Verification of a Mouth Simulator by in Vivo Measurements

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The volatile content of the effluent from the retronasal aroma simulator (RAS) was compared with that of human breath using mass spectroscopy (MS−Nose). The ratios of volatile compounds from the RAS were closely related to those from the panelists' breath with the correlation coefficients ranging from 0.97 to 0.99 from model food systems. A greater sensitivity using the RAS was achieved because higher concentrations of volatiles in the MS−Nose were produced from the RAS than from the breath. In analyzing the effects on volatility of RAS parameters including airflow rate, temperature, saliva ratio, and blending speed, airflow rate had the greatest effect. The correlation coefficients for the real food systems studied ranged from 0.83 to 0.99. The RAS gives a good approximation of time-averaged flavor release in the mouth as defined by breath-by-breath measurements.

Keywords: *MS−Nose; retronasal aroma simulator; RAS; breath-by-breath analysis; aroma; flavor release*

INTRODUCTION

Mixtures of aroma compounds in the vapor phase of foods approaching the olfactory epithelium through the nostrils (orthonasal route) have compositions different from mixtures from the same food entering from the mouth (retronasal route), thus imparting different perceptions. Although the flavor composition in the food remains the same, the compositional differences are attributed to conditions in the mouth that affect flavor release, such as temperature, saliva, mastication, and semi-dynamic air flow (*1*−*3*). The perceptions from the retronasal route are associated more strongly with the flavor experienced during consumption (*4*). Thus, the composition of the retronasal flow from foods is of interest in understanding the flavor that is associated with foods.

Trapping the breath contents during eating has been attempted; however, the direct measurement of retronasal aroma using soft ionization mass spectrometers with detectors that can accommodate moisture and atmospheric pressure is more direct and less time-consuming (*5*). Taylor and Linforth reviewed methods for measuring in vivo volatile release (*6*).

Many mathematical models have been proposed to predict flavor release and to give insight into the reasons for the difference between ortho- and retronasal aroma (*2*, *3*, *7*−*11*). Most theories are based on equilibrium thermodynamics but few have been rigorously tested with experimental data. Because foods have diverse compositions and complex structures, empirical tests are necessary for predicting flavor release; no simple combination of chemical characteristics based on equilibrium measures can predict flavor release during eating.

Some mouth simulators incorporating mixing, dilution, and temperature have been designed to simulate eating and drinking dynamics (*7*, *12*−*17*). The simulators represent continuous exhaling; thus, they do not represent the time profile of eating and drinking. These models have much less variation than is exhibited by human subjects and they eliminate concerns with palatability and safety. Flavor release simulators including the Roberts and the van Ruth systems can work with a broad range of foods including all liquids and most solid foods. The retronasal aroma simulator (RAS) developed by Roberts and Acree is unique in the use of blades for shearing, stainless steel to reduce adsorption, large size to increase sensitivity, and gas flow rates similar to those of human breathing. Simulation settings are based on the actual parameters in the mouth.

Though, in theory, the RAS represents conditions in the human mouth, only direct comparison with retronasal flavor release can verify how well it mimics mouth conditions. Only recently has this been possible with the development of breath-by-breath analysis and the MS−Nose by Taylor and Linforth. This paper discusses the verification of the RAS by breath-by-breath analysis. The experiment can be divided into four stages. In stage 1, breath-by-breath analysis was correlated with the RAS profiles, both measured with the MS−Nose using a simple controllable food system: imitation cheese (*18*). Stage 2 involved investigating the effect of RAS parameters on flavor release from the imitation cheese. Correlation of the RAS with humans, using a diverse group of flavor compounds added to chocolates, was evaluated in stage 3. Verification of the method was achieved in stage 4 by comparing breath-by-breath analysis and RAS effluent of real foods.

