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In vitro Study of the Influence of Physiological Parameters on Dynamic In-mouth Flavour Release from Liquids

Swen Rabe, Ulrich Krings and Ralf G. Berger

Institut für Lebensmittelchemie im Zentrum Angewandte Chemie der Universität Hannover, Wunstorfer Straße 14, 30453 Hannover, Germany

Correspondence to be sent to: Ralf G. Berger, Institut für Lebensmittelchemie im Zentrum Angewandte Chemie der Universität Hannover, Wunstorfer Straße 14, 30453 Hannover, Germany. e-mail rg.berger@lci.uni-hannover.de

Abstract

Influences of shear rate (surface extension), airflow, in-mouth headspace volume, synthetic saliva and human epithelial cells (modelling mucosa) on the initial dynamic flavour release from liquids were analysed. Simulating physiological mouth parameters, initial dynamic flavour release experiments over a time period of 30 s were carried out using a proven mouth model apparatus. Flavour compounds of different chemical classes were dissolved in water or in aqueous starch hydrolysate in concentrations typically present in food (μg/l to mg/l). Forced by increasing shear rates the enlargement of the gas–liquid interface (vortex formation) caused an increased release of flavour molecules. The release of less soluble compounds was reduced by increasing shear forces due to an improved dissolution. Increasing volumetric airflow rates resulted generally in higher release rates and in a change of pattern of release kinetics. Maximum flavour release was found at a ratio of 1:1 for in-mouth headspace and liquid volume. Neither addition of saliva alone nor the combination of saliva and mucosa showed significant influence on in-mouth flavour release from liquids in the model mouth.

Key words: airflow, headspace volume, in-mouth flavour release, mucosa, saliva, shear rate

Introduction

Human olfaction is a complex process and involves a catenation of specific reactions before perceptual identification of aromas is possible (Kini and Firestein, 2001). The mechanisms of 'odour image' discrimination flavour perception also depends on the input from taste receptors (Shepherd *et al.*, 2003; Taylor *et al.*, 2003). Looking at the retronasal pathway of flavour molecules to reach the olfactory epithelium, volatiles have to travel through the food matrix and a film of saliva (in low moisture foods) until they evaporate into the airspace in the mouth. This release process during food consumption is dependent on the individual physiological parameters of the human's mouth cavity. Temperature and hydration, the surface area of the food, presence of enzymes, saliva, binding phenomena and phase inversions are all potentially relevant for the changes in retronasal flavour release, and consequently, perception (Taylor, 1996).

The process of mastication, for example, has a large influence on the perception of flavour. Bakker (1995) and Brown and Wilson (1996) reported large deviations of mastication patterns and chewing times between individuals, resulting in significant perceptual differences. Similar results were reported by Buettner and Schieberle (2000), who showed that the duration of mastication had a considerable influence on the retardation of flavour molecules in the mouth. Both the total amount of odorants released and the general profile were affected.

The retronasal airflow through the mouth cavity was also identified as an important factor determining retronasal flavour release. Comparing orthonasal and retronasal flavour perception using a time-intensity method, Voirol and Daget (1986) found the perception of flavours to be affected by the volume of air reaching the regio olfactoria. The higher the flow rate of air through the nose (inhaling airflow > exhaling airflow), the lower the perception threshold. This implicated that higher volumetric flow rates resulted in higher quantities of flavours reaching the olfactory epithelium. However, Burdach *et al.* (1984) did not find any difference between orthonasal and retronasal perception.

Regarding volatile release studies classical *in vivo* approaches are bound to encounter the human limits of chemoreception: lack of reproducibility, dependency from non-controllable factors, and perceptual identification of at best three compounds of a mixture at the same time (Laing and Jinks, 2001). Bearing in mind the limitations of model mouth devices, the particular advantage of *in vitro* approaches is the possibility to study single parameters

influencing volatile release (Margomenou *et al.*, 2000). The present work separately studied *in vitro* the significance of physiological mouth parameters on flavour release during liquid consumption in an attempt to overcome the experimental difficulties of sensorial volatile release studies. Simultaneous detection of release kinetics of different flavours from liquids in real time and at simulated mouth conditions were performed using a proven mouth model apparatus (Rabe *et al.*, 2002).

Materials and methods

Model flavour mix

Thirteen volatile flavours from different chemical classes were chosen for their broad physicochemical properties (numbers in brackets represent final concentrations in the liquid in mg/l): diacetyl (0.784), isobutyl acetate (0.024), ethyl 2-methylbutyrate (0.029), (*Z*)-3-hexenyl acetate (0.383), 2,3-dimethylpyrazine (1.933), (*Z*)-3-hexenol (0.963), 2-isobutylthiazole (0.883), furfuryl acetate (0.978), linalool (0.972), 2-pentylpyridine (1.463), D-carvone (1.448), β-damascenone (1.951) and γ-nonalactone (4.854). GC-MS analysis confirmed the purity of the flavour compounds. An aroma stock solution in propylene glycol was prepared to spike the liquids analysed in release experiments.

Artificial saliva

Artificial saliva was composed (g/l) of the recommended ingredients (Jenkins, 1978): 2.8 mucin, 0.11 lysozyme, 0.5 α-amylase, 0.01 glucose, 0.13 urea, 0.03 uric acid, 0.5 cyclo-AMP, 0.68 K_2HPO_4, 0.94 KCl, 0.16 $CaCl_2$, 0.75 NaCl and 1.09 $NaHCO_3$ (all supplied by Sigma-Aldrich, Steinheim, Germany). Dissolution of the listed compounds in distilled water with vigorous stirring resulted in synthetic saliva with a pH of 7.4. The saliva was freshly prepared and brought to 37°C prior to experimentation.

