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Persistence of aroma volatiles in the oral and nasal cavities: real-time monitoring of decay rate in air exhaled through the nose and mouth

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Abstract

The persistence of aroma compounds in breath after swallowing is an important attribute of the overall aroma experience during eating and drinking. It is mainly related to the coating of the oral tract with food residues and the interaction between volatile compounds and airway mucosa. We have studied the persistence of eight compounds (2,5-dimethylpyrazine, guaiacol, 4-methylguaiacol, phenylethylalcohol, ethylbutanoate, ethyloctanoate, isoamylacetate and 2-heptanone) both in-nose and in-mouth after administration of volatiles in gas phase (vapor) to five different panelists. By using volatiles in the gas phase, only the interaction with the mucosa is highlighted and the formation of a liquid coating in the oral and tracheal airway is avoided. The physicochemical properties of the compounds, mainly polarity and vapor pressure, determine the interactions of the volatiles with the airway mucosa. The use of different breathing protocols allowed the study of the differences between nasal and oral mucosa in volatile retention, with higher persistence of volatiles obtained in-mouth. Initial concentration also affected persistence, but only for compounds with high volatility and at low concentration.

1. Introduction

A consumer's flavor experience is a highly dynamic process. Considering beverages as one of the 'simplest' scenarios—as no mastication is involved—the consumer's olfactory sensation will start with the orthonasal aroma smelled from above the beverage, before it is introduced into the mouth. After a sip is taken, the retronasal aroma starts. Compounds are released from the beverage into the mouth cavity and transported by the airflow to the olfactory epithelium in the nose. During swallowing, volatile organic compounds (VOCs) are released as they pass by the throat, producing the so-called 'swallow-breath' that results in high intensities of some VOCs in the air exhaled just following swallowing. Some volatile compounds can remain in the breath airflow for seconds or minutes after swallowing, resulting in a prolonged persistence of the aroma in the oral cavity, and producing the sensation commonly known as 'after-smell' or 'after-odor' [1, 2]. The food industry, as well as chefs, is increasingly coming to appreciate the importance of the quality, intensity and duration of such long-lasting sensations once a food or beverage has been swallowed, and considers them as an integral part of the food experience and quality. Particularly with beverages, where the liquid is often swallowed quickly, the after-odor may be the major element of the overall aroma perception. Hence, and in order to better understand the consumer's experience when drinking a beverage, more systematic and detailed studies are needed to elucidate the processes governing the persistence of aroma compounds in breath air.

Besides their obvious relevance to food and aroma science and applications, these fundamental studies are also of great significance to health and medical applications. Indeed, diagnostic applications of breath VOCs will be affected equally by the persistence of VOCs

originating from the lungs and the alveoli. Hence a better understanding of the link between the physical properties of VOCs and their persistence in breath is of importance to both food aroma persistence and medical breath studies.

Aroma persistence in breath depends on a number of factors, where physiological effects, characteristics of the food matrix and physicochemical properties of the aroma compounds seem to play a key role [3]. Of these three, physiological factors are characteristic of each person and contribute to inter-individual differences. These factors include naso-oropharyngeal volumes, saliva composition, mucus composition, soft-palate opening and breath flow, among others. In the case of aqueous solutions swallowed immediately after consumption, aroma persistence was found to be independent of the panelist, suggesting that physiological factors have little influence on those systems [3, 4].

The composition of the food matrix determines the partitioning of volatiles between the food product and the gas phase present in the oral cavity. Oral processes (chewing, tongue movements, salivation) disrupt the food matrix, affecting the release of volatiles to the exhaled airflow and contributing to the overall sensation. After swallowing, a viscous layer containing residues of the food product diluted in saliva can form on the oral and oropharyngeal mucosa. This coating keeps releasing traces of odorants over time, thereby affecting persistence [5]. Aroma persistence will therefore depend both on the partition of volatiles between the coating film and exhaled air and the adhesion degree of the matrix material to the oral and nasopharyngeal mucosa. Camacho *et al* found that the fraction deposited on the anterior tongue surface increased linearly with the oil content of an oil and water emulsion, with more coating resulting in higher concentration of lipophilic aroma compounds [6]. Some authors compared odorant persistence in breath after drinking an aqueous solution of the aroma compound ('wet swallow') or swallowing a gaseous aliquot of the compound ('dry swallow'). They observed the persistence effect for isoamylacetate [7] and diacetyl [8] only in the case of the wet swallow, suggesting the existence of liquid residues on the oral tract and the weak interaction of those compounds and the mucosa when they are in gas phase.

The physicochemical characteristics of the aroma compounds also determine their release from the food matrix and the interaction of those compounds with mucosa. In order to reduce matrix effects, several authors have studied aroma persistence using aqueous model solutions. Linforth and Taylor analyzed the persistence of 41 compounds in water and identified polarity, volatility, length of the carbon chain and ether linkage as the main factors controlling aroma persistence [3]. Other studies also using water solutions pointed to volatility as the main contributor to aroma persistence [4, 7, 9].

The study of aroma persistence using volatile compounds in gas phase eliminates all possible interactions between volatiles and the food matrix and also the possibility of food debris coating the walls of the oral tract after swallowing that could act as an aroma reservoir and release volatiles over time. Therefore, by using volatiles in gas phase, only interactions with mucosa or saliva would be revealed. These interactions are also important in breath research as inhaled compounds can be retained in the airway mucosa and released back to the breath flow, impacting the measurement of endogenous compounds [10].

In this study, the differences in persistence between the nasal and oral cavities are examined for several VOCs. For that purpose, aromatized air containing eight volatile compounds was administered to five panelists breathing according to four different breathing protocols and the exhaled breath was analyzed on-line by proton transfer reaction mass spectrometry (PTR-MS). The high sensitivity of PTR-MS allowed the real-time analysis of volatiles in single breath exhalations [11, 12]. The breath-by-breath data were then fitted to a power curve as done previously by Hodgson [9] and the decay rate was calculated. The volatiles studied were selected based on their differences with respect to physicochemical properties, mainly polarity, volatility and chemical class.

2. Materials and methods

2.1. Preparation of aromatized air

An aroma cocktail containing 2-heptanone (57 mg), guaiacol (349 mg), 4-methylguaiacol (594 mg), ethyloctanoate (202 mg), ethylbutanoate (210 mg), 2,5-dimethylpyrazine (112 mg), isoamylacetate (286 mg) and phenylethylalcohol (375 mg) was prepared. All compounds were mixed without solvent and a liquid aliquot of the cocktail (300 μl) was injected in a 40 l Tedlar bag filled with compressed air. The bag was left at 60 °C for 3 h to evaporate and equilibrate the volatile compounds. The same bag was used for all the experiments evaluating differences between compounds, panelists and breathing protocols. The concentration of all compounds in the bag was above their odor threshold and was high enough to ensure the monitoring of nose-space intensity changes over time. A different bag was prepared for evaluating differences in persistence as a function of compound concentration. Five panelists (three male and two female, aged 30–50) gave their consent to participate in the experiments.

2.2. Breathing protocols

Aromatized air (500 ml) was sampled from the Tedlar bag with a gas-tight syringe and injected in the nose or mouth of the panelist, depending on the breathing protocol. Four protocols were selected which defined on the one hand the mode by which the aroma cocktail was administrated to the panelist and, on the other hand, how the air was inhaled and then exhaled for subsequent on-line measurements by PTR-MS (figure 1). For all protocols, the volatiles were administered once during the first inhalation. Afterwards, the panelists continued breathing lab air for at least 5 min.

