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Differences in the Effect of Bolus Weight on Flavor Release into the Breath between Low-fat and High-fat Products

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The maximum intensity of flavor release increased as the weight of food introduced into the mouth (the bolus) was increased for a range of different foods. The relationship was not directly proportional (1:1) but followed a power law function. Low-fat (≤1 g/100 g) foods showed a different relationship than high-fat (≥5 g/100 g) foods, but all low-fat and all high-fat foods were broadly similar irrespective of food type or flavor molecule chemistry. For low-fat foods the intensity of flavor release increased with increasing bolus weight to a greater extent than high-fat foods. This may be associated with the capacity of fat to selectively adhere to the surfaces of the oral cavity, thereby changing the effective surface area for the release of lipophilic flavors.

KEYWORDS: APCI; MS; lipid

INTRODUCTION

There have been many studies devoted to studying flavor release in vivo. These have considered several aspects, such as the influence of matrix composition or the relationship between flavor release and perception. The effect of perhaps the most fundamental feature of any food sample, the amount consumed, has however received little attention.

Studies of flavor release from solutions of different volumes (3−18 mL) showed little difference in the maximum intensity of flavor release (*1*). In this aqueous system mass transfer from the liquid to the gas phase occurs through partition, and it is therefore the concentration in the liquid phase that determines the maximum concentration transported to the nose.

For solid foods mass transfer takes place from the solid matrix to the mouth liquid phase (saliva) and then to the gas phase (breath). In this case the amount of matrix and flavor present in the mouth may have significant effects on aroma release, while mastication will determine the degree of matrix breakdown and the effective surface area. This might be expected to vary for foods which exhibit different types of in-mouth behavior (melting, hydrating, fracturing, or dissolving) as a result of their basic structure, which may result in diverse bolus size−flavor release relationships.

When food is consumed the amount of a food introduced into the mouth is often dependent on the foodstuff itself (*2*), which reflects expectations of the ease, or complexity of oral processing required. Bolus weight has been shown to be an important factor affecting the way chewing gum is processed in mouth (*3*). In addition to affecting the way in which we eat, bolus weight can also impact sensory perception (*4*), which is why it is often controlled in such analyses. Consequently, the physical weight of the bolus is a key food characteristic.

The purpose of the current study was to investigate the relationship between bolus weight and flavor release. By using a range of different food types, panelists would use different oral processing strategies and allow investigation of the question: does each food type (e.g., foods that hydrate rather than melt) behave differently or are there similarities between types of foods?

MATERIALS AND METHODS

Food Samples. Samples used in these experiments are shown in **Table 1**. Some of the samples were commercial products purchased from a local supermarket (mint sweets, cornflakes, fruity chewing gum, cheese). Biscuit dough was flavored and baked in house, flavored ice was prepared by the addition of flavor compounds to water that was then frozen, and gelatin gels were prepared using gelatin, sucrose, and glucose syrup. The chocolate samples were prepared by adding flavor to molten chocolate and allowing it to solidify. Low-fat yogurt and mashed potato (prepared from dried potato powder) were purchased from a local supermarket and flavored, and the fat content was adjusted by the addition of cream (an equivalent amount of water was added to the corresponding low-fat samples).

The samples were consumed by a panel of six people in a range of portion weights, typically dependent on the amount that could reasonably be consumed. For some of the samples, such as mint sweets and cornflakes, the smallest samples weighed less than 150 mg. These weights were not abnormally small but were in the range of normal consumption. The smallest sample corresponded to two cornflakes or one whole mint sweet.

Table 1 shows the largest and smallest sample portion consumed, which were typically 8-fold different (samples 2, 3, and 5 times the size of the smallest sample were also consumed). For the mashed potato the sample range was greater; for these experiments the samples

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Table 1. Food Sample Type, Aroma Compound, Ion Monitored, Fat Content, and Weight Range

sample	compound	*m/z*	fat content (g/100 g)	weight range (g)
biscuit	carvone	151	18	1.0–8.0
cheese	heptan-2-one	115	35	0.5–4.0
chewing gum	3-methylbutyl acetate	131	0	0.67–5.3
chocolate	3-methylbutyl acetate	131	30	1.45–11.6
cornflake	methylpropanal	73	1	0.13–1.0
gel	limonene	137	0	1.0–7.6
ice	octan-2-one	129	0	0.5–4.0
mint sweets	menthol	139	0	0.14–1.2
LF yogurt[a]	ethyl hexanoate	145	0.1	1.0–8.0
HF yogurt[b]	ethyl hexanoate	145	5	1.0–8.0
LF mashed potato	cymene	134	0	0.25–12
HF mashed potato	cymene	134	5	0.25–12
LF mashed potato	pyrazine	81	0	0.25–12
HF mashed potato	pyrazine	81	5	0.25–12

