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Volatile Release from Liquids: A Comparison of *In Vivo* APCI-MS, In-mouth Headspace Trapping and *In vitro* Mouth Model Data

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Abstract

In-mouth volatile release from flavoured water was followed using atmospheric pressure chemical ionization–mass spectrometry (APCI-MS) or using a hand-held, computer-controlled device based on sequential trapping of flavours on Tenax traps. The present results verify recent *in vitro* data obtained with a sophisticated, fully computerized mouth model apparatus and confirm its validity for the simulation of in-mouth dynamic volatile release. In-nose APCI-MS measurements showed considerable person-to-person variability in non-trained individuals during drinking due to subconscious control of muscles during swallowing and subsequent breathing. Data showed a 'swallow breath' volume reaching the nasal cavity from the throat, not from the mouth cavity. Flavour enriched air from the mouth was shown to be transported to the nose (via exhalation) immediately after the swallowing event, but the dynamic process of volatile equilibration between residuals of the swallowed liquid and the exhaled air predominantly determined volatile in-nose concentration. Owing to its dynamic character, the process of volatile equilibration and release in the throat upon exhalation should be similar to the in-mouth process studied in the present work. A full mechanical simulation of retronasal volatile transport, however, will remain difficult.

Key words: APCI-MS, dynamic flavour release, in-mouth, model mouth, perception, retronasal, volatile

Introduction

Perception during the consumption of food is governed by cognitive effects between tastants sensed at taste buds on the tongue and volatiles perceived at the regio olfactoria in the nose (Taylor, 2002). Odorant receptors are activated upon the binding of an odour molecule, resulting in a cascade of signal transduction in olfactory neurons (Kini and Firestein, 2001; Laing and Jinks, 2001). This binding is selective for different odours and entails odour maps in the olfactory bulb presumably governing odour perception (Shepherd *et al.*, 2003).

Flavour molecules perceived at the olfactory epithelium need to be volatilized from the food matrix to reach the olfactory receptors by either the retro- or ortho-nasal route. As to the retronasal pathway of flavour delivery, studying in-mouth release of flavour molecules is of great interest as the released quantity of a volatile determines, among other variables, the intensity of aroma sensation. Sensory measurements (Piggott *et al.*, 1998), mass spectrometers coupled with a human mouth or nose (Soeting and Heidema, 1988; Taylor *et al.*, 2000; Mayr *et al.*, 2003) or mouth modelling devices (van Ruth *et al.*, 1994; Roberts and Acree, 1995; Rabe *et al.*, 2002) were applied for the study of this process. Additionally, breath can be sampled through flavour traps containing adsorbing materials, which are analysed off-line (Linforth and Taylor, 1993; Delahunty *et al.*, 1996; Buettner and Schieberle, 2000).

Although mouth models can never simulate the full complexity of the eating/drinking process [i.e. muscle actions in the mouth/throat during chewing and swallowing, or the tidal airflow into and out of the lungs with the continuous exchange of air from/into the mouth (Buettner *et al.*, 2001)], or volatiles released from residuals of the food matrix adhering to the throat and the pharyngeal part of the tongue after swallowing (Taylor, 1996; Buettner *et al.*, 2001), they do combine a number of advantages for the study of release mechanisms occurring in the oral cavity itself. Increased sensitivity (Roberts and Acree, 1995; Deibler *et al.*, 2001; Rabe *et al.*, 2002), high reproducibility (Roberts and Acree, 1995; Deibler *et al.*, 2001; Rabe *et al.*, 2002), no selectivity problems and the ability to distinguish between a comparably large number of analytes in one gas chromatographic run (Piggott and Schaschke, 2001) are

advantageous in comparison to sensory or semi-sensory approaches involving human panels.

Remaining questions are (i) whether data provided by *in vitro* measurements reflect the real release process occurring *in vivo* in the mouth cavity; and (ii), how and to what extent these data can be correlated to the amount of volatiles reaching the olfactory epithelium through the retronasal pathway.

The present work studied *in vivo* volatile release from liquids using APCI-MS and a hand-held, computer-controlled sampling device, and compared in-mouth data with those of a sophisticated computerized mouth model apparatus (Rabe *et al.*, 2004b) to determine the validity of qualified *in vitro* approaches. Additionally, nose space analysis was aimed at the further assessment of the significance of *in vitro* data.

Materials and methods

Model flavour mix

Propylene glycol was used as a co-solvent for diacetyl, isobutyl acetate, ethyl 2-methylbutyrate, (*Z*)-3-hexenyl acetate, 2,3-dimethylpyrazine, (*Z*)-3-hexenol, 2-isobutylthiazole, furfuryl acetate, linalool, 2-pentylpyridine, D-carvone, β-damascenone and γ-nonalactone to prepare the stock solution applied in the release experiments. All aroma compounds were of analytical and sensory grade. Flavour concentrations in the liquid used for experiments ranged from µg/l to mg/l (cf. Rabe *et al.*, 2004b). In-nose volatile concentration during continuous drinking was studied using a commercial blackcurrant juice. Fruit juice and flavoured water were brought to 22°C prior to the start of experiments.

Trapping and analysis of dynamic headspace of blackcurrant juice

Blackcurrant squash (50 ml) containing 10% of fruit were filled in a 100 ml flask. Using N_2 as carrier gas, the headspace was continuously drawn through a Tenax trap at a flow of 40 ml/min for 10 min. The trap was then thermodesorbed at 240°C for 5 min in an injector (CHISA; SGE, Milton Keynes, UK) mounted on an HP 5890 gas chromatograph (GC) (Hewlett-Packard, Manchester, UK) using He as carrier gas at ~2.5 ml/min. The first loop of the analytical column (BP-1: 25 m × 0.22 mm i.d. × 1 µm film; SGE) was placed in liquid nitrogen during the desorption process to achieve the cryogenic focussing of flavours prior to gas chromatography. For chromatography, the oven temperature was programmed from 30°C, held for 2 min, then increased to 250°C at 8°C/min and held for 5 min. Flavours were detected in electron impact mode using a MSD 5970 mass spectrometer (Hewlett-Packard). Continuous scanning was carried out from *m/z* 35 to 210, with a scan time of 0.4 s and an interscan delay of 0.1 s at an ionization voltage of 70 eV.

