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Persistence of aroma compounds in human breath after consuming an aqueous model aroma mixture

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Abstract

Persistence of aroma compounds in the breath was studied from a model mixture containing five chemicals in an aqueous medium at two concentration levels. The breath of five subjects was monitored by atmospheric pressure ionization-mass spectrometry (API-MS) and the persistence of each compound computed as the ratio of instrumental responses between first and second breaths. Persistence was modelled, based on relevant physicochemical parameters of the compounds in the mixture. Vapour pressure was found to be the most significant parameter. Persistence was not influenced by panellists, volatile concentration levels or replications within subjects, although there were large differences in persistence among chemicals.

Keywords: Aroma release; APCI-MS; Human breath

1. Introduction

The persistence of food volatiles in the breath depends on three main factors: physicochemical properties of the volatiles, human physiology and characteristics of the food matrix (Linforth & Taylor, 2000). Most flavours are mixtures of compounds of diverse chemical nature; therefore, properties related to their molecular size, shape and polarity will decisively influence their individual availability to the sensory system (Nahon, Harrison, & Roozen, 2000).

Factors of human physiology, such as mouth volume, saliva flow rate and air flow will also alter the volatile profile that reaches the receptors. Thus, the same proportion of aroma compounds originally released from a food on eating may not reach the olfactory epithelium. Compounds may become absorbed during transport, resulting in an altered temporal profile and concentration (Overbosch, Afterof, & Marring, 1991). The transport and uptake of inspired odorant molecules in the human nasal cavity were determined by Keyhani, Scherer, and Mozell (1997) using a three-dimensional finite element model. Increase in nasal flow rate at a constant inlet concentration resulted in an increase in total olfactory uptake and, consequently, in a higher perceived odour intensity for all chemicals. However, with increase in flow rate, the fractional uptake, that is, the total olfactory flux normalized by convective flux at the inlet, decreased for poorly soluble odorants but increased for highly soluble odorants. The pattern of flux that carries information about odour intensity across the olfactory mucosa was determined as a function of transport parameters. There was an overall decrease in odorant flux according to the location of the olfactory surface.The flux pattern became more uniform as the mucus solubility of the odorants decreased. In addition, flux decreased approximately exponentially with time, and so did perceived odour intensity with cessation of nasal airflow.

Odour-active volatiles may be free, entrapped, adsorbed or complexed in the food matrix (Nahon, Roozen, & De Graaf, 1996). For example, Baines and Morris (1987) reported that thickening agents affect

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aroma release above a critical concentration, c^*, at which coil overlap and entanglement occur with an abrupt increase in viscosity. They found that sweetness and strawberry aroma perception were progressively suppressed at thickener concentrations above c^*. In a follow-up study, Baines and Morris (1988) proposed that perceptual changes may be linked to inefficient mixing in solutions above c^*, inhibiting the transport of small taste and aroma molecules to their respective receptors. Hollowood, Linforth, and Taylor (2002) used real-time analysis of in-nose volatile release by atmospheric pressure ionization mass spectrometry (API-MS) to show that retronasal benzaldehyde release was not substantially changed by hydroxypropylmethyl cellulose (HPMC) at up to 2.1 times the c^* concentration, although almond flavour perception declined sharply above in accordance with Baines and Morris (1988). This was explained in terms of a taste–aroma interaction, with the perceived drop in sweetness of the system driving the decline in perceived aroma, even though the concentration of aroma reaching the olfactory epithelium remained broadly constant. Later, Cook, Hollowood, Linforth, and Taylor (2003) found oral shear stress, determined from rheological measurements, to be responsible for the taste–aroma interaction, supporting the hypothesis that somatosensory tactile stimuli can interact with taste and aroma signals to modulate their perception.

Persistence of volatile compounds in the breath was monitored by Linforth and Taylor (2000) after their consumption in aqueous solutions. Factors studied were variation in volatile release patterns between panellists, effect of adding HPMC, and differences among compounds. For any given compound, persistence was broadly similar for all panellists, and not significantly affected by adding HPMC at concentrations in excess of c^*. The largest persistence differences corresponded to those among compounds (>20-fold). A quantitative structure-property approach was used to model persistence, which depended primarily on hydrophobicity and vapour pressure of the corresponding odorant.