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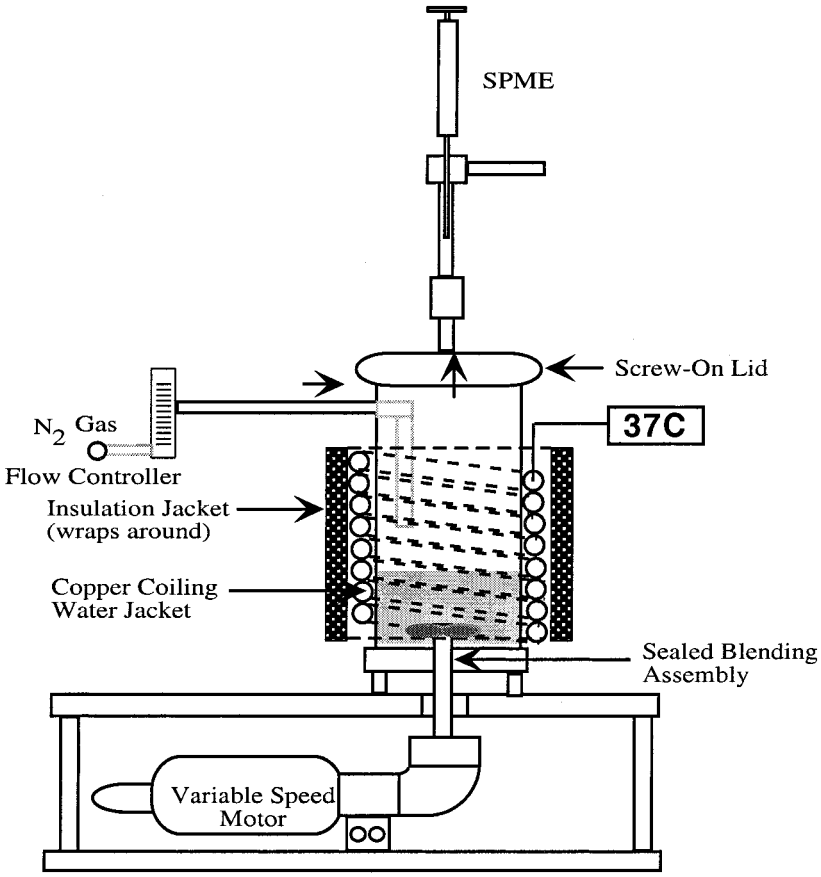

Figure 1. Diagram of mouth simulator, RAS.

Table 1. Mouth Simulation Conditions of the RAS

condition	setting
temperature	37 °C
air or N_2 flow	20 mL/s
blending rate	2.5 rps
saliva-to-food ratio	1/5, v/w

MATERIALS AND METHODS

Stage 1 − Correlation of RAS with Breath-by-Breath Analysis. Sixteen panelists (ages 22 to 39, 6 males and 10 females) were instructed to consume 5 g of imitation cheese as they would normally eat while resting one nostril on the plastic tube attached to the MS−Nose. Consumption took approximately 1 min. The RAS effluent flowing past the sampling tube of the MS−Nose was monitored from 150 g of imitation cheese in triplicate. The RAS settings were as listed in Table 1. The ions monitored are listed later in Table 3. Authentic standards were used for calibration and quantification.

MS−Nose Parameters. The MS−Nose is a Platform LCZ quadrupole mass spectrometer (Micromass, Altrincham, U.K.) operating in the API positive ion mode fitted with a proprietary air-sampling interface (MS−Nose, Micromass) (*5*). The operating parameters of the API source were optimized while headspace of each of the selected volatiles was continuously introduced. The cone voltage was adjusted to give maximum sensitivity for the MH+ ion. For all compounds the corona pin voltage used was 4 kV. Dwell time was 0.01 s for breath-by-breath analyses and 0.1 s for RAS experiments.

For the breath-by-breath analysis experiments, panelists trained in the use of the instrument ate portions of the sample while resting one nostril at one end of a plastic tube (12 mm × 50 mm). The tidal flow of air from the nostril passed back and forth through the tube. Part of this air stream was sampled (flow rate 25 to 72 mL/min) onto the API source through a capillary tube (0.53 mm i.d.) inserted through the wall of the plastic tube at right angles to the direction of flow. As the subject breathed out, expired air was sampled, but, upon inspiration, laboratory air was sampled. The RAS, with a 150-g sample, was connected to the API such that part of the gas stream leaving the RAS was sampled into the API.

The MS−Nose was fitted with a calibration port so that authentic compounds could be introduced into the gas stream. Known concentrations of cyclohexane solutions of the volatiles were introduced via a microsyringe (10 μL) on a syringe pump into the heated flow of nitrogen (10 L/min) at 1.5 μL/min. The quantity associated with the corresponding peak height could thus be calculated on the basis of the standard's peak height and sampling flow rate.