[a] LF Low fat. [b] HF High fat.

weighed 0.25, 0.5, 1, 2, 4, 8, and 12 g. Each panelist consumed one portion of all sample weights of one foodstuff during a session. Panelists were given water to cleanse their pallet during breaks of at least 5 min between samples; individual sessions were at least 1 week apart.

The panelists were asked to consume the foods in a normal manner appropriate to the type of food. The majority of the foods were chewed, except for the mint sweets and the ice sweets, which were sucked. Panelists were not instructed to consume the samples at a specified rate but to swallow the samples when they would normally.

Analysis of Flavor Release. As the foods were consumed, the release of aroma compounds into the breath was measured using a Platform II mass spectrometer (Waters, Manchester, U.K.) fitted with an atmospheric pressure chemical ionization (APCI) source. The corona pin discharge was set at 4 kV in positive-ion mode, which resulted in the formation of the protonated molecular ion. The only exception to this was menthol, which formed the dehydrated, protonated molecular ion.

Breath expired from the nose was sampled into the source at 30 mL/min through a heated (140 °C) deactivated fused silica tube. The maximum intensity of flavor release (I_{max}) was recorded for each sample. For each foodstuff these values were normalized by dividing I_{max} for each sample by either I_{max} of the smallest sample consumed or I_{max} for the 1 g sample.

Statistical analysis was performed using Design Expert 6.0.6 (Statease, Minneapolis).

RESULTS AND DISCUSSION

Flavor Release from Chewing Gum and Small Mint Sweets. The first two samples tested were fruit-flavored chewing gum containing isoamyl acetate and small (average weight 140 mg) mint-flavored sweets containing menthol. These represented very different sample types. Chewing gum is typically localized between the teeth on one side of the mouth and chewed, whereas the small mints are moved around on the tongue as they dissolve (panelists were instructed to suck the mint sweets and not to chew them). Different eating patterns affect not only interaction with saliva, but also mouth movements, which may affect volatile transmission from the mouth to the nose (*5*, *6*). The two samples also have different surface area-to-volume ratios. Increasing the number of small mints results in an equal increase in the initial surface area and volume of the product. The chewing gum bolus could however be thought of as a single sphere, where an increase in volume does not increase the surface area of the bolus to the same extent. In addition, there were also differences in the weight range of two products (**Table 1**) and the properties of the flavor compounds themselves, which may affect flavor delivery.

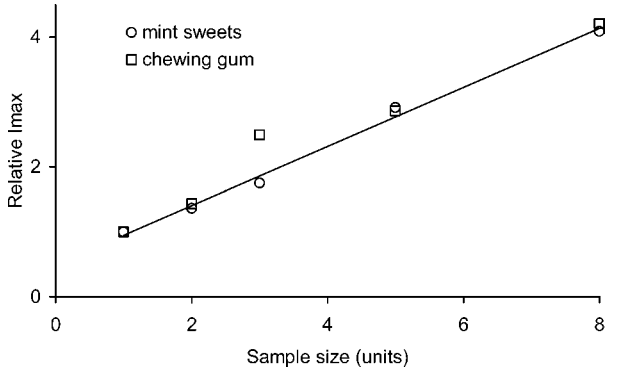

Figure 1. Influence of bolus size on the maximum intensity of flavor release for small mint sweets and chewing gum. The data were normalized to I_{max} of the smallest sample in each case. Each value is the mean of six replicate samples, each consumed by a separate panelist.

