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In Vivo Flavor Release from Gelatin–Sucrose Gels Containing Droplets of Flavor Compounds

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Gelatin–sucrose gels containing the same amount of flavor compounds present as either suspended droplets or homogenously distributed in the gel (dissolved) were eaten, and the in vivo flavor release was studied using atmospheric pressure chemical ionization–mass spectrometry. The maximum intensity of release was higher from all droplet-containing samples as compared with the dissolved sample (by a factor of 4–2500-fold). When the flavor was dispersed as a greater number of smaller droplets rather than one 1 μL droplet, the intensity of in vivo release was slightly lower. The release of 16 of the flavor compounds varied in their Log *P* (range 0.26–4.83) and vapor pressure (Log vapor pressure ranged from −1.09 to 1.99). The differences in release for flavors present as either droplets or dissolved in the gel matrix were strongly influenced by both of these factors. This suggested a different mechanism for flavor release from droplets as compared to the classical partition mechanism established for dissolved flavors.

KEYWORDS: Aroma release; APCI-MS; nosespace; droplets; aroma dispersions; flavor; I_{max}; sensory; gelatin–sucrose gels

INTRODUCTION

Flavor release from real foods, as well as from model systems, has been studied in vitro and in vivo using a variety of analytical techniques (see ref *1* for a review). The conventional mechanism for flavor release assumes that during mastication or consumption, the flavor compounds are solubilized in the liquid phase and are transferred to the gas phase in the mouth through mass transfer, diffusion, and partition (*2*). Gas from the mouth is then transported to the tidal air flow in the throat due to chewing or swallowing (*3, 4*), and "pulses" of flavor find their way to the olfactory epithelium where the sensing process starts. Various models of flavor release have been proposed (see ref *5* for a review), all of which assume solubilization in the liquid phase and gas–liquid partition as the driving force for transfer to the gas phase.

However, many flavor compounds are sparingly soluble in water, and it can be difficult to prepare an aqueous solution of hydrophobic flavor compounds. For this reason, cosolvents such as ethanol or propylene glycol are often used. These, however, can also influence the release of flavor compounds from food matrices (*6*), as can flavor–flavor interactions (*7*).

When other solutes are present (e.g., sugars), then the solubility of many flavor compounds in water can be affected (*8*). The same effect occurs when foods are dehydrated during food processing, and it is pertinent to question in what form the flavor compounds exist in products such as hard-boiled candies (water content less than 1%) or in dry cereal foods (water content below 10%). For foods that contain fat, then it is likely that the flavor compounds are located in the fat phase. However, for some food products, it is probable that the flavor compounds are not in solution but phase separated. Some potential examples are confectionery products where the flavor compounds are in a high sugar matrix and encapsulated flavors where the initial flavor solution is spray dried.

The purpose of this study was to study flavor release from droplets of the pure compounds as compared to release from solubilized flavor. A simple gel system was used as it had a relatively high water content (ca. 45%) but could also hold droplets due to the gel network. Release was investigated in vivo as gels were eaten.

MATERIALS AND METHODS

Materials. Flavor compounds (>97% pure) used were amyl acetate and ethyl butyrate obtained from Firmenich (Geneva, Switzerland); octanol, 2-octanone, limonene, and hexanal from Acros Organics (Loughborough, United Kingdom); 2-butanone, methyl acetate, 2,3-diethylpyrazine, citral, linalool, ethyl hexanoate, ethyl octanoate, menthofuran, and carvone from Aldrich (Gillingham, United Kingdom); and ethyl acetate from Fisher Scientific UK Ltd. (Loughborough, United Kingdom). These compounds were selected for their wide range of physicochemical properties (**Table 1**) and used without any further treatment. β-Carotene and propylene glycol were obtained from Aldrich.

Sucrose was obtained from British Sugar (Peterborough, United Kingdom), glucose syrup 42DE was from Cargill Sweeteners (Tilbury, United Kingdom), gelatin A type (225 Bloom) was from Gelatines Weishardt (Cedex, France), and citric acid was from Aldrich.

