Simulation of Retronasal Aroma Using a Modified Headspace Technique: Investigating the Effects of Saliva, Temperature, Shearing, and Oil on Flavor Release

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A device that simulated retronasal aroma was constructed from a 1 L blender incorporating purge-and-trap, synthetic saliva addition, temperature regulation to 37 °C, and blending at shear rates reported to occur during eating. Volatiles were collected on a silica trap, solvent desorbed, and quantitated by GC/FID or GC/MS with high precision (CV < 5%) and sensitivity (micrograms per liter). Increasing the temperature from 23 to 37 °C and adding shear increased volatility. The addition of synthetic saliva to a model grape beverage (pH 2.6) increased the pH and the volatility of the bases, 2-acetylpyridine, methyl anthranilate, *o*-aminoacetophenone, and 2-methoxy-3-methylpyrazine, relative to a model neutral compound, 1,8-cineole. The data were consistent with a sensory test that showed a significant shift in the perception of "minty" to "nutty" upon the addition of synthetic saliva to a mixture of 1,8-cineole and 2-acetylpyridine in an acid medium. The volatility of eight flavor compounds was investigated in a soybean oil versus water matrix. The volatilities of α-pinene (log P = 3.75), ethyl 2-methylbutyrate, 1,8-cineole, 2-methoxy-3-methylpyrazine, and methyl anthranilate decreased by factors of 8000, 130, 100, 7, and 3 upon oil addition; however, butyric acid did not decrease, and polar maltol (log P = 0.02) actually increased.

Keywords: *Retronasal; dynamic headspace; purge and trap; flavor profile; shear rate; log P; mastication; volatility; precision; sensitivity*

INTRODUCTION

Retronasal and Orthonasal Aroma. Contributions of the olfactory experience to flavor are demonstrated by the striking decrease in flavor one experiences while pinching the nose during eating. Retronasal aroma, the odor sensation experienced during food consumption, is caused by flavor molecules traveling from the mouth to the nasal cavity via the nasopharynx and the lungs. Orthonasal aroma, however, occurs during sniffing as odorants enter the nasal cavity through the external nares. Unlike orthonasal aroma, retronasal aroma is affected by salivation, chewing, and temperature change of the food after it enters the mouth.

For example, the high polarity and neutral pH of saliva can change the volatility of some flavors, especially in foods with high fat or low pH. As a mucus, proteinaceous, and enzymatic solution, saliva can also modify flavor by emulsification or by breaking down starch or esters (Hussein et al., 1983) through the action of amylases and esterases. These enzymatic reactions along with oxidation may be accelerated when mastication mixes parts of the food and combines it with air. Mastication will also change the flavor profile by accelerating mass transfer (Burdach and Doty, 1987)—increasing the surface area and reducing the diffusion path from a solid matrix to the vapor phase. Flavor compounds physically entrapped can be released as chewing breaks down solid particles. Further, the temperature change that a food undergoes when placed in the mouth can cause melting and other phase changes modifying volatility and changing flavor perception.

The sensation of retronasal aroma is different from orthonasal aroma, and it is influenced by the conditions in the mouth. For example, when the aroma sensations of citral and vanillin flavored solutions were compared during sipping, sniffing, and retronasal inhaling, the thresholds were lowest for sipping and highest for retronasal inhaling (Voirol and Daget, 1986). This shows that the mouth conditions during sipping can increase the detection of the odorants. Similarly, sipping a meat flavoring showed a better concentration discrimination than sniffing (Voirol and Daget, 1989). However, in a time intensity study with citral and vanillin, the orthonasal aroma temporal pattern had a shorter onset time, a shorter extinction time, and a higher maximum intensity than retronasal aroma (Kuo et al., 1993). The maximum intensity for vanillin retronasal aroma intensity was only slightly reduced from that for orthonasal aroma; however, citral's retronasal aroma was much reduced. This orthonasal/retronasal difference may be specific for each particular odorant or subject. For example, in a study comparing the orthonasal to retronasal intensity of benzaldehyde, more variation was seen between the subjects than between the two routes (Marie et al., 1987).

In vivo measurements of flavors released were performed by having subjects chew strong mints (Linforth and Taylor, 1993; Ingham et al., 1995) and tomatoes (Linforth et al., 1994) while vapors exhaled though the nose were measured by several methods. The method that showed the most sensitivity was trapping the "nosespace" volatiles on a Tenax trap during exhaling. While this technique was not able to detect volatiles at very low levels, it was useful for determining differences from headspace analysis. The mints showed a methone/menthol ratio for headspace of 0.4, yet for nosespace this ratio was 1.9. For the tomatoes, the nosespace had a greater relative proportion of 2-isobutylthiazole, 2-methylaldehydes, and 3-methylnitrobutane, but headspace had a greater relative proportion of hexanal, which is

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Table 1. Comparison of Retronasal Aroma Simulator to Other Methods of Headspace Analysis

	release parameters				features for high applicability	
headspace method	salivation	mastication	temp of mouth	air flow	volatile detection (major = mg/L; trace = ng/L to μg/L)	foods
equilibrium headspace	−	−	−	−	major only	all
stirred headspace	−	+	+/−	−	major only	liquids
purge and trap	−	−	+	+	trace	all
Lee's apparatus (1986)	+	+	+	+	major only	liquids
van Ruth's apparatus (1994)	+	+	+	+	trace	all
RAS	+	+[a]	+	+	trace[b]	all

[a] Shear rate known and can be controlled. [b] With precision (average CV < 5%).