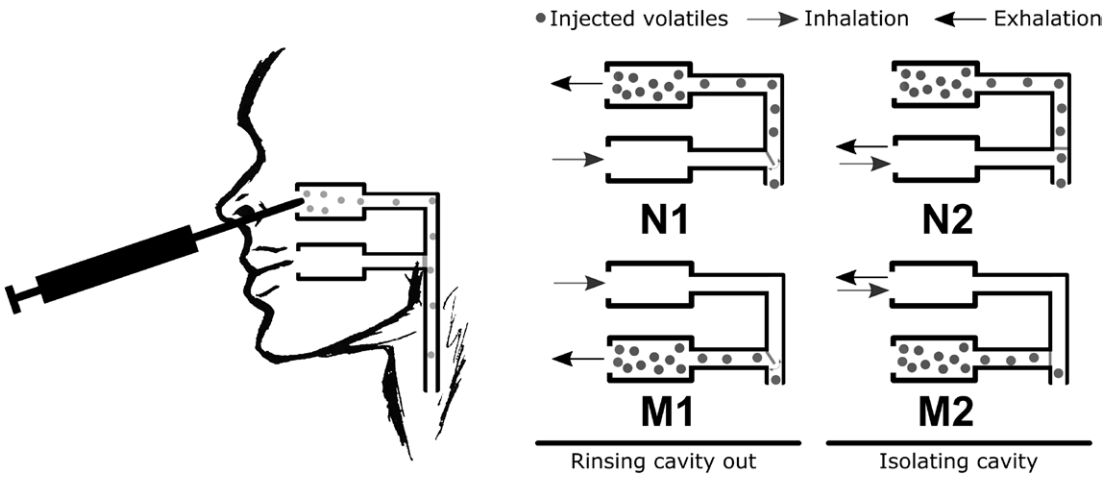


Figure 1. Representation of the aromatized gas administration (left) and scheme of the four different breathing protocols used in the study (right). After the administration of aromatized air in the first inhalation, the panelist freely breathed room air.

The breathing protocols were: M1, the volatiles were injected in and inhaled through the mouth, then air was exhaled through the mouth into the sampling device and fresh air was inhaled through the nose for each breath; M2: the volatiles were inhaled through the mouth and then, with a closed mouth, air was exhaled through the nose into the sampling device and air was inhaled through the nose; N1: the volatiles were inhaled through the nose and then air was exhaled through the nose and inhaled through the mouth; N2: the volatiles were inhaled through the nose and then, while the nose was closed with a clip, air was both exhaled and inhaled through the mouth. For all protocols, the breath rhythm was defined as 3 s inhalation and 3 s exhalation, and was paced with a metronome during the experiments. All five panelists performed each breathing protocol in triplicate.

2.3. Dose–response

Different concentrations were prepared by dilution of the aromatized air, bringing the compound concentration closer to real food situations. Dilution was made directly into the syringe prior to administration: different volumes (50, 100, 200, 300, 400, 500 ml) were taken from the bag containing aromatized air and then the syringe was filled back up to 500 ml with non-aromatized lab air. Blanks containing lab air were analyzed to ensure that it was free of VOCs that could interfere with the measurements. Two replicates of each concentration were administrated to two different panelists.

2.4. Exhaled air sampling

Volatile concentration in the exhaled air was monitored via proton transfer reaction quadrupole mass spectrometry (PTR-QMS) (Ionicon GmbH, Austria). Two inlet systems were used, depending on whether the air was exhaled through the nose or through the mouth. For nose sampling, a commercial nosespace air sampling extension (Ionicon GmbH, Austria) was used. For mouth sampling, a custom-built setup was used. The setup contained a removable mouth tip that was changed for each panelist, mounted on a one-way respiratory valve (Hans Rudolph Inc., USA) equipped with a membrane to prevent saliva from passing through the system. The valve was connected by a T-piece to the PTR-QMS inlet sampling 40 ml min^{-1} and the other side was left open to expel the excess of the exhaled air. The whole system was heated to 90 °C to prevent a condensation of the volatiles. To check for volatile retention on the sampling setups, compressed air was connected to the setup immediately after exhalation of the volatile mixture. It was observed that all mass signals instantaneously returned to background levels.

2.5. PTR-MS conditions

A commercial PTR-MS with a quadrupole detector was used. The PTR conditions were as follows: 160 °C inlet temperature, 80 °C drift tube temperature, 2.2 mbar drift tube pressure, 495 V drift voltage, yielding an E/N value of 120 Td. The same dwell time (50 ms) was used for all the m/z measured: 71 (isoamylacetate), 105 (phenylethylalcohol), 109 (2,5-dimethylpyrazine), 115 (2-heptanone), 117 (ethylbutanoate), 125 (guaiacol), 139 (4-methylguaiacol), 173 (ethyloctanoate). The headspace of each compound was analyzed to determine its fragmentation under the selected PTR-MS conditions and the data were corrected accordingly. No overlap was observed between the selected m/z, with the exception of an ethyloctanoate fragment of m/z 109 (<1%) which overlapped with 2,5-dimethylpyrazine. The data obtained for 2,5-dimethylpyrazine were corrected for the ethyloctanoate contribution.

2.6. Data processing

Exhalations were identified by the increase of the acetone signal (m/z 59). Intensity was measured during the 3 s of each exhalation, resulting in five data points for each compound (550 ms scan rate). Since the signal intensity was essentially constant over the 3 s exhalation

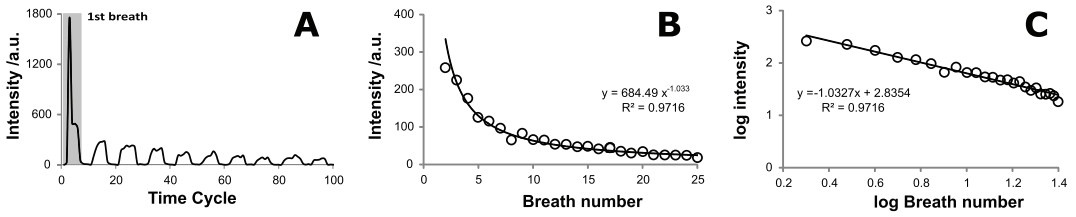
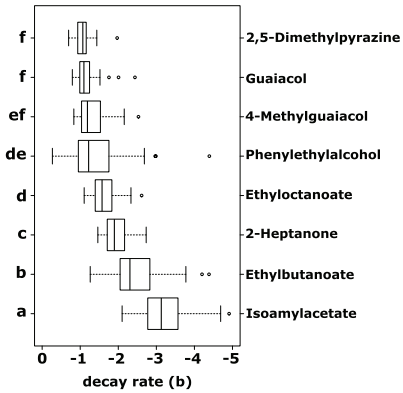


Figure 2. Ten breath-by-breath intensity profiles for 2,5-dimethylpyrazine to show the raw data (A). Plot of 25 intensity averaged exhalations (150 s) fitted to a power curve (B) and with logarithmic axes (C).



	Mw	log $K_{o/w}$	log $K_{a/w}$	Vapor pressure /Pa
2,5-Dimethylpyrazine	108	0.63	-3.84	533
Guaiacol	138	1.32	-4.31	13
4-Methylguaiacol	122	1.88	-4.26	13
Phenylethylalcohol	124	1.36	-4.98	3
Ethyloctanoate	172	3.81	-1.28	27
2-Heptanone	114	1.98	-2.16	627
Ethylbutanoate	116	1.85	-1.79	1853
Isoamylacetate	130	2.25	-1.62	760

Figure 3. Boxplots for the decay rate (b) of the eight compounds considering all panelists and breathing protocols with letters marking significant differences in decay rate among the compounds (Tukey's test, $p < 0.01$). Physicochemical data of the compounds were obtained via ChemSpider, an open-access online chemical database hosted by the Royal Society of Chemistry (www.chemspider.com).

window, the intensity for each compound was given as the average intensity over the five data points measured for each exhalation. Before administration of aromatized air, five exhalations were recorded each time to determine the background levels. The first exhalation was discarded from data analysis; starting with the second exhalation, the background corrected intensities of the exhalation signals were fitted to a power curve $I = at^{-b}$ where I was the intensity at time t. The two parameters obtained from the fitting represented the intensity at the beginning (a) and the decay rate (b) of the volatile compound.

Analysis of variance (ANOVA) was performed to assess the effect that compounds, panelists, breathing protocols or concentration had on the power curve parameters. Significant differences were obtained using Tukey's honest significant difference post hoc test ($p < 0.01$). All analyses and the creation of graphs were performed with packages and scripts developed in R [13].

3. Results

The concentration of volatiles in the exhaled breath was monitored on-line by PTR-MS for 5 min following the one-time administration of aromatized air. Two minutes after inhalation of the aroma mixture, volatile levels in the exhaled air were close to background levels. Therefore, only the first 2 min were used for further calculations. For each breath, the concentration in the exhaled air was averaged and plotted against the time at the end of the exhalation. The first exhalation contained aromatized air that remained in the dead space of the airway (where no gas exchange takes place), resulting in a sharp peak of high intensity followed by a shoulder (figure 2(A)). This first exhalation was therefore discarded and only the following ones, containing those volatiles that had been retained on the respiratory tract and then released breath by breath, were considered [9]. As previously done by Hodgson *et al* using aqueous solutions, we described the long-time persistence of volatiles by fitting the concentrations of the second breath onwards to a power curve $I = at^{-b}$ (figure 2(B)), where the factor b represents the decay rate.