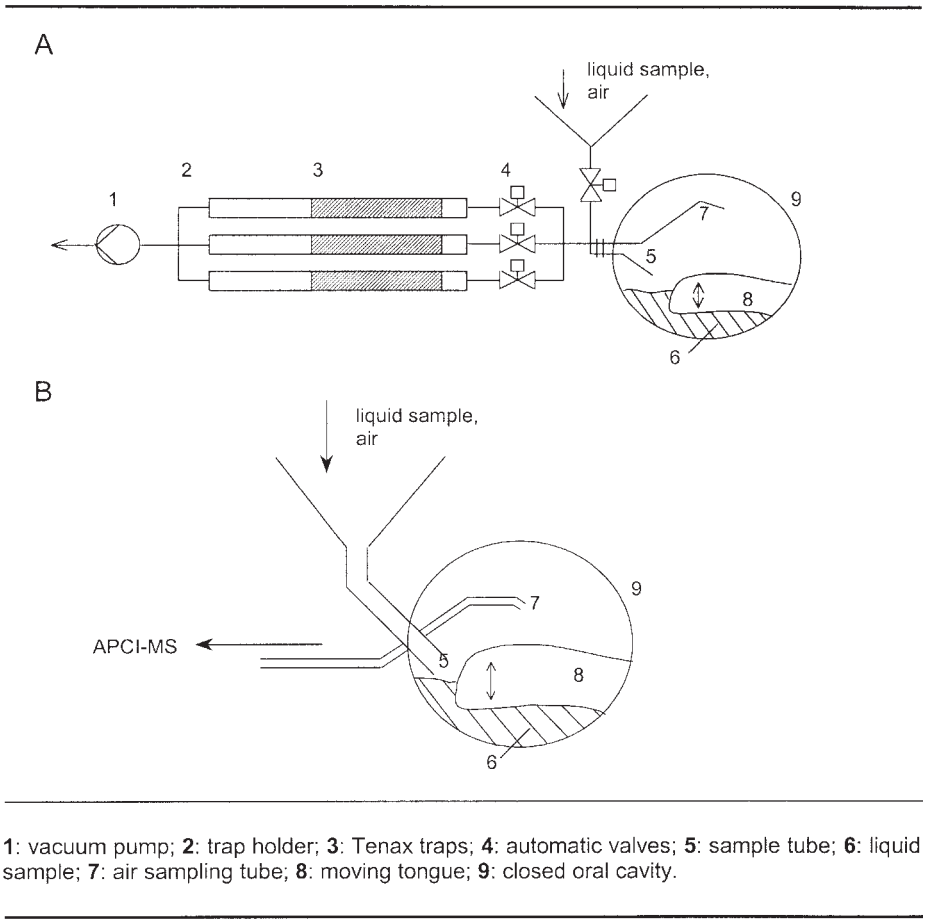
Thermodesorption–gas chromatography–flame ionization detection (TDS-GC-FID)

Thermodesorption of the Tenax traps (in-mouth release; cf. below) was carried out using a thermal desorption device (Gerstel TDS2, Gerstel, Mühlheim an der Ruhr, Germany) mounted on a HP 6890 GC (Agilent Technologies, Böblingen, Germany) equipped with a temperature programmable vaporization inlet (CIS 4 PTV; Gerstel). The PTV inlet incorporated a Tenax packed liner (glass liners—Tenax TA; Gerstel) and was cooled by liquid nitrogen. Analytical conditions were as follows. Thermal desorption: 30°C at 60°C/min to 260°C (held for 8 min); splitless mode; 50 ml/min desorption gas flow (N_2). PTV: 1°C (cryofocusing temperature) at 12°C/min to 260°C (held for 10 min); splitless; split mode (1/50) after 1.5 min; gas saver mode (1/20) after 3 min; column: 30 m × 0.25 mm i.d. × 0.25 µm INNOWAX (J&W Scientific, Folsom, CA); carrier gas flow 52 cm/s hydrogen; oven temperature: 40°C (held for 1.5 min) at 4°C/min to 130°C, then at 8°C/min to 180°C and at 25°C/min to 250°C (held for 10 min); detection: FID; 250°C.

Measuring in-mouth volatile release using Tenax traps

Volatile release in the mouth was studied using a novel computer-controlled sampling device. Figure 1A schematically illustrates the construction of the sampling unit: It consists of silanized 6 mm o.d. glass tubes (Rabe *et al.*, 2002), a glass funnel, three 6 mm o.d. Tenax traps (desorption tubes—Tenax TA 60/80; Gerstel), a glass Tenax trap holder, and four automatic valves (Automation Systems, Versoix, Switzerland). Glass tubes, the funnel and one end of each Tenax trap were fitted into the valves using Cajon Ultra Torr fittings (Sunnyvale Valve and Fitting, Sunnyvale, CA). The second end of each trap was fixed in a trap holder using conventional screw caps with a centric drill hole and silicon rubber sealing coated with PTFE (LAT, Garbsen, Germany).

After pouring 25 ml of flavoured water [the flavour concentration was exactly ten times higher than in the previous work (Rabe *et al.*, 2004b) to obtain similar GC-FID signals] into the funnel, and positioning the sample tube (liquid) and the air sampling tube (Figure 1A) in the mouth, a computer program started the automated experiment (cf. Rabe *et al.*, 2002). Within 3 s the sample entered the mouth and filled the area below the tongue. The oral cavity formed a closed chamber around the sample (Figure 1A). A vacuum pump (Ilmvac MP052Z, Ilmenau, Germany) was started, and air was sampled successively through the three Tenax traps at a flow of 200 ml/min, while the panellist's tongue moved up and downwards at regular intervals. The sampling time for each trap was 10 s. Flavours trapped on Tenax were thermodesorbed and analysed by GC-FID. Peak areas of the single time periods were accumulated to obtain the kinetics of the first 30 s of dynamic release. Experiments were repeated at least three times.



1: vacuum pump; **2**: trap holder; **3**: Tenax traps; **4**: automatic valves; **5**: sample tube; **6**: liquid sample; **7**: air sampling tube; **8**: moving tongue; **9**: closed oral cavity.