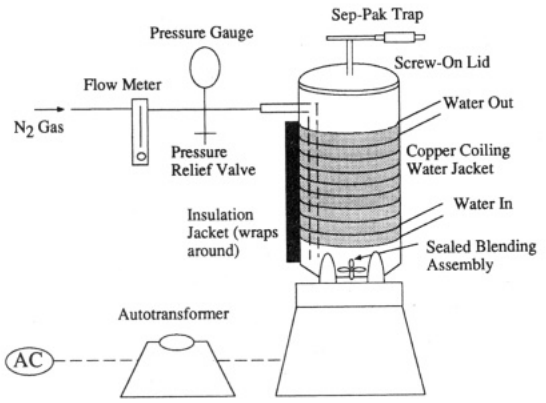

Figure 1. Diagram of the retronasal aroma simulator (RAS) incorporating synthetic saliva addition and regulated shearing, gas flow, and temperature.

usually cited as a major flavor component of tomato. For Cheddar cheeses, a similar nosespace method was developed that had a different volatile profile from conventional dynamic headspace analysis (Delahunty et al., 1994).

Retronasal aroma is caused by a different volatile profile than is simulated in most purge-and-trap or headspace systems. Therefore, this paper describes a method that attempts to simulate more closely the relative volatilities of flavorants during the consumption of heterogeneous foods.

Sampling Procedures for Aroma Volatiles. *Orthonasal Aroma.* Simple equilibrium headspace and purge-and-trap studies simulate the flavors that are released when food is sniffed prior to eating (orthonasal aroma) more precisely than they simulate retronasal aroma. Sensitivity problems are commonly present using equilibrium headspace sampling because only the most volatile compounds, present at 10^{-5} g/L, are detectable by GC/MS (Reineccius, 1994). Purge-and-trap devices have a greater sensitivity than does sampling the equilibrium headspace because a larger effective headspace is sampled. Purge-and-trap devices are usually used with liquid foods such as grapefruit juice (Cadwallader and Xu, 1994) and milk (Cormier et al., 1991; Vallejo-Cordoba and Nakai, 1993) but have also been used for solid foods such as potato chips (Kuo et al., 1989), slurries of pork, beef, and chicken (Ramarathnam et al., 1993a,b), and others (Heikes, 1987).

Table 1 summarizes the relevance to eating of the main methods and new novel methods of flavor release. A background in the area can be found in de Roos and Wolswinkel (1994) and Overbosch et al. (1991).

Retronasal Aroma. In actual eating situations, odorant concentrations are determined kinetically rather than thermodynamically because equilibrium is not very likely. Although equilibrium data have useful implications for storage and packaging issues, more dynamic methods employing shearing and air flow are needed to simulate the events associated with retronasal aroma perception. Only a few analytical methods of flavor release have incorporated the crushing, mixing, dilution, and temperature required to adequately simulate retronasal volatile release. Although having a very small sample size (2.5 mL) limited its sensitivity, the elements of synthetic saliva addition, stirring with metal balls, and heating were incorporated in a device that could be used for liquid foods (Lee, 1986). For the analysis of solid foods, the incorporation of a blending device in the apparatus can improve the sensitivity, relevance to actual eating, and losses from sample handling (Page and Avon, 1989). A mastication plunger was used with dynamic headspace sampling, synthetic saliva, and temperature regulation for rehydrated red bell pepper cuttings (van Ruth et al., 1994).

The current equilibrium headspace and purge-and-trap devices simulate orthonasal better than retronasal aroma. Past research and common experience showed that often a food has a different flavor during eating than when sniffed. There is a need for a device that produces headspaces similar to those occurring in the mouth during eating. A large-scale retronasal aroma simulator (RAS) was thus designed to simulate the mouth conditions of mastication, salivation, and temperature change and to provide sensitivity to detect odorants active at 10^{-9} g/g in foods. This study reports on the design parameters of the device and several initial studies with model systems.

MATERIALS AND METHODS

Investigations during the development of the RAS included several necessary but diverse studies. Initially, the shear rate determination was necessary to develop an accurate model of chewing. Investigating the significance of the RAS parameters (shearing, temperature regulation, and saliva addition) also gave an estimate of precision. Lastly, the RAS was used to investigate the effect of saliva addition on the volatility of ionic bases and the effect of oil on the volatility of a range of flavor compounds.