3.1. Differences in persistence between compounds

Differences in persistence were observed for the eight compounds studied (2,5-dimethylpyrazine, ethylbutanoate, ethyloctanoate, guaiacol, 2-heptanone, isoamylacetate, 4-methylguaiacol, phenylethylalcohol). Figure 3 shows the boxplots of the calculated decay rates for each compound, including the results from all experiments carried out (5 panelists × 4 protocols × 3

replicates = 60 measurements for each compound). The calculated decay rates (b) presented statistically significant differences among the compounds even when all measurements were considered, indicating that compound physicochemical properties had a higher impact on persistence than the panelist or the breathing protocol used (figure 3). Two main groups of compounds can be differentiated according to their decay rate: those with fast decreasing intensities, which correspond to compounds with lower water solubility and high volatility (2-heptanone, isoamylacetate and ethylbutanoate), and those presenting a much slower decay, which are highly soluble in water (2,5-dimethylpyrazine, guaiacol, 4-methylguaiacol and phenylethylalcohol).

3.2. Differences in persistence between panelists

Inter-individual differences were observed in the decay rate (b) for most of the compounds and they were dependent on the breathing protocol used. To determine those differences among panelists, an ANOVA test was performed for each of the compounds and protocols used. A complete list of the decay rates with statistically significant differences is shown in table 1. In this section, only the general trends within each of the breathing protocols are discussed.

Breathing protocol M1 resulted in significant differences between panelists for all compounds except for isoamylacetate. For the other compounds, the highest decay rate was found for P3 and the lowest for P1 and P2, which showed higher persistence. For protocol M2, no significant differences were found for guaiacol, 4-methylguaiacol, phenylethylalcohol and ethylbutanoate. In the case of ethylbutanoate, one of the replicates of P5 was most probably an outlier as it yielded an extremely high decay rate, which affected the ANOVA and resulted in no differences between the five panelists. After removal of the outlier, a significantly higher decay rate was observed for panelist 4 with no significant differences between the other four panelists (table 1). Regarding the rest of the compounds, P1 and P2 had generally higher persistence. Protocol N1 also showed no differences for guaiacol, 4-methylguaiacol and phenylethylalcohol. P1 again showed lower values of decay for the other compounds, but this time P2 had the fastest decay. The last protocol revealed no differences for ethyloctanoate, 2-heptanone and isoamylacetate. P2 and P3 had the slowest decay for guaiacol, 4-methylguaiacol, 2,5-dimethylpyrazine and isoamylacetate.

It is interesting to note that for M2 and N1, the two protocols that involved breathing through the nose after volatile inhalation, no differences were found between panelists for those compounds with lower values of K_{ow} and K_{aw} (with the exception of 2,5-dimethylpyrazine). In the case of N2, where breathing was done by the mouth, the picture is the opposite, with no differences found for compounds with high values of K_{ow} and K_{aw} (with the exception of ethyloctanoate).

3.3. Differences between protocols

Differences both in measured intensity and decay rate were found between the compounds. Figure 4 shows the decay curves of all the compounds and protocols for one of the panelists (P1). Regarding intensity, M1 presents the highest intensity for all the compounds independently of the panelist, but the rest of the protocols did not show statistically significant differences. This trend can be observed by comparing the intensity at time 1 s, calculated from the fitted data (table 1—parameter a). By comparing the intensity of the volatiles measured in the second breath, interesting trends could be observed. Compounds with low values of K_{ow} and K_{aw} (2,5-dimetylpyrazine, guaiacol, 4-methylguaiacol and phenylethylalcohol) presented intensities for M2 in the range of 5%–17% of those obtained for the M1 protocol. For the rest of the compounds (heptanone, ethylbutanoate, ethyloctanoate and isoamylacetate), the intensities for the M2 protocol were between 10%–70% of M1. Similarly, for protocols N1 and N2, the highly water-soluble group of compounds showed in N2 intensities that were lower than or in the same range as those of N1 (20%–100% of the N1 intensity) while the group containing fewer polar and highly volatile compounds generally presented higher intensities for N2 than for N1 (80%–410% of the N1 intensity).

Differences in persistence between the breathing protocols are less pronounced. Decay rates are similar for all the protocols with the only exception of N1 that shows higher decay for some compounds (guaiacol, methylguaiacol and phenylethylalcohol), indicating lower persistence on this breathing protocol. Table 1 shows the decay rate for all breathing protocols. Again, we can cluster the compounds in two main groups with similar behavior: compounds with high K_{ow} and K_{aw} and low vapor pressure presented differences in fewer than two panelists and compounds with low K_{ow} and K_{aw} and high vapor pressure showed differences between protocols for at least three panelists. In order to get a better understanding of the effect of the breathing protocol on persistence, we considered all the panelists together for each of the protocols. No significant differences among the breathing protocols were observed for the decay rates of ethyloctanoate, ethylbutanoate and 2-heptanone. In contrast, isoamylacetate and methylguaiacol presented significant differences, with the protocols involving mouth breathing (M1 and N2) being the ones with lower decay rates. For phenylethylalcohol and guaiacol, the only significant difference was found for the N1 protocol in which the decay rate was higher. 2,5-Dimethylpyrazine had a significantly higher decay rate on the M1 and N1 protocols.

3.4. Dose–response

To assess the influence of volatile concentration on aroma persistence, different volumes of aromatized air were sampled with the gas-tight syringe and diluted with clean air prior to administration to two panelists.

Table 1. Decay rate (b) and initial intensity (a) for all compounds, panelists and breathing protocols. Data are presented as the average of the three replicates with the standard deviation.