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Table 1. Compounds Added to the Gelatin Gels, the Ions (*m*/*z*), and Cone Voltages (V) Used for Their Analysis[a]

compound	ion (cone voltage)	Log *P*[b]	Log vapor pressure[b]	I_{max} ratio droplet/dissolved[c]
2-butanone	73 (20)	0.26	1.99	4.2
methyl acetate	75 (22)	0.37	1.72	4.3
ethyl acetate	89 (18)	0.86	1.99	14.3
hexanal	101 (18)	1.80	0.98	83.7
ethyl butyrate	117 (18)	1.85	1.16	666
2,3-diethyl pyrazine	137 (28)	2.02	−0.11	12.5
octanone	129 (20)	2.22	0.37	421
amyl acetate	131 (18)	2.26	0.62	1793
octanol	113 (24)	2.73	−0.62	6.6
ethyl hexanoate	145 (18)	2.83	0.26	2432
carvone	151 (15)	3.07	−0.89	7.6
linalool	137 (18)	3.38	−0.80	388
citral	153 (18)	3.45	−1.04	9.9
ethyl octanoate	173 (23)	3.81	−0.63	764
menthofuran	151 (18)	4.29	−1.09	88.0
limonene	137 (20)	4.83	0.16	11.3

[a] Their physical properties [Log *P* and Log vapor pressure (mm Hg)] and the ratio between the breath volatile content when the flavor was added to the gels as either a droplet or dissolved. One panelist consumed four replicate samples of each gel. [b] Values calculated using EPI Suite, U.S. Environmental Protection Agency and Syracuse Research Corp. [c] Each result is the mean of four replicates samples consumed by one panelist.

Sample Preparation. To visualize flavor compounds added as droplets, they were colored with β-carotene (20 mg) dissolved in pure flavor compound (2 mL). For dissolved samples, propylene glycol was used as a carrier solvent to increase flavor compound dispersion (2% propylene glycol in final gel). Flavor compounds were added to give a concentration in the gel of 125 mg/kg.

Gelatin Sucrose Gels. Gelatin (30 g) was presoaked for 30 min in 100 mL of distilled water at room temperature. It was then dissolved at 60 °C to produce a gelatin solution. A sugar syrup containing 150 g of sucrose in 75 mL of water was prepared, and then, 200 g of glucose syrup was added. The syrup was heated to 112 °C and stirred to achieve complete dissolution; it was then cooled to 75 °C, and the gelatin solution was then added and stirred in, followed by 5 g of citric acid.

To prepare gels with solubilized flavor compounds (referred to as dissolved gel samples hereafter), aliquots (250 g) of the hot gelatin–sucrose acid gel (45 °C) were weighed, and the appropriate flavor compound in propylene glycol (31.25 mg in 5 mL) was added and stirred throughout the gel for a period of at least 20 s. The hot liquid was then poured into an aluminum tray to a depth of 20 mm and allowed to set for 1 h at 20 °C and then stored at 4 °C for a maximum of 1 week prior to use. Samples were covered to reduce dehydration.

For gel samples containing droplets, 250 g of hot gel was weighed and stirred for 20 s to ensure the same treatment as the dissolved sample. The gel was poured into an aluminum tray and allowed to cool for 15 min. Then, droplets of the neat flavor compounds were injected into the gel (35 °C) using a 5 μL syringe. Droplets were injected at this stage as this was found to be the optimum point at which droplets were retained at the injection site in the gel. Samples with 1 drop (1 μL), 2 drops (0.5 μL /drop), or 4 drops (0.25 μL/drop) were also prepared. Gels were then allowed to set for a further 45 min at 20 °C before storage at 4 °C for a maximum of 1 week prior to use. Gel samples were cut into cubes (20 mm × 20 mm × 20 mm; approximately 8 g) for consumption. The droplets were still visible in the gels at this point, facilitating the cutting of the gels with the droplets in the centre of each sample. The dissolved gels were in effect control samples against which the release from the droplet-containing samples could be judged.