RAS Parameters. The RAS was composed of a 1-L stainless steel blender container and assembly, a voltage controller and high torque variable-speed motor to give precise control of blender speed to simulate chewing and crushing of food, a controlled gas supply to sweep over the food, the addition of artificial saliva, and a copper coil with a water flow to control the temperature (Figure 1). Table 1 shows the parameters used for simulating mouth conditions. The artificial saliva was composed of $NaHCO_3$ (20 mmol/L), K_2HPO_4 (2.75 mmol/L), KH_2PO_4 (12.2 mmol/L), NaCl (15 mmol/L), and α-amylase (200 U/mL, from *Aspergillus oryzae*); all purchased from Sigma-Aldrich Corp., St. Louis, MO.

Imitation Cheese Preparation. A model cheese was used to simulate a complex food system containing fat, protein, and carbohydrates that are known to affect flavor release, and to have the ability to adjust ingredients in future studies. Ingredients were used in the amounts listed in Table 2. Aroma compounds were selected on the basis of their literature importance in various cheeses and were added at approximate levels found in cheese (*19*). Distilled water (75% of total), vegetable oil (75% of total), and emulsifying salts were heated to 85 °C, while stirring, in a 2-L Erlenmeyer flask. The hot mixture was poured into an 8-L mixer with a stainless steel bowl. The casein was added and mixed on the low setting with a paddle attachment for 3 min. Water (85 °C) was added and

Table 2. Ingredients and Quantities for Imitation Cheese

ingredient, source	percentage
water	41.4
vegetable oil, Crisco Puritan Canola Oil	24.6
casein from bovine milk, Sigma-Aldrich	24.0
whey, WPC, Cornell University Dairy plant	2.7
sodium citrate, Sigma-Aldrich	2.0
sodium choride, Sigma-Aldrich	1.9
$Na_8Al_2(OH)_2(PO_4)_4$, KASAL, Rhodia Inc., Cranbury, NJ	1.0
adipic Acid, Sigma-Aldrich	1.0
sorbic Acid, Sigma-Aldrich	0.4
trisodium phosphate, Sigma-Aldrich	0.6
guar gum, Sigma-Aldrich	0.4

Table 3. Flavor Compounds at 1% in Ethanol Added to Imitation Cheese Prepared at 5 μg/g Cheese

flavor compound	CAS no.	ion monitored[a]
butryic acid	107-92-6	89.3
ethyl hexanoate	123-66-0	145.3
nonanal	124-19-6	143.4
hexanal	66-25-1	101.3
2-nonenal	18829-56-6	141.4
1-octen-3-one	106-69-3	129.4
2-nonanone	821-55-6	143.4
isoamyl acetate	123-92-2	131.3
ethyl nonanoate	123-29-5	187.5
benzaldehyde	100-52-7	107.3

[a] Ion monitored is for MS−Nose measurements.

mixed for 1 min. The remaining oil with flavor components (Table 3), whey, and guar gum were added and mixed for 2 min on the middle setting. Acids were added and mixed for one additional min. The product was air sealed in aluminum bags and used within 2 days.

Stage 2A − Parameter Effects Experiment. Imitation cheese was prepared as described above except a commercial cheddar cheese flavor (0.05%) was used instead of individual flavor compounds (Virginia Dare, Brooklyn, NY). A full factorial design for four variables was conducted to determine the effect of temperature (24, 37 °C), saliva-to-cheese ratio (0.5, 0.4), blending rate (17, 37 rps), and airflow rate (10, 50 mL/s) on flavor release from the imitation cheese. The air leaving the RAS was sampled by three SPME fibers: two carbowax/divinyl benzene-coated (CW/DVB) and one divinyl benzene/polydimethyl siloxane-coated (DVB/PDMS). The location of the fibers and desorption order were randomized. SPME fibers were stored at −10 °C before being desorbed in the gas chromatograph mass spectrometer (GC/MS).

GC/MS Conditions. The GC/MS used was a Hewlett-Packard 5890 GC/MS with an electron impact ion source equipped with a 25 m × 0.20 mm cross-linked methyl silicone fused silica capillary column (film thickness, 0.33 μm). The GC/MS oven was programmed to start increasing its temperature three minutes from the initial temperature of 35 °C to 225 °C at a rate of 4 °C/min.

SPME Parameters. Before initial use, the fibers were conditioned as instructed by the supplier (Supelco, Bellefonte, PA). Before each extraction the fiber was held at 225 °C for five minutes and allowed to come to room temperature for 10 min. The plunger depth was set at 3 cm to allow for maximum desorption into the GC by injecting into the hottest part of the injection port. The fibers were exposed to the effluent from the RAS with imitation cheese at 5, 10, 15, and 20 min to determine minimal exposure time. The shortest time in which 95% of maximum total peak area was achieved was determined to be the optimum exposure time for the fibers (10 min).