The maximum intensity of release (I_{max}) for the chewing gum and small mints samples was normalized to I_{max} of the smallest sample consumed. This allowed easier comparison of the additional flavor released from the two samples as their weight increased. The relationship between bolus weight and I_{max} of flavor release was approximately linear as the sample size was increased by a factor of 8 (**Figure 1**). Despite the variation between panelists (typical %CV; %CV = 100 × mean/standard deviation = 50% for a sample) the average values were all close to the trendline (**Figure 1**). Six panelists appeared to have been sufficient to minimize any influence of outliers, allowing the influence of sample size to be observed clearly. Surprisingly there were no significant differences (at the level $P < 0.05$) in the relative increase in flavor release for the two sample types as each sample increased in size, despite the large number of differences between the two samples.

A 1:1 relationship between bolus weight and flavor delivery might be expected, particularly when the samples are physically small. This assumption appears in the flavor release models of Hills and Harrison (*7*, *8*), where flavor release from chewing gum and boiled sweets (similar to the small mint sweets) was considered to be directly proportional to the surface area of the sample. This should be the case at least for the small mint sweets where an increase in bolus weight was achieved by consuming a greater number of sweets (one, two, three, five, or eight at a time). However, flavor delivery only increased by a factor of 4, while the bolus weight increased by a factor of 8. This was a much smaller increase than expected.

Increasing the sample weight might result in an increase in the concentration of volatile compounds in saliva if they were released into one pool of saliva. Alternatively, if flavor was released into localized pools of saliva, there would be an increase in the surface area of saliva involved in flavor release. Either option should, in turn, result in a proportional increase in volatile delivery into the breath.

It is possible that the samples themselves directly affected flavor release via a feedback mechanism. The increase in saliva volatile (and nonvolatile) concentration with increasing sample weight may have affected the in-mouth volatile/nonvolatile concentration gradients (from food to saliva) reducing dissolution of the small mint sweets, restricting further release. However, this mechanism should result in a nonlinear relationship between bolus weight and release as larger amounts of sample inhibit further flavor release to a greater extent. This was not observed.

In addition, the shape of the release time course for the mint sweets was similar as the bolus weight increased (**Figure 2**).

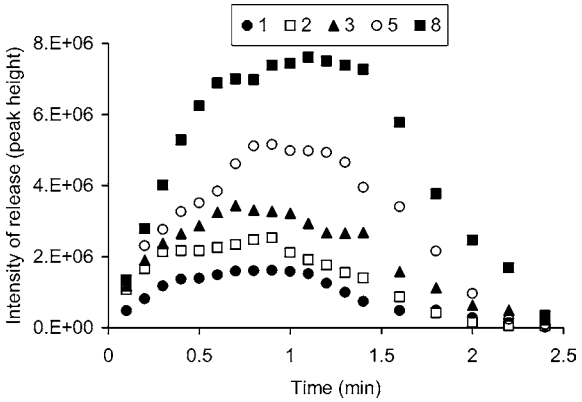

Figure 2. Influence of bolus size on the time course of flavor release from small mint sweets. Each value is the mean of six replicate samples, each consumed by a separate panelist.

This shape would not be expected if there was substantial inhibition of sample dissolution by solutes in saliva, which would cause the time course of release to increase substantially with bolus weight. These observations are consistent with the finding that abrasion, rather than the rate of dissolution, can be a major factor affecting release in such systems (*7*, *8*). The release mechanism most affected by solute concentration would be rate of dissolution.

The increase in flavor release as the number of sweets increased may have been due to imperfect mixing of saliva and the portions of bolus in mouth. This may result in increases in saliva flavor concentration only at the junctions between the pools of saliva into which each sweet dissolved. Consequently, the increase in flavor release would not increase in direct proportion to the amount of bolus present.

It is also known that food consumption can have a substantial effect on the saliva flow rate (*9*). This can depend on the in-mouth sugar concentration (*10*) but will also increase even if an unflavored chewing gum base is chewed (*11*). Direct measurement of sugar release in mouth (*12*) showed that sugars released from chewing gum were at their highest concentration in saliva close to the bolus, decreasing sharply further away in the mouth (volatile compounds would be expected to show a similar pattern). This pattern was evident over a 5-min time course, implying substantial dilution and removal of saliva (and the compounds within it) during consumption. Otherwise the in-mouth concentration would have progressively increased, becoming more homogeneous throughout the oral cavity as saliva circulated around in the mouth. It is possible that the increase in sample weight stimulated an increase in the saliva flow rate, limiting the increase in saliva volatile concentration (through dilution and removal during swallowing) and hence release overall. This is the most likely mechanism to explain the relationship between bolus weight and flavor release into the breath.