Design of the RAS. The RAS, based on a modified blender, is shown in Figure 1. This apparatus simulated only the necessary conditions of the mouth that affect flavor release from food. It did not attempt to simulate the dimensions or the timing of eating. A dynamic device, the RAS incorporated controlled gas flow, regulated mixing, vapor phase sampling, and temperature control. As in purge-and-trap systems, N_2 gas flowed through the sample and the volatiles were collected on one or a series of 690 mg silica trap(s), 25 × 10 mm with 5 mm o.d. (Sep-Pak, Millipore, Milford, MA). A copper coiled water jacket controlled the temperature in the RAS to 37 °C. The screw-top lid and the blending assembly provided a sealed chamber (Eberbach, Ann Arbor, MI). Synthetic saliva was added immediately before the analysis began. Overloading of the trap was checked by sniffing the effluent of the trap and also by attaching an additional trap in tandem and analyzing the volatiles. The trap was desorbed with 3 mL of

ethyl acetate and analyzed by one of three methods: (1) Hewlett-Packard 5890A gas chromatograph with a 0.32 × 10 m fused silica capillary column, coated with 0.33 μm of methyl silicone, with a flame ionization detector; (2) same as (1) but with a 15 m × 0.32 mm i.d. Carbowax (Innowax column); or (3) a HP 5970 GC/MS system with a 0.20 mm × 25 m fused silica capillary column, coated with 0.33 μm of methyl silicone. A standard curve using dodecane or tetradecane (4 mg/L) as an internal standard was used for quantitation. The temperature program increased from 35 or 50 (Innowax) to 225 °C at 4 (GC/MS) or 6 °C/min.

Saliva. The operational parameters for RAS were chosen to most closely match what is known about the mouth. Given that 2 mL/min is the average stimulated saliva flow rate (Bourne, 1982) and 5 g of food is in the mouth for 30 s, 1 mL of saliva would be produced. This is a 1/5 sample volume of saliva. However, larger amounts of saliva could be added to simulate the aroma portion of "aftertaste" as volatiles are released from residual food after swallowing. The synthetic saliva was chosen to contain the buffering system of simulated saliva (Roth and Calmes, 1981): 20 mmol/L $NaHCO_3$, 2.75 mmol/L K_2HPO_4, 12.2 mmol/L KH_2PO_4, and 15 mmol/L NaCl with a pH of 7.0. The flavor-releasing effects of amylase and mucin in saliva have been shown to be minimal (van Ruth et al., 1995) but will be tested in the future.

Air Volume Flow. The air volume flow rate during inhaling through the external nares is 100 mL/s (Voirol and Daget, 1986). It is not known what the air volume flow rate over food in the mouth during eating is, but it was assumed to be less than 100 mL/s. Given that the apparatus is a much larger than actual size mouth, the operating N_2 flow rate is much less than the sniff volume flow rate. The flow rate was regulated using a Brooks-Mite flow indicator (O. R. Laurence, Syracuse, NY). The flow rate of N_2 was 20 mL/s with a 10 min collection time. As compared to simply flowing N_2 over the food, the purge-and-trap method was chosen to increase sensitivity of the apparatus without changing the volatile profile (van Ruth et al., 1994).

Shear Rate in the Mouth. This apparatus did not attempt to simulate the geometry of chewing in the mouth. What it did simulate was the flow of food induced by chewing. During mastication, a force is applied by the tongue and teeth, causing shear stress and the break up of food. Shear rate is the velocity gradient established in a fluid as a result of an applied shear stress (Bourne, 1982). The shear rate operating in the mouth during eating is not constant (Shama and Sherman, 1973) and varies over several orders of magnitude depending on the food. The shear rates in the mouth for various foods ranged from 10 to 500 s^{-1} (Elejalde and Kokini, 1992) and for milk, a liquid food similar to the samples analyzed here, the shear rate was 416 s^{-1}. The shear rate in the RAS was chosen to be in this range.

The equation used for estimation of shear rate in the RAS was

$$\dot{\gamma} = kN \quad (1)$$

where $\dot{\gamma}$ is the average shear rate of the impeller, k is a proportionality constant for the impeller, and N is the rotational speed of the impeller (rps) (Rao and Cooley, 1984).

To estimate the proportionality constant for the complex geometry of the blender impeller, the Rieger and Kovak method was used (Rao, 1975).

Estimation of Shear Rate. Aqueous solutions of guar gum (Sigma, St. Louis, MO) (0.3, 1.0, and 1.5% w/v), hydroxypropyl methylcellulose (Dow, Midland, MI) (0.25 and 2.5% w/v), and xanthan gum solutions (2 and 5% w/v) were prepared using agitation. These non-Newtonian fluids and the Newtonian fluid, corn syrup (Karo, Best Foods, Englewood Cliffs, NJ), were analyzed the next day at room temperature (23 °C).

The blender impeller and 1 L vessel (RAS) were used in conjunction with a Deer Rheometer III (Van Bremen, Niewleusen, The Netherlands) to measure the impeller velocity as a function of power. The impeller, placed into the blender 1 in. above the normal impeller, was covered 4.5 cm by 600 mL of the gum solutions. Fifty impeller speed measurements were taken between 120 and 3000 rps.