		2,5-Dimethylpyrazine		Ethylbutanoate		Ethyloctanoate		Guaiacol		2-Heptanone		Isoamylacetate		4-Methylguaiacol		Phenylethylalcohol	
		a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
P1	M1	3980 ± 385	0.79 ± 0.04	7150 ± 821	1.90 ± 0.13	1880 ± 113	1.3 ± 0.02	2590 ± 268	0.77 ± 0.02	49 400 ± 2200	1.62 ± 0.03	124 000 ± 11 700	2.50 ± 0.06	942 ± 137	0.80 ± 0.03	270 ± 30.3	0.66 ± 0.05
		b/mn	*a/n*	*b/m*	*a/n*	*c/m*	*a/n*	*b/o*	*b/mn*	*b/m*	*a/n*	*b/m*	*a/m*	*b/n*	*b/mn*	*b/mo*	*b/mn*
	M2	330 ± 40	0.68 ± 0.05	664 ± 25.0	1.38 ± 0.09	842 ± 30.9	1.35 ± 0.03	267 ± 36.2	0.97 ± 0.05	27 500 ± 1530	1.77 ± 0.10	63 100 ± 15 500	3.06 ± 0.25	116 ± 3.97	1.2 ± 0.00	22.61 ± 1.17	0.78 ± 0.04
		a/mn	*a/n*	*a/m*	*a/n*	*ab/m*	*a/o*	*a/o*	*a/m*	*ab/o*	*a/n*	*ab/m*	*a/mn*	*a/n*	*a/m*	*a/m*	*ab/m*
	N1	1040 ± 650	0.85 ± 0.18	148 ± 55.6	1.52 ± 0.18	626 ± 94.4	1.21 ± 0.07	885 ± 577	1.10 ± 0.14	16 800 ± 2930	1.64 ± 0.08	35 400 ± 13 400	3.17 ± 0.22	286 ± 164	1.19 ± 0.07	96.2 ± 94.9	1.30 ± 0.52
		a/m	*a/n*	*a/m*	*a/n*	*a/mn*	*a/n*	*a/mn*	*a/m*	*a/mn*	*a/n*	*a/no*	*a/n*	*a/mn*	*a/m*	*a/m*	*a/m*
	N2	424 ± 120	0.69 ± 0.10	924 ± 612	1.96 ± 0.49	1230 ± 464	1.34 ± 0.21	287 ± 76.2	0.98 ± 0.04	37 400 ± 18 900	1.82 ± 0.35	104 000 ± 55 500	3.18 ± 0.49	103 ± 37.3	1.10 ± 0.08	25.25 ± 8.18	0.63 ± 0.10
		a/mn	*a/n*	*a/m*	*a/mn*	*b/m*	*a/m*	*a/n*	*a/m*	*ab/mn*	*a/m*	*ab/mn*	*a/m*	*a/m*	*a/m*	*a/*	*b/*
P2	M1	2400 ± 474	0.76 ± 0.09	7460 ± 1960	2.28 ± 0.27	1470 ± 173	1.43 ± 0.08	1420 ± 189	0.68 ± 0.05	39 500 ± 5990	1.73 ± 0.07	97 600 ± 23 200	2.70 ± 0.17	562 ± 113	0.74 ± 0.08	153 ± 20.8	0.63 ± 0.05
		b/m	*a/n*	*b/m*	*a/mn*	*b/m*	*a/mn*	*c/mn*	*b/n*	*b/m*	*a/mn*	*b/m*	*b/m*	*b/m*	*b/n*	*b/m*	*a/n*
	M2	185 ± 48	0.70 ± 0.12	1840 ± 2300	1.814 ± 0.50	587 ± 319	1.44 ± 0.22	161 ± 56.8	0.96 ± 0.18	8600 ± 3390	1.64 ± 0.24	31 900 ± 21 500	3.17 ± 1.08	67.2 ± 25.3	1.20 ± 0.22	15.70 ± 2.63	0.76 ± 0.11
		a/m	*a/mn*	*a/m*	*a/n*	*a/m*	*a/no*	*a/mn*	*ab/m*	*a/m*	*ab/n*	*a/m*	*ab/mn*	*a/m*	*ab/m*	*a/m*	*a/m*
	N1	599 ± 230	0.97 ± 0.05	644 ± 322	2.76 ± 0.66	1030 ± 401	1.77 ± 0.27	495 ± 191	1.28 ± 0.31	19 700 ± 6340	2.24 ± 0.37	64 800 ± 15 000	4.23 ± 0.34	223 ± 102	1.58 ± 0.31	—	—
		a/m	*a/mn*	*a/n*	*a/m*	*ab/n*	*a/m*	*b/m*	*a/m*	*a/n*	*a/m*	*ab/o*	*a/m*	*a/mn*	*a/m*		
	N2	276 ± 118	0.51 ± 0.16	791 ± 484	1.82 ± 0.23	720 ± 401	1.31 ± 0.22	163 ± 58.1	0.62 ± 0.14	20 700 ± 12 900	1.55 ± 0.16	34 100 ± 27 800	2.41 ± 0.42	64.7 ± 21.7	0.90 ± 0.17	—	—
		a/m	*b/n*	*a/m*	*a/mn*	*ab/m*	*a/m*	*a/mn*	*b/n*	*a/m*	*b/m*	*a/mn*	*b/m*	*a/m*	*b/mn*		
P3	M1	9270 ± 2050	1.06 ± 0.03	20 100 ± 6700	2.78 ± 0.28	3670 ± 762	1.81 ± 0.12	2040 ± 349	0.93 ± 0.03	96 600 ± 15 200	2.12 ± 0.13	217 000 ± 62 600	2.90 ± 0.12	715 ± 106	0.98 ± 0.03	341 ± 42.1	0.92 ± 0.02
		b/p	*a/m*	*b/n*	*a/m*	*b/n*	*a/m*	*b/no*	*ab/m*	*b/n*	*a/m*	*b/n*	*a/m*	*b/mn*	*a/m*	*b/no*	*a/m*
	M2	538 ± 98	0.74 ± 0.08	2060 ± 231	2.11 ± 0.09	862 ± 153	1.74 ± 0.04	154 ± 16.5	0.90 ± 0.03	23 000 ± 8570	2.24 ± 0.17	25 000 ± 5520	2.80 ± 0.01	54.25 ± 7.50	0.86 ± 0.13	21.33 ± 2.44	0.66 ± 0.04
		a/op	*b/mn*	*a/m*	*a/mn*	*a/m*	*a/mn*	*a/mn*	*ab/m*	*a/no*	*a/m*	*a/m*	*a/n*	*a/m*	*a/m*	*a/m*	*b/m*
	N1	7200 ± 3130	1.14 ± 0.19	502 ± 204	2.27 ± 0.50	434 ± 185	1.36 ± 0.08	1680 ± 866	1.08 ± 0.22	22 900 ± 9950	1.95 ± 0.34	36 200 ± 20 400	2.80 ± 0.52	565 ± 283	1.13 ± 0.20	292 ± 125	1.49 ± 0.24
		b/n	*a/m*	*a/mn*	*a/mn*	*a/m*	*b/mn*	*b/n*	*a/m*	*a/n*	*a/mn*	*a/no*	*a/n*	*b/n*	*a/m*	*b/n*	*a/m*
	N2	541 ± 9.69	0.59 ± 0.04	4370 ± 2780	2.73 ± 0.77	1200 ± 313	1.52 ± 0.18	148 ± 13.3	0.71 ± 0.08	61 600 ± 29 200	2.06 ± 0.34	124 000 ± 78 100	3.06 ± 0.75	52.64 ± 0.90	0.77 ± 0.04	—	—
		a/n	*b/n*	*a/n*	*a/m*	*a/m*	*ab/m*	*a/m*	*b/n*	*ab/n*	*a/m*	*ab/n*	*a/m*	*a/m*	*a/n*		

(*Continued*)

Table 1. (*Continued*)

		2,5-Dimethylpyrazine		Ethylbutanoate		Ethyloctanoate		Guaiacol		2-Heptanone		Isoamylacetate		4-Methylguaiacol		Phenylethylalcohol	
		a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
P4	M1	5160 ± 1020	1.04 ± 0.13	6950 ± 4401	2.44 ± 0.62	2350 ± 1060	1.64 ± 0.32	1300 ± 326	0.96 ± 0.17	48 800 ± 22 400	1.99 ± 0.34	86 300 ± 47 200	2.76 ± 0.48	535 ± 166	1.01 ± 0.21	232 ± 79.1	0.90 ± 0.20
		b/no	*a/n*	*b/m*	*a/mn*	*b/mn*	*a/mn*	*b/m*	*a/m*	*b/m*	*a/mn*	*a/m*	*a/m*	*b/m*	*b/m*	*b/mn*	*a/m*
	M2	642 ± 96.8	0.86 ± 0.10	2100 ± 397	2.62 ± 0.38	1350 ± 221	1.79 ± 0.11	197 ± 26.9	1.04 ± 0.12	30 500 ± 3220	1.96 ± 0.09	75 800 ± 32 600	3.47 ± 0.35	102 ± 26	1.19 ± 0.15	26.8 ± 5.72	0.68 ± 0.08
		a/p	*a/m*	*ab/m*	*a/n*	*ab/n*	*a/m*	*a/no*	*a/m*	*ab/o*	*a/mn*	*a/m*	*a/mn*	*a/mn*	*ab/m*	*a/m*	*a/m*
	N1	547 ± 9.64	0.92 ± 0.02	221 ± 38.3	1.81 ± 0.11	688 ± 56.0	1.47 ± 0.02	178 ± 18.7	1.12 ± 0.3	16 100 ± 4910	1.87 ± 0.01	30 600 ± 10 600	3.28 ± 0.15	85.9 ± 11.6	1.25 ± 0.06	—	—
		a/m	*a/mn*	*a/m*	*a/mn*	*a/mn*	*a/mn*	*a/m*	*a/m*	*a/mn*	*a/mn*	*a/mn*	*a/n*	*a/m*	*a/m*		
	N2	622 ± 95.2	0.99 ± 0.02	184 ± 98.9	1.84 ± 0.29	724 ± 181	1.41 ± 0.12	153 ± 46.6	1.04 ± 0.06	17 200 ± 7340	2.04 ± 0.26	27 500 ± 9390	3.20 ± 0.38	57.4 ± 19.1	1.01 ± 0.12	—	—
		a/no	*a/m*	*a/m*	*a/mn*	*a/m*	*a/m*	*a/m*	*a/m*	*a/m*	*a/m*	*a/mn*	*a/m*	*a/m*	*b/mn*		
P5	M1	7470 ± 1420	0.95 ± 0.12	11 100 ± 4790	2.47 ± 0.35	2180 ± 672	1.48 ± 0.17	1570 ± 292	0.92 ± 0.07	34 400 ± 10 400	1.95 ± 0.17	60 400 ± 28 000	2.48 ± 0.48	716 ± 142	0.97 ± 0.07	414 ± 87.9	0.93 ± 0.08
		b/op	*a/mn*	*b/mn*	*a/mn*	*b/m*	*a/mn*	*b/mn*	*a/m*	*b/m*	*a/mn*	*a/m*	*b/m*	*b/mn*	*a/mn*	*b/o*	*a/m*
	M2	421 ± 77.4	0.85 ± 0.06	216 ± 108	1.99 ± 0.40	398 ± 0	1.34 ± 0	84.4 ± 39.0	0.96 ± 0.17	12 100 ± 5310	2.21 ± 0.19	79 400 ± 7600	4.35 ± 0.76	—	—	14.3 ± 1.32	0.57 ± 0.05
		a/no	*ab/m*	*a/m*	*ab/mn*	*a/m*	*a/o*	*a/m*	*a/m*	*a/mn*	*a/m*	*a/m*	*a/m*			*a/m*	*ab/m*
	N1	1670 ± 340	1.04 ± 0.10	53.6 ± 26.9	1.32 ± 0.40	406 ± 60.0	1.26 ± 0.15	452 ± 177	1.16 ± 0.22	4980 ± 557	1.81 ± 0.29	4280 ± 1710	3.00 ± 0.58	194 ± 75.2	1.18 ± 0.30	70.2 ± 11.7	1.06 ± 0.20
		a/m	*a/mn*	*a/m*	*b/n*	*a/m*	*a/n*	*a/m*	*a/m*	*a/m*	*a/mn*	*a/m*	*ab/n*	*a/m*	*a/m*	*a/m*	*a/m*
	N2	768 ± 63.2	0.68 ± 0.8	94.2 ± 35.2	1.46 ± 0.26	673 ± 97.2	1.38 ± 0.14	169 ± 35.3	0.95 ± 0.10	10 000 ± 1650	1.84 ± 0.16	8180 ± 6370	2.50 ± 0.86	—	—	—	—
		a/o	*b/n*	*a/m*	*b/n*	*a/m*	*a/m*	*a/m*	*a/m*	*a/m*	*a/m*	*a/m*	*b/m*				