Breath-by-Breath Analysis by Atmospheric Pressure Chemical Ionization–Mass Spectrometry (APCI-MS). The panelists (*4*) were instructed to consume each of the gel samples (at room temperature ca. 22 °C) whilst placing their nostril on a piece of tubing connected via a heated transfer line (160 °C) to the APCI-MS. The panelists were asked to chew the samples, with their mouths closed, for a period of 45 s and then to swallow and to continue breathing into the APCI-MS for a further 15 s. This reflected the time at which all samples could be swallowed, clearing residues of chewed gel from the mouth. Each panelist consumed three replicate samples of each gel per sampling session. The panelists were given water to drink during breaks between samples (5 min minimum).

The volatiles from the exhaled air were sampled at a flow rate of between 5 and 25 mL/min (depending on the compound) into the APCI-MS source (Waters, Manchester, United Kingdom). Once inside the source, flavor compounds were ionized by a +4 kV corona discharge before being sampled into the vacuum region of the mass spectrometer. The ions were measured, and the cone voltage was applied, depending on the flavor compound being investigated; typically, the protonated molecular ion $[MH]^+$ was monitored (**Table 1**).

Gel Flavor Content Verification. To confirm that flavor concentrations in the two types of gel samples were the same, samples of each preparation type were taken and dissolved in 1 L of distilled water at 50 °C in sealed flasks. Samples were then allowed to equilibrate at 20 °C for 24 h before the headspace above each of the samples was measured. The headspace was sampled for approximately 40 s at a flow rate of 5 mL/min into the APCI-MS interface using the settings in **Table 1**.

Data Processing. Data were processed using MassLynx 3.2 (Waters, Manchester, United Kingdom) and Microsoft Excel (Microsoft Corp., United States), and statistical analyses were performed using Design Expert 6.06 (StatEase Inc., Minneapolis, MN).

RESULTS AND DISCUSSION

Preliminary Experiments. Insertion of droplets into gels was best achieved using a microliter syringe, injecting the appropriate volume of flavor into the gel as it started to set. This created a spherical droplet suspended in the viscous matrix, which then became embedded in the gel. Attempts to inject the droplet after the gel had set resulted in fissures, with the result that the flavor compound was in the form of an irregular shape. In addition, the fissures may act as weak points facilitating release; hence, this approach was not used.

There was the possibility that the amounts of flavor added to gels via injection or solubilization might be different due to the differences in gel temperature during flavor addition. This was checked by dissolving gels in water and measuring the flavor content of the headspace using APCI-MS. There were no significant differences in the equilibrium headspace concentration observed between the two types of samples for any of the compounds used (data not shown) and, hence, no differences in flavor content.

When gels containing droplets of ethyl butyrate were eaten, a very high intensity of flavor release into the nasal airway occurred and the signal recorded on the APCI-MS exceeded the maximum detection limit (**Figure 1a**). In contrast, when the release profile of the gels containing the dissolved flavor were eaten, the maximum ethyl butyrate signal was far smaller (**Figure 1b**). Indeed, the release profile could only be clearly seen if it was presented on a *Y*-axis increased by a factor of approximately 25-fold (**Figure 1c**). Clearly, the release from the gels containing droplets was much greater than that for the samples with dissolved flavor. The true extent of the differences could, however, only be determined by altering the MS operating conditions to ensure that the MS produced data within the APCI-MS detection limits. The possibility that the droplet would not necessarily be ruptured during mastication and could be swallowed intact (i.e., without releasing flavor) did not occur in any of the samples tested. For all of the droplet experiments, release was observed, indicating that mastication had exposed the droplet.

