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# The effect of eating on aroma release from strawberries

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The aroma of strawberries was sampled directly from the expired air of people eating strawberries and by a headspace method which had been designed to mimic maceration in the mouth. Comparison of the aroma profiles showed that, using the modified headspace technique, it was possible to produce headspace profiles for the esters, very similar to the profile observed in the nosespace. The C6 aldehydes hexanal and (*E*)-2-hexenal were present at higher amounts in the headspace than the nosespace. The amounts of these volatiles in the headspace of strawberries increased as the homogenisation period increased. Reducing the duration of homogenisation before headspace collection resulted in lower amounts of C6 aldehydes and headspace profiles virtually identical to the nosespace profiles. The rate of release of aroma volatiles from strawberries during eating was also investigated. This demonstrated that the maximum concentration of aroma volatiles occurred at the point of swallowing. Prior to this, volatile concentrations were 40–60% of the maximum level observed: this is likely to have been associated with swallowing the juice and saliva present in the mouth at this time, resulting in transfer of volatiles to the nasal cavity. The volatiles which persisted the longest in the nosespace were the smaller esters, methyl acetate and ethyl acetate. This may be due to the differences in polarity between these compounds and other less-polar esters such as methyl butanoate.

## INTRODUCTION

The analysis of the volatile compounds that produce the aroma of foods has been extensively studied using gas chromatography, often in conjunction with mass spectrometry. The aroma compounds that contribute to the aroma of different foods are known and, by using techniques such as steam distillation or purge and trap, the absolute amounts of each compound present can be determined. However, it is often difficult to relate these instrumental analyses to sensory data. Considerable attention has been focused on improving the statistical analysis of instrumental and sensory data, or the conversion of instrumental data into units which take into account the odour thresholds of aroma components. This may, however, adjust rather than solve the problem.

One fundamental problem could be the way in which samples are prepared for analysis. Steam distillation and purge and trap are ideal as methods for the analysis of the total volatile composition of aroma compounds in food. They do not, however, yield any information about the composition of volatiles released from food and sensed at the olfactory epithelium as it is eaten (Taylor & Linforth, 1994). Yet it is this combination of volatiles which is perhaps the most important, since it is this volatile profile which is perceived by consumers or taste panellists. It may therefore prove useful to develop techniques for the determination of the volatile profile at the olfactory epithelium during eating.

Despite considerable interest in flavour release, very few papers have been published in which aroma volatiles have been collected from the breath (Soeting & Heidema, 1988; Haring, 1990). The analysis of aroma compounds in air expired from the nose during the eating of mint-flavoured sweets was described by Linforth and Taylor (1993), who termed the aroma profile 'nosespace'. They found that, using a simple food and collecting breath over 1 min (to produce a time-averaged profile), reproducible volatile profiles could be obtained. Further work on mints showed that the time-averaged profile was similar for different people (Ingham *et al.*, 1995*a*).

In addition to the time-averaged profile, the time course of flavour release in the mouth has been investigated. Ingham *et al.*, (1995*b*) measured the changes in the aroma profile as mints are eaten, whilst Legger and


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Roozen (1994) have shown correlations between the amount of 2-methyl butanal present on the breath and the perceived intensity of chocolate flavour. Time intensity studies have shown that some volatiles persist longer in the breath than others (Ingham *et al.*, 1995*b*); however, it was not possible to determine which properties of the volatiles caused changes to the aroma profile. Consequently, it is necessary to investigate flavour release in other systems.

Many of the volatiles that contribute towards strawberry aroma are esters (Hirvi & Honkanen, 1982), which are chemically similar (relative to the mint volatiles studied) and cover a range of boiling points. Consequently, strawberries provide a useful food system which may allow further insight into the factors that affect flavour release. This current paper describes time-averaged volatile profiles obtained by sampling breath from people eating strawberries and the time course of flavour release.

## MATERIALS AND METHODS

### Headspace samples

Samples of strawberries (100 g; purchased from a local supermarket) were placed in a stomacher bag and stomached (Seward M50-110, London, UK) for 10–600 s. A sample of the headspace (40 $cm^3$) was then drawn through a Tenax trap (60/68 mesh, SGE, Milton Keynes, UK) over 1 min, using a vacuum line.