Note. Letters indicate significant differences among the breathing protocols (a–c) and panelists (m–p) (Tukey's test, $p < 0.01$).

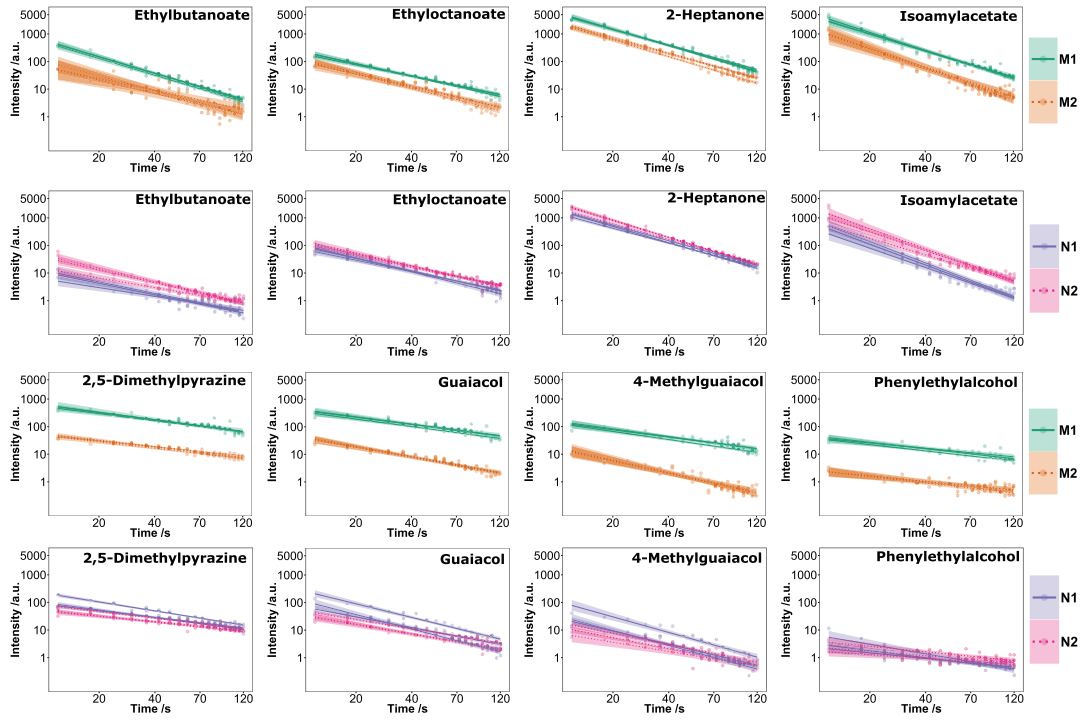


Figure 4. Decay curves for panelist P1. Lines represent the fit of three replicates and color ribbons the 95% confidence level of the fit.

Table 2 shows the differences in power fit parameters for all compounds and breathing protocols. Figure 5 shows the decay curves for 2,5-dimethylpyrazine for the four protocols and two panelists. Differences in measured intensity were observed for the different concentrations administered, but the decay rate seemed to be affected only for the lowest concentrations considered. This is discussed further in the next section.

The curve fitting parameters for all compounds and protocols are shown in table 2. The parameter *a*, which was the calculated intensity at the beginning, increased with increasing concentration (volume of aromatized air), although differences were not always statistically significant. Parameter *a* represented the estimated concentration at time 1 s using the power fit and therefore was expected to be proportional to the concentration administered. The lack of significance observed may have resulted from inter-individual differences between the two panelists, as the high values for the standard deviation seemed to indicate.

This effect was particularly high for protocol M2, resulting in no significant differences for any of the compounds. To eliminate inter-individual differences and to check the effect of administering different concentrations of aroma compounds in the exhaled breath, we performed a linear regression on the measured intensity in the second exhalation for each of the compounds, protocols and panelists. Values for the regression coefficient (r^2) are shown in table 3. Although the data did not always perfectly fit a linear regression, a correlation between the concentration delivered to the panelist and the intensity of the compounds in the exhaled breath was clear.

The effect of concentration on persistence was less pronounced. Only the decay rate of one compound (2,5-dimethylpyrazine) was significantly affected by the concentration independently of the breathing protocol (table 2). Higher concentrations resulted in faster decay rates for this compound with the decay rate at the highest concentration doubling that at the lowest one. Other compounds presented similar behavior, but only in certain protocols with significant differences in persistence between low and high concentrations for ethylbutanoate (protocol M1), ethyloctanoate (protocols M1 and N2) and 2-heptanone (protocols M1 and N1).

4. Discussion

4.1. Aroma persistence mainly depends on the physicochemical properties of the volatile compounds when delivered in the gas phase

The impact of compound physicochemical properties on the decay rate, and therefore on the persistence of the compounds in breath, when in the gas phase, was proven to be higher than the differences between different panelists or breathing protocols. A trend was observed between compound persistence and hydrophilicity (K_{aw}, K_{ow}) and volatility (vapor pressure). The less hydrophilic but highly volatile compounds (i.e. isoamylacetate) showed a faster decay than the highly water-soluble and less volatile ones (i.e. 2,5-dimethylpyrazine). This relationship is to be expected as it has already been reported in

Table 2. Decay rate (b) and initial intensity (a) for all compounds and concentrations administered to the panelists. Data are presented as the average of the two replicates for two panelists with the standard deviation.