A Carri-Med CSL 100 rheometer (Carri-Med Americas, New Castle, DE) with a cone (2°, 4 cm diameter, 52 μm gap) and plate system was used to measure the ascending and descending flow curves of shear stress vs shear rate at room temperature over 10 min. The Carri-Med 50 software program was used to calculate the power law parameters K (consistency index) and n (flow behavior index). These, as well as the Deer Rheometer parameters (impeller diameter, d, 0.06 m; the power, P, in N m/s; and the rotational speed of the impeller, N, in s^{-1}), were used to calculate the values for the plot of log $(P/KN^{n+1}d^3)$ vs $1 - n$. This plot, with an equation of $y = -1.095x - 0.069$, $R^2 = 0.73$, gave a proportionality constant $(10^{-\text{slope}})$ of 12.44. The impeller speed of 26.7 rps was measured with a Digital Phototach (Cole Parmer Instrument, Chicago, IL) and regulated with a Superior Electric Volt Box autotransformer. Thus, by eq 1, the shear rate in the retronasal aroma simulator was 332 s^{-1}, which was near estimates published for the shear rate of liquid foods in the mouth.

Shearing, Temperature, and Saliva. *Model Grape Beverage.* A pH 2.6 grape beverage (flavor mix A) was made with 8% w/v sucrose and 0.012 M citric acid monohydrate (Fisher, Pittsburgh, PA). An ethanolic solution with flavors (100 μL) was added to each 400 mL beverage sample and shaken just prior to analysis. The final flavor (Aldrich, Milwaukee, WI) concentrations were 40 mg/L methyl anthranilate, 20 mg/L *o*-aminoacetophenone, 5 mg/L 2-acetylpyridine, 2 mg/L 2-methoxy-3-methylpyrazine, and 0.5 mg/L 1,8-cineole. All effects were tested in triplicate.

Procedure. The general effects of temperature, shearing, and saliva addition were separately assessed using the RAS (Figure 1), and each was compared to the baseline condition: 400 mL of sample at room temperature (23 °C), without saliva and shearing. To test the effect of temperature, a 400 mL sample was assessed at body temperature (37 °C). To test the effect of shearing, the sample was sheared at 26.7 rps. To test the effect of saliva addition, 100 mL of 37 °C synthetic saliva was added to the 400 mL sample. The results were analyzed by the Dunnett test for difference from control at $\alpha = 0.05$. A more in-depth analysis of saliva addition was made using a heated vessel and shearing at 26.7 rps. To the same 400 mL sample were added different amounts of 37 °C saliva, corresponding to sample volumes of 1/6 (67 mL), 1/4 (100 mL), 1/2 (200 mL), and 1 (400 mL).

Sensory Test Samples. Distilled, HPLC grade water was used for all samples, which were prepared approximately 2 h prior to testing. Flavored solutions were made by adding the appropriate amounts of an ethanolic solution of 50 mg/mL 2-acetylpyridine and 1,8-cineole (Aldrich). The following solutions were used for the screening triangle tests (Meilgaard et al., 1991): 1,8-cineole (5 mg/L), 2-acetylpyridine (2 mg/L), 1,8-cineole mix (5 mg/L 1,8-cineole and 1 mg/L 2-acetylpyridine), and 2-acetylpyridine mix (2 mg/L 2-acetylpyridine and 2.5 mg/L 1,8-cineole). The concentrations were chosen in preliminary trials so that the mixed solutions were moderately easy to distinguish from the pure solutions. The flavored beverage samples (flavor mix B) for the sensory test contained 8% w/v sucrose and 0.012 M citric acid monohydrate, 5 mg/L 1,8-cineole, and 2 mg/L 2-acetylpyridine. These levels represented a moderate aroma sensation. The flavored beverage plus synthetic saliva contained 1/4 volume of synthetic saliva.

Sensory Test Procedure. Visually identical samples (25 mL) were presented at room temperature and labeled with random three-digit codes with random presentation order. They were presented in wine glasses with Petri dish covers and were sniffed but not ingested. Testing took place in individual, partitioned booths under fluorescent lighting. Panelists were chosen by their ability to correctly identify 4/4 or 6/8 odd samples in a series of triangle tests. For the first two triangle tests, 1,8-cineole and the 1,8-cineole mix were tested, with each as the odd sample in one test. For the next two triangle tests, 2-acetylpyridine and the 2-acetylpyridine mix were tested. If the panelist did not get all four correct, another randomly coded and presented four trials were performed. Twenty-two panelists were screened and 21 (13 females and 8 males, aged

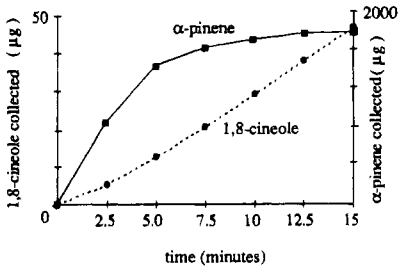

Figure 2. Example of the different volatility elution curves over time showing the amount of α-pinene and 1,8-cineole in water collected by the traps.

22–50) were selected for the panel. A sample line scale (as below) was then presented with the predicted answer given. The next day, the actual sensory test took place.

