthrough experiments. It was easy to use and cheap to set up. However, the need to remove liquified gases prior to GC was a concern as it was not certain whether volatiles were lost at the same time. Although the profiles of the four compounds were consistent when

expressed on a percentage area basis, the actual amounts of material on each trap were variable despite the standardisation of the sample collection and analysis procedure. It seems that the removal of liquefied gases is accompanied by loss of volatiles, although the effect on the profile of these four closely related compounds was minimal. Compounds with a wider range of volatility might not be so amenable to this loss. In addition, cryogenic trapping accumulated water in the traps, which could block the traps if sampling was continued for longer periods or if the traps were not sealed after sampling. The presence of water also affects chromatography and, while not important in the analysis of these compounds, may be a problem with other volatiles.

Tenax trapping of mint volatiles

Tenax is a popular adsorbent for volatiles and while it has certain disadvantages (for instance the volatile profile changes according to the time of sampling, Wyllie *et al.*, 1978), it does have the advantage of retaining little water. Since the sampling time was short and constant, it was thought that Tenax would give consistent profiles which would provide comparative data for the different sampling methods. The previous experiment had shown marked differences in profiles from headspace and nosespace, so a system was set up which drew air from these samples through a Tenax trap at a known flow rate using a vacuum pump. The Tenax traps were then desorbed and chromatographed to give the results in Table 2.

Again there was a difference in the profiles obtained from headspace (A 49·0%; D 36·5%) and nosespace (A 74·2%; D 10·2%) and these are highly comparable with the results of cryogenic trapping (Table 1). The variation between replicates was acceptable and of a similar order (CV 7·75%) to that seen for cryogenic trapping. The actual amounts of material trapped on Tenax were measured as the peak area and, in Table 2, it can be seen that there was variation in these amounts (10–30% CV). However, this was less than the variation seen on cryogenic trapping, which was of the order of 100%. The amount of water trapped on Tenax was much lower than that trapped in the cryogenic method, which resulted in 'cleaner' chromatograms. Although some workers have recommended purging traps with dry nitrogen to remove excess of water prior to chromatography (Boyko *et al.*, 1978; Ishihara & Honma, 1992), this may result in the loss of very volatile components and was not attempted in these experiments.

Estimation of menthone and menthol in headspace and nosespace

Solutions containing menthol and menthone at know concentrations were applied to Tenax traps and analysed by GC-MS, using the same chromatographic and detection conditions as for the samples (Table 3). Authentic menthone (80 ng) separated into two peaks which were thought to represent menthone (Peak A, 78%) and its isomer, carvomenthone (Peak B, 22%), as the mass spectra for Peaks A and B were almost identical. Chromatography of the optical isomers of menthol (46·5 ng) gave a single peak (D). The amounts of menthone and menthol in whole mints and in the nosespace and headspace of Tenax trapped samples (Table 2) were estimated. For menthone, it was assumed that, since 80 ng was applied and 78% was found in Peak A, the amount in Peak A was 62 ng. The content of menthone and menthol in whole mints was estimated as 0·76 and 1·7 mg kg^{-1}, respectively. The amounts in the Tenax trapped headspace and nosespace were 166 and 487 ng (menthone) and 450 and 249 ng (menthol), respectively. These correspond to concentrations of 8·32 and 24·3 mg m^{-3} air (menthone) and 22·5 and 12·5 mg m^{-3} air (menthol), respectively. A comparison with published threshold values (Gembert & Nettenbreijer, 1977) shows thresholds of 42·9 mg m^{-3} for menthone and 2·1 mg m^{-3} for menthol. From these crude data, it would appear that menthone concentrations in the nose are below the threshold, but menthol concentrations are six to ten times threshold, suggesting that menthol is the more important aroma compound of the two.

Table 3. Relative amounts of menthone and menthol in the Tenax trapped headspace (HS) and nosespace (NS) and a methanol extract (MeOH) of extra strong mints

Sample	Menthone peak (A)	Menthol peak (D)
MeOH extract (mg kg^{-1})	760	1 690
HS (mg m^{-3} air)	8·32	22·5
NS (mg m^{-3} air)	24·3	12·5

It is recognised that many other food aromas (for example, diacetyl; threshold 0·003 mg m^{-3}) have thresholds substantially lower than the values for menthone and menthol and, for the analysis of nosespace in foods containing diacetyl, the sensitivity needs to be increased by a factor of about 1000.

CONCLUSIONS

The MSD is not suitable for direct sampling of air as the sensitivity is greatly affected. Mass spectrometers with greater pumping capabilities may be more amenable to this technique although the potential for burning filaments when air is introduced should not be ignored. The cryogenic and Tenax trapping methods produced comparable results which show that the volatile profile of mints in the headspace, mouthspace and nosespace are very different. Mints, however, contain high amounts of volatiles (about 2·5 mg kg^{-1}) and the sensitivity of the systems to other foods needs investigation.

These experiments show that volatile profiles in the mouth and nose can be measured during eating and they also show that, in this system, the profiles do change as the mint is masticated. Further development

is required to improve sensitivity so that the method can be applied to other food systems.

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