	V	2,5-Dimethylpyrazine		Ethylbutanoate		Ethyloctanoate		Guaiacol		2-Heptanone		Isoamylacetate		4-Methylguaiacol		Phenylethylalcohol	
	(ml^{-1})	a	−b	a	−b	a	−b	a	−b	a	−b	a	−b	a	−b	a	−b
M1	50	81 ± 38	0.62 ± 0.14	323 ± 151	2.98 ± 0.81	232 ± 111	1.67 ± 0.44	56 ± 27	0.75 ± 0.07	883 ± 165	1.67 ± 0.10	3920 ± 647	3.89 ± 0.36	41 ± 18	0.83 ± 0.09	23 ± 12	0.71 ± 0.2
		a	b	a	b	a	c	a	a	a	b	a	a	a	a	a	a
	100	133 ± 54	0.69 ± 0.13	1540 ± 766	4.38 ± 0.50	343 ± 154	2.07 ± 0.25	55 ± 23	0.76 ± 0.10	2220 ± 696	2.03 ± 0.13	11 900 ± 4620	4.20 ± 0.19	43 ± 14	0.89 ± 0.07	20 ± 8.3	0.70 ± 0.14
		a	ab	a	ab	a	bc	a	a	a	ab	a	a	a	a	a	a
	200	287 ± 88	0.89 ± 0.13	3380 ± 3610	4.38 ± 1.22	509 ± 181	2.35 ± 0.58	83 ± 18	0.87 ± 0.10	4690 ± 1400	2.26 ± 0.37	21 400 ± 11 400	4.12 ± 0.67	47 ± 14	0.94 ± 0.12	20 ± 3.8	0.76 ± 0.06
		ab	ab	ab	ab	a	ac	a	a	ab	ab	ab	a	a	a	a	a
	300	453 ± 103	0.90 ± 0.11	29 800 ± 502	4.35 ± 0.55	667 ± 103	2.34 ± 0.12	120 ± 22	0.8. ± 0.05	1950 ± 303	2.16 ± 0.16	28 900 ± 3920	3.73 ± 0.20	68 ± 13	0.92 ± 0.06	31 ± 8.1	0.82 ± 0.09
		ab	ab	a	ab	a	ac	a	a	ab	ab	ab	a	a	a	a	a
	400	773 ± 185	1.02 ± 0.19	13 800 ± 4660	5.78 ± 0.37	1900 ± 390	3.33 ± 0.42	211 ± 48	0.90 ± 0.13	14 300 ± 2290	2.58 ± 0.41	61 400 ± 16 300	4.00 ± 0.36	127 ± 27	1.07 ± 0.12	54 ± 19	0.94 ± 0.21
		b	ab	ab	a	ab	ab	ab	a	bc	ab	bc	a	ab	a	ab	a
	500	1304 ± 451	1.22 ± 0.46	18 600 ± 14 900	5.48 ± 1.29	3030 ± 1980	3.45 ± 1.02	338 ± 159	1.06 ± 0.44	21 200 ± 11 400	2.77 ± 0.93	79 600 ± 44 000	4.10 ± 1.08	195 ± 79	1.17 ± 0.44	86 ± 46	1.16 ± 0.61
		c	a	b	a	b	a	b	a	c	a	c	a	b	a	b	a
M2	50	10.8 ± 4.7	0.45 ± 0.09	70 ± 30	2.90 ± 0.78	63 ± 28	1.35 ± 0.24	8.3 ± 5.8	0.86 ± 0.20	403 ± 215	1.77 ± 0.25	1310 ± 530	4.19 ± 0.63	6.5 ± 3.3	1.03 ± 0.19	5.10 ± 0	0.81 ± 0
		a	b	a	a	a	a	a	a	a	a	a	a	a	a	a	a
	100	17 ± 9	0.61 ± 0.09	171 ± 190	3.12 ± 1.26	73 ± 23	1.41 ± 0.22	8.7 ± 5.1	0.93 ± 0.17	922 ± 513	1.87 ± 0.19	3420 ± 2030	4.18 ± 0.26	2.9 ± 0.7	0.73 ± 0.04	2.9 ± 0.5	0.69 ± 0.05
		a	ab	a	a	a	a	a	a	a	a	a	a	a	a	a	a
	200	54 ± 43	0.76 ± 0.10	133 ± 60	2.87 ± 0.44	121 ± 42	1.45 ± 0.13	29 ± 31	0.99 ± 0.18	2160 ± 465	2.18 ± 0.46	8950 ± 2470	4.46 ± 0.97	14 ± 12	1.06 ± 0.24	9.5 ± 3.7	1.17 ± 0.16
		a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
	300	58 ± 30	0.71 ± 0.08	2660 ± 2950	4.70 ± 1.32	108 ± 85	1.65 ± 0.22	24 ± 14	0.99 ± 0.11	4650 ± 2680	2.29 ± 0.15	20 900 ± 14 000	4.21 ± 0.32	16 ± 8.6	1.18 ± 0.15	10.7 ± 0.03	1.31 ± 0.04
		a	ab	a	a	a	a	a	a	a	a	a	a	a	a	a	a
	400	75 ± 24	0.77 ± 0.05	2790 ± 2910	4.34 ± 1.72	197 ± 81	1.53 ± 0.09	33 ± 14	1.14 ± 0.19	8250 ± 2540	2.87 ± 0.79	37 700 ± 16 100	4.70 ± 1.03	16 ± 6.4	1.19 ± 0.07	11.3 ± 2.5	1.51 ± 0.24
		a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
	500	94 ± 59	0.69 ± 0.20	2560 ± 3820	3.90 ± 2.23	496 ± 620	1.91 ± 0.96	38 ± 29	0.89 ± 0.23	8480 ± 8730	1.89 ± 0.70	32 500 ± 37 000	3.51 ± 0.74	23 ± 13	1.03 ± 0.20	12.8 ± 7.3	1.09 ± 0.33
		a	ab	a	a	a	a	a	a	a	a	a	a	a	a	a	a

(*Continued*)

Table 2. (*Continued*)

	V	2,5-Dimethylpyrazine		Ethylbutanoate		Ethyloctanoate		Guaiacol		2-Heptanone		Isoamylacetate		4-Methylguaiacol		Phenylethylalcohol	
	(ml^{-1})	a	−b	a	−b	a	−b	a	−b	a	−b	a	−b	a	−b	a	−b
N1	50	32 ± 7.6	0.62 ± 0.05	28 ± 6.1	3.62 ± 0.66	43 ± 24	1.50 ± 0.12	25 ± 12	1.08 ± 0.10	372 ± 75	1.65 ± 0.16	886 ± 219	3.97 ± 0.50	19 ± 8.4	1.24 ± 0.03	11.70 ± 0	1.44 ± 0
		a	c	a	a	a	a	a	a	a	b	a	a	a	a	ab	a
	100	53 ± 8.0	0.74 ± 0.02	52 ± 26	2.87 ± 0.54	41 ± 35	1.27 ± 0.40	36 ± 27	1.14 ± 0.28	733 ± 78	1.67 ± 0.38	2160 ± 834	3.81 ± 1.08	28 ± 13	1.38 ± 0.19	6.44 ± 2.05	0.95 ± 0.18
		ab	bc	a	a	a	a	a	a	a	b	a	a	a	a	a	a
	200	155 ± 21	1.03 ± 0.13	121 ± 70	3.24 ± 0.96	51 ± 37	1.39 ± 0.13	80 ± 20	1.32 ± 0.13	2170 ± 257	2.10 ± 0.28	7680 ± 1410	4.29 ± 0.74	42 ± 12	1.42 ± 0.29	18 ± 5.6	1.43 ± 0.33
		ab	ac	a	a	a	a	a	a	ab	ab	ab	a	a	a	a	a
	300	303 ± 104	1.07 ± 0.16	372 ± 309	3.57 ± 1.45	73 ± 42	1.66 ± 0.26	105 ± 29	1.14 ± 0.13	4930 ± 1830	2.40 ± 0.21	18 400 ± 6540	4.44 ± 0.15	58 ± 17	1.31 ± 0.26	27 ± 9.3	1.65 ± 0.36
		ab	ab	a	a	a	a	a	a	bc	a	bc	a	ab	a	ab	a
	400	402 ± 216	1.11 ± 0.22	1170 ± 1250	4.63 ± 0.59	167 ± 172	1.66 ± 0.22	139 ± 79	1.18 ± 0.19	6940 ± 1760	2.23 ± 0.21	24 900 ± 8940	3.85 ± 0.43	75 ± 47	1.32 ± 0.14	29 ± 14	1.49 ± 0.31
		bc	ab	a	a	a	a	ab	a	c	ab	c	a	ab	a	ab	a
	500	718 ± 213	1.28 ± 0.23	346 ± 459	2.94 ± 1.37	196 ± 183	1.96 ± 0.61	226 ± 86	1.28 ± 0.30	7920 ± 2090	2.59 ± 0.33	28 800 ± 9070 c	4.26 ± 0.48	130 ± 49	1.50 ± 0.28	51 ± 19	1.79 ± 0.42
		c	a	a	a	a	a	b	a	c	a		a	b	a	b	a
N2	50	24 ± 11	0.53 ± 0.05	265 ± 191	4.58 ± 1.37	108 ± 81	1.49 ± 0.48	13 ± 12	0.75 ± 0.14	654 ± 167	1.80 ± 0.17	2280 ± 1100	4.07 ± 0.15	15 ± 7.9	0.97 ± 0.19	—	—
		a	b	a	a	ab	b	ab	a	a	a	a	a	a	a		
	100	29 ± 6.1	0.72 ± 0.05	108 ± 73	3.38 ± 0.68	92 ± 9.9	1.33 ± 0.08	7.5 ± 3.0	0.83 ± 0.12	1050 ± 392	1.67 ± 0.28	3490 ± 3850	3.67 ± 0.58	4.4 ± 1.9	0.84 ± 0.17	3.24 ± 0	0.65 ± 0
		a	ab	a	a	a	b	a	a	a	a	a	a	a	a	a	a
	200	41 ± 11	0.70 ± 0.11	299 ± 192	3.92 ± 0.82	154 ± 53	1.53 ± 0.21	11 ± 3.7	0.91 ± 0.18	2540 ± 664	1.89 ± 0.31	9540 ± 3750	3.82 ± 0.57	5.8 ± 1.4	1.02 ± 0.06	4.59 ± 0	0.86 ± 0
		a	ab	a	a	ab	b	ab	a	a	a	ab	a	a	a	a	a
	300	68 ± 22	0.8 ± 0.21	1050 ± 900	4.25 ± 1.50	207 ± 42	1.48 ± 0.15	18 ± 3.6	0.97 ± 0.16	5480 ± 2060	2.02 ± 0.48	22 800 ± 10 500	3.54 ± 0.81	9.6 ± 1.8	1.17 ± 0.12	5.83 ± 0.05	0.97 ± 0.09
		ab	ab	ab	a	ab	b	ab	a	ab	a	ab	a	a	a	a	a
	400	97 ± 16	0.82 ± 0.09	2200 ± 1150	5.40 ± 0.77	460 ± 111	2.12 ± 0.19	24 ± 7.1	0.84 ± 0.16	8060 ± 1610	2.28 ± 0.28	31 200 ± 7010	3.76 ± 0.37	11 ± 3.3	1.10 ± 0.13	6.59 ± 1.29	1.00 ± 0.08
		bc	ab	ab	a	b	ab	ab	a	b	a	bc	a	a	a	a	a
	500	136 ± 23	0.90 ± 0.12	4840 ± 3690	5.50 ± 1.59	847 ± 344	2.75 ± 0.80	30 ± 10	0.98 ± 0.14	12 900 ± 3870	2.47 ± 0.54	53 000 ± 21 500	3.81 ± 0.81	17 ± 6.8	1.16 ± 0.22	8.00 ± 1.12	1.00 ± 0.10
		c	a	b	a	c	a	b	a	c	a	c	a	a	a	a	a