Selection of Fiber Coating. Poly(dimethylsiloxane) (PDMS); PDMS/divinyl benzene (DVB); carbowax (CW)/DVB; and carboxen/PDMS fibers were tested for optimal selectivity of volatiles from the imitation cheese. Each fiber was exposed to the effluent from the RAS with artificial saliva and imitation cheese for 15 min and GC/MS, and gas chromatography olfactometry (GC/O) single sniffs were compared for each fiber. CW/DVB gave the largest number of odor-active compounds and the maximum extraction of volatile acids. Though most compounds were extracted by all fibers to varying degrees, the carboxen/PDMS extracted much fewer odor-active compounds. A combination of CW/DVB and DVB/PDMS extracted the maximum number of odor active compounds as shown by GC/O single sniffs.

Stage 2B − Parameter Effects Experiment. The effect of RAS parameters was also evaluated using MS−Nose measurements. Because of limited sample quantity only three factors were evaluated using the MS−Nose. A full factorial design for temperature (24 and 50 °C), blending rate (17 and 37 rps), and airflow rate (5 and 35 mL/s) effects was conducted on the imitation cheese. Ions from Table 3 were monitored as described above in the MS−Nose Parameters section.

Stage 3 − Diverse Compounds Correlation with RAS and Breath-by-Breath Analysis. To find limitations of the applicability of the correlation of the RAS and breath-by-breath analysis, a more diverse group of compounds was selected to vary along five physicochemical factors (Table 4) at three levels with correlations between molecular weight and Log *P*, and Log *P* and dielectric energy. The physicochemical factors were calculated by CAChe 3.2 software (Oxford Molecular, Oxford, U.K.). Five flavor solutions in absolute ethanol were prepared to avoid combinations of compounds with identical molecular ions and to produce palatable samples. The solutions are denoted by letters in the "set" column in Table 4 and the quantity is given in parts per million weight in the flavor solution denoted in the "stock ppm" column. Five panelists between the ages of 22 and 39 conducted breath-by-breath analysis as described above on 5-g samples from each flavor group and a control. Samples (150 g) were also measured from the RAS in triplicate.

Chocolates Preparation. Heavy cream (296 mL; 1/3 milk fat weight to total volume, g/mL) and glucose (50 mL) were heated to a boil. Then, chunks of milk chocolate (650 g) were added and stirred until melted. Once a smooth consistency was achieved, 50 mL of pure ethanol (control) or ethanol flavor solution was added. Portions (5 g) were rolled into balls and cooled.

Stage 4 − Real Food Verification. RAS effluents from foods were measured by the MS−Nose under mass scan conditions and the resulting maximum peaks were monitored for each food as listed in Table 5. Foods were consumed in pre-cut bite-size portions or drunk through a straw, and breath-by-breath analysis was conducted as described above with five panelists. The foods (150 g) were sampled in the RAS in triplicate. Food samples used were Tropicana Pure Premium Orange Juice, Nabisco Chips Ahoy chocolate chip cookies, and a sandwich made of white bread, peanut butter, and orange marmalade.

RESULTS AND DISCUSSION

Stage 1 − Correlation of RAS with Breath-by-Breath Analysis; Initial Experiment. For the imitation cheese, the RAS produced a ratio of odorants similar to that produced by the 16 panelists with a 200-fold greater intensity (Figure 2). The correlation coefficient of maximum intensity (Imax) ratio of the monitored odorants from the imitation cheese from the RAS and Imax ratios of odorants from the average of panelists was 0.99. Thus the relative ratios of concentration compounds released from the RAS and from the panelists are very similar. Variation between the panelists was between 41 and 49% for the compounds monitored and variation within a panelist was 25%. Variation for the RAS was 1 to 5%. The magnitude of change in odorant concentration required for a human to perceive a difference has been estimated to be between 10 and 30% (*20, 21*).