Figure 1 Schematic illustration of the novel computer-controlled sampling device for in-mouth measurement of volatile release (A) and the experimental assembly used for in-mouth APCI-MS measurements (B).

Atmospheric pressure chemical ionization–mass spectrometry (APCI-MS)

The volatile content in air during in-mouth measurements and breath-by-breath experiments were measured using a modified APCI-MS source (Linforth and Taylor, 1998). The dwell time of the mass spectrometer (Platform II; Micromass, Manchester, UK) in selected ion mode was 0.011 s and the compounds were ionized by a 4 kV corona discharge. A constant cone voltage of 15 V was used for the majority of flavours except for diacetyl (18 V) and 2,3-dimethylpyrazine (22 V).

Measuring in-mouth volatile release using APCI-MS

Flavoured water (for flavour concentrations, cf. Rabe *et al.*, 2004b) was poured into the front of the lower mouth cavity via a 8 mm o.d. Teflon tube (sample inlet) connected with a glass funnel (Figure 1B). After the sample reached the mouth cavity, regular movements of the tongue resulted in approximately constant shear force input. Similar to the above study (measuring in-mouth release with Tenax traps), the lips sealed the sample inlet tube and a second 3 mm o.d. air-sampling tube (Figure 1B) connected to the heated (120°C) transfer line (3.18 × 50 mm o.d.) of the APCI-MS. Thus, the mouth cavity formed a closed chamber. Flavour enriched air was constantly (in real time) drawn into the MS after having entered the funnel, moved above the liquid in the mouth and finally passed through the inlet of the air-sampling tube in the upper back of the mouth cavity (cf. Figure 1B).

Sample volumes (25 ml) were analysed at different air sampling flow rates (68, 100, 150 and 200 ml/l) to study effective impacts on dynamic in-mouth volatile release. Experiments were run by one trained panellist at least in triplicate. The area below the MS-signal curve was used to compare release rates (cf. Figure 2 for an exemplary signal trace). Areas of the first three 9 s time intervals (0–9 s, 9–18 s, 18–27 s; preset by the data processing software) representing volatile release within 27 s were accumulated and kinetics were compared with those of a mouth model system (Rabe *et al.*, 2004b).

Breath-by-breath measurements using APCI-MS

In-nose volatile concentration was measured during the continuous consumption of blackcurrant juice through a

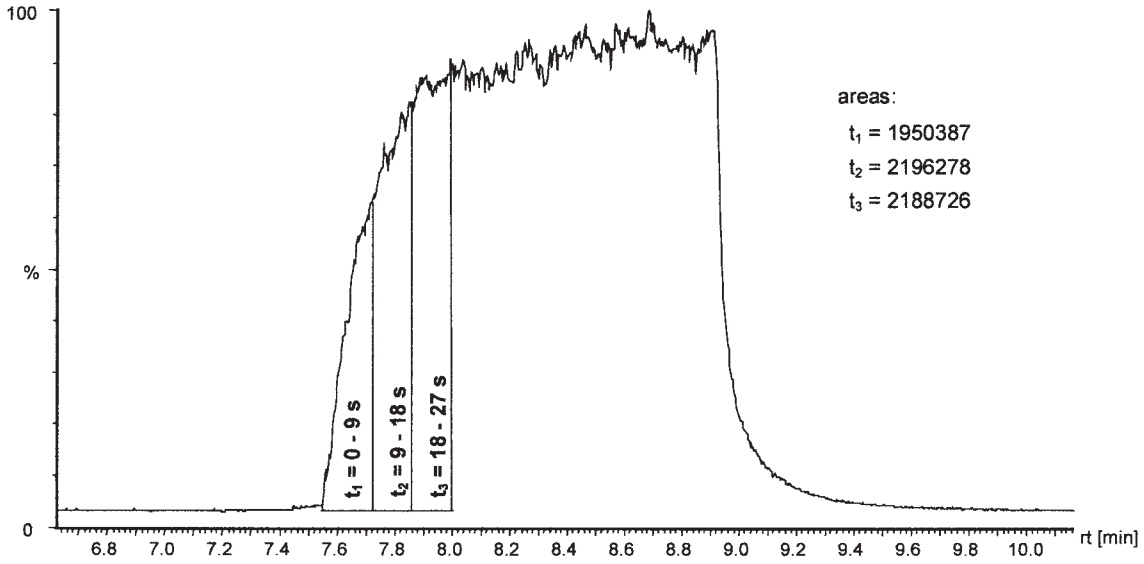


Figure 2 Typical signal trace (m/z 87, diacetyl) obtained during in-mouth APCI-MS measurements. The areas of the first three 9 s time intervals below the signal curve were used for further data processing.

Table 1 Panelist instructions for the breath-by-breath experiments

1	Regular breathing
2	Breathing in through the nose, sucking liquid (undefined volume) into the mouth cavity
3	Pause for 2 s (avoid movement of the liquid/headspace)
4	Swallow all of the liquid at once
5	Pause for 2 s (avoid further swallowing and breathing)
6	Exhale through the nose
7	Pause for 2 s
8	Start immediately with action 2

straw. Five panellists, one of whom was trained, were asked to drink 200 ml of the liquid following the instructions given in Table 1, while exhaled breath was continuously sampled (200 ml/min) through a tube (8 mm i.d.) connected to the end of the MS sampling line. Accordingly, panellists started with regular breathing. At a certain time, when breathing in, they simultaneously sucked an undefined portion of liquid into the mouth cavity, paused about two s (avoiding liquid and/or air movement), and then swallowed the gulp in one action followed by another 2 s pause, and subsequently, exhalation. This process (starting with inhalation and simultaneous sucking of liquid) was repeated until the 200 ml were consumed. Experiments lasted ~1–2 min.

Statistical analysis

Analysis of variance (ANOVA) was performed on values of the in-mouth release studies. Duncan's multiple-range (DMR) test was carried out to determine significant differences among different airflow rates in the mouth. A significance level of $P = 0.05$ was applied.