Note. Letters indicate significant differences among the concentrations (Tukey's test, $p < 0.01$).

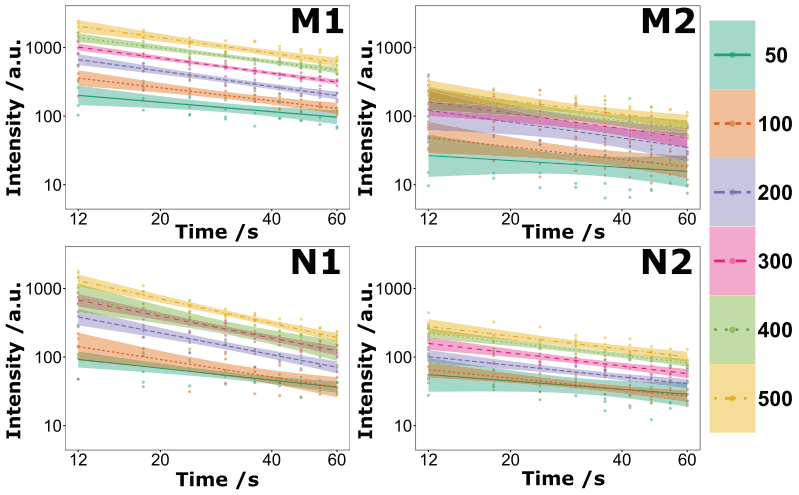


Figure 5. Decay curves for 2,5-dimethylpyrazine at six different concentration levels for the four breathing protocols studied. Lines represent the fit of four measurements (2 replicates × 2 panelists) and color ribbons the 95% confidence level of the fit.

Table 3. Linear fit regression coefficients. A linear fit was performed for each panelist and compound using the intensity of the compound exhaled on the second breath (in duplicate) as a function of the concentration administered.

Regression coefficient (r^2)		2,5-Dimethylpyrazine	Ethylbutanoate	Ethyloctanoate	Guaiacol	2-Heptanone	Isoamylacetate	4-Methylguaiacol	Phenylethylalcohol
Panelist 1									
	M1	0.9400	0.8222	0.9087	0.8616	0.9672	0.9148	0.7770	0.7467
	M2	0.6268	0.4582	0.8616	0.5884	0.9375	0.9293	0.7160	0.6557
	N1	0.9245	0.7682	0.9672	0.8440	0.8892	0.9455	0.3103	0.8738
	N2	0.9134	0.8982	0.9148	0.8254	0.9413	0.8611	0.7765	0.8417
Panelist 2									
	M1	0.9463	0.7726	0.9004	0.9126	0.9304	0.8715	0.8841	0.8738
	M2	0.9226	0.3965	0.9126	0.8510	0.9605	0.5451	0.8125	0.4518
	N1	0.7844	0.8187	0.9304	0.7356	0.9542	0.9747	0.6808	0.6057
	N2	0.6709	0.6669	0.8715	0.7249	0.7366	0.6658	0.6678	0.2053

aqueous solutions [9, 14], in theoretical models of volatile transport in the respiratory tract [15, 16] and in studies on the impact of inhaled compounds on their concentrations in exhaled breath [10]. In studies with solutions, persistence depends not only on the absorption and depletion of volatiles from the mucus, but also on the release of volatiles from beverage residues coating the throat [7]. In fact, Hodgson *et al* found that swallowing an aqueous solution of isoamylacetate (wet swallow) resulted in the persistence of the aroma compound in subsequent exhalations, whereas a swallow of the same compound in gas phase (dry swallow) did not show any evidence of persistence. The same results were obtained for diacetyl in a different study [8]. Therefore, they attributed most of the persistence effect to volatile release from a liquid film remaining within the oral tract and containing residues of the beverage product. In our case, isoamylacetate inhaled in gas phase did show evidence of persistence, although its decay rate was the fastest of all compounds analyzed. This discrepancy in results might come from the difference in volatile administration as in Hodgson's work the gas phase was swallowed: the volatiles passed from the pharynx to the esophagus and could not go back to the air-tract during breathing. In ours the gas phase was inhaled: the volatiles passed from the pharynx to the larynx and trachea, where they could be absorbed on the nasal mucosa and were released again during respiration. Furthermore, swallowing will remove an important fraction of saliva from the oral cavity, together with dissolved volatile compounds.

The importance of the coating layer in volatile persistence, and therefore for after-smell sensations, is also indicated by the differences in persistence between our work with aromatized air and those with aqueous solutions found in the literature. Linforth and Taylor measured the volatile persistence of dimethylpyrazine, guaiacol, isoamylacetate and ethylbutanoate expressed as the ratio between the first and second exhalations after swallowing an aqueous solution [3]. Comparing Linforth's ratios with those obtained using the closest breathing protocol to real consumption (M2—where the volatiles were injected into the mouth and then breathed in and out through the nose), we observed that persistence was 15%–35% higher on aqueous solutions for all compounds except ethylbutanoate. For this compound, persistence values were comparable to those of Linforth and Taylor [3] and Wright *et al* [17], but

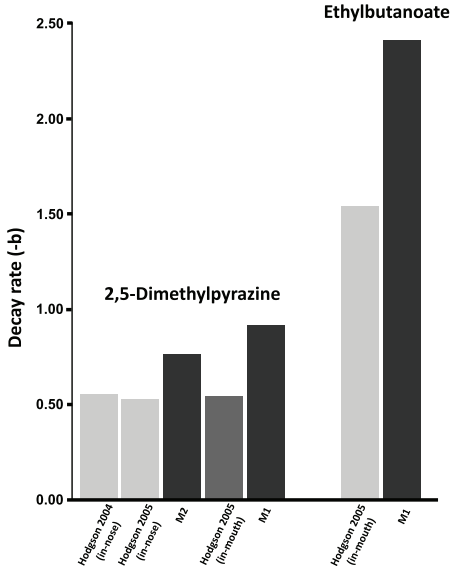


Figure 6. Comparison between the measured decay rates for 2,5-dimethylpyrazine and ethylbutanoate in gas phase and those reported in literature for aqueous solutions.

much lower than the persistence reported by Buffo also using an aqueous solution [4]. Similar observations can be made by comparing the decay rates measured in this work with those reported by Hodgson *et al* [7, 9] as depicted in figure 6. They measured decay rates for 2,5-dimethylpyrazine (0.53 in-nose and 0.55 in-mouth) and ethylbutanoate (1.60 in-mouth), while the decay rates obtained in our study after inhaling aromatized air by the mouth were 0.77 (M2, in-nose) and 0.98 (M1, in-mouth) for 2,5-dimethylpyrazine and 2.37 (M1, in-mouth) for ethylbutanoate. The higher persistence (lower decay rate) of the compounds in aqueous solutions could again be attributed to residues of the aqueous solution that remained in the oral tract and acted as aroma reservoirs.