Artificial oral mucosa

Human oral mucosa was simulated by epithelial tissue cells of a human *cervix* carcinoma (HELA, DSMZ ACC 57; DSMZ, Braunschweig, Germany) first isolated by Gey *et al.* (1952). Adherently growing cells were cultivated in baffled roller-bottles (2125 cm^2 surface area; Greiner Bio-One, Frickenhausen, Germany) at 0.5 r.p.m. during the first 2 days and 1 r.p.m. afterwards, using Dulbecco's modified Eagle's medium (Life Technologies, Paisley, Scotland) containing 10% (v/v) fetal bovine serum (PAA Laboratories, Cölbe, Germany) at 37°C and 5% CO_2 atmosphere until confluence was obtained (Figure 1). Diameter and height of the roller bottles were 122 and 267 mm (cylindrical shell: 235 mm), respectively.

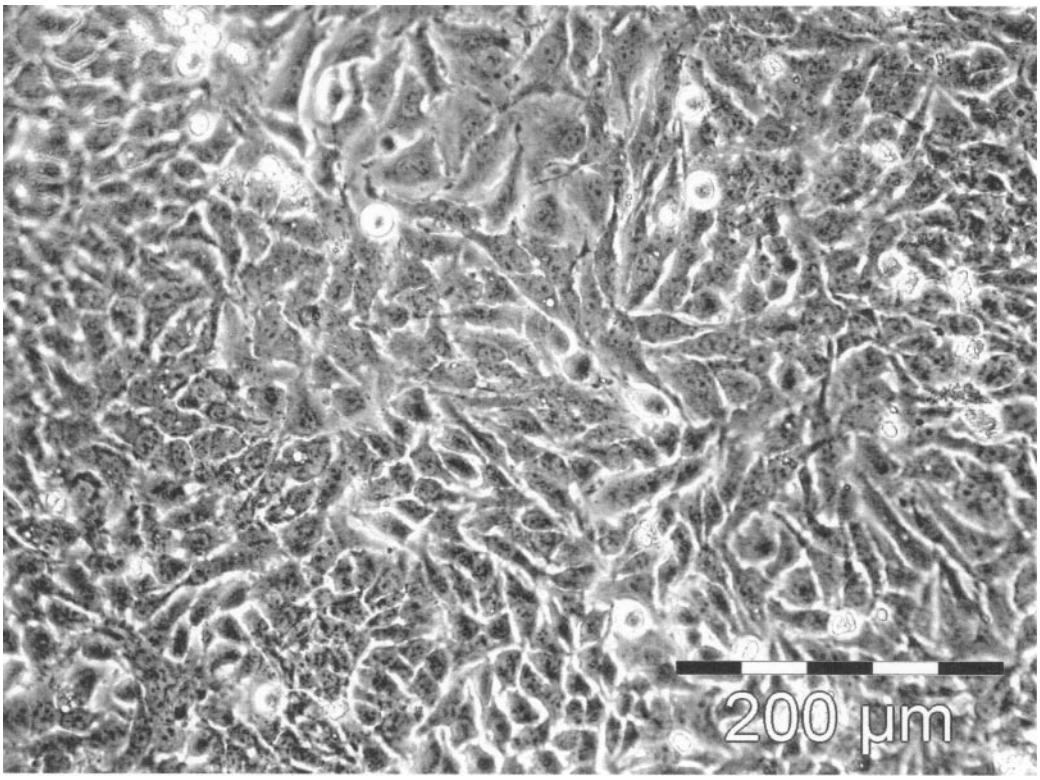

Figure 1 Adherent epithelial mucosa cells of a human cervix carcinoma (HELA) at confluent state (growing in a roller bottle) used for the simulation of oral mucosa.

Preparation of flavoured solutions

Solutions of maltodextrin 6–10DE (MD; Roquette, Lestrem, France) at 200 g /l were prepared adding the solute stepwise to water and used for the study of enzymatic activity affecting initial flavour release. Vigorous stirring using a conventional stirring device (IDL, Nidderau, Germany) supported the dissolution. All other studies of the present work were conducted in water for the sake of effect separation. After the addition of the flavour stock solution premix the above compound concentrations were obtained.

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

Analysis of the Tenax traps 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 programmable temperature vaporization (PTV) inlet (CIS 4 PTV; Gerstel). The PTV inlet incorporated a Tenax packed liner (Tenax TA; Gerstel) and was cooled by liquid nitrogen. Analytical conditions were as follows. Thermal desorption: from 30°C at 60°C/min to 260°C (held for 8 min); splitless mode; 50 ml/min desorption gas flow (N_2). PTV: from 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^{-1} 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. Chromatograms were evaluated using HP ChemStation Software (Agilent Technologies). Quantification of each flavour compound was done by external calibration.