### Nosespace samples

For the time-averaged profiles, breath expired from the nose was sampled onto a Tenax trap (40 $cm^3$ $min^{-1}$) whilst eating nine strawberries (typical weight, 90 g total) over 3 min as described previously (Ingham *et al.*, 1995*a*).

For the nosespace time course, strawberries were placed in the mouth and chewed for 10 s before the solids were swallowed. During the first 10 s it was not possible to avoid swallowing altogether, so subjects were allowed to swallow the strawberry juice and saliva when necessary, but, the solids were retained for the 10 s period. The mouth was kept closed from the point of placing the strawberry in the mouth until the end of the breath sampling period. Breath was sampled onto Tenax traps (400 $cm^3$ $min^{-1}$) over a 180 s period for the following times; 0–10, 5–15, 10–20, 15–25, 20–40, 30–50, 40–60, 60–90, 90–120 and 120–180 s. Rather than sampling breath from just one individual, samples of breath were collected on the same Tenax trap from five individuals (for the relevant time interval) consecutively. It was hoped that this would reduce any variations in the results associated with variation between individual strawberries.

### Volatile analysis

Volatiles were analysed on a VG MD800 bench top mass spectrometer (Fisons Scientific, Manchester, UK) connected to a Hewlett Packard 5890 Series II gas chromatograph fitted with a headspace injector trap (Chisa injector, SGE, Milton Keynes, UK). The volatiles were desorbed from the traps for 2 min at 240°C (column head pressure 18 psi (1 psi ≈ 6·9 kPa); carrier gas helium) and re-focused on a 400 mm length of the column (25 m × 0·22 mm i.d. BP-1; 1·0 μm film thickness, SGE) which was cooled with liquid nitrogen. The volatiles were then chromatographed from 40 to 200°C at 4°C $min^{-1}$ after a 2 min delay. Volatiles were identified in preliminary chromatograms on the basis of their mass spectra and retention indices. Subsequently, data were collected for sample chromatograms using selected ion monitoring; *m/z* 44, hexanal; *m/z* 55, (*E*)-2-hexenal; *m/z* 61, ethyl acetate; *m/z* 71, ethyl butanoate and butyl butanoate; *m/z* 74, methyl acetate, methyl butanoate, methyl-3-methylbutanoate and methyl hexanoate; *m/z* 88, ethyl hexanoate.

## RESULTS AND DISCUSSION

### Comparison of headspace and nosespace techniques for the analysis of aroma volatiles

Many of the methods used for the analysis of aroma volatiles using headspace sampling involve sweeping the compounds present in the gas phase above the food onto porous polymer traps (such as Tenax). The time taken for sample collection can range from a few minutes, up to several hours, with variable sweep gas flow and different sampling temperatures. Typically, the main objective is the collection of sufficiently large samples of volatiles to allow detection and quantification of as many volatiles as possible. These samples are unlikely to have a similar volatile profile to that present at the olfactory epithelium during eating.

Using modern analytical equipment it is possible in some cases to obtain sufficient volatiles for analysis from as little as 10 $cm^3$ of headspace; consequently it is possible to modify the way in which headspace is collected in order to obtain a profile that may be closer to the profile present at the olfactory epithelium. When food is eaten it is chewed briefly (2–30 s) after which it is swallowed. As the food is swallowed, some of the volatiles in the gas phase in the mouth are forced past the soft palate into the nasal cavity and over the olfactory epithelium in the nose. In an attempt to simulate the chewing action (and the partitioning of volatiles into the nasal cavity) we have developed a headspace collection system in which food is homogenised for short periods of time in a stomacher (where the food in a plastic bag is compressed alternately by two metal plates); then some of the headspace is drawn onto a Tenax trap using a vacuum line. Unfortunately, the stomacher bags used in sample preparation also contributed volatiles to the headspace; however, none of these had similar mass spectra or retention times to the compounds of interest.