Flavor Release from Eight Food Samples. The initial study of chewing gum and small mint sweets was extended to include a wider range of food types with contrasting flavor release mechanism. Some of these samples were ones that hydrate upon consumption (biscuits and cornflakes), while others would melt (ice, chocolate, and gelatin gels). It was anticipated that these samples might show a different relationship between bolus weight and flavor release than that observed for chewing gum and mint sweets.

The samples with the lowest lipid content showed a broadly similar relationship between bolus weight and flavor release (**Figure 3**) despite the differences in in-mouth processing and

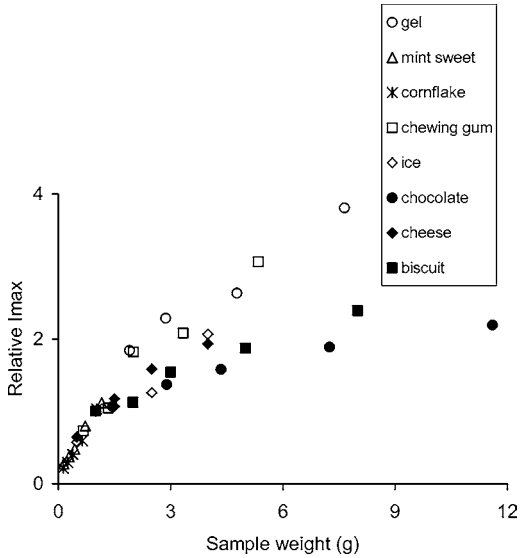

Figure 3. Influence of bolus size on the maximum intensity of flavor release for a range of food samples. The data were normalized to I_{max} for a sample size of 1 g from curves fitted to each data set. Each value is the mean of six replicate samples, each consumed by a separate panelist.

the physical chemistry of the aroma compounds themselves. The trends in the flavor release curves were close to the linear relationship observed for the small mint sweets and chewing gum (**Figure 1**), although there did appear to be a slight deviation for the heavier samples. A decrease in flavor release might be anticipated as the bolus weight increases more and more due to the limited potential for increasing the surface area for flavor release as the mouth becomes fuller. In addition, there might be a direct impact of the volume of sample in mouth physically influencing the eating action itself. It is important to remember that the mouth is optimized more for food management and ingestion rather than flavor delivery.

The samples with the highest fat contents showed a similar relationship to each other, different from that of the low-fat foods. These samples showed a much smaller increase in flavor release as the bolus weight increased (**Figure 3**). These differences could not be attributed to differences in in-mouth processing since high-fat and low-fat food types both included foods that hydrated and those that melted upon consumption. Neither could the differences be attributed to the physical chemistry of the flavor compounds involved since both high-fat and low-fat samples contained ketones, esters, and cyclic molecules (**Table 1**). There appeared to be an effect of fat content on the relationship between bolus weight and flavor delivery for a series of largely unrelated food types. This was further evaluated using pairs of samples which were similar, except for their fat content.

Flavor Release from High- and Low-Fat Samples. Samples of yogurt and mashed potato were prepared which differed primarily in their fat content (**Table 1**). Other factors such as protein content would also be different as cream was used to increase the fat content. However, no obvious differences associated with other compositional differences were observed for a range of foodstuffs (**Figure 3**) and were consequently considered to be less significant. The low-fat samples had fat contents close to zero, while the high-fat samples contained 5 g/100 g fat, lower than the fat content of the previous samples.

The relationship between bolus weight and flavor release for both systems showed that there was a different bolus weight−flavor release relationship for the high-fat and low-fat samples (**Figures 4** and **5**), both of which were significantly different

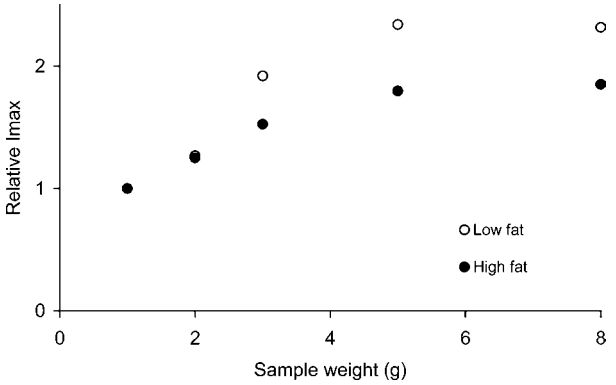

Figure 4. Influence of bolus size on the maximum intensity of flavor release for a low fat (0.1 g/100 g) and high fat (5 g/100 g) yogurt. The data were normalized to I_{max} for a sample size of 1 g. Each value is the mean of six replicate samples, each consumed by a separate panelist.