Wright, Hills, Hollowood, Linforth, and Taylor (2003) have recently reported on additional work focused on mathematically modeling aroma persistence in the breath when swallowing an aqueous flavor sample. They found that a good model can be obtained for the persistence effect (after the first breath) using basic principles of interfacial mass transfer. Their model included the interfacial area of the saliva film:gas phase, the volume of saliva thin film, the interfacial mass transfer coefficient, a constant reflecting the loss of aroma compounds from the breath via other mechanisms, breathing amplitude and frequency, and aroma compound concentration in the liquid. It was much more difficult to obtain a valid model for the first (i.e. swallow) breath. Factors such as sample temperature changes upon entering the mouth and dynamics of swallowing complicated the task. However, using either experimentally or empirically derived persistence values (R values, based largely on structural and partitioning parameters of aroma compounds), reasonable modeling was done for the maximum intensity of the first breath as well. Thus, Wright, Hills, Hollowood, Linforth and Taylor (2003) were able to offer models that predicted volatile concentrations in both the first breath and the decay in concentration in subsequent breaths. They noted however that this model does not account for interactions between aroma compounds and the food, or any influence of rheological properties of the food. This is the topic of future research.

This paper studies the relationship between physicochemical parameters of aroma compounds and their persistence in the breath after being released from an aqueous model mixture during eating. Persistence of these aroma compounds is also analyzed statistically with respect to differences among mixture constituents, mixture concentration and experimental replications.

2. Materials and methods

2.1. Chemicals and sample preparation

Five aroma compounds of dissimilar chemical structures were chosen to use in this study: ethyl butyrate, 2-methyl pyrazine, benzaldehyde, 2-octanone and *cis*-3-hexenol. They were provided by Robertet Flavors, Inc. (Piscataway, NJ, USA). A 10 ml mixture of the chemicals was obtained by directly mixing 2 ml of each species. Then, 0.1 ml of the flavour mixture was diluted in 10 ml of food grade ethanol. Next, 1.0 ml of the flavour/ethanol mixture was added to either 1 or 2 l of bottled water to get final total concentrations of 100 and 50 ppm, respectively.

2.2. Subjects and testing protocol

Five subjects (three female, two male, ages 25–38) were recruited from the Department of Food Science and Nutrition at the University of Minnesota. Subjects were seated in front of the modified API-MS source, as described below, and the inlet capillary tube was positioned at nose height. They were given a small plastic container with 20 ml of the aqueous solution, instructed to swallow it in one gulp and then breathe normally into the inlet for about 60 s. All subjects participated in one training session, performing at least two practice runs prior to analysis to familiarize them with the protocol. Both 50 and 100 ppm total concentration samples were analyzed twice by each subject.

2.3. API-MS analysis

An API-MS system (LC-Q ion trap, Finnigan MAT/ThermoQuest, San Jose, CA, USA) was modified to introduce gaseous samples (i.e. samples of human breath) and to allow humidified sheath and auxiliary gases, as described by Zehentbauer, Krick, and Reineccius (2000). Air was sampled into the modified source at a flow rate of 90 ml/min. Operating conditions were as follows: vaporizer temperature, 400 °C, discharge current, 5 μA, capillary temperature, 150 °C, capillary voltage, 31.3 V, tube lens offset 5 V, sheath gas: nitrogen at 80 arbitrary units (about 5.7 l/min), auxiliary gas: nitrogen at 60 arbitrary units (about 7.7 l/min), full MS scanning in positive ion mode for a mass range of 50–200.

2.4. Characteristic ions

The parent ion (MW + 1) and the most significant fragments were used to monitor each compound. Two ions were observed for ethyl butyrate: the protonated molecule $m/z = 117$ and the protonated butyric acid fragment $m/z = 89$. Both, 2-methyl pyrazine an benzaldehyde, showed only their molecular protonated forms, $m/z = 95$ and $m/z = 107$, respectively. In the case of 2-octanone and *cis*-3-hexenol, two ions were identified: the parent protonated molecule and the fragment corresponding to the loss of one water molecule. They were $m/z = 129$ and $m/z = 111$ for the former, and $m/z = 101$ and $m/z = 83$ for the latter. Each compound in each run was quantified by adding the areas of their parent and fragment ions (the latter, when present). For all subjects and replications, an API-MS signal was observed only after intake of the mixture and first exhalation into the probe; there were no interferences from compounds naturally present in the breath.

2.5. Calculation of persistence and statistical computations

Persistence values were computed individually for each aroma compound as the ratio between the corresponding intensities of second and first exhalations after swallowing, times 100. In other words, persistence was quantified by expressing the peak height for the volatile in the second exhalation as a percentage of the first (Linforth & Taylor, 2000). The MacAnova statistical package (Oehlert & Bingham, 1997) was used to compute simple statistics, *t*-tests for comparison of means and regression models.

3. Results and discussion

Persistence values of flavour compounds were obtained from a model mixture in an aqueous medium (as opposed to a real food) in order to minimize matrix interactions and mimic an actual flavour system that typically contains a number of chemicals of diverse nature rather than isolated compounds.