The sensory test was performed using a 15 cm line scale (Lawless and Malone, 1986) where the end anchors were the standard aromas A (5 mg/L 1,8-cineole) and B (2 mg/L 2-acetylpyridine). "Aroma A" and "Aroma B" were written on either end of the line as were the panelists' personal description for the aroma. Four samples were presented at once, the two standard aromas and the two unknowns (flavor mix B and flavor mix B plus synthetic saliva). The panelists were instructed to briefly sniff each sample, taking ample time between sniffs; and then to place a mark on the line for the relative aroma sensation perceived for the two unknowns. The panelists took at least a 10 min break between each of the three replicates. The results were analyzed by repeated measures ANOVA.

Effect of Oil. *RAS Method.* For experiment 2, a prototype apparatus was used which was very similar to the apparatus in Figure 1. Several details present in this "effect of oil" experiment were different from previous ones. The blender was larger (4 L), as was the air flow rate (32 mL/s). The synthetic saliva added was water, 500 mL. The shear rate was not measured but could be estimated given the mixing rate of 300 rpm and the k value determined in the other apparatus. A shear rate of approximately 60 s^{-1} was estimated, which is within the range that occurs in the mouth. Silica traps were activated by heating at 125 °C for 16 h prior to the experiment. Activating increased the retention of butyric acid but did not change the retention of the other compounds. To measure the dynamic nature of the volatility, the traps were changed every 2.5 min for 15 min.

Flavor mix C in water (2.5 L) contained ethyl 2-methylbutyrate, α-pinene, 1,8-cineole, maltol, vanillin, butyric acid, methyl anthranilate, and 2-methoxy-3-methylpyrazine (Aldrich) at levels of 0.1, 6.0, 15, 19, 2.0, 15, 4.0, and 11.0 mg/L, respectively. The flavors were chosen for their range of chemical properties and importance to flavor in food. The levels were chosen to simulate the levels found in natural products. Flavor mix C in water was compared in duplicate to flavor mix C in soybean oil, purchased locally.

Log P Determination. The octanol–water partition coefficients (P) (Leo et al., 1971) were measured for the flavor compounds:

$$P = \text{concn in octanol phase/concn in water phase}$$

The measurement of log P for all of the flavor compounds was performed in triplicate by the traditional shake-flask method at 23 °C. Concentrations in the octanol and water phases were measured by GC/MS and GC/FID.

Determination of Rate Constants. The collection of flavors on six silica traps over 15 min showed the dynamics of volatility. As seen in Figure 2, a large part of the α-pinene in water volatilized immediately. However, 1,8-cineole (Figure 2) released less than 1% during the 15 min sampling period. For α-pinene in water, the rate constant could be estimated using the equilibrium first-order rate equation

$$\ln \frac{[A] - [A_\infty]}{[A_0] - [A_\infty]} = -kt \quad (2)$$

where $[A_\infty]$ is the final concentration of volatile in solution (mg/L), [A] is the concentration of volatile in solution (mg/L), $[A_0]$ is the initial concentration of volatile in solution (mg/L), k is the first-order rate constant (min^{-1}), and t is time (min). $[A_\infty]$ was determined according to the method of selected points where values of $[A_\infty]$ were chosen so that a plot of $\ln([A] - [A_\infty])$ vs time was linear (Friess and Weissberger, 1953).

All other rate constants were estimated from the initial rate of adsorption on the trap, where

$$d[A]/dt = -k[A] = -(d[A]/dt)_{trap} \quad (3)$$

For all of the compounds but α-pinene in water, the amount volatilized was negligible compared to the amount remaining in solution, leading to the following assumption:

$$[A] \approx [A_0]$$

Thus

$$k = (d[A]/dt)_{trap}/[A_0] \quad (4)$$

The slopes of the graphs plotting micrograms of flavor compound collected from the trap vs time (Figure 2) determined $(d[A]/dt)_{trap}$. The rate constants, k, were calculated using eq 4 for the compounds in the oil plus "saliva" and the water plus "saliva" mixtures.

RESULTS AND DISCUSSION

Testing of the RAS with model systems has shown that it is a sensitive, reproducible device which produced a measurable flavor release similar to the mouth. The parameters included in the RAS changed the release from that of a device without the parameters. Incubation to body temperature approximately doubled the flavor release from that at room temperature. The saliva addition caused a flavor profile change in a grape beverage due to its buffering action. Shearing, while causing little difference in liquid model systems, gives the RAS the potential to study the release of flavor from actual foods. The sensory test showed that the flavor profile shift measured using the RAS was actually observed with people. This lends credence to the hypothesis that flavor release in the RAS is similar to release in the mouth. The first actual study using the RAS showed that some flavor compounds volatilize markedly differently in oil and water and log P or vapor pressure can only partially explain these differences among compounds.