Results

In-mouth volatile release: influence of volumetric airflow rate

In-mouth APCI-MS measurements showed significant differences in dynamic volatile release at different airflow rates in the mouth (ANOVA and DMR test, $P < 0.05$). Increasing volumetric airflow rates through the mouth, that is increasing sampling rates into the MS, resulted in significantly increased volatile release rates for all aroma compounds (Table 2). The enhancement of volatile release upon changing airflow rates from 68 ml/l to 200 ml/l differed from compound to compound and ranged from factor 1.04 to 4.68 (Table 2). Good reproducibility of the APCI-MS method with mean coefficients of variation (CV) ranging from 6.5 to 10.1 was obtained (Table 2).

Linear release kinetics (accumulated MS signal areas (cf. Figure 2) with time) were found within experiments. Table 3 compares linear regression coefficients of accumulated APCI-MS signal areas of 9 s time periods obtained within the first 27 s. Accordingly, R2 ranged from 0.9459 to 0.9991. Although not significant for most of the compounds (ANOVA and DMR test, $P < 0.05$) a trend of improved linearity of kinetics with increasing volumetric airflow rate was noted. This resulted in a considerable change of release pattern of (*Z*)-3-hexenyl acetate, 2,3-dimethylpyrazine, 2-isobutylthiazole, 2-pentylpyridine and γ-nonalactone.

Linear release kinetics were also found within in-mouth Tenax trapping experiments characterized by R^2 ranging from 0.951 to 0.993.

Table 2 Influence of in-mouth volumetric air flow rate on dynamic

Volatile	Volumetric air flow rate in the mouth cavity (mean ± SD, ml/min)				Ratio[1] (%)
	68	100	150	200	
Diacetyl	60.7 ± 1.7^{ac}	74.4 ± 3.3^{b}	66.7 ± 3.7^{c}	63.4 ± 4.7^{c}	1.0
Isobutyl acetate	4.2 ± 0.3^{a}	4.3 ± 0.4^{a}	5.7 ± 0.3^{b}	6.8 ± 0.6^{c}	1.6
Ethyl 2-methylbutyrate	55.1 ± 1.2^{a}	66.2 ± 5.3^{b}	85.1 ± 6.5^{c}	115.4 ± 4.7^{d}	2.1
(*Z*)-3-Hexenyl acetate	41.3 ± 2.8^{a}	71.7 ± 5.3^{b}	93.3 ± 8.9^{c}	121.5 ± 8.9^{d}	2.9
2,3-Dimethylpyrazine	239.1 ± 34.8 ^{a}	419.5 ± 9.6^{b}	514.7 ± 33.4^{c}	614.5 ± 40.9^{d}	2.6
(*Z*)-3-Hexenol	217.8 ± 4.3^{a}	329.9^{b} ± 17.5	446.1 ± 30.6^{c}	571.1 ± 18.7^{d}	2.6
2-Isobutylthiazole	314.3 ± 27.1^{a}	569.3^{b} ± 50.5	773.2 ± 73.5^{c}	976.4 ± 69.9^{d}	3.1
Furfuryl acetate	94.6 ± 4.9 ^{a}	138.2 ± 5.2^{b}	157.0 ± 9.3^{c}	193.9 ± 3.8^{d}	2.0
Linalool	43.6 ± 2.8^{a}	76.3 ± 3.9^{b}	100.9 ± 10.1^{c}	129.0 ± 9.7^{d}	3.0
2-Pentylpyridine	92.5 ± 22.7^{a}	184.4 ± 42.1^{b}	327.3 ± 74.4^{c}	432.8 ± 34.2^{d}	4.7
D-Carvone	90.1 ± 7.3^{a}	151.0 ± 11.2^{b}	195.0 ± 25.0^{c}	242.2 ± 11.0^{d}	2.7
β-Damascenone	257.0 ± 16.1^{a}	443.4 ± 22.2^{b}	598.8 ± 65.3^{c}	737.6 ± 59.1^{d}	2.9
γ-Nonalactone	11.8 ± 1.7^{a}	21.1 ± 2.4^{b}	30.0 ± 5.7^{c}	35.6 ± 3.6^{c}	3.0
CV(%)[2]	8.3	7.8	10.1	6.5	

Values represent APCI-MS signal × 10^{-5}. Data are based on not less than three replicates of release experiments, and values with different superscripts within a line are significantly different (ANOVA and DMR test, $P < 0.05$). Experiments were conducted by one trained panellist.
[1]Between released volatile quantities at highest and lowest flow rate.
[2]Average coefficient of variation of volatile release experiments.

Table 3 Effect of in-mouth volumetric air flow rate on linear regression coefficients (R^2) of release kinetics

Volatile	Volumetric air flow rate in the mouth cavity [ml/min]			
	68	100	150	200
Diacetyl	0.9920 ± 0.005^{a}	0.9941 ± 0.002^{ab}	0.9956 ± 0.004^{ab}	0.9991 ± 0.001^{b}
Isobutyl acetate	0.9907 ± 0.003^{a}	0.9870 ± 0.006^{a}	0.9819 ± 0.005^{a}	0.9838 ± 0.009^{a}
Ethyl 2-methylbutyrate	0.9933 ± 0.003^{a}	0.9951 ± 0.002^{a}	0.9903 ± 0.004^{a}	0.9911 ± 0.005^{a}
(*Z*)-3-Hexenyl acetate	0.9757 ± 0.006^{a}	0.9757 ± 0.005^{a}	0.9814 ± 0.008^{a}	0.9821 ± 0.004^{a}
2,3-Dimethylpyrazine	0.9747 ± 0.013^{a}	0.9802 ± 0.005^{a}	0.9849 ± 0.010^{a}	0.9900 ± 0.004^{a}
(*Z*)-3-Hexenol	0.9904 ± 0.005^{a}	0.9903 ± 0.002^{a}	0.9866 ± 0.005^{a}	0.9908 ± 0.004^{a}
2-Isobutylthiazole	0.9630 ± 0.007^{a}	0.9652 ± 0.008^{a}	0.9762 ± 0.010^{a}	0.9766 ± 0.005^{a}
Furfuryl acetate	0.9919 ± 0.005^{a}	0.9924 ± 0.002^{a}	0.9882 ± 0.006^{a}	0.9908 ± 0.003^{a}
Linalool	0.9920 ± 0.002^{a}	0.9901 ± 0.001^{a}	0.9908 ± 0.005^{a}	0.9886 ± 0.004^{a}
2-Pentylpyridine	0.9511 ± 0.015^{a}	0.9459 ± 0.014^{a}	0.9593 ± 0.018^{a}	0.9650 ± 0.009^{a}
D-carvone	0.9911 ± 0.002^{a}	0.9862 ± 0.001^{a}	0.9885 ± 0.004^{a}	0.9877 ± 0.004^{a}
β-Damascenone	0.9820 ± 0.007^{a}	0.9871 ± 0.001^{ab}	0.9932 ± 0.006^{b}	0.9939 ± 0.003^{b}
γ-Nonalactone	0.9603 ± 0.015^{a}	0.9608 ± 0.006^{a}	0.9738 ± 0.011^{a}	0.9752 ± 0.007^{a}