Comparison of the headspace profile with the nosespace profile will indicate the contribution of the maceration/partitioning process to the overall release of

**Table 1. Mean percentage peak areas for selected volatiles in the headspace (samples homogenised for 1 min) and nosespace (nine strawberries eaten over 3 min) of strawberries**

| Compound | Headspace | Nosespace |
|---|---|---|
| Methyl acetate | 41 | 47 |
| Ethyl acetate | 3·3 | 3·5 |
| Methyl butanoate | 38 | 41 |
| Methyl 3-methylbutanoate | 0·91 | 0·62 |
| Hexanal | 2·8** | 0·35 |
| Ethyl butanoate | 1·9 | 1·7 |
| (*E*)-2-Hexenal | 4·1** | 0·0 |
| Methyl hexanoate | 6·1 | 4·7 |
| Butyl butanoate | 0·06 | 0·09 |
| Ethyl hexanoate | 0·37 | 0·49 |

**Amounts of volatiles present in the nosespace and headspace are significantly different (*t*-test; $n = 4$; $P < 0{\cdot}01$).

aroma in the mouth. However, the similarity of the headspace and nosespace profiles will depend on the relative importance of the physical breakdown of the food, compared with other factors such as air flow, swallowing and volatile binding. The headspace of stomached strawberries and nosespace profiles of strawberries during eating were found to be broadly similar (Table 1), suggesting that maceration is one of the most important factors affecting the volatile profile present at the olfactory epithelium when strawberries are eaten. There was, however, a significant difference between the two profiles in the amount of C6 aldehydes observed. Hexanal and (*E*)-2-hexenal were present at much lower amounts in the nosespace relative to the headspace ($P < 0{\cdot}01$).

Hexanal and (*E*)-2-hexenal are typical products of enzymic oxidation of lipids (Hatanaka, 1993). They may have been present in the intact fruit or generated as the strawberries were homogenised. If they were generated on homogenisation, then the concentration of these two volatiles would be expected to increase with progressively longer periods of homogenisation. The amounts of hexanal and (*E*)-2-hexenal were found to increase relative to the amount of esters as the duration of homogenisation was increased (Fig. 1; $R = 0{\cdot}7479$, $P < 0{\cdot}01$). This confirms that these compounds are generated upon homogenisation, either by chemical degradation, or more likely enzymic oxidation of lipids. The significance of these two compounds would clearly be over-estimated in samples which were homogenised for several minutes, or if headspace was collected for long periods of time without inhibition of the processes which give rise to these compounds.

The amounts of hexanal and (*E*)-2-hexenal in the headspace profiles were most similar to those in nosespace if strawbewrry samples were only homogenised for 10–30 s. This is similar to the time taken to eat a strawberry. The ratio of the esters within the headspace samples did not vary significantly over the duration of the time course. The profiles of esters in the headspace appears to be dependent on release rather than generation.

The similarity of the headspace and nosespace profiles of strawberries (Table 1) was a direct result of the way in which the headspace was collected. Strawberries are, however, a high moisture food and such similarities in volatile profile might not occur for other foods which have a lower water content. When the headspace of mints was collected from above dry crushed mints, or mints crushed in water, it was the profile of the mints crushed in water that most resembled the nosespace profile (Ingham *et al.*, 1995*a*). This suggests that, for mints, it is hydration, rather than physical breakdown which may have the strongest influence on the volatile profile present at the olfactory epithelium.

### The dynamics of volatile release from strawberries as they are eaten

Previous studies have shown that, even for simple foods such as mint flavoured sweets, the major volatiles

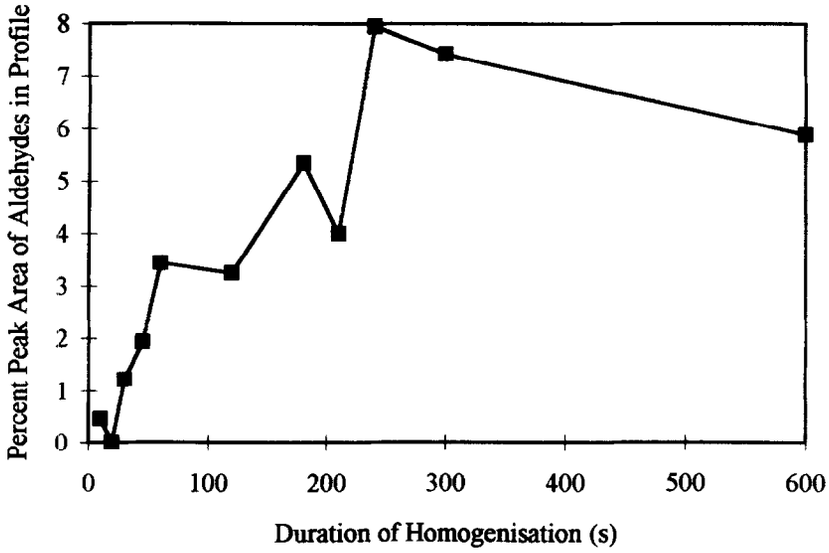


**Fig. 1.** Percentage peak area of the C6 aldehydes (hexanal and (*E*)-2-hexenal) as a proportion of the headspace aroma profile of strawberries as they are homogenised for progressively longer time periods.