Precision and Sensitivity. The precision of the RAS (average CV for Tables 2 and 3 = 4.6%) for the flavor compounds in the model acidic beverage was very good and comparable or better than that of other methods of flavor analysis. A consistent % CV was seen between the flavor compounds, even with their range of volatilities. Contributing to the high precision is the RAS's one-step analysis, not requiring previous sample homogenization, mixing, or cutting. Solutions containing levels of 100 μg/L (ethyl 2-methylbutyrate) and 500 μg/L (1,8-cineole) were very easily detected by the RAS. An estimation, using the FID quantitation limit of 1,8-cineole and the ability to concentrate the ethyl acetate elution, will give the RAS the sensitivity to measure flavors with the volatility of 1,8-cineole in solution down to 1 μg/L. Quantitative gas chromatography olfactometry (GCO) techniques such as CharmAnalysis (Acree

Table 2. Volatility Rate Constants[a] ($k \times 10^{-5}$, min^{-1}) of Flavor Compounds Showing the Effects of Shearing, Temperature, and Saliva Addition

flavor	base (25 °C, no saliva, no shear)	shearing (25 °C, no saliva)	temp (37 °C, no saliva, no shear)	saliva (25 °C, no shear)
1,8-cineole	965 (40)	1130 (64)[b]	2025 (99)[b]	705 (12)[b]
2-methoxy-3-methylpyrazine	86.4 (3.3)	95.8 (4.1)[b]	195 (11)[b]	71.4 (0.67)[b]
2-acetylpyridine	9.5 (0.075)	9.9 (0.31)	18.2 (1.1)[b]	11.4 (0.31)[b]
methyl anthranilate	5.41 (0.16)	6.63 (0.32)[b]	11.9 (0.57)[b]	5.6 (0.13)
o-aminoacetophenone	2.99 (0.11)	3.56 (0.071)[b]	5.63 (0.32)[b]	2.98 (0.20)

[a] Values are mean of three independent determinations with standard deviations in parentheses. [b] Significantly different from base at $\alpha = 0.05$ (Dunnett).

Table 3. Volatility Rate Constants[a] ($k \times 10^{-5}$, min^{-1}) of Flavor Compounds in Sheared Samples at 37 °C Showing the Effect of Dilution with Saliva

	saliva volumes				
flavor	0	1/6	1/4	1/2	1
1,8-cineole	1360 (120)	1255 (100)	1330 (48)	1110 (2.5)[b]	970 (35)
2-methoxy-3-methylpyrazine	116 (9.5)	117 (10)	113 (4.8)	104 (2.2)	91.6 (0.93)
2-acetylpyridine	11.9 (0.91)	15.0 (1.1)	16.6 (0.89)	15.8 (0.78)	13.4 (0.60)
methyl anthranilate	8.19 (0.50)	9.81 (0.65)	9.5 (0.64)	8.5 (0.25)	7.25 (0.17)
o-aminoacetophenone	4.14 (0.21)	4.7 (0.41)	4.53 (0.29)	3.73 (0.11)	3.34 (0.18)
pH of resulting solution	2.6	2.96	3.13	3.78	5.11

[a] Values are mean of three independent determinations with standard deviations in parentheses. [b] Mean of two independent determinations.

and Barnard, 1994) are more highly sensitive to odor-active compounds than the GC/FID system and can be used in combination with the RAS to give a measure of the potent odors released from food during eating.

Shearing and Temperature Effects. The RAS has several parameters that make it different from simple purge-and-trap devices: mixing, saliva addition, and temperature regulation. An evaluation without these parameters would be closer to orthonasal aroma. The effects of these parameters on a simple model beverage were tested for their significance. Table 2 shows the amount of flavor compounds collected from these trials. Shearing produced a slight increase in volatility of these flavor compounds. Similarly, shearing increased the release rate of diacetyl from an aqueous system but not the final equilibrium headspace concentration (Bakker et al., 1994). The shearing effect would be much greater for a solid food that had to be broken up for the release of flavor compounds. The utilization of a blender instead of a stir bar allows such solid foods to be analyzed. Analyzing the samples at 37 °C instead of 23 °C caused about a 2-fold increase in the flavor volatility. The amount of time a food is in the mouth will determine the amount of temperature increase. For a cold food, such as ice cream, the temperature increase is likely to have a substantial effect.

Saliva Effects. From Table 2, the addition of synthetic saliva had a differential effect on the flavor compounds. While 1,8-cineole and 2-methoxy-3-methylpyrazine decreased in volatility upon the addition of synthetic saliva, 2-acetylpyridine increased.

These effects were studied more closely by investigating the effect of synthetic saliva volume (Table 3). The relative flavor profile changed upon the addition of saliva. Foods are mixed with various amounts of saliva depending on the type of food and the salivary flow of the individual. Here, there is a range of synthetic saliva volumes in which the higher volumes could correspond to the high dilution with saliva which occurs to the residual food in the mouth after swallowing. The residual flavor often found with foods after swallowing could be due to this dilution.

pK_a and Molar Concentration Influences. Saliva addition caused two opposing forces which affected the volatility of the bases. At a pH close to their pK_a values, a certain amount of basic flavor compounds would be protonated. The addition of a buffered synthetic saliva (pH 7.0) to the pH 2.6 grape beverage caused a pH increase, resulting in an increase in the volatility of the four ionic bases. Contrary to the effect of changing the pH, the dilution with saliva caused a decrease in the molar concentration of the flavor compounds and therefore, by Henry's law, a decrease in volatility.