Data are based on not less than three replicated and means with different superscripts within a line are significantly different (ANOVA and DMR test, $P < 0.05$). Experiments were conducted by one trained panellist.

Breath-by-breath measurements using APCI-MS

Compounds identified by analysis of the dynamic headspace of blackcurrant juice were compared with *m*/*z* values obtained by a full scan of the static headspace above the juice squash using APCI-MS. Among others, *m*/*z* 113 and 117, representing (*E*,*E*)-2,4-hexadienoic acid and ethyl

butyrate, respectively, were found. Including also *m*/*z* 59 of acetone, these ions were followed during single ion monitoring in breath-by-breath experiments.

Person-to-person variability

Figure 3A–C shows real time MS spectra of three volunteers (trained person: Figure 3C) continuously drinking blackcurrant juice squash through a straw following the guidelines given in Table 1. The ion traces of the individuals (data of two more panellists are not shown) showed large differences of the swallowing event, of the exchange of air from the mouth into the throat, and of the breathing rhythm during drinking, although panellists tried to objectively follow the instructions for the experiment. Acetone (*m*/*z* 59) is generated in the liver, and considerable amounts are transferred in exhaled breath. It is therefore useful as a marker for exhalation events (Linforth *et al.*, 2002). Accordingly, *m*/*z* 59 in Figure 3A–C illustrate the person-to-person variability of subconscious body functions (e.g. timing and course of exhalation after swallowing) and the difficulty to control these without intensive training.

The traces of *m*/*z* 113 and 117 consistently showed considerable differences in maximum intensity as represented by the peak heights of the single ions. Furthermore, exhalation time of individuals differed resulting in different signal areas for each exhalation. As a result, the length of exhalation and the intensity of released flavour during exhalation, both determining the quantity of volatiles reaching the regio olfactoria, varied between the three individuals (Figure 3A–C).

'Swallow breath' and exhaled breath volatile concentration

Figure 3C shows the MS spectra of a trained person consuming blackcurrant juice. After the initial breathing period, juice previously sucked into the mouth was swallowed at approximately 1.315 min. After the swallowing action ended at ~1.325 min an easily visible peak of the acetone trace lasting until 1.345 min revealed an exchange of air from the throat (initially coming from the lungs) to the nasal cavity. No signal for (*E*,*E*)-2,4-hexadienoic acid and ethyl butyrate was obtained. At 1.35 min the first exhalation started, and acetone and the two volatiles were detected during the whole exhalation period. The ester showed a sharp rise at the beginning of exhalation indicating an initial flavour burst. This peak was followed by a plateau phase with a constant release signal, a course, which was repeated during ongoing drinking, that is swallowing and subsequent exhalation. (*E*,*E*)-2,4-Hexadienoic acid showed a different behaviour. A continuous increase in release during one exhalation and again upon exhalations during further drinking was found (Figure 3C).

The acetone trace also showed that breathing in (i.e. at 1.390 min), sucking of liquid into the mouth simultaneously, and pausing of ~2 s subsequently caused no exchange of air. Flavour molecules from the mouth and/or the throat were not transferred into the nasal cavity. After the second swallowing event ending at 1.405 min (Figure 3C) the acetone peak from the exchange of air into the nasal cavity occurred again. This time, however, both *m*/*z* 113 and 117 ions also showed a peak matching exactly the timing of the acetone signal (1.407–1.425 min). The same observation was made with the following swallowing events. Although not as regular as in Figure 3C, the small acetone and flavour signals immediately following the swallowing actions were found in the traces of all panellists having participated in the study (Figure 3A,B).

Discussion

In-mouth release

Volumetric airflow rate was found to significantly influence dynamic volatile release from liquids in the mouth. The release of individual compounds was specifically enhanced depending on their physicochemical properties. This resulted in a change of the overall flavour profile upon changing airflow rates through the mouth. Furthermore, linearity of linear release kinetics found for the first 27 s of release increased with increasing airflow rate as indicated by linear regression coefficients approaching 1 (Table 3). Both effects including the change of overall release profiles are in agreement with results obtained with a computerized model mouth (Rabe *et al.*, 2004b) simulating mouth conditions (Rabe *et al.*, 2002), and demonstrated therefore its validity to simulate and describe in-mouth volatile release. Reactor volume to airflow rate ratios of 5 l to 3.3 l/min, 5 l to 4.8 l/min, 5 l to 7.2 l/min and 5 l to 9.6 l/min (Rabe *et al.*, 2004b) were similar to those applied in the present study for the in-mouth release experiments—100 ml (assumed mouth volume; Mayer, 1966) to 68 ml/min, 100 ml to 100 ml/min, 100 ml to 150 ml/min and 100 ml to 200 ml/min—and therefore represented mouth conditions. Figure 4 illustrates the influence of different volumetric airflow rates on the release of hydrophilic 2,3-dimethylpyrazine (log*P* = 0.64; Banavara *et al.*, 2002) and hydrophobic 2-isobutylthiazole (log*P* = 2.51; Banavara *et al.*, 2002) from water in the mouth and in the mouth model apparatus. Accordingly, the improved linearity and increasing quantity of released flavour with increasing volumetric airflow rates matched well.