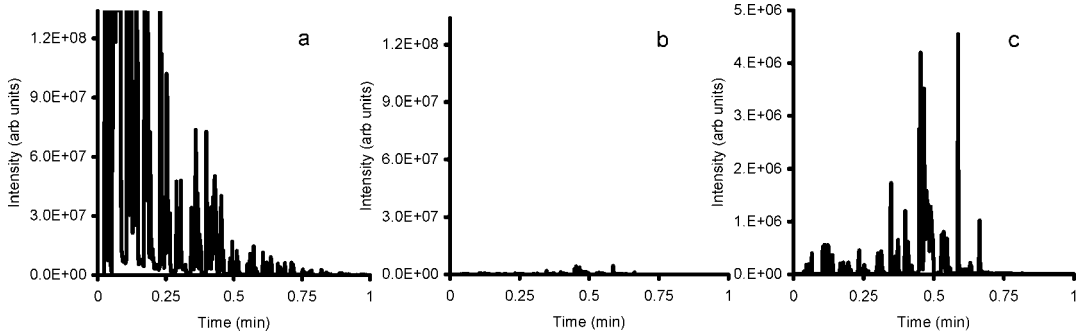

Figure 1. Breath release profile from the consumption of gelatin gels containing ethyl butyrate as a single droplet (**a**), dissolved in the gel matrix and presented on the same scale as panel **a** (**b**), or on its own scale (**c**).

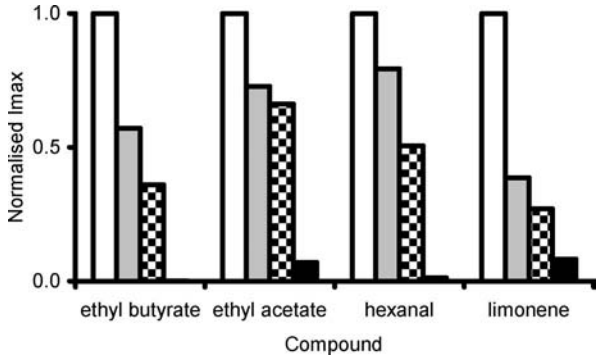

Figure 2. Breath I_{max} values (normalized to the release for gel samples containing one droplet) for gels containing ethyl butyrate, ethyl acetate, hexanal, or limonene. The flavor was added as either 1 (white), 2 (gray), or 4 (checked) droplets or dissolved in the gel matrix (black). Each result is the average of 12 values (four panelists each consuming three replicate samples). The average coefficient of variation for three replicates samples consumed by each panelist was 54%.

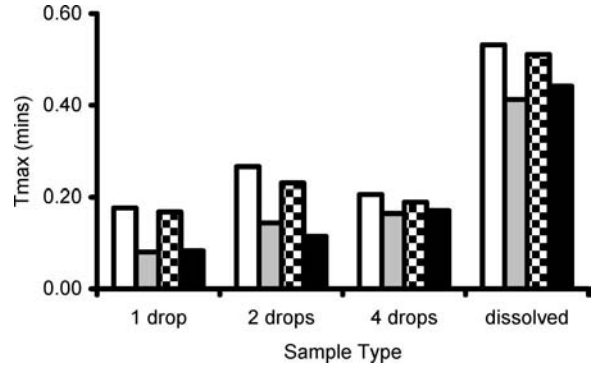

Figure 3. Breath T_{max} values for gels containing ethyl butyrate (white), ethyl acetate (gray), hexanal (checked), or limonene (black). The flavor was added as either 1, 2, or 4 droplets or dissolved in the gel matrix. Each result is the average of 12 values (four panelists each consuming three replicate samples).

There was the possibility that the propylene glycol added to the dissolved flavor gel system may have influenced flavor release. Studies have shown an impact of propylene glycol on flavor delivery (*6*). However, these effects were much smaller than the differences observed resulting from changes in flavor dispersion in the food matrix.

The time course of the flavor release profile also appeared to be different between the droplet and the dissolved gel samples (**Figure 1**). For the dissolved flavor, the ethyl butyrate breath concentration gradually increase over 30 s of the eating profile, consistent with release as the gel was fractured and dissolved in-mouth. The droplet-containing gel gave a much earlier (and intense) release than the gel with dissolved flavor, and the breath flavor content then declined as the gel was consumed further, such that the intensity of flavor in the breath was relatively low after 30 s.