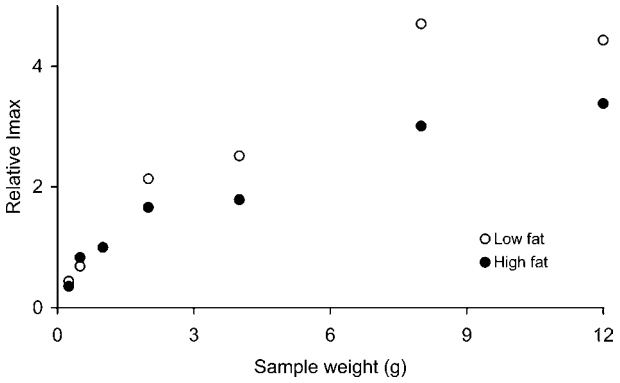

Figure 5. Influence of bolus size on the maximum intensity of flavor release for a low-fat (0.0 g/100 g) and high-fat (5 g/100 g) mashed potato. The data were normalized to I_{max} for a sample size of 1 g. Each value is the mean of six replicate samples, each consumed by a separate panelist.

($P < 0.01$). For both foods the low-fat food showed a much greater increase in flavor release as bolus weight increased compared with the equivalent high-fat food. This trend was consistent with those observed for the previous food samples. In addition, these two sets of samples also showed a curved relationship between the bolus weight and flavor release, with progressively smaller increases in flavor release with further increases in the bolus weight.

When these data sets were combined with the previous data sets on one graph (**Figure 6**) the curved nature of the bolus weight−flavor release relationship and its dependence on fat content can clearly be seen. High-fat foods appeared to release less additional flavor as bolus weight increases compared with low-fat food samples. The two trendlines fitted to the samples were power functions of the form $y = ax^b$ ($b = 0.67$ for low-fat samples and 0.44 for high-fat samples). The overall correlation coefficients (R^2) were greater than 0.9, a reasonable correlation given the diversity of the samples.

Flavor Release of Pyrazine from Low- and High-Fat Mashed Potato. The influence of fat may have depended on the interaction of flavor molecules with the fat itself and the redistribution of the fat within the foodstuff. This might involve the fat partially separating from the bolus and spreading around the mouth. Hence, there would be different behaviors in mouth of fat and nonfat phases during eating. Alternatively, the presence of fat may have physically altered the structure and behavior of the entire food product in mouth and hence flavor release. In this case fat would enhance oral coating by the whole product. It was impossible to discriminate between the two

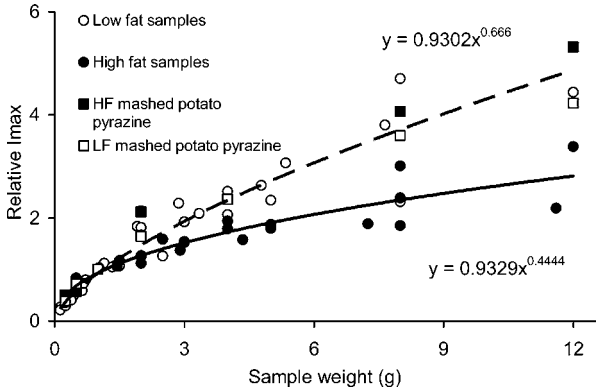

Figure 6. Influence of bolus size on the maximum intensity of flavor release for a low-fat and high-fat sample (data from **Figures 3**–**5**) and the release of pyrazine from low-fat and high-fat mashed potato. The data were normalized to I_{max} for a sample size of 1 g. Each value is the mean of six replicate samples, each consumed by a separate panelist.

possibilities given the samples already tested. All of the high-fat food samples contained compounds that were lipophilic (lowest log $P = 1.73$) and were consequently likely to partition into the fat present.