Persistence values are presented in Tables 1 and 2 for the 50 ppm and 100 ppm mixtures, respectively. Differences in persistence among the five chemicals studied were evident, with ethyl butyrate, benzaldehyde and 2-octanone showing lower values than 2-methyl pyrazine and *cis*-3-hexenol. Persistence values greater than 100 for some compounds were the result of a more intense response in the second breath than in the first breath. Effectively, more persistent compounds were released at a slower pace and reached the API-MS source later with respect to the less persistent ones that were 'exhausted' on the first exhalation (the original mixture had similar concentrations of all species).

Table 1
Persistence values at 50 ppm total concentration

	Subjects								
		I	II	III	IV	V	Mean	SD[a]	CV%[b]
Ethyl butyrate	1[c]	53	54	53	59	42	53.5	9.0	16
	2	64	65	59	49	37			
2-Me pyrazine	1	155	213	171	137	101	141	36.0	25
	2	147	163	108	112	105			
Benzaldehyde	1	77	76	76	69	70	76.5	7.4	10
	2	89	67	88	74	79			
2-Octanone	1	75	73	85	105	110	92.6	12.7	14
	2	94	98	105	95	86			
Cis-3-Hexenol	1	242	98	135	217	191	175	58.6	33
	2	201	253	99	125	90			
Mean		120	126	97.9	104	91.1			
SD[a]		63.7	73.6	35.2	49.0	42.9			
CV%[b]		53	58	36	47	47			

[a] Standard deviation.
[b] Coefficient of variation (percentage).
[c] Numbers 1 and 2 indicate replications within subjects.

Table 2
Persistence values at 100 ppm total concentration

	Subjects							
		I	II	III	IV	V	Mean	SD[a]	CV%[b]
Ethyl butyrate	1[c]	61	64	41	47	25	42.7	20.8	49
	2	17	77	48	33	14			
2-Me pyrazine	1	127	152	91	93	99	115	34.6	30
	2	159	163	67	123	78			
Benzaldehyde	1	56	80	126	72	60	72.2	26.1	36
	2	54	42	99	86	47			
2-Octanone	1	119	113	61	89	62	79.6	23.8	30
	2	70	90	78	72	42			
Cis-3-hexenol	1	98	91	150	146	78	132	50.0	38
	2	229	163	136	167	64			
Mean		99.0	104	89.7	92.8	56.9			
SD[a]		62.0	42.8	37.7	42.0	25.7			
CV%[b]		53	58	36	47	47			

[a] Standard deviation.
[b] Coefficient of variation (percentage).
[c] Numbers 1 and 2 indicate replications within subjects.

Our persistence values were higher than those given by Linforth and Taylor (2000) and Wright, Hills, Hollowood, Linforth and Taylor (2003). For example, their ethyl butyrate values were in the single-digit range whereas the ones reported here are in the double-digit range. However, the statistical parameters (that ultimately characterize data distribution) were similar. Thus, they obtained a 50% coefficient of variation (CV) (i.e., the ratio between mean and standard deviation) for ethyl butyrate versus 49% at 100 ppm volatiles concentration in this study, and a 33% CV versus 36% for benzaldehyde. Even more, the persistence values for our 50 ppm concentration results were much lower, namely 17% for ethyl butyrate and 9% for benzaldehyde. Differences in persistence values could have been related to different sets-ups in API-MS data collection, as well as on the model system itself, as they ran each compound individually whereas we ran them simultaneously as a model mixture.

Tables 1 and 2 also give statistics to compare persistence values within subjects. In general, there was a smaller variation among panellists than among flavour compounds. Due to the varied chemical nature of the components of the model mixture and their rather dissimilar APCI-MS responses, we expected much higher coefficients of variation for each subject individually than for each flavour compound, but these values showed lower variability across subjects than across chemicals. Thus, taking the highest and the lowest coefficients of variation for chemicals and subjects within each sample concentration, there was a 347% variation within chemicals versus a 162% variation within subject for the 50 ppm sample, and a 164% variation within chemicals versus a 151% variation within subjects for the 100 ppm sample. There were also important differences in API-MS responses for each subject within each flavour compound (data not shown), evidently related to different breathing patterns and breathing intensities.

Table 3
Statistical comparison of persistence values

(A) Each compound across sample concentration and within sample replication

Compound	*p*-value
Ethyl butyrate	0.150
2-Methyl pyrazine	0.117
Benzaldehyde	0.623
2-Octanone	0.170
Cis-3-hexenol	0.211

(B) All possible pairs of mixture components within sample concentration and replication

	2-Methyl pyrazine	Benzaldehyde	2-Octanone	*Cis*-3-hexenol
Ethyl butyrate	8.8×10^{-11}**	3.6×10^{-5}**	11×10^{-7}**	5.4×10^{-9}**
2-Methyl pyrazine		1.0×10^{-6}**	5.5×10^{-5}**	0.189
Benzaldehyde			0.069	3.0×10^{-6}**
2-Octanone				4.4×10^{-5}**

No *p*-value is significant at the 95% level ($p < 0.05$).
Each number in the table is the *p*-value of the corresponding *t*-test ($\mu_1 = \mu_2$).
No asterisk means lack of significance ($p > 0.05$).
** Highly significant ($p \ll 0.001$).