Effect of Flavoring Method on the Release Profile. The maximum intensity of flavor release (I_{max}) was measured for four panelists each consuming three replicate samples of gels flavored with ethyl acetate, ethyl butyrate, limonene, or hexanal. The flavor (total volume 1 μL) was either dissolved in the gel matrix or added as 1, 2, or 4 droplets. All of the compounds showed significantly greater release ($P < 0.05$, t test) for the samples containing one droplet than those where the flavor was dissolved in the matrix (**Figure 2**). When the flavor was present as a droplet, the flavor release was the most intense for the gels containing just 1 droplet with I_{max} decreasing overall as the droplet number increased ($P < 0.01$, regression analysis). The difference between the droplet and the dissolved samples appeared to vary for each compound. The greatest differences

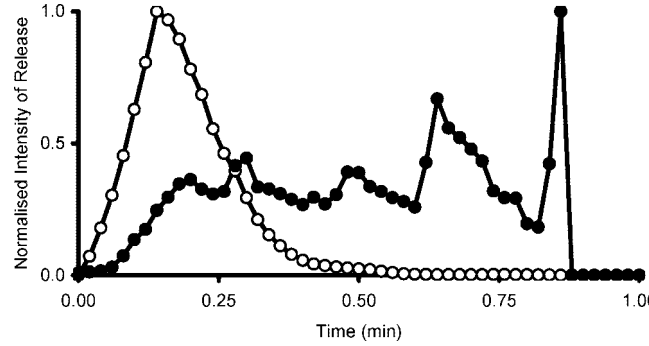

Figure 4. Normalized (to I_{max}) average time–course profiles for the release of ethyl butyrate from gelatin gels when present as droplets (○) or dissolved (●) in the gel matrix. Each curve is the average of 12 eating profiles (four panelists each consuming three replicate samples).

were observed for ethyl butyrate and limonene; ethyl butyrate release was 660 times greater when present as a droplet, as compared with only 11 times for limonene. Such differences in release behavior may be related to the physical properties of the compounds themselves.

The temporal dimension of flavor release was also affected by the flavor addition method. When the flavor was present as droplets, the time to maximum intensity (T_{max}) was much shorter ($P < 0.05$) than when the flavor was dissolved in the matrix (**Figure 3**). However, there were no significant differences in T_{max} between gels containing different numbers of droplets.

Average time–course profiles typical for the droplet and dissolved flavor systems showed temporal release differences (**Figure 4**). The droplets produced an early intense peak in flavor release, which then declined, despite the fact that the gel was

still present in-mouth. The gel matrix was, however, not the source of flavor; this had come from the droplet and was now either less available or exhausted. The release profile of the dissolved sample was longer and gradually increased with time due to associated gel breakdown and dissolution, since the matrix was the primary source of flavor molecules.

It was interesting to observe that the gels released the flavor droplets early in the eating time course (**Figures 1**, **3** and **4**). This would occur if the droplet imparted a structural weakness to the gel, resulting in fracture through the droplet itself. However, the structure of the gel was not considered structurally imperfect since the flavor droplet had been added to the molten gel, which then solidified around it. Alternatively, the presence of a droplet in an otherwise perfectly formed gel may form a weak point that will enhance fracture.

Irrespective of the exact mechanism of droplet release, the key finding was that this resulted in a greater intensity of flavor release earlier in the time course. An increase in the intensity of release would be expected to increase the intensity of sensory perception, particularly when the increased delivery is 10–1000-fold greater. However, changes to the temporal dimension of release should also increase the intensity of perception, since the rate of change of the aroma stimulus affects the intensity at which it is perceived (*9*), with faster increases in flavor delivery producing more intense sensory experiences. Sensory difference tests were not performed on the droplet and dissolved gel systems. However, the four panelists reported an obvious difference in flavor intensity between the gel systems, with the droplet-containing samples producing a very strong flavor experience relative to the samples with dissolved flavor.

The number of droplets appeared to have a limited effect on the timing of release (**Figure 3**). However, the number of droplets appeared to have a clear effect on the I_{max} (**Figure 2**), with an increased number of droplets reducing the maximum release intensity. This should be related to the mechanism of droplet release itself. It could be that all of the flavor (from gels with increased droplet numbers) released at approximately the same time during consumption but that its delivery into the breath was less efficient as dispersed pools of flavor. Alternatively, smaller droplets may have resulted in less structural weaknesses, which may be harder to fracture, particularly as the time course advanced. Some of the flavor may have been swallowed entrapped within the gel and was not released or released later in the time course.