The high precision of the RAS can be used to determine the effects of flow rate, temperature, and, to some

Table 4. Flavor Compounds Used in Stage 3 for Correlation of RAS with Breath-by-breath Analysis Using More Diverse Compounds

compound	set[a]	stock ppm	CAS no.	ion[b]	cone voltage	Log *P*[c]	dipole	electron affinity	dielectric energy	MW[d]
ethyl hexanoate	a	20	123-66-0	145	18	2.02	1.75	−1.09	−0.51	144.21
cymene	a	200	99-87-6	134.3	18	3.71	0.08	−0.36	−0.12	134.22
octanal	a	200	124-13-0	139.1	18	2.03	2.78	−0.83	−0.57	128.21
furfuryl acetate	b	200	623-17-6	141.3	18	0.45	2.30	−0.44	−0.69	140.14
ethyl lactate	b	200	97-64-3	119.1	15	0.19	2.49	−0.88	−0.65	118.13
isoamyl acetate	b	200	123-92-2	131.3	18	1.40	1.86	−1.05	−0.57	130.19
linalool	c	200	126-91-0	137	18	2.52	1.66	−0.90	−0.28	154.25
benzothiazole	c	200	95-16-9	136	25	1.44	1.56	0.73	−0.45	135.18
decanal	c	20	112-3-2	157.1	26	2.82	2.79	−0.83	−0.57	156.27
2,5-dimethyl furan	c	20	625-86-5	96.8	18	1.92	0.16	−0.58	−0.18	96.13
diethyl-5-methyl pyrazine	c	200	18138-04-0	123.3	18	2.31	0.42	0.32	−0.31	150.22
anethole	d	200	4180-23-8	148.1	23	2.79	1.26	0.07	−0.29	148.20
carvone	d	400	6485-40-1	151.3	15	1.61	3.40	0.08	−0.55	150.22
eugenol	d	400	97-53-0	165.1	18	2.55	1.80	−0.17	−0.49	164.20
menthol	d	200	89-78-1	138.4	24	2.78	1.50	−3.02	−0.20	156.27
1,2-propane diol	e	1000	57-55-6	75.3	18	−0.30	0.69	−2.96	−0.49	76.10
α-terpineol	e	400	10482-56-1	136.4	18	2.02	1.35	−1.16	−0.25	154.25
α-damascenone	e	200		192.4	19	3.08	2.38	−0.01	−0.39	192.30
diethyl succinate	e	400	123-25-1	175.2	15	0.37	0.09	−0.89	−0.94	174.20
2-octanol	e	20	5978-70-1	112.7	28	2.53	1.53	−3.04	−0.30	130.23

[a] Set indicates which mixture the compound was found in. [b] Ion refers to the ion monitored for that compound by MS−Nose. [c] Log *P* is the log of the octanol−water partition coefficient (*12*). [d] MW is the compound molecular weight.

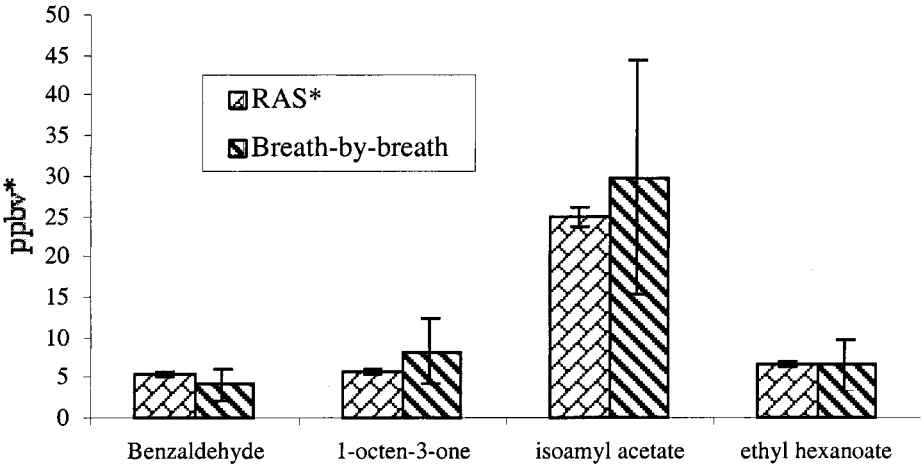

Figure 2. Correlation of flavor release from RAS relative to breath-by-breath analysis. RAS values are divided by 200.