Real time flavour release experiments

Initial flavour release was measured using a recently developed computerized apparatus (Rabe *et al.*, 2002). The 'from zero start' technique was applied to obtain release kinetics in real time. The procedure of release experiments was exactly the same as reported previously (Rabe *et al.*, 2002). After the complete filling of the reactor with flavoured liquid (5 l), a defined headspace (0.85 l) was created within 3 s. From this time on-line sampling of air at a volumetric flow rate of 9 l/min started. Within 10 s a defined volume of dynamic headspace was sampled in a cascade comprising of four high precision syringes. Using three cascades, the flavour release was kinetically measured in the course of 30 s, described by three measurement points. The volatiles collected in each cascade were then directed off-line onto corresponding Tenax traps by a low vacuum at a flow rate of 60–80 ml/min. After quantitative accumulation of the flavour molecules on Tenax, the polymer traps were thermodesorbed and analysed by GC-FID. Each experiment was repeated at least three times.

Effect of different shear rates (gas–liquid surface area)

The influence of different shear rates on dynamic flavour release from water (26°C) was examined. Stirring speeds in the reactor ranging from 50 to 450 r.p.m. were applied to study the effects of surface extension caused by shear rate on flavour release. Shear rate values were calculated as reported earlier (Rabe *et al.*, 2002). Synthetic saliva and simulated mucosa were not applied. Airflow during experiments was constant at 9.6 l/min (1.6 l headspace sampled in each cascade of syringes).

Effect of different airflow rates

Release experiments from water (22°C) were conducted using airflow rates through the reactor of 3.3, 4.8, 7.2, 9 and 9.6 l/min (0.55, 0.8, 1.2, 1.5 and 1.6 l headspace sampled in each cascade of syringes). Artificial saliva and mucosa were not considered during experimentation. Stirring speed was maintained at 450 r.p.m., resulting in a shear rate of 150 s^{-1}.

Effect of saliva

Potential effects of saliva on initial dynamic flavour release were determined in water (26°C) and aqueous solutions of MD (26°C). Two sets of release experiments with and without addition of saliva were carried out. Artificial saliva was continuously pumped into the reactor after creation of headspace. A flow rate of 175 ml/min was used during experiments to fit real mouth conditions (Rabe *et al.*, 2002). Stirring speed during experiments was maintained at 450 r.p.m. A constant airflow of 9.6 l/min was used within experiments. Artificial oral mucosa was not applied.

Effect of the ratio of headspace to liquid volume

The influence of headspace volume above a liquid on flavour release under dynamic conditions was investigated using headspace:liquid volume ratios of 1:3, 1:1 and 3:1. In contrast to the previous experimental procedure, the exact volume of liquid was added into the reactor within seconds immediately followed by the start of the experiment. This was necessary, as the depletion process of the liquid would have taken far more than 3 s (cf. 'from zero start' technique; Rabe *et al.*, 2002) and would probably have led to a considerable increase of headspace flavour concentration prior to the experiment start and on-line sampling of headspace. Artificial saliva and oral mucosa were not applied. An airflow rate of 9.0 l/min (1.5 l headspace sampled in each cascade of syringes) and stirrer rotation of 450 r.p.m. were kept constant during experiments.

Effect of artificial oral mucosa

To investigate effects of mucosa/flavour interactions on dynamic flavour release from liquids in the mouth, roller bottles, inside colonized with confluent epithelial cells, were placed onto the reactor wall of the apparatus in contact with the liquid. For this, the bottom and the top of a roller bottle were removed and the remaining cylindrical shell covered

with mucosa cells was placed into the reactor after it had been immersed in a bath of artificial saliva at 37°C. Release experiments were carried out with flavoured water which was immediately filled into the reactor after the replacement of the reactor lid (Rabe *et al.*, 2002). Saliva was continuously added at 175 ml/min. Stirring rate and volumetric airflow were kept constant, at 450 r.p.m. and 9.0 l/min respectively. For comparison, flavour release from water was measured using the above parameters, but no saliva was added and a plain roller bottle shell (without mucosa cells) was applied.

Statistical analysis

Release data of saliva and mucosa experiments were subjected to Student's *t*-test. Analysis of variance (ANOVA) was performed on data of the shear rate, flow rate and headspace volume study. Duncan's multiple-range (DMR) test was carried out to determine significant differences among means. A significance level of $P = 0.05$ was applied throughout the studies.

Results

Influence of saliva

The comparison of initial dynamic flavour release data from water with and without addition of saliva showed no significant difference for each compound of the flavour mix (Student's *t*-test, $P > 0.05$). The effect of salivation on initial flavour release from high-starch liquids was also studied using flavoured solutions of a starch hydrolysate. Again, no significant effect on the initial release process was found (Student's *t*-test, $P > 0.05$). Consequently, enzymatic reactions were irrelevant for the initial process of volatile release (up to 30 s). Furthermore, dilution of liquid food matrices at physiological conditions was found to be negligible and no significant salting out effect by the electrolytes was observed.

Influence of shear

Increasing shear rates in the reactor ranging from 0 to 150 s^{-1} (0–450 r.p.m.) affected the release of flavour compounds differently (Table 1). Most of the volatiles showed increased flavour release rates, which were significant at stirring speeds above 200 r.p.m. and partly significant below or equal 200 r.p.m. (Table 1; ANOVA and DMR test, $P < 0.05$). Figure 2 shows the correlation between the shear rate and the absolute release of 2-isobutylthiazole. The graph presents both, the increased interfacial area with increasing shear rates caused by vortex formation in the reactor and the parallel positive trend of the relative release. The parallel tendency of these lines was found for every flavour compound showing increasing flavour release rates at increasing shear rates (data not shown). Accordingly, in these cases, the shear rate is directly correlated with the interfacial surface extension of the liquid. Comparing the release rates of these compounds at shear rates of 0 and 150 s^{-1} (0 and 450 r.p.m. respectively), the absolute change in