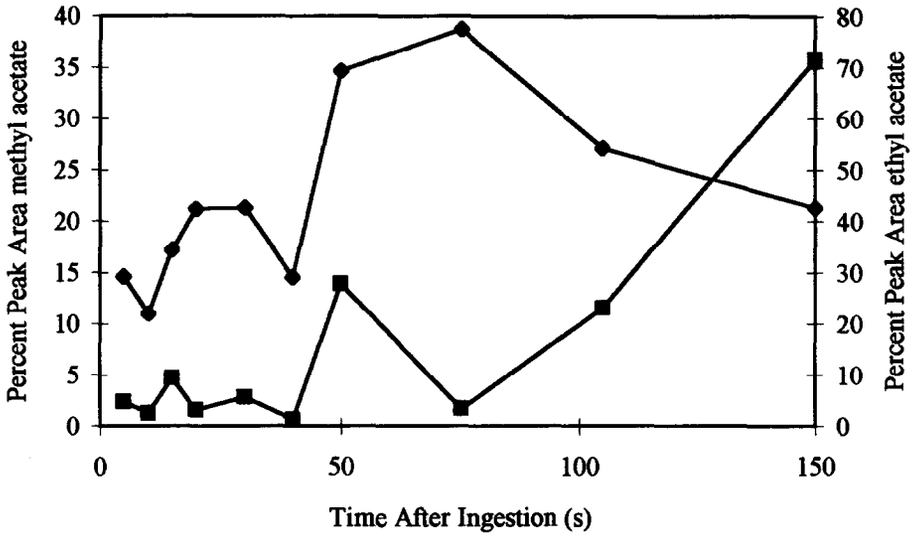


**Fig. 2.** Percentage peak area of methyl acetate (◆—◆) and ethyl acetate (■—■) as a proportion of the nosespace aroma profile, during the time course of eating strawberries.

(limonene, menthone and menthol) have different patterns of release and persistence (Ingham *et al.*, 1995*b*), which seemed to be related to their different polarities and boiling points. Many of the volatiles observed in the headspace and nosespace of strawberry samples, however, all belong to the same chemical class (esters). Thus it may be easier to observe trends in the pattern of volatile release and to relate these trends to chemical differences.

From preliminary experiments it was evident that strawberries contained lower amounts of volatiles than did mints and were less uniform as a food, which may increase experimental variation. Consequently it was necessary to improve the method of sample collection used for the time course experiments. Sampling times were chosen such that they were of short duration (10 s) at the start, becoming progressively longer (up to 60 s) as the time course progressed. This provided more detailed information on the amounts of volatiles present at the start of the time course (when concentrations were at their highest and greatest changes might occur), whereas longer sampling periods allowed adequate detection of volatiles even though their concentrations were much lower towards the end of the time course. At the beginning of the chromatogram, overlapping sampling times were used (0–10, 5–15 s, etc.), to obtain more estimates of the changes in the amounts of volatiles present in the nosespace around the time at which the strawberries were swallowed. The resulting data points represented the volatiles collected over specific time windows, and were plotted at a time corresponding to the mid-point of the time window (for example, the sample collected from 0 to 10 s was plotted at the 5 s time point).