For 1,8-cineole, the model neutral compound, only the effect of a decreasing molar concentration in solution was present. Consequently, 1,8-cineole was used as a standard by which to compare the ionic bases. Neutral flavor compounds in a food would behave similarly to 1,8-cineole. When compared to 1,8-cineole, 2-methoxy-3-methylpyrazine exhibited a slight increase in volatility as the pH increased with larger synthetic saliva volumes. Its pK_a is not known but is probably quite low, given that 2-methoxypyrazine and 2-methylpyrazine have pK_a values of 1.45 and 0.75, respectively (Weast, 1975; Liu et al., 1991). *o*-Aminoacetophenone, with a higher pK_a of 2.44 (Sykulski et al., 1979), showed an increase in volatility after the addition of 1/6 and 1/4 saliva volumes. Methyl anthranilate, with a pK_a of 2.32 (Buckingham, 1994), showed a 20% increase in volatility after the addition of 1/6 volume of saliva. 2-Acetylpyridine had the highest pK_a (2.85) (Novakovskii and Provotar, 1968) and, consequently, showed the largest increase in volatility with the addition of saliva, a 40% increase after 1/4 volume of saliva was added.

Basic flavor compounds will increase in volatility relative to neutral aroma compounds upon salivation, resulting in an increase in the perception. Likewise, the relative volatility of basic 3-methylnitrobutane was greater after one placed the food into the mouth and breathed than in headspace analysis (Linforth et al., 1994). When flavor compounds are at levels around their threshold, this change can result in a basic flavor being perceived in the mouth. For example, the retronasal aroma of wine in the mouth is often very different from the sniffing bouquet (Baldy, 1995), which may be partly caused by the increased volatility of basic flavors such as methyl anthranilate, methoxypyrazines, *o*-aminoacetophenone, or tetrahydropyridines (Acree and

Table 4. Flavor Compound Measured Values: Volatility Rate Constants ($k \times 10^{-5}$, min^{-1}) at 25 °C with Shearing plus Saliva Determined with a Prototype RAS[a] and log P[b] (Octanol–Water Partition Coefficient)

flavor	$k_{water + saliva}$	$k_{oil + saliva}$	log P
α-pinene	33000 (6800)[d]	4.3 (1.6)	3.75
ethyl 2-methylbutyrate	1400 (140)[d]	11 (0.71)	1.19
1,8-cineole	230 (43)[d]	2.4 (0.15)	1.34
butyric acid	62[c]	64 (36)	0.79
2-methoxy-3-methylpyrazine	14 (1.9)[d]	2.1 (0.064)	1.30
methyl anthranilate	1.2 (0.11)	0.46 (0.25)	2.23
vanillin	<0.1	<0.1	0.93
maltol	<0.1	2.2 (2.0)	0.02

[a] Values are mean of two independent determinations with standard deviations in parentheses. [b] Values are mean of three "before shaking" octanol concentrations and three "after shaking" octanol or water concentrations. [c] Value is from one determination. [d] Significantly different from $k_{oil + saliva}$ at $\alpha = 0.05$ (Dunnett).

Lavin, 1990; Acree, 1981; Heresztyn, 1986). The volatility of acidic flavors is also dependent upon pH, as demonstrated by the increase in the threshold of butyric and other acids as the pH increased (Baldwin et al., 1973).

Flavor Profile Change. If the flavor profiles for the 1/6 volume saliva addition are compared to zero saliva in Table 3, we can see that while 1,8-cineole has a lower volatility, 2-methoxy-3-methylpyrazine has the same volatility and *o*-aminoacetophenone, methyl anthranilate, and 2-acetylpyridine have increased volatility. The perceived quality of the mixture may indeed change. These differences among the flavors' volatility become even larger at 1/2 volume saliva addition. The sensory test using 2-acetylpyridine and 1,8-cineole with 1/4 volume saliva addition confirmed the instrumental results that upon saliva addition, the volatility of 1,8-cineole decreases but that of 2-acetylpyridine increases. The line scale markings were converted to values from 0 to 15, where 0 equals the aroma of 1,8-cineole and 15 the aroma of 2-acetylpyridine. Flavor mix B without saliva had a mean value of 6.2, while the flavor mix B with saliva had a mean value of 9.1. ANOVA showed a significant difference between the two samples ($p < 0.00005$). There were no significant ($\alpha = 0.05$) interaction or replication effects. The saliva addition to the beverage resulted in a move of perception from more minty/menthol to more smoky/nutty. The RAS was able to detect a flavor profile shift that was also detected by a panel, indicating that they may be producing a similar flavor release.

Effect of Oil. Since the advent of reduced-fat foods, the importance of fat level to the flavor profile of a food has been recognized. Often, an off-aroma note will come above threshold in a reduced-fat food in which it was previously solvated by oil. A better understanding of flavor compound properties that cause water as compared to oil volatility will help to better predict flavor release and formulate flavors for a food's oil level.

The volatility rate constants in water and in oil for the eight flavors as well as their log *P* values are shown in Table 4. These values and the precision are not the same as in the previous experiment (Tables 2 and 3) because the prototype apparatus and different conditions were used. Log *P* was used in this study as a general measurement of compound polarity.