Table 1 Influence of stirrer speed on flavour release (μg) from water within 30 s

Volatile	r.p.m.						Ratio (%)[1]
	0	50	100	200	325	450	
Diacetyl	0.27 ± 0.01^{a}	0.29 ± 0.00^{a}	0.31 ± 0.01^{a}	0.34 ± 0.00^{a}	0.49 ± 0.01^{b}	0.63 ± 0.06^{c}	2.3
Isobutyl acetate	0.04 ± 0.01^{a}	0.04 ± 0.01^{ab}	0.05 ± 0.01^{b}	0.07 ± 0.00^{c}	0.21 ± 0.00^{d}	0.31 ± 0.00^{e}	8.0
Ethyl 2-methylbutyrate	0.06 ± 0.01^{a}	0.04 ± 0.01^{a}	0.06 ± 0.01^{a}	0.08 ± 0.00^{b}	0.24 ± 0.01^{c}	0.38 ± 0.00^{d}	6.8
(*Z*)-3-Hexenyl acetate	3.17 ± 0.04^{a}	2.15 ± 0.12^{b}	2.31 ± 0.50^{ab}	2.66 ± 0.68^{ab}	4.48 ± 0.33^{c}	5.77 ± 0.60^{d}	1.8
2,3-Dimethylpyrazine	0.12 ± 0.00^{a}	0.14 ± 0.00^{ab}	0.14 ± 0.00^{ab}	0.13 ± 0.01^{ab}	0.15 ± 0.00^{b}	0.19 ± 0.01^{c}	1.6
(*Z*)-3-Hexenol	0.40 ± 0.02^{a}	0.45 ± 0.00^{ab}	0.47 ± 0.01^{bc}	0.46 ± 0.04^{bc}	0.52 ± 0.02^{c}	0.63 ± 0.03^{d}	1.6
2-Isobutylthiazole	1.73 ± 0.10^{a}	1.60 ± 0.02^{a}	1.79 ± 0.09^{a}	1.94 ± 0.28^{a}	2.75 ± 0.26^{b}	3.56 ± 0.20^{c}	2.1
Furfuryl acetate	0.60 ± 0.04^{a}	0.68 ± 0.02^{ab}	0.70 ± 0.03^{ab}	0.72 ± 0.08^{b}	0.87 ± 0.05^{c}	1.06 ± 0.05^{d}	1.8
Linalool	2.54 ± 0.17^{a}	1.79 ± 0.30^{b}	1.75 ± 0.57^{b}	1.56 ± 0.49^{b}	1.58 ± 0.22^{b}	1.63 ± 0.28^{b}	0.6
2-Pentylpyridine	1.59 ± 0.30^{a}	1.22 ± 0.10^{a}	1.50 ± 0.13^{a}	1.34 ± 0.38^{a}	1.51 ± 0.27^{a}	2.03 ± 0.43^{a}	1.3
D-carvone	1.74 ± 0.20^{a}	1.25 ± 0.25^{a}	1.23 ± 0.38^{a}	1.06 ± 0.34^{a}	1.06 ± 0.15^{a}	1.17 ± 0.24^{a}	0.7
β-Damascenone	34.48 ± 6.56^{a}	27.36 ± 8.44^{ab}	22.45 ± 9.53^{bc}	17.47 ± 6.52^{bc}	14.48 ± 3.35^{c}	13.31 ± 5.85^{c}	0.4
γ-Nonalactone	0.15 ± 0.02^{a}	0.14 ± 0.02^{a}	0.15 ± 0.02^{a}	0.12 ± 0.05^{a}	0.08 ± 0.01^{a}	0.11 ± 0.03^{a}	0.7
CV[2] (%)	9.7	11.3	15.1	18.4	9.1	13.5	

Values with different superscripts within a line are significantly different, ANOVA and DMR test ($P < 0.05$).
[1]Between flavour quantities released at 450 and 0 r.p.m.
[2]Mean coefficient of variation.

flavour release ranged from factor 1.3 to 8.0 for isobutyl acetate (Table 1).

Less volatile compounds (sequence of the aroma compounds in Table 1 equals the chronological order of elution during GC analysis), namely linalool, D-carvone, β-damascenone and γ-nonalactone showed an opposite behaviour. Their release decreased at increasing shear rates, was partly significant (Table 1; ANOVA and DMR test, $P < 0.05$), and the absolute difference for these compounds at stirring rates between 0 and 450 r.p.m. ranged from factor 0.4 to 0.7.

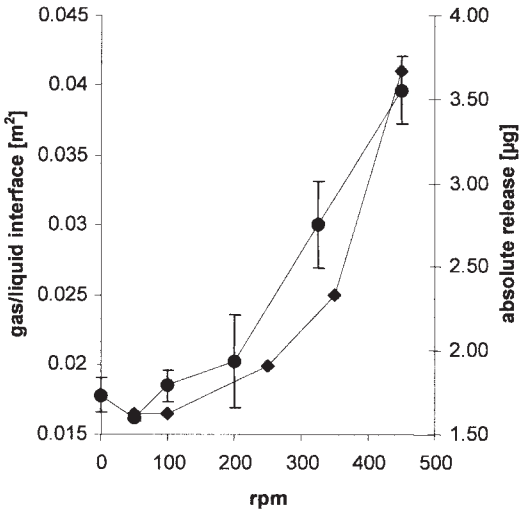

Figure 2 Influence of stirrer speed and gas–liquid interface on dynamic release of 2-isobutylthiazole from water.