Table 5. Foods Used in Stage 4 for Correlation of the RAS with Breath-by-Breath Analysis

food	sample size	ions monitored
orange juice	50 mL	82, 94, 118, 137, 166
chocolate chip cookie	1/4 cookie	70, 74, 87, 104
banana	0.5 g	84, 100, 131, 145, 159
Dr. Pepper	50 mL	107, 108, 123, 137, 166
peanut butter and jelly sandwich	0.5 g	93, 107, 119, 133, 137

Table 6. Range of Parameters from Stage 2B Which Would Produce Volatiles Within 30% of Amount Representative of Human Breath

parameter	range
temperature	13−61 °C
airflow rate	13−27 mL/s
blender speed	1.4−3.6 rps

degree, shear on flavor release, whereas the breath-by-breath measurements can be used to determine the effects of aroma composition on perception. In addition, the greater sensitivity caused by the higher concentration of volatiles in the RAS effluent enhances the detection limit of the mass spectrometer.

Stage 2A − Parameter Effects of RAS on Volatility; Use of SPME. The compounds from the artificial cheese flavor in the imitation cheese could be classified into three groups on the basis of how they were affected by the RAS parameters. Blending rate had a relatively insignificant ($p > 0.01$) effect on volatility of the compounds. Group 1 consisted of ethyl acetate (141-78-6), ethyl hexanoate (123-66-0), ethyl butanoate (105-54-4), and isoamyl acetate (123-92-2). Increasing the airflow rate by 5-fold reduced the volatility of compounds in group 1 by approximately 10-fold. Saliva ratio also affected group 1 volatility but to a lesser degree: increasing the saliva ratio resulted in an increase in volatility. Group 2 consisted of benzaldehyde (100-52-7), isoamyloctanoate (2035-99-6), 2-(*Z*)-decenal (2497-25-8), isoamyl butyrate (106-27-4), 2-nonanone (821-55-6), and ethyl octanoate (106-32-1). Increasing temperature and airflow rate resulted in an increase in volatility for group 2 compounds. Group 3 included the straight-chained aldehydes pentanal (110-62-3), octanal (124-13-0), nonanal (124-19-6), and decanal (112-31-2). Group 3's volatility was not significantly ($p < 0.01$) affected by any of the parameters. This shows that conditions in the mouth affect aroma compounds selectively.

Table 7. Results from Correlation of Breath-by-Breath (BBB) and RAS of Chocolates Containing Diverse Flavor Compounds

compound	CAS no.	set	BBB μg/L	RAS μg/L	BBB ratios[a]	RAS ratios[a]	% difference[b]
ethyl hexanoate*	123-66-0	a	61.2	527	1.00	1.00	0%
cymene	99-87-6	a	8.06	97.93	0.13	0.19	6%
octanal	124-13-0	a	6.33	131.28	0.10	0.25	15%
furfuryl acetate*	623-17-6	b	2.53	10.96	1.00	1.00	0%
ethyl lactate	97-64-3	b	2.53	670	1.00	61.10	34%
isoamyl acetate	123-92-2	b	4720	6148.9	1865.6	560.79	19%
linalool*	126-91-0	c	77.4	535	1.00	1.00	0%
benzothiazole	95-16-9	c	21.9	732	0.28	1.37	23%
decanal	112-3-2	c	13.3	22.9	0.17	0.04	21%
2,5-dimethyl furan	625-86-5	c	20.5	13.2	0.26	0.02	29%
ethyl-5-methyl pyrazine	18138-04-0	c	126	3250	1.63	6.07	20%
anethole*	4180-23-8	d	2.29	11.4	1.00	1.00	0%
carvone	6485-40-1	d	102	888	44.54	77.89	10%
eugenol	97-53-0	d	11	22.6	4.80	1.98	15%
menthol	89-78-1	d	2.17	11.4	0.95	1.00	1%
1,2-propane diol*	57-55-6	e	25.8	59.4	1.00	1.00	0%
a-terpineol	10482-56-1	e	1.78	7.05	0.07	0.12	9%
damascenone		e	2.19	3.58	0.08	0.06	6%
diethyl succinate	123-25-1	e	171	1720	6.63	28.96	22%
2-octanol	5978-70-1	e	9.51	2.04	0.37	0.03	29%

[a] Ratios are determined for each set relative to the compound indicated with *. [b] Percent difference was calculated as (BBB concentration − RAS concentration)/RAS concentration.