To minimise variation that would result from short (10 s) sampling times and the variation between strawberries, nosespace was collected from five different individuals, for the same time interval, using one trap. This also

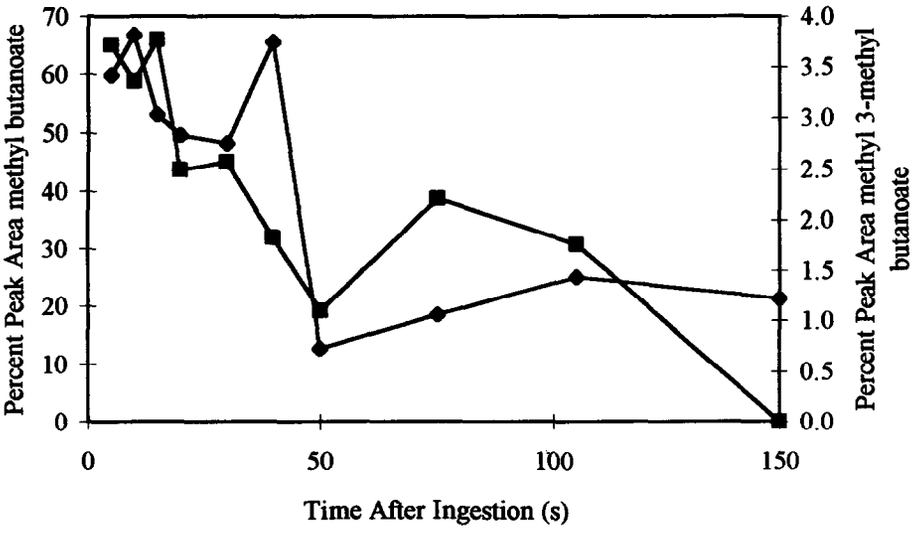


**Fig. 3.** Percentage peak area of methyl butanoate (◆—◆) and methyl 3-methylbutanoate (■—■) as a proportion of the nosespace aroma profile, during the time course of eating strawberries.

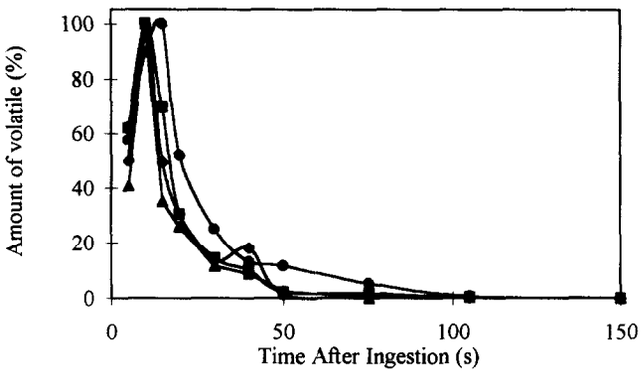


**Fig. 4.** Amounts of methyl acetate (●—●), methyl butanoate (◆—◆), methyl 3-methylbutanoate (■—■) and methyl hexanoate (▲—▲), in the nosespace profile relative to the maximum amount (100%) of each volatile in the breath, over the time course of eating strawberries.

increased the amount of volatiles collected on each trap, which helped overcome any difficulties with sensitivity.

One of the objectives of the time course experiment was to look for changes in the volatile profile over time. If the results are plotted as absolute amounts, it is often difficult to observe the change in relative proportions of volatiles within the profile as the concentration changes. Consequently, the results for each volatile were plotted as a percentage of the total peak area of the profile, such that the changes in the proportions of each compound can easily be observed.

The percentage peak areas of the major aroma compounds released in the strawberry nosespace time course experiments are shown in Figs 2 and 3. Methyl acetate increased relative to the other compounds over the first 90 s of the time course ($R$ = 0·7063, $P < 0·05$) and then decreased slightly. The proportion of ethyl acetate in the aroma profile also increased ($R$ = 0·8296, $P < 0·01$), principally towards the end of the time course. The relative amounts of the higher boiling point volatiles (b.p. > 100°C) in the nosespace profile all decreased over the duration of the time course (methyl butanoate $R$ = –0·7287, $P < 0·05$; methyl-3-methylbutanoate $R$ = –0·8454, $P < 0·01$; ethyl butanoate $R$ = –0·6616, $P < 0·05$; methyl hexanoate $R$ = –0·6607, $P < 0·05$). However, the changes observed in the release of esters from strawberries with time were much less than the changes in the aroma profile of mints as they were eaten (Ingham *et al.*, 1995*b*).