Influence of airflow rate

Increasing flow rates of air streaming above the liquid through the reactor resulted in significantly increased amounts of released flavour (ANOVA and DMR test, $P < 0.05$). Table 2 shows a comparison of the amounts released at different volumetric flow rates. Accordingly, the absolute difference in flavour release comparing the lowest and the highest flow rate, 3.3 and 9.6 l/min, respectively, ranged from factor 1.9 to 4.0. Less volatile compounds (sequence of the aroma compounds in Table 2 is equal to the chronological order of elution during GC analysis), namely linalool, 2-pentylpyridine, D-carvone, β-damascenone and γ-nonalactone, showed partly non-significant changes among single flow rates, but consistently, slightly increased values were obtained within experiments using higher flow rates. A linear correlation between volumetric airflow rate and increasing flavour release was found for each compound (R^2 ranged from 0.962 to 0.997, mean = 0.976).

Using a constant flow rate of 9.6 l/min, the release over time was linear for each compound measured. Linear regression coefficients ranging from 0.97 to 0.9999 were calculated. Decreasing volumetric flow rates resulted in curved runs of the corresponding release curve and significantly decreased linear regression coefficients (Table 3). Thus, the volumetric airflow rate influenced the pattern of flavour

Table 2 Influence of volumetric air flow rate through the reactor on dynamic flavour release (after 30 s, µg)

Flavour	Volumetric air flow rate through the reactor (l/min)					Ratio[1] (%)
	3.3	4.8	7.2	9.0	9.6	
Diacetyl	0.56 ± 0.00[a]	0.80 ± 0.00[b]	1.10 ± 0.01[c]	1.22 ± 0.06[d]	1.35 ± 0.00[e]	2.4
Isobutyl acetate	0.43 ± 0.01[a]	0.54 ± 0.01[b]	0.70 ± 0.02[c]	0.81 ± 0.01[d]	0.87 ± 0.01[e]	2.0
Ethyl 2-methylbutyrate	0.69 ± 0.01[a]	0.86 ± 0.02[b]	1.10 ± 0.04[c]	1.22 ± 0.01[d]	1.30 ± 0.01[e]	1.9
(*Z*)-3-Hexenyl acetate	3.09 ± 0.12[a]	4.00 ± 0.15[b]	5.59 ± 0.18[c]	6.22 ± 0.22[d]	6.58 ± 0.14[e]	2.1
2,3-Dimethylpyrazine	0.09 ± 0.00[a]	0.14 ± 0.01[b]	0.24 ± 0.00[c]	0.28 ± 0.00[d]	0.29 ± 0.01[d]	3.1
(*Z*)-3-Hexenol	0.29 ± 0.01[a]	0.43 ± 0.02[b]	0.64 ± 0.01[c]	0.74 ± 0.01[d]	0.79 ± 0.03[e]	2.7
2-Isobutylthiazole	2.03 ± 0.06[a]	2.71 ± 0.09[b]	3.89 ± 0.10[c]	4.50 ± 0.05[d]	4.74 ± 0.18[e]	2.3
Furfuryl acetate	0.48 ± 0.01[a]	0.72 ± 0.03[b]	1.04 ± 0.02[c]	1.23 ± 0.07[d]	1.34 ± 0.06[e]	2.8
Linalool	0.78 ± 0.05[a]	1.09 ± 0.09[b]	1.79 ± 0.05[c]	1.98 ± 0.10[d]	1.98 ± 0.02[d]	2.5
2-Pentylpyridine	1.05 ± 0.20[a]	1.42 ± 0.13[b]	2.55 ± 0.08[cd]	2.36 ± 0.14[c]	2.66 ± 0.15[d]	2.5
D-carvone	0.44 ± 0.06[a]	0.65 ± 0.05[b]	1.14 ± 0.03[c]	1.31 ± 0.0[d]1	1.35 ± 0.06[d]	3.0
β-Damascenone	1.44 ± 0.37[a]	1.94 ± 0.32[a]	4.46 ± 0.34[b]	4.79 ± 0.43[b]	4.41 ± 0.23[b]	3.1
γ-Nonalactone	0.03 ± 0.01[a]	0.04 ± 0.00[a]	0.09 ± 0.01[b]	0.11 ± 0.02[c]	0.11 ± 0.01[bc]	4.0
CV[2] (%)	7.5	6.1	3.1	4.2	3.4	

Values with different superscripts within a line are significantly different, ANOVA and DMR test ($P < 0.05$).
[1]Between flavour quantities released at the highest and the lowest flow rate.
[2]Mean coefficient of variation.