Stage 2B − Parameter Effects of RAS on Volatility; Measurements by MS−Nose. The parameter effects on volatility were similar for benzaldehyde (100-52-7), 1-octen-3-one (4312-99-6), isoamyl acetate (123-92-2), and ethyl hexanoate (123-66-0) added to the imitation cheese and measured by the MS−Nose in stage 2b. Temperature, airflow rate, and blending speed significantly affected volatile concentration in decreasing order of magnitude, respectively. There was additionally an interactive effect of temperature and airflow rate. An increase in temperature resulted in an increase in volatility, whereas increases in airflow rate or blending speed resulted in a decrease in volatility. In an attempt to visualize the magnitude of effect from the parameters, for each parameter, ranges which would produce a change in volatility less than 30% were calculated (R^2 of 0.94) and are given in Table 6. For olfaction, the Weber ratio, which describes the relationship between the perceived intensity of a sensation and the physical amount of the stimulus, has been found to be approximately 30% (*20*−*22*). Thus, it is likely that if the RAS were operated within the ranges listed in Table 6, the ratios would still be representative of what a human could perceive. The airflow rate had the smallest range and thus must be carefully controlled.

Results from stages 2a and 2b are in good agreement with the exception of the direction of the effect (increased or decreased volatility) of airflow rate on benzaldehyde. Airflow rate had an interactive effect with temperature in stage 2b experiments that may explain the directional difference of the effect of airflow on benzaldehyde. Under certain temperature conditions the effects would be in the same direction. This interaction was undetectable in stage 2a experiments.

Stage 3 − Correlation of RAS with Breath-by-Breath Analysis; Diverse Compounds. Chocolates were used as the delivery system for the flavors in stage 3 for two reasons. First the semi-soft system was simple to prepare reproducibly, yet it had complexity of fats and proteins. Second, the chocolate system was more acceptable to the panelists than the imitation cheese. Initially gelatin gels were used for stage 3; however, the tear resistance was too great for the blades in the RAS to overcome. This demonstrated that the RAS is limited to foods with low tear resistance.

The correlation and percent difference of relative volatility for each of the tested compounds appear in Table 7. The correlation between the breath-by-breath analysis and the RAS was described by a correlation coefficient of 0.97. Compounds with relatively high dielectric energy had the greatest percent difference between the RAS and panelists; thus, the RAS is most representative of humans for compounds with moderate to low dielectric constants. The effluent from the RAS was 50 times more concentrated than that from the humans. This is a smaller increase in concentration than was found in stage 1 with the imitation cheese, indicating that magnitude of increase is matrix dependent.

Stage 4 − Verification with Real Foods. The RAS produced an effluent with a higher concentration than that of the breath-by-breath analysis, yet with similar ratios of volatiles. The correlations between the RAS and breath-by-breath analysis of food samples were described with the following correlation coefficients: orange juice, 0.83; peanut butter and jelly sandwich, 0.92; Dr. Pepper, 0.99; chocolate chip cookie, 0.95; and banana, 0.99. Volatiles in the RAS effluent from cheddar cheese, brie, and vanilla ice cream were measurable, but insignificant signals were produced during breath-by-breath analysis. The increased concentration from the RAS would allow one to study the flavor of foods that otherwise would not be measurable by breath-by-breath analysis. Potent odorants are often found in foods at very low concentrations.

CONCLUSIONS

The RAS gave a good approximation of time-averaged flavor release in the mouth as defined by the in-mouth or breath-by-breath measurements. The control of shear rate, temperature, airflow rate, and saliva ratio allows the RAS to be used to simulate a range of release conditions. Although the RAS is not applicable to the study of temporal dimension of release, it is a good benchmark, giving precise and sensitive estimates of average flavor release. The concentrations of odorants

in the effluent from the RAS are greater than those found in the human breath upon consumption; however, the compounds are present at similar ratios. Thus, using the RAS can increase sensitivity and can be used to measure potent odorants that would not be found by breath-by-breath analysis. The RAS is applicable to a large range of compounds. The process of eating and drinking combines closed and open systems, though the system is always under nonequilibrium conditions. This close correlation of volatile ratios from the RAS and in vivo indicates that an open system with a flow rate that allows volatiles to return to the food matrix is a better in vivo representation than either a static equilibrium system or a truly dynamic system in which no volatiles return to the matrix. There are defined variations of the RAS conditions that continue to produce in vivo comparable flavor release ratios. The application to some real foods shows that flavor release from many foods can be measured from the RAS.

ABBREVIATIONS USED

RAS, retronasal aroma simulator; API, atmospheric pressure ionization; PDMS, poly(dimethylsiloxane); DVB, divinyl benzene; CW, carbowax; BBB, breath-by-breath.

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