Table 4
Physicochemical parameters of aroma compounds

	MW[a,e]	h_{vap}[b] (kJ/mol)[f]	VP_{37}[c] (Pa)[f]	log P[d,g]
Ethyl butyrate	116	35.5	4.26	1.85
2-Me pyrazine	94	42.1	2.89	0.49
Benzaldehyde	106	50.3	2.85	1.71
2-Octanone	128	39.8	3.27	2.22
Cis-3-hexenol	100	51.3	1.03	2.19

[a] Molecular weight.
[b] Enthalpy of vaporization.
[c] Vapour pressure at 37 °C.
[d] Octanol–water partition coefficient.
[e] From Burdock (1995).
[f] From Lide (2001).
[g] From Syracuse Research Corporation (2003).

Table 5
Persistence models

	p-value	Regression coefficient (r^2)
Persistence $= 317.0 - 2.0$ MW	0.061	0.371
Persistence $= -60.4 + 3.6h_{vap}$	0.095	0.308
Persistence $= 189.7 - 32.0VP_{37}$	0.002**	0.710
Persistence $= 117.7 - 11.6 \log P$	0.610	0.034

Physicochemical parameters as described in Table 4.
No asterisk means lack of significance ($p > 0.05$)
** Highly significant ($p < 0.01$).

Persistence was regressed against subject, concentration and replication. The corresponding regression coefficients were extremely low ($r^2 = 0.056$ for "Persistence = Subject", $r^2 = 0.031$ for "Persistence = Concentration", and $r^2 = 0.001$ for "Persistence = Replication"), another indication that differences within subjects were not significant compared to those within chemicals. In addition, there were no differences among replications within subjects or concentration levels of the model across subjects.

Statistical t-tests of comparisons of means were performed for each compound between the 50 and 100 ppm samples and for each possible pair of compounds across subjects, replications and concentrations (i.e. pooling all persistence values in one set per compound). Results are shown in Table 3. No differences existed for any of the mixture constituents between the two model concentrations. Persistence differences among compounds were confirmed by the t-test. For example ethyl butyrate, the least persistent chemical, showed extremely significant differences from all other compounds. On the other hand, there was no difference between 2-methyl pyrazine and *cis*-3-hexenol, the two most persistent chemicals.

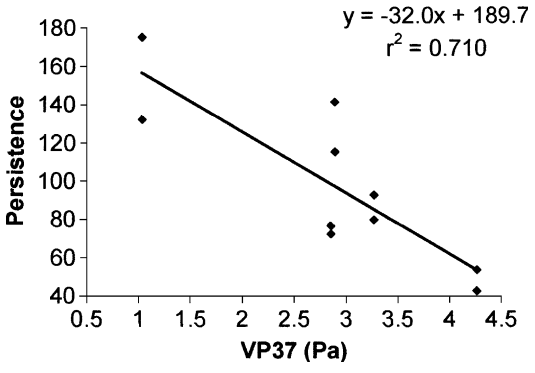

Fig. 1. Persistence vs. vapour pressure at 37 °C.

Persistence was modelled by linear regression analysis, using four physicochemical parameters of the aroma compounds: molecular weight (MW), enthalpy of vaporization (h_{vap}), vapour pressure at 37 °C (VP_{37}) (i.e. the normal body temperature), and octanol–water partition coefficient (log P). These parameters are given in Table 4. Persistence was modelled separately for each parameter. Since there was no statistical difference between 50 and 100 ppm samples, the entire data set was used for this analysis, based on the mean values across subjects, replications and concentrations for each compound in the mixture. Models, p-values and regression coefficients are presented in Table 5.

Vapour pressure was the only parameter with statistical significance ($p < 0.01$) and the one that rendered the highest regression coefficient ($r^2 = 0.710$). The corresponding model is also depicted in Fig. 1. Expectedly, vapour pressure showed an inverse relationship with persistence (as indicated by its negative coefficient in the model), that is, the higher the vapour pressure, the lower the persistence, and vice versa. The other parameters modelled persistence rather poorly, and ultimately, were considered not relevant.

4. Conclusions

Different flavour compounds show a broad range of persistence values, even with almost no matrix interaction (as is the case for an aqueous medium), but no significant differences were found among subjects, replicates or concentration levels of the model mixture.

According to the results of this study, it is feasible to attempt modelling persistence of aroma compounds in human breath based on relevant physicochemical parameters, such as vapour pressure at body temperature.

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