Samples of high- and low-fat mashed potato containing pyrazine were prepared. Pyrazine has a log P of −0.06 and consequently would be expected to distribute itself equally between the fat and nonfat phases of the samples. When these samples were consumed, both sets of samples showed the same bolus weight to flavor release relationship irrespective of fat content (**Figure 6**), the same relationship as previously seen for the low-fat samples. The difference between the low-fat and high-fat samples was therefore dependent on the interaction of the flavor molecules with the lipid. The bolus weight to flavor release relationship for hydrophilic compounds was not influenced by the presence of lipid. This indicates that the effect of fat was not due to a general increase in oral coating by the whole product.

Mechanism of Bolus Weight−Flavor Release Relationships. For certain sample weight ranges there appears to be a linear relationship between bolus weight and flavor release (**Figure 1**). However, when a greater range of samples was examined, the curves describing the relationship between bolus weight and flavor release were found to have a power law shape (**Figures 5** and **6**). For low sample weights this resulted in a near linear increase in flavor release, with less and less additional flavor released as bolus weight increased further.

These trends should be related to factors such as the surface area for release and the bolus to in-mouth gas volumes. For many of the foods the initial increases in bolus weight would have resulted in an approximately proportional increase in bolus surface area. Then additional increases in bolus weight would contribute less and less to the effective surface area for flavor release, a factor which will be highly dependent on the physiology of the mouth and the way it adapts to control and manipulate the bolus. This would be consistent with the bolus weight−flavor release relationship. Equally, the in-mouth bolus to gas volume relationship would follow the same trend. Both of these relationships might be expected to be the same for high-fat and low-fat foods, so why were they different?

The bolus to gas to saliva volume relationship should not be influenced by the presence of lipid. However, the effective surface area of the bolus might be influenced by fat content. The presence of fat might increase oral coating and adhesion of the bolus to the oral cavity, resulting in a larger surface area

for release than expected for a given sample weight. Increasing the bolus weight would have less effect in this case (as seen in **Figure 6**), with oral coating approaching a maximum with even a small bolus. Simply increasing oral coating would not explain the behavior of the pyrazine in the high-fat mashed potato. If the effect of fat was simply bolus adhesion increasing surface area, then these samples should have shown the same relationship as the other high-fat foods.

Therefore, the effect of the lipid appears to have been dependent not only on the fat content, but also on the capacity of the flavor compound to partition into it. This may be related to the observation that different components of food products (fat, protein, and carbohydrate) are retained to different extents in the oral cavity during consumption. de Jongh et al. (*13*) found that after consumption the fat from mayonnaise was present on the surfaces of the oral cavity at higher concentrations than in the original product.

If the fats present in the food bolus selectively adhere to the surfaces of the oral cavity, they can enhance the surface area available for flavor release, compared with low-fat foods which have components (protein, carbohydrate) with lower adhesion properties. Flavor molecules dissolved in the fat would be more dispersed around the oral cavity with the potential for release from both the bolus- and fat-coated surfaces. This will, however, depend on the capacity of these molecules to dissolve in the lipid. Hydrophilic flavor molecules present in either low-fat or high-fat foods would be expected to behave as if they were present in low-fat foods. They are not present at higher concentrations in the fat, and hence, the fat-coated surfaces would not form a reservoir for their release. Only the hydrophobic molecules will preferentially partition into the fat, enhancing their surface area for release.

The effect of bolus weight on flavor release has several implications for flavor delivery. A low-fat product designed to deliver the same amount of flavor as a high-fat product will achieve this at a given bolus weight. Increasing or decreasing the bolus weight will result in differences in flavor delivery. If flavor delivery for high- and low-fat products is the same for a 1 g sample, it will be 60% higher for the low-fat sample relative to the high-fat sample when 8 g samples are consumed. The low-fat sample increases its delivery by a factor of 4 as the sample weight increases from 1 to 8 g, while the comparable high-fat samples increase delivery by a factor of only 2.5.

In addition, for a fat-containing product there will be differences in the relative release of hydrophobic and hydrophilic compounds with bolus weight. In the case of pyrazine and cymene in high-fat mashed potato, if they are released in equal quantities from a 1 g sample, the pyrazine will be released at higher levels relative to cymene as the sample weight increases. The relative proportions of the flavor molecules in the breath, and hence those delivered to the olfactory epithelium, will be altered.

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