Table 3 Linear regression coefficients (R^2) of release kinetics in the apparatus at different volumetric air flow rates

Flavour	Volumetric air flow rate through the reactor (l/min)				
	3.3	4.8	7.2	9.0	9.6
Diacetyl	0.9732 ± 0.0042[a]	0.9954 ± 0.0024[b]	0.9997 ± 0.0001[c]	0.9994 ± 0.0007[c]	0.9997 ± 0.0000[c]
Isobutyl acetate	0.9701 ± 0.0039[a]	0.9942 ± 0.0032[b]	0.9994 ± 0.0003[c]	0.9999 ± 0.0001[c]	0.9999 ± 0.0000[c]
Ethyl 2-methylbutyrate	0.9653 ± 0.0029[a]	0.9936 ± 0.0034[b]	0.9996 ± 0.0000[c]	0.9999 ± 0.0000[c]	0.9999 ± 0.0000[c]
(*Z*)-3-Hexenyl acetate	0.9663 ± 0.0035[a]	0.9926 ± 0.0030[b]	0.9996 ± 0.0002[c]	1.0000 ± 0.0000[c]	0.9999 ± 0.0000[c]
2,3-Dimethylpyrazine	0.9680 ± 0.0046[a]	0.9910 ± 0.0049[b]	0.9988 ± 0.0005[c]	0.9997 ± 0.0002[c]	0.9991 ± 0.0002[c]
(*Z*)-3-Hexenol	0.9721 ± 0.0050[a]	0.9929 ± 0.0043[b]	0.9997 ± 0.0000[c]	0.9999 ± 0.0000[c]	0.9998 ± 0.0001[c]
2-Isobutylthiazole	0.9654 ± 0.0056[a]	0.9920 ± 0.0036[b]	0.9995 ± 0.0004[c]	1.0000 ± 0.0000[c]	0.9997 ± 0.0002[c]
Furfuryl acetate	0.9618 ± 0.0135[a]	0.9934 ± 0.0041[b]	0.9997 ± 0.0000[b]	0.9998 ± 0.0000[b]	0.9996 ± 0.0002[b]
Linalool	0.9446 ± 0.0085[a]	0.9780 ± 0.0041[b]	0.9957 ± 0.0018[c]	0.9988 ± 0.0006[c]	0.9974 ± 0.0012[c]
2-Pentylpyridine	0.9346 ± 0.0193	0.9659 ± 0.0062[b]	0.9899 ± 0.0038[c]	0.9981 ± 0.0007[c]	0.9902 ± 0.0058[c]
D-carvone	0.9381 ± 0.0127[a]	0.9718 ± 0.0057[b]	0.9927 ± 0.0032[c]	0.9976 ± 0.0007[c]	0.9954 ± 0.0025[c]
β-Damascenone	0.9152 ± 0.0204[a]	0.9471 ± 0.0089[b]	0.9758 ± 0.0069[c]	0.9918 ± 0.0072[c]	0.9840 ± 0.0064[c]
γ-Nonalactone	0.9701 ± 0.0360[a]	0.9640 ± 0.0094[a]	0.9632 ± 0.0071[a]	0.9905 ± 0.0066[a]	0.9835 ± 0.0133[a]
CV[1] (%)	1.13	0.50	0.19	0.13	0.23

Values with different superscripts within a line are significantly different (ANOVA and DMR-test, $P < 0.05$).
[1]Mean coefficient of variation.

release. For example, Figure 3 shows the release kinetics of linalool from water at different volumetric flow rates.

Influence of headspace volume/liquid volume ratio

Table 4 illustrates flavour amounts released at different headspace volumes in the reactor. For most of the compounds release was highest at the headspace/liquid volume ratio 2.5/2.5 (v/v). Release rates at ratios 1.25/3.75 (v/v) and 3.75/1.25 (<v/v) were mostly significantly smaller than those for the medium ratio (ANOVA and DMR test, $P < 0.05$) or showed at least the same trend. In turn, comparing the quantities of released volatiles at ratios of 1.25/3.75 and 3.75/1.25, differences were not significant for most of the volatiles.

Influence of artificial oral mucosa

Dynamic flavour release from water was measured after the reactor of the apparatus was equipped with a cylindrical shell of a roller bottle, coated on its inside with the model mucosa. The shell was immersed in synthetic saliva prior to experimentation, and saliva was continuously added during the experiment. For comparison, flavour release from water was analysed using a plain shell of a roller bottle and no artificial saliva was added. Mean coefficients of variation (CV) were 8.7 and 12.3% for experiments with and without mucosa, respectively. Student's *t*-test at $P > 0.05$ showed no significant difference between the release rates from water with and without application of artificial mucosa and saliva.

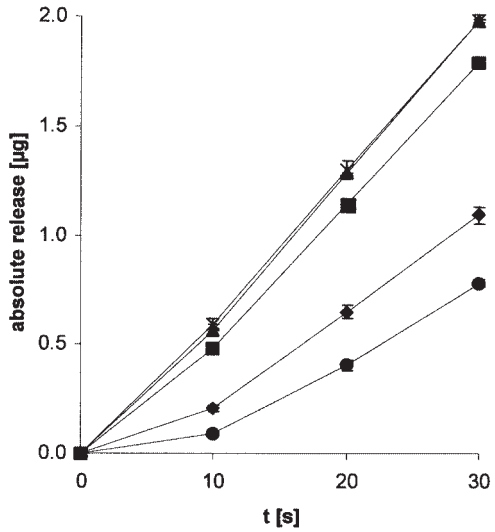

Figure 3 Effect of volumetric airflow rate on the release kinetics of linalool from water (circles, 3.3 l/min; diamonds, 4.8 l/min; squares, 7.2 l/min; stars, 9.0 l/min; triangles, 9.6 l/min; error bars represent variance of repeated measurements, $n \geq 3$).