Nonpolar Flavor Compounds. α-Pinene, $C_{10}H_{16}$, is a very nonpolar flavor without functional groups. Its high volatility in the water matrix is drastically reduced (8000 times) upon the presence of oil. Likewise, with α-ionone and naphthalene, increases of 2000- and 3500-fold were found for the air–water from the air–oil partition coefficients (Graf and de Roos, 1994). Limonene and styrene also showed strong depressions of volatility with oil (Schirle-Keller et al., 1994). The volatility of hydrocarbon flavors will be greatly influenced by the presence of oil in food. α-Pinene was able to show these substantial differences in volatility for the two matrices because it has a rather large vapor pressure in the pure state (4.7 mm at 25 °C) (Weast, 1975). This high volatility of α-pinene in water, where the compound is almost completely volatilized, was also seen in *in vivo* measurements over time, where limonene was released much more rapidly than menthone or menthol (Ingham et al., 1995).

Methyl anthranilate, however, has a very low volatility in the pure state [0.2 mm at 25 °C, extrapolated from higher temperatures (Weast, 1975)]. Even in water, it had a very low volatility rate constant. Thus, the nonpolar nature of methyl anthranilate caused only a 3-fold reduction in the oil phase. The low volatility of this flavor, which is often used in fruit flavor compositions, is likely the reason it is often added at high levels of 20–50 mg/L in candy and consumer products and as much as 2200 mg/L in chewing gum (Arctander, 1969).

Ethyl 2-methylbutyrate had the second highest volatility in water, due in part to its high vapor pressure in the pure state (8.8 mm at 25 °C) as calculated by the Hass/Newton equation (Weast, 1975). 1,8-Cineole had a high volatility in water and decreased 100-fold in oil. This is similar to the 130-fold decrease seen by ethyl 2-methylbutyrate. 2-Methoxy-3-methylpyrazine showed a decrease of only 7-fold in oil, probably because it already had low volatility in water.

Polar Flavor Compounds. The more polar compounds represent different scenarios. Butyric acid had a similar moderate volatility in oil and water. Vanillin, because of its very low vapor pressure in the pure state [about 0.0005 mm at 25 °C, estimated from higher temperatures (Weast, 1975)], was not detected with the RAS. Similarly, 100% of vanillin in oil and in water was retained during dynamic air flow conditions (Graf and de Roos, 1994). Very polar maltol also has a low vapor pressure in the pure state (estimated) and was not detected in the water matrix. However, it was detected in the oil matrix. This higher volatility in oil than water was unlike all of the other flavor compounds tested and was due to maltol's high polarity.

Indeed, the addition of oil had different and sometimes opposite effects on the volatility of flavor compounds, which partially explains the different flavor profiles of reduced-fat and full-fat foods.

Relation to Threshold. Threshold data (Fazzalari, 1978) are reported for some of these compounds. Vanillin had a much higher reported threshold in water (200 and 4000 μg/L) than in air (1.1×10^{-6} and 2×10^{-4} μg/L). However, butyric acid had only about a 100-fold lower threshold in air, 1,8-cineole had a 10-fold lower threshold in air, and α-pinene had similar thresholds in both. The k_{water} values can explain these differences. Almost all of α-pinene in water was volatilized, thus giving similar thresholds. However, very little vanillin in water was volatilized, thus the much higher threshold in water than in air. Even though vanillin in the gas state is often found to be a potent aroma compound in GCO dilution techniques, its contribution to aroma is unlikely if not present at high levels.

Volatility Predictors. Several chemical properties of the flavor compounds were evaluated for their ability

to predict log k_{water} and log $k_{oil + saliva}$. These compounds did not represent a homologous series and had quite diverse natures. Most of the properties measured had very low correlations, indicating that other controlling factors were present. Boiling point was found to be inversely correlated ($R^2 = 0.5$) with volatility for both the log k_{water} and log $k_{oil + saliva}$. Log P values were found to have a very weak positive correlation with log k_{water} ($R^2 = 0.2$), similar to the lack of correlation found between polarizability and the air–water partition coefficient for several hundred compounds (Schuurmann and Rothenbacher, 1992). Retention time on an OV101 column, however, had a weak inverse relationship to log k_{water} ($R^2 = 0.4$), yet a strong inverse relationship to log $k_{oil + saliva}$ ($R^2 = 0.8$). This last relationship is logical because both are measurements of the partition of the volatiles between a nonpolar stationary phase and air or helium. To predict volatility in a food, many factors of the flavor and the food in combination need to be investigated.

Conclusions. The retronasal aroma simulator is a flavor analysis method that more closely simulates mouth conditions than existing headspace methods. Its large size improves sensitivity, and its blending action will allow foods with many different physical forms to be analyzed. The features of the device (saliva addition, temperature regulation, and shearing) were shown to result in different headspaces from dynamic headspace trapping. Synthetic saliva addition changed the flavor profile of ionic bases, which may be important in many foods. Because of the large differences seen in oil and water volatility, the development of modified-fat foods would benefit from studies using the RAS.

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