Figure 5 further verifies the validity of the model mouth. It compares dynamic volatile release measured in the mouth using either APCI-MS or the presented device applying automated Tenax trapping with data of the recent mouth model apparatus (Rabe *et al.*, 2004b). Applying similar headspace to liquid volume ratios and mouth/reactor volume to volumetric airflow rate ratios of 75 ml to 25 ml, and 100 ml to 200 ml/min for *in vivo* measurements, and 3.75 l to 1.25 l and 5 l to 9.6 l/min for *in vitro* measurements, respectively, the release kinetics of (*Z*)-3-hexenol, 2-isobutylthiazole and linalool agreed well (Figure 5). Minor

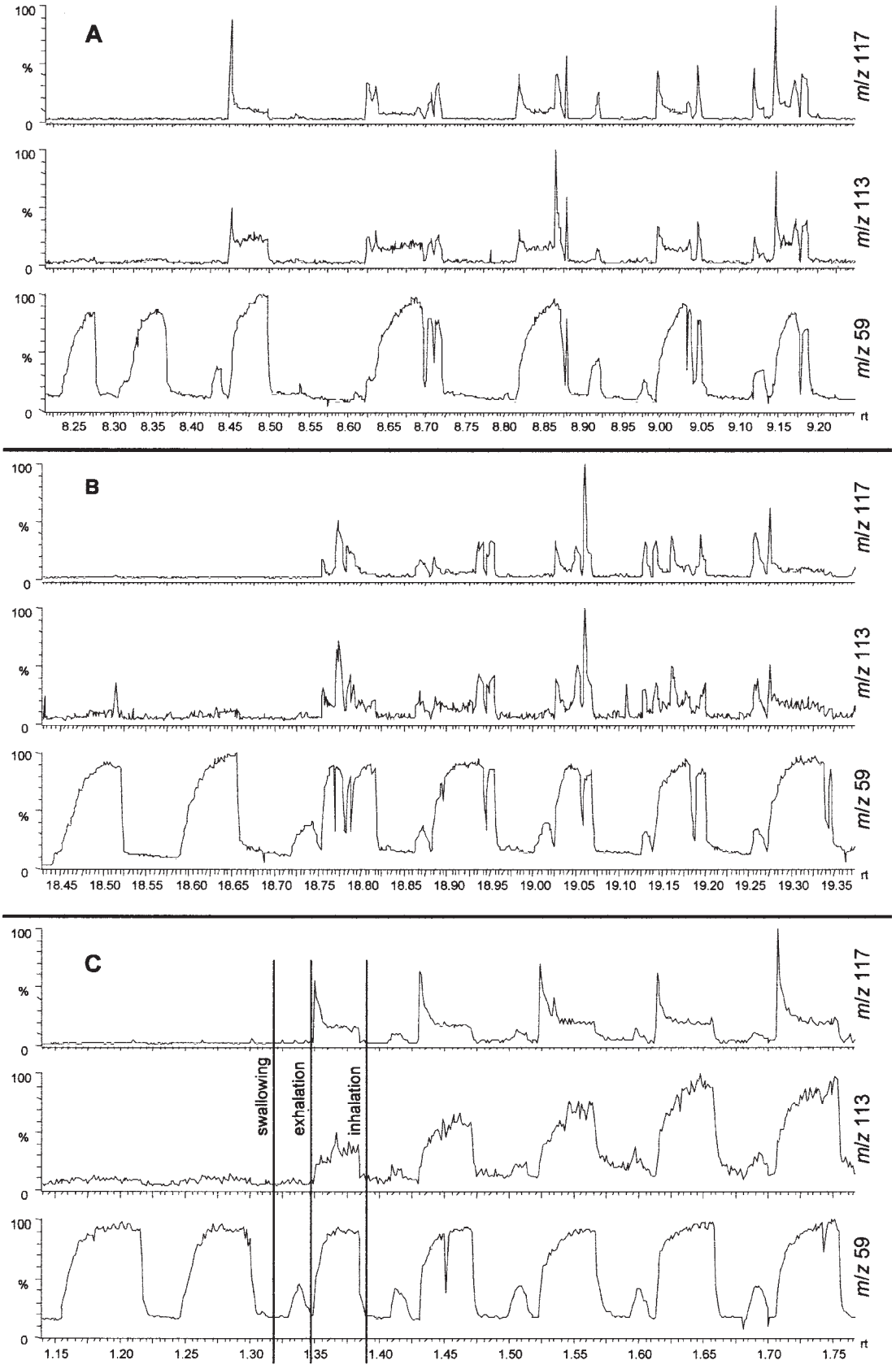


Figure 3 Breath-by breath profiles of two non-trained **(A, B)** and one trained **(C)** panellist drinking blackcurrant juice following the instructions given in Table 1. *m/z* 59, 103 and 117 represent molecular ions of acetone, (*E*,*E*)-2,4-hexadienoic acid and ethyl butyrate, respectively.

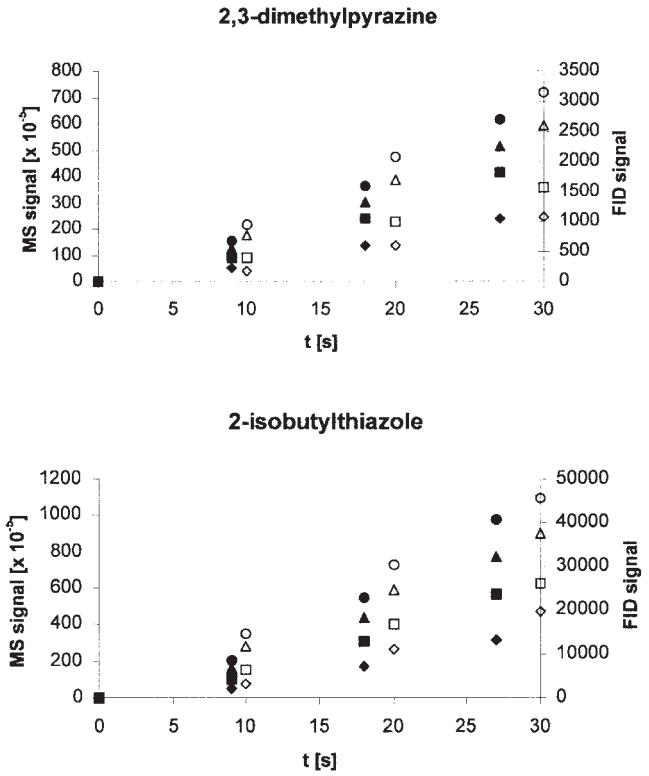


Figure 4 Influence of different volumetric airflow rates on the initial dynamic flavour release in the mouth simulated by a computerized mouth model apparatus (open symbols: FID signal; data from Rabe *et al.*, 2003a) and measured by APCI-MS (solid symbols: MS signal). Mouth/reactor volume: volumetric flow rate ratios of ~1.5 (diamonds), 1 (squares), 0.675 (triangles) and 0.5 (circles) are compared.