Discussion

CV among the data of repeated experiments confirmed the good reproducibility of the apparatus, especially, as food-like flavour concentrations were applied (cf. CV in Tables 1–4). Furthermore, even considerably lower concentrations

Table 4 Effect of liquid:headspace volume ratio on dynamic flavour release [after 30 s, FID signal ($\times 10^{-3}$)]

Flavour	Ratio between sample and headspace volume (l/l) in the reactor of the apparatus		
	1.25:3.75	2.5:2.5	3.75:1.25
Diacetyl	4.6 ± 0.2[a]	4.9 ± 0.1[b]	4.6 ± 0.1[a]
Isobutyl acetate	6.8 ± 0.1[a]	8.5 ± 0.1[b]	7.4 ± 0.0[c]
Ethyl 2-methylbutyrate	12.2 ± 0.6[a]	17.2 ± 0.3[b]	14.2 ± 0.5[c]
(*Z*)-3-Hexenyl acetate	72.1 ± 8.1[a]	97.4 ± 1.9[b]	77.2 ± 3.9[a]
2,3-Dimethylpyrazine	3.1 ± 0.5[a]	4.0 ± 0.1[b]	3.5 ± 0.2[ab]
(*Z*)-3-Hexenol	6.9 ± 0.5[a]	7.7 ± 0.0[b]	6.8 ± 0.3[a]
2-Isobutylthiazole	56.2 ± 2.9[ab]	67.2 ± 0.4[a]	47.3 ± 9.4[b]
Furfuryl acetate	9.3 ± 0.7[a]	8.7 ± 1.3[a]	9.4 ± 0.1[a]
Linalool	31.8 ± 4.0[a]	43.4 ± 1.3[b]	32.5 ± 1.4[a]
2-Pentylpyridine	52.9 ± 5.1[a]	76.1 ± 2.3[b]	44.6 ± 5.8[a]
D-carvone	18.4 ± 2.0[a]	25.3 ± 0.0[b]	18.5 ± 0.1[a]
β-Damascenone	76.0 ± 10.0[a]	147.4 ± 7.2[b]	83.3 ± 2.2[a]
γ-Nonalactone	1.2 ± 0.2[ab]	1.6 ± 0.1[a]	1.0 ± 0.3[b]
CV[1] (%)	9.4	3.1	6.7

Values with different superscripts within a line are significantly different (ANOVA and DMR-test, $P < 0.05$).
[1]Mean coefficient of variation.

were well within the sensitivity range of the FID (data not shown).

Saliva effects

Comparative flavour release experiments showed no significant difference between the applications of synthetic or human saliva (van Ruth and Roozen, 2000; Friel and Taylor, 2001). For this reason artificial saliva was applied in the present study. The data showed that artificial saliva and its ingredients had no influence on the flavour release from liquids during initial consumption time. Using a saliva flow/mouth volume ratio similar to that in the human mouth (Rabe *et al.*, 2002), neither dilution nor the salivary ingredients influenced initial flavour release. These results are in agreement with *in vivo* data reported by Linforth *et al.* (2002). Applying APCI-MS coupled with human panellists, they also found the breath volatile concentration released from liquids to be unaffected by saliva. Static headspace experiments showed that dilution as well as mucin significantly affected the headspace concentration of volatiles above liquids (Friel and Taylor, 2001; van Ruth *et al.*, 2001). Additionally, when using non-static conditions in a comparison of different mouth models, van Ruth *et al.* (van Ruth *et al.*, 1995, 1996; van Ruth and Roozen, 2000) and Odake *et al.* (1998) also found effects of saliva on flavour release from different foods. However, one-point measurements after 15 min up to 60 min were used to arrange with the instruments sensitivity. This type of measurement represents a thermodynamic approach and can hardly describe the initial dynamic release process in the mouth cavity. On the other hand, these data can give information about the aftertaste phenomenon perceived by humans. After swallowing a food, residuals of the food matrix left in the mouth cavity, especially parts adhered to the pharyngeal part of the tongue or the throat (Buettner *et al.*, 2001) will be influenced by dilution and pH changes by saliva reacting as a buffer system (Roberts and Acree, 1995). Slow processes, such as adsorption to salivary proteins and mucous membranes, or enzymatic reactions (Hussein *et al.*, 1983; van Ruth *et al.*, 1996; Buettner, 2002a,b) will also affect the release from food residuals and will determine the persistence and overall profile of flavour molecules in the breath. In contrast to liquids, dilution and hydration are important factors for the retronasal flavour release from low moisture foods. Clawson *et al.* (1996) showed that the hydration of dry cereal foods with water had a strong impact on flavour release. This was related to the conditions found in the mouth. Brauss *et al.* (1999) also demonstrated the influence of hydration of dry foods. They found this effect to be determined by the physicochemical properties of the flavour molecules.

Shear effects

The results of the shear rate experiments are in good agreement with literature. Comparing different types of mouth models, Odake *et al.* (1998) and van Ruth and Roozen (2000) found, although not in real consumption time, that the addition of simulated mastication increased the release of many flavour compounds from vegetables and cream style dressings, respectively. However, the present results also revealed the opposite effect with less volatile compounds (Table 1). A plausible explanation for these data might be found in the solubility of these compounds. They possess high log*P* values, and their aqueous solubility is considerably lower than that of the other flavours. Vigorous mixing at high stirring rates might have improved the distribution of the compounds initially existing as droplet dispersions (Taylor, 2002) resulting in an improved dissolution or a real solution, which in turn caused decreased release rates. The degree of shear rate influence accordingly depended on the type of molecule and its specific physicochemical properties, which in turn caused considerably changed overall release profiles. Similar release values between 0 and 100 r.p.m. (0 and 33 s^{-1}) for most of the compounds in Table 1 can be explained by the constant interfacial area, as no vortex formation took place up to 100 r.p.m. Even though shear forces applied were small, depleted flavour concentrations at the liquid–gas interface are rapidly equilibrated, as first released flavour fractions are very small compared with the bulk concentration during initial flavour release (Rabe *et al.*, 2002, 2003a–c, 2004; Roberts *et al.*, 2003), and as flavour diffusion in the liquid phase is considered to be very fast (Harrison and Hills, 1997). Special sipping techniques, as

developed for the sensorial analysis of wine or tea, underline the importance of shear and the associated surface extension (air movement velocity and headspace volume in the mouth are discussed later) on retronasal volatile release from liquids.