In the experiments on mints (Ingham *et al.*, 1995*b*), the high boiling point compounds with the greatest polarity were the most persistent in the breath; however, it was not clear which factor was most important due to the large differences between the volatiles present. For the strawberry volatiles, the compounds with the greatest persistence appear to be the low boiling point compounds (b.p. < 100°C) which were slightly more polar. This indicates that polarity may be more important than boiling point, in determining the persistence of volatiles in the breath and hence in the after-taste.

If the most significant factor in the retention of methyl acetate is polarity, then this is likely to be associated with the greater affinity of methyl acetate for the aqueous phase (and possibly other polar compounds such as proteins). It might be expected that the higher boiling point volatiles would be less volatile and consequently the most persistent. However, from the air–water partition coefficients of methyl acetate and methyl hexanoate ($4·7 \times 10^3$ and $15 \times 10^{-3}$, respectively, Buttery *et al.*, 1969), it is more reasonable to expect a greater release of methyl hexanoate as strawberries are eaten and consequently less retention in an essentially polar environment.

Whether such changes have a significant effect on the after-taste of strawberries is uncertain, since the majority of volatiles were present at less than 20% of their maximum amounts 20 s after swallowing (a total of 30 s after the start of the time course) and less than 5%, 20 s later (Fig. 4). The greater persistence of methyl acetate relative to other esters can clearly be seen in Fig 4. Figure 4 also shows the sharp rise in the amount of individual volatiles around the point of swallowing (10 s time point, breath sampled from 5 to 15 s). The relatively high amounts of volatiles present in the breath (40–65% of maximum) prior to the swallowing of the fruit solids, is probably due to volatile transfer, associated with swallowing the juice released from the fruit (and saliva). A major part of the volatile release process may therefore involve the release of the juice from the fruit as it is crushed and subsequent partitioning of volatiles between the juice/saliva and gas phase. This situation is very different from the mint system where a dry food is broken down and combined with saliva.

**Table 2. Percentage peak areas for volatiles in the headspace (samples homogenised for 30 s) and nosespace (average of time course 5, 10 and 15 s time points from Fig. 4) of strawberries**

| Compound | Headspace | Nosespace |
|---|---|---|
| Methyl acetate | 19 | 27 |
| Ethyl acetate | 14 | 2·5 |
| Methyl butanoate | 51 | 61 |
| Methyl 3-methylbutanoate | 7·1 | 3·5 |
| Hexanal | 0·11 | 0·06 |
| Ethyl butanoate | 0·72 | 0·19 |
| (*E*)-2-hexenal | 0·21 | 0·05 |
| Methyl hexanoate | 6·1 | 5·0 |
| Butyl butanoate | 0·02 | 0·02 |
| Ethyl hexanoate | 0·01 | 0·01 |

**Comparison of time-averaged and time course maximum aroma profiles**

The maximum volatile concentration in the breath occurred around the point of swallowing (5, 10 and 15 s data points). It may be better to determine the aroma profile over this time period rather than the time-averaged profile, as this would represent the aroma profile at the point of maximum release and excludes any contribution of the after-taste. The percentage peak areas of the volatiles in this nosespace aroma profile (Table 2) are, however, fairly similar to those in the time-averaged

profile (Table 1), although there are some differences. Higher proportions of methyl 3-methylbutanoate were observed in the 'time course nosespace volatile profile' and the methyl acetate:methyl butanoate ratio was approximately 1 : 2 compared with 1:1 in the time-averaged profiles (Table 1). However, comparison of the headspace profiles (Tables 1 and 2) suggests that these differences may be due to variation between the batches of strawberries analysed. There are clearly greater differences between strawberries than the methods of volatile collection used and changes in the nosespace profile are paralleled by changes in the headspace profile.

## CONCLUSION

Headspace sampling under defined conditions can produce similar volatile profiles to nosespace sampling in the analysis of strawberry aroma. In the application of this headspace method to other food systems, it is important to consider the effects of enzymes, lipids, rheology and hydration, all of which may alter flavour release and necessitate an alternative headspace system. The similarity of these alternative artificial-mouth/headspace methods to the actual flavour release in the mouth, should be confirmed by comparison of the headspace profiles obtained, with time-averaged nosespace profiles and by investigation of changes in the nosespace profile over time.

It is now clear that modern analytical equipment can be used to measure volatile release directly. We now need to discover whether this technique produces data which correlate better with sensory analysis and can measure, analytically, the smallest differences detectable by sensory panels.

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