4.2. Volatile persistence depends on the breathing protocol

The effect of the breathing protocol on both intensity and persistence was compound-dependent. The highest intensity for all compounds was measured during the M1 breathing protocol. The M1 and M2 protocols shared the way the aromatized air was administered to the panelist: the volatiles were injected into the mouth and inhaled. Inhaled volatiles could be absorbed at any point of the airway from the oral cavity to the alveoli, where they could be drawn into the blood stream. Before the first exhalation, the dead space of the airway was filled with a fraction of the aromatized air supplied. From this point onward, both protocols differed. On the M1 protocol, air was exhaled through the mouth. On the first exhalation, the volume of air in the dead space was flushed out by air from the lungs and expelled through the mouth. After the first exhalation, air was inhaled through the nose and exhaled through the mouth in the whole duration of the experiment. During inhalation, only compounds absorbed on the respiratory tract, from the pharynx to the lungs, could be released into the air flow and incorporated into the blood flow to some extent. During exhalation, it cannot be excluded that a fraction of the compounds formerly transferred to the blood was transferred back to the exhaled breath flow, together with a portion of the compounds absorbed in the whole respiratory tract, including the mouth cavity. Therefore, the fraction absorbed in the mouth cavity would only be depleted during exhalations and not during inhalations. On breathing protocol M2, the first exhalation was done through the nose. Aromatized air in the dead space was also flushed out by the air coming from the lungs, but in this case, a fraction of the volatiles were expected to be retained by the nasal mucosa which was free of the volatile mix before the first exhalation. Subsequent breathings were performed only through the nose. Nose breathing necessitates closing of the velum with the back of the tongue, to prevent volatiles in the mouth cavity from passing to the airflow (Buettner *et al* [18]). Therefore, during exhalation, volatiles measured in the breath corresponded to the fraction released from the respiratory tract, including the nasal cavity, but not the mouth cavity. That would result in M1 being higher than M2 as it included volatiles from the mouth cavity and did not illustrate loss through the nasal mucosa. In fact, M1 resulted in the highest intensity of all the breathing protocols tested, but the ratio M2/M1 depended on the compound. The lower M2/M1 ratio observed for the higher-polarity compounds indicates that those compounds were highly retained in the oral cavity and released in high concentrations to the exhaled breath in the M1 protocol, and that a big fraction were retained during nose exhalation in M2, due to absorption into the nasal mucosa. The less polar compounds presented lower M2/M1 ratios, indicating less retention of these compounds in the mucosa. Van Ruth and co-workers measured the concentration at the nostrils and compared it with the concentration at the nasopharynx for diacetyl, ethylbutanoate and ethylhexanoate. In all cases, only 60%–70% of the concentration in the nasopharynx reached the nostrils [19], and this retention by nasal mucosa is expected to be higher for more polar and less volatile compounds [20].

A similar situation was expected with breathing protocols N1 and N2, with N1 including volatiles retained in the nose and respiratory tract and N2 containing only volatiles absorbed in the respiratory tract during inhalation or in the mouth cavity during the first exhalation. Interestingly, the intensity on the N1 protocol was higher than that of N2 for the polar compounds, but the N2 breathing protocol resulted in higher concentrations than N1 for the less polar ones. This finding implies that the retention of less polar and highly volatile compounds in the nose is lower than that in the mouth cavity. Higher retention in the mouth might be explained by the presence of saliva. Saliva composition can affect the partition coefficient of the volatiles, with the salting out of hydrophilic compounds and the

non-covalent binding of hydrophobic ones by proteins [21, 22].

Differences in decay rate were much lower. Despite their different intensities among the protocols, three compounds presented no significant differences in decay rate (ethylbutanoate, ethyloctanoate and 2-heptanone). This result implies that although the concentration retained in the mouth mucosa was higher than that in the nose, the mechanisms of absorption and release of the compounds must be similar, therefore not affecting the decay rate. For the rest of the compounds, N1 always exhibited the fastest decay. The lower persistence might be an effect of lower absorption in nasal mucosa or loss of volatiles by incorporation into the blood that will preferably affect the most polar compounds [20, 23]. Some compounds presented decay rates that were not significantly different from those of N1 in other breathing protocols: isoamylacetate and methylfuran on the M2 protocol, the other protocol that involved nose exhalation; and 2,5-dimethylpyrazine for M1. As we have presented in section 3.4 and will discuss in the next section, 2,5-dimethylpyrazine was the compound that exhibited higher differences in persistence with concentration. In both the M1 and N1 protocols, the 2,5-dimethylpyrazine concentration was significantly higher than in the other two protocols and that might be the reason for the higher decay rate obtained for this compound.

4.3. Concentration of volatiles has low impact on persistence

Administration of higher concentration of volatiles resulted in higher intensities in exhaled breath for all compounds and protocols, but only 2,5-dimethylpyrazine showed significant differences in decay rate, independently of the breathing protocol used. A lower decay rate for menthone with low concentrations has been reported after swallowing of aqueous solutions [7], but no differences have also been reported for other compounds [17]. In this study, this change of behavior with low concentrations has also been observed for ethylbutanoate (protocol M1), ethyloctanoate (protocols M1 and N2) and 2-heptanone (protocols M1 and N1), the most volatile compounds—and only for the lowest concentration applied.

5. Conclusions

In this study we have analyzed for the first time the aroma persistence of volatile aroma compounds administered in the gas phase. With the approach taken here, we eliminated the interactions of the volatile compounds with the food matrix and the prolonged release from food residues that remain in the oral tract after swallowing. Therefore, persistence was only dependent on the interaction between the volatile compounds and mucosa. Three main learnings can be drawn from this systematic study: (i) The persistence of aroma compounds in the oral and nasal cavities is mainly dependent on the physicochemical properties of the volatile compounds. The compounds with high volatility and lower water solubility were the least persistent in the breath. (ii) The impact of a compound's physicochemical properties on its persistence in breath was higher than the differences between different panelists and breathing protocols. Still, the persistence of volatiles was shown to vary among breathing protocols. By using different breathing protocols, differences in aroma retention and subsequent release between the nasal and oral cavities were highlighted, with higher concentrations found in the oral cavity for all the compounds and longer persistence in the mouth for the most water-soluble compounds. (iii) The concentration of the aroma compound slightly affected persistence, but only for the most volatile compounds and in the lowest concentration range administered to the panelist. Otherwise, persistence showed barely any dependence on concentration.

This study has obvious implications to aroma persistence in food applications. But it is equally relevant in the health and medical context. Indeed, VOCs will be equally affected, irrespective of whether they originate from foods in the mouth or are transported by air coming from the lungs and the alveoli. The retention of compounds in the airway has several implications. The most obvious is that the amount of volatiles measured in exhaled air would be lower than that in the lungs, as some compounds will be retained by the mucosa. Also, the release of those volatiles back into the breath flow means that they will be detected over time even in the absence of metabolic reactions—production or degradation of the compound. Therefore, the persistence of compounds in breath due to interactions with the mucosa needs to be taken into account when studying dynamic processes by breath analysis (i.e. metabolic rate, pharmacokinetics). A better understanding of the link between the compound properties of VOCs and their persistence in the exhaled air will therefore assist as well in the application and interpretation of health-related and medically-related breath analysis.

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