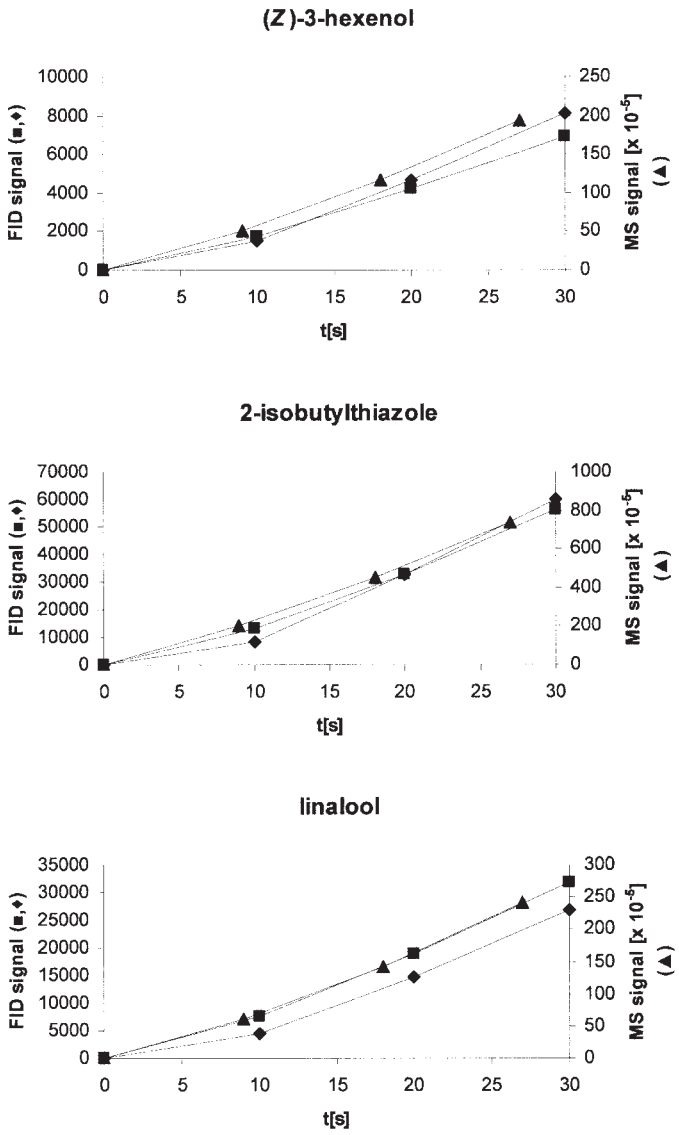


Figure 5 Comparison of in-mouth release kinetics from water measured *in vitro* using a mouth simulator (squares; data from Rabe *et al.*, 2003a), or *in vivo* by automated sequential headspace trapping on Tenax traps (diamonds) or APCI-MS (triangles).

deviations were found for the release kinetics obtained with *in vivo* Tenax trapping (cf. linalool in Figure 5). Its 10 s sampling points were mostly considerably lower (also in the case of 2-isobutylthiazole in Figure 5) than those obtained with the other two sampling methods. The next two measurement points together with the 10 s measurement point run parallel to those of the other lines indicating a constant sampling rate. It appears that the sampling process takes a certain time to reach a constant air velocity through the Tenax material. This adsorbent possessed a considerable flow resistance at the beginning of the sampling process (actuation of the first automatic valve) due to its dense packing in the glass tubes. Furthermore, the small dead volume of air of the device (cf. Figure 1A) might have contributed due to a dilution effect. In summary, kinetics as produced by both in vivo methods fit those measured *in vitro* by the mouth model apparatus.

Breath-by-breath experiments

The results of the present study (Figure 3A–C) are in line with the previously observed large person-to-person variability upon eating/drinking foods. Buettner *et al.* concluded from their observations that the velum–pharyngeal performances during consumption considerably influence volatile perception (Buettner *et al.*, 2001). The differences in velum movements, that is the opening and closing off the oral cavity to the airways and the nasal cavity should be therefore one variable explaining human variability in flavour perception.

In this study the concentration of flavour compounds in the nose during continuous drinking was followed using APCI-MS with panellists considering a simple experiment protocol (Table 1). Ensuring that the mouth cavity was closed off from the airways (using a straw), different portions of volatile loaded air reached the nose during drinking, intermediate breathing and after swallowing, respectively (Figure 3C): flavour was not detected during swallowing, as there was no nasal airflow at this particular