Effects of volumetric airflow rate

Mastication not only increases flavour release in the mouth, but also influences the velum-pharyngeal performance during consumption (Buettner *et al.*, 2001). Investigating the oropharyngeal deglutition, Buettner *et al.* concluded that the movements of the velum, by closing off and opening the oral cavity to the airways and the nasal cavity, and thus inducing a certain flow of flavour loaded air, distinctively influence the aroma perception. High variability in these velum–pharyngeal performances between humans is considered to lead to the observed differences in aroma perception. The data of the present flow rate study verified these proposals. The volumetric flow rate in the mouth cavity towards the nasal cavity below the regio olfactoria is an important factor influencing the retronasal flavour release. Representing a constant drinking process, flow rates through the reactor of between 3.3 and 9.6 l/min corresponded to ~5–20 ml of exchanged flavour loaded air per swallow (swallow breath; Land, 1996; Rabe *et al.*, 2002). Depending on the volume of air, which was exchanged within initial consumption time, not only was the absolute amount of released flavour affected but so too was the overall flavour profile of the food (Table 2). This was due to the physicochemical properties of the molecules resulting in different degrees of release changes at changing volumetric flow rates of air. This specific behaviour of flavour compounds was also found by Deibler *et al.* (2001).

The pattern of flavours released depended on the volumetric flow rate of air. High flow rates resulted in linear release kinetics describing a constant transport of volatiles out of the simulated mouth cavity. At lower flow rates, this behaviour was only found after 10 s of release. Before, an enrichment of the headspace above the liquid took place. This pseudo-thermodynamic process should also occur when a food is placed in the mouth; ultimately, at the very start of consumption. Consistently, a larger headspace above the liquid in the reactor led also to the above described enrichment process and the corresponding kinetics (data not shown). As a result, flavour release rates were lower than those with medium headspace volume (Table 4). However, experiments with a medium headspace volume also showed larger release rates than those with small headspace volume, indicating the existence of an optimum headspace volume with maximum flavour release. An explanation for this behaviour could again be the velocity of air exchange in the reactor. Instead of being too slow, as in the case of larger headspace volumes causing the enrichment process, the exchange of air is too fast for the flavour's mass transfer velocity, so, in comparison to the situation with the medium headspace volume in the reactor, less flavour is transferred from the liquid into the gas phase causing a dilution effect.

Effects of artificial oral mucosa

The effect of epithelial cells possibly binding flavours during liquid consumption was shown to have no significant influence on the initial dynamic flavour release from water (Student's *t*-test, $P < 0.05$). This should be true as long as the liquid volume is large in comparison with the volume of saliva. On the other hand, when the volume of the consumed liquid is/becomes very small, that is, if the main portion is swallowed and only residuals are left in the mouth cavity, the pharyngeal part of the tongue or the throat, volatile binding to the corresponding mucosa layers and dilution with saliva will change the flavour release in terms of released flavour quantities and continuance of release. Due to flavour adsorption to the mucous layers (Hussein *et al.*, 1983; Linforth and Taylor, 2000) and/or mucin of saliva (van Ruth *et al.*, 1994; Friel and Taylor, 2001), and the improved solubility of flavours owing the increased volume of free water upon salivation available for the dissolution (Rabe *et al.*, 2003b,c), flavour release from residuals should decrease in comparison to that from the bulk liquid. On the other hand, previously volatilized or dissolved flavours adsorbed to the mucosa act as a reservoir which is continuously depleted with time, resulting in the aftertaste phenomenon. This effect of flavour adsorption/desorption considerably depends on the physicochemistry of the corresponding aroma compound (Linforth and Taylor, 2000).

Conclusion

The effects of physiological mouth parameters on dynamic flavour release from liquids were separately studied. A mouth model apparatus provided kinetic data of the initial 30 s of dynamic release under simulated mouth conditions. The results demonstrated that three main factors—(i) shearing/mastication (surface area; improved distribution or solubilization of non-dissolved flavour molecules), (ii) headspace volume and (iii) the volumetric airflow rate—were of significant importance for initial dynamic flavour release. Shear rate and volumetric airflow rate affected the amount of released flavour, with their extent depending on the type and physicochemical parameters of the flavour molecule. The compound-specific change of release resulted in a significant change of the overall flavour profile and might consequently influence flavour perception. In liquid food, artificial saliva and oral mucosa had no influence on the initial dynamic flavour release process.

Although the simulated mouth model constitutes an idealized situation, it is believed that the data produced with this apparatus contribute to the description and understanding of the large variations within *in vivo* flavour release measurements and individual flavour perception. As a result, the

present data convey insight into the factors influencing the human perception of food aromas.

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