Physical Chemistry of Droplet Release. A wider range of flavor compounds with different properties (**Table 1**) were consumed as either a droplet within the gel or dissolved in the matrix, to try and determine why compounds behaved differently (**Figure 2**). These showed that there could be substantial differences between the release of flavor compounds from the dissolved and droplet gel systems. Ethyl hexanoate showed the maximum differences in release, with an I_{max} over 2000-fold higher, when it was present as a droplet (**Table 1**). There did not appear to be a simple relationship between enhanced release and physical properties such as Log *P* (related to hydrophobicity and aqueous solubility) or the vapor pressure alone. Instead, both factors appeared important.

Overall, the greatest differences between droplets and dissolved samples were observed at intermediate values of Log *P* (Log *P* between 2 and 4) for compounds with the highest vapor pressures. At one end of the scale, where Log *P* was low and vapor pressure was high, compounds such as butanone and methyl acetate showed only a four-fold increase in release intensity. As Log *P* increased, there was a general increase in the intensity ratio (droplet I_{max}/dissolved I_{max}) up to a point where a balance between hydrophobicity and vapor pressure was reached (**Table 1**). Ethyl hexanoate showed the highest ratio of release, and this was considered to be due to its relatively high hydrophobicity (low water solubility), but it still had a reasonably high vapor pressure. Compounds with higher Log *P* values but correspondingly low vapor pressures also showed a lower intensity release ratio with carvone and citral, showing increases of only 7–10 fold. As Log *P* increased further, the overall intensity ratios of release declined. Limonene with a Log *P* of 4.83 and a relatively high vapor pressure showed an increase of only 11-fold for droplets as compared to dissolved samples.

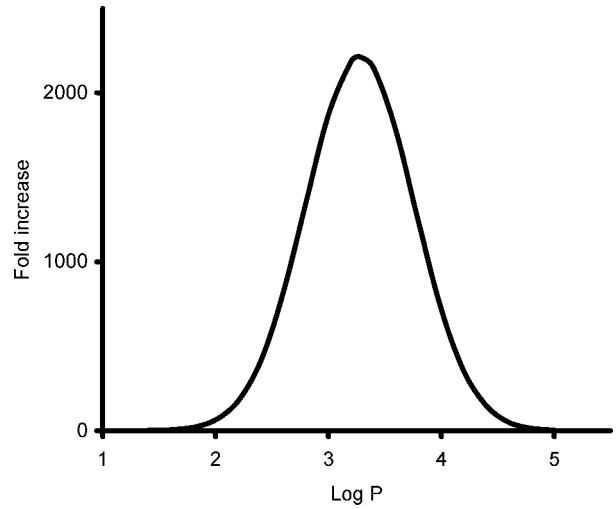

Figure 5. Effect of changing Log *P* on the difference between flavor release from droplet and dissolved flavor systems (fold increase = droplet I_{max}/dissolved I_{max}). The curve was generated from the Design Expert model at a Log vapor pressure of 0.15.

The relationship between enhanced release, Log *P*, and Log vapor pressure was modeled in Design Expert (both terms significant at $P < 0.01$; correlation coefficient $R^2 = 0.91$), with both Log *P* and Log vapor pressure exhibiting nonlinear relationships (eq 1). The model predicted that the greatest difference in flavor release between a gel containing a droplet of flavor or one where it was dissolved would be observed for a compound with a Log *P* of 3.27 and a Log vapor pressure of 0.15. The relationship for both Log *P* and Log vapor pressure showed distinct maxima at this point declining at lower and higher values (**Figures 5** and **6**).

$$\mathrm{Log10}(l_{max}\ \mathrm{droplet}/l_{max}\ \mathrm{dispersed}) = -7.9 + 6.5 \times \mathrm{LogVP} - 0.94 \times \mathrm{Log}\,P^2 - 1.8 \times \mathrm{Log}P^2 - 2.1 \times \mathrm{Log}P \times \mathrm{LogVP} \quad (1)$$