time (cf. Hodgson *et al.*, 2003). After swallowing, a considerable peak of the acetone trace indicated air initially originating from the lungs, which was displaced into the nasal cavity by complex muscle contractions (Buettner *et al.*, 2001). The missing peaks in the traces of (*E*,*E*)-2,4-hexadienoic acid and ethyl butyrate after the first swallowing event (Figure 3C; 1.325–1.345 min) of the continuous drinking process clearly demonstrated that direct retronasal volatile release from the mouth cavity into the nasal cavity, the so-called 'swallow breath' volume of air as defined by Land (1996), did not originate from the mouth, but is a displaced volume of air from the throat. This volume contained flavour molecules after the second swallowing event (1.41–1.425 min; Figure 3C), which should have been generated from the residuals of the previously swallowed liquid covering the throat and the pharyngeal part of the tongue (Buettner *et al.*, 2001). The main volatile quantity reaching the nose is transported through exhalation after swallowing (cf. also Hodgson *et al.*, 2003). In agreement with Linforth and Taylor (2000) and Linforth *et al.* (2002), volatile signals differed depending on the physicochemical properties of the compounds, which also determined their adsorption to the human oral, pharyngeal and nasal mucosa (Hussein *et al.*, 1983; Linforth and Taylor, 2000). After an initial burst, the trace of ethyl butyrate showed a constant intensity during one single exhalation and remained constant during the following swallowing and exhalation events. An explanation for the flavour burst could be volatile-enriched air from the mouth cavity which is passed into the throat during swallowing and which is transferred via exhalation into the nasal cavity. Absorption of the ester into the mucous layers is fast, but also limited, as quantities above saturation seemed to be transferred to the nose as one sharp peak, whereas dilution with air seemed not to occur (cf. Linforth *et al.*, 2002). The steady state after the burst peak found with the ester should represent a dynamic equilibration of flavour between the liquid residuals covering the throat and the flavour absorbing mucous layers, and the flowing air during exhalation (similar to the dynamic in-mouth process analysed in the present work). As the absorption and desorption of ethyl butyrate is an equilibrium process, its intensity is similar even after numerous swallowing events. No burst signal was obtained for (*E*,*E*)-2,4-hexadienoic acid (Figure 3C). This behaviour might be explained by its equilibration and accumulation with and in the mucous layers during transport via exhalation. Continuous dilution of this compound with air might also have alleviated its initial intensity (Linforth *et al.*, 2002). According to Figure 3C, the intensity of the (*E*,*E*)-2,4-hexadienoic acid signal increased constantly from swallowing event to swallowing event not only during one exhalation, but also during ongoing drinking. Consistently, the intensity of (*E*,*E*)-2,4-hexadienoic acid contained in the air volume displaced from the throat into the nose cavity upon swallowing increased with time and proved, according to the above theory of dynamic equilibration, the accumulation of the compound in the mucous layers. Such a persistent behaviour was previously found for various flavour compounds (Linforth and Taylor, 2000).

In vivo versus *in vitro* measurements—an ongoing matter of dispute

Release data of a computerized mouth model apparatus were validated by the present in-mouth release data of *in vivo* measurements (Figure 5). The main advantages of the *in vitro* approach (Rabe *et al.*, 2002) are good reproducibility and high sensitivity enabling the study of minute changes of release (Rabe *et al.*, 2003a,b, 2004a). Furthermore, the isolation of single physiological variables (for example saliva, mucosa), and their exact control (shear rate, airflow) enabled statements about their significance on the initial dynamic release of volatiles from liquids (Rabe *et al.*, 2004b). The validation also verified the applicability of a mathematical model, developed for the prediction of volatile release from water (Banavara *et al.*, 2002), oil-in-water emulsions (Rabe *et al.*, 2004a) and different solutions (Rabe *et al.*, 2003a,b) in the apparatus (Rabe *et al.*, 2002), for the estimation of volatile release in the mouth. Both the model mouth and the mathematical model can help to reduce the time- and cost-intensive empirical composition work of flavourists, which becomes necessary when the matrix of a flavoured food is changed.

The present breath-by-breath experiments also showed that in-mouth release is not the only process determining the flavour concentration at the regio olfactoria over time. It furthermore depends on how this flavour portion is transferred into the breath stream, on the fraction released from liquid residuals in the throat upon exhalation and on the flavour quantity absorbed by the different mucous layers over which the air is flowing. Although parts of these processes should be similar to that analysed in-mouth in the present work representing dynamic equilibration between flowing air and liquid, the complex process of swallowing and air movement going along with a catenation of different muscle actions (Buettner *et al.*, 2001) cannot be simulated at present with any mechanical device. As a result, comparisons of mouth model data with those obtained *in vivo* in-nose, as done by Deibler *et al.* (2001) and van Ruth and Buhr (2003), should be carefully interpreted.

To calculate the perceivable quantity of volatiles reaching the regio olfactoria, many other variables need to be considered: for example, the flavour fraction released from the liquid film in the throat after swallowing, the dilution of this film with saliva including the resulting solubility improvement of flavours (water volume for dissolution increases; cf. Rabe *et al.*, 2003a,b), the repartitioning and improved release of flavours in diluted oil-in-water emulsions (Rabe *et al.*, 2004a), pH changes upon dilution (Roberts and Acree, 1995; Rabe *et al.*, 2003c) and the physicochemical properties of flavours that determine the extent of absorption into the

mucous layers. Yet prediction of flavour perception will still remain difficult, due to the high person-to-person variability during consumption (Taylor, 2002), enzymatic activity in the mouth (Hussein *et al.*, 1983; Buettner, 2002a,b) and the congenitally differing flavour perception thresholds in humans (Friedrich and Acree, 1999). In a study by Damm *et al.* (2002), the latter was also shown to be dependent on the volumes of the anterior part of the lower and upper meatus of the right nasal cavity. This implies that the morphology of the nasal cavity and different segment volumes influence airflow and volatile transport towards the olfactory epithelium, with intranasal volumes below the cibriform plate and the anterior, lower meatus apparently being of special importance to the sense of smell (Damm *et al.*, 2002).

Conclusion

In vivo measurements presented in this study verified data previously produced by a sophisticated mouth model apparatus designed to simulate in-mouth volatile release. Breath-by-breath experiments underlined that, besides in-mouth release, the volatile concentration in the nose also depends on various additional physiological and physicochemical factors, which are difficult to control in humans. To complicate the situation further, data from previous studies give good reasons to suppose that volatile thresholds depend not only on the concentration of volatiles in the nose, but also on the structure of the nasal cavity (Damm *et al.*, 2002), the human genetics and associated expression of olfactory receptor proteins (Friedrich and Acree, 1999), and cognitive effects between flavours and tastants (Davidson *et al.*, 1999; Dalton *et al.*, 2000; Taylor *et al.*, 2002). Only the comprehensive understanding of (i) release mechanism from food matrices, (ii) factors causing variability of flavour release and perception in humans, (ii) final volatile concentrations at the place where the aroma is sensed and (iv) cognitive effects apparently responsible for the modulation of flavour sensations due to simultaneous nerve stimulation by volatiles at the regio olfactoria and tastants sensed at taste buds on the tongue will enable the successful estimation of food flavour perception in humans.

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