Mechanism of Droplet Release. The timing of release (**Figure 3**) and the dependency of enhanced release on Log *P* (**Figure 5**) and Log vapor pressure (**Figure 6**) suggested a potential mechanism of flavor delivery from droplets. In the droplet system, fracture of the gel would have resulted in flavor release. This took place early in the eating time course (**Figures 3** and **4**), effectively releasing a pool of flavor in-mouth. Observations of flavor compounds during the preparation of solutions in the laboratory show that flavor compounds can behave like oil on the surface of aqueous media (such as saliva) and spread out to form a thin layer in response to surface tension. However, unlike oil, they are volatile and will volatilize directly into the gas phase; from here, the in-mouth flavor laden air can be pumped into exhaled breath by chewing actions (*3*) and delivered to the nose. This avoids the normal route of flavor

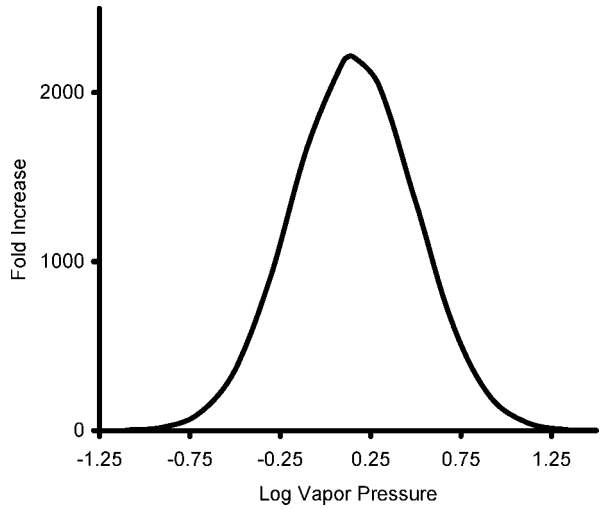

Figure 6. Effect of changing Log vapor pressure on the difference between flavor release from droplet and dissolved flavor systems (fold increase = droplet I_{max}/dissolved I_{max}). The curve was generated from the Design Expert model at a Log *P* of 3.27.

delivery from the food product into the saliva (as a solution) and from here into the gas phase (*10*). The later mechanism relies on volatile partitioning rather than direct volatilization to deliver flavor molecules into the gas phase, which is clearly less efficient.

Droplets of flavor compounds are often seen to spread across the air–water interface during the preparation of solutions. This can be visualized by injecting 1 or 2 μL of compounds such as ethyl butyrate into water, coated with a layer of activated charcoal. The droplet of flavor rises to the surface, spreads out, and pushes away the charcoal, resulting in clear zone approximately 10 cm across, clearly providing a thin layer of the compound, which may dissolve into the water or volatilize into the air depending on its hydrophobicity and vapor pressure.

At low values of Log *P*, the compounds typically have high vapor pressures, which should enhance their delivery into the gas phase. However, these compounds did not exhibit the highest droplet/dissolved ratios. These compounds may have been too water soluble and partially dispersed in saliva before they could pass to the air/saliva interface for volatilization.

Alternatively, their more hydrophilic nature may have allowed them to partially disperse and dissolve in the gel during storage. This would have reduced the differences in release between the dissolved and the droplet-containing gels.

As the hydrophobicity of the compounds increased, their capacity to dissolve in saliva (and diffuse or disperse through the high sugar gel) decreased but, so too, did their vapor pressure. Those compounds with intermediate Log *P* values and the highest vapor pressures showed the greatest enhanced delivery.

Further increases in the Log *P* of a compound would generally result in further decreases in their vapor pressure as the compounds became larger, limiting their capacity to volatilize directly into the gas phase. It is also possible that the high Log *P* compounds such as limonene (which has a reasonably high vapor pressure) may have been partially phase separated in the dissolved samples, enhancing delivery and decreasing their droplet I_{max} to dissolved I_{max} ratio.

The presence of phase-separated flavor molecules in a food product may enhance flavor delivery for compounds with intermediate Log *P* values and the highest vapor pressures. Few (if any) food products exist with such extreme phase separation as the samples used in these experiments. However, some encapsulated flavor systems do contain droplets of flavor (Blake, A. Personal communication), and this may account for their enhanced flavor delivery properties. It may be possible by careful manipulation of flavoring and flavor addition that the potential for enhanced flavor delivery can be incorporated into food products.

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