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Computerized Apparatus for Measuring Dynamic Flavor Release from Liquid Food Matrices

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A fully computer-controlled apparatus was designed. It combines a glass reactor with a temperature-controlled hood, in which headspace volatiles are captured. Flavored liquids can be introduced into the reactor and exposed to conditions of temperature, air flow, shear rate, and saliva flow as they occur in the mouth. As the reactor is completely filled before measurements are started, creation of headspace just before sampling start prevents untimely flavor release resulting in real time data. In the first 30 s of flavor release the concentrations of the volatiles can be measured up to four times by on-line sampling of the dynamic headspace, followed by off-line trapping of the samples on corresponding Tenax traps and analysis using GC-TDS-FID. Flavor compounds from different chemical classes were dissolved in water to achieve concentrations typically present in food (micrograms to milligrams per liter). Most of the compounds showed constant release rates, and the summed quantities of each volatile of three 10 s time intervals correlated linearly with time. The entire method of measurement including sample preparation, release, sampling, trapping, thermodesorption, and GC analysis showed good sensitivity [nanograms $(10\ s)^{-1}$] and reproducibility (mean coefficient of variation = 7.2%).

KEYWORDS: Computerized apparatus; mouth conditions; real time; dynamic flavor release; sensitivity; reproducibility

INTRODUCTION

Historically, flavor release in the mouth was followed merely by sensorial techniques as reviewed by Piggott et al. (*1*).

Due to the disadvantage that, using pure aroma compounds, humans are able to follow the release of a maximum of only three to four flavor compounds at the same time (*2*), in vivo measurements were developed. A major breakthrough was achieved by coupling a human nose to the ion source of a mass spectrometer, enabling real time measurements for the first time (*3*, *4*). As an alternative option, nose-space was sequentially sampled onto a set of polymer traps to obtain a time-resolved flavor release. Off-line GC analysis of the traps was carried out after thermodesorption (*5*−*7*) or solvent desorption (*8*). Breath samples were also collected in special Tedlar bags followed by proton-transfer-reaction mass spectrometry (*9*).

Numerous flavor compounds can now be measured at the same time, but problems with the reproducibility of breath-by-breath experiments still exist. Different ways and duration of mastication, different composition and flow of saliva (*10*, *11*), and different tidal air flow and breathing characteristics (*3*) are responsible for variations between and, to a lesser extent, within panelists. Brown and Wilson (*12*) analyzed the flavor release from different gelatin gels in time intensity studies. Perceptual differences resulted from a high variation of chewing times. Buettner and Schieberle (*13*) showed that the duration of mastication had a large influence on the retardation of flavor molecules in the mouth. Not only the total amount of released odorants but also the profile in general was affected. The variation of one individual in replicates also depends on psychological and physiological factors such as mood, tiredness, hunger, and the biorhythm of a person's body, for example, different saliva flows at different times during the day (*11*). Also, selectivity problems using MS-nose techniques may occur due to the properties of the separation membrane used or overlapping single ions of different compounds in complex food flavors (*14*).

Investigating breath-by-breath analysis by coupling a human nose with an MS, Soeting and Heidema found variabilities between subjects expressed by a coefficient of variation (CV) of ∼80% and within one subject a CV of ∼20% (*3*). CVs ranging from 11 to 86% for the sampling of nose-space of strawberries (*15*), from 5 to 62% in nose-space analysis during the chewing of mints (*16*), and from 41 to 49% between panelists and ∼25% within one panelist eating imitation cheese (*17*) were reported by Taylor's group. These variations were due not only to the physiological factors given above but also to the complexity of the food matrices and the interactions

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between these variables. However, data on chewing gum with a mean CV of 32% for 11 panelists showed that variability of nose-space analysis can be improved if standardized eating, breathing, and swallowing protocols are applied (*18*).

Another approach of analyzing the retronasal flavor is the in vitro simulation of dynamic flavor release using devices that try to copy the environment of the human mouth as closely as possible. Trapping on polymers (*8*, *19*−*21*), cryofocusing on capillary columns (*22*), and direct MS coupling of the mouth simulating device (*17*, *23*−*25*) were applied to measure the volatiles released. A detailed summary of in vitro approaches is given by Piggott and Schaschke (*14*). Advanced devices were developed, for example, by van Ruth et al. (*19*) and Roberts and Acree (*21*). The advantages of in vitro devices are the increased reproducibility of flavor release data and the higher sensitivity in comparison to MS-nose techniques (*17*). Disadvantages are the considerably lower resolution of time/intensity data and the restriction of their use mainly to liquids, although the retronasal aroma simulator (RAS) is capable of analyzing solid and semisolid foods (*17*).

The concept of the apparatus presented was developed from the idea to represent an idealized situation of food consumption, whereas no attempt was made to simulate the oral mucosa. Full computer control of all mechanical and electronic parts of the apparatus allowed the monitoring of high-quality flavor release data in real time.

MATERIALS AND METHODS

Propylene glycol (PG) and 13 volatile flavors [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] were kindly provided by Dragoco (Holzminden, Germany). Molecules were selected from different chemical classes covering a broad range of polarity from log $P = -1.33$ for diacetyl to log $P = 4.04$ for β-damascenone. Log P values were calculated by ACD/LogP Database 4.5 software (Science Serve, Pegnitz, Germany). The purity of the compounds (>99%) was proved by means of GC-MS.

A 10% (w/w) stock solution was prepared using propylene glycol, a common solutizer, as a solvent for the flavor mix (*26*). Preliminary experiments showed that different initial concentrations of the flavor mix had no influence on the relative release of the compounds, indicating that interactions among the volatiles and between PG and the flavor molecules did not occur (data not shown). The composition of the aroma mix, the final concentrations of each compound in the bulk phase, and maximum concentrations commonly applied in nonalcoholic beverages are given in **Table 1**.

Artificial saliva consisted of mucin (2.8 g L^{-1}), lysozyme (0.11 g L^{-1}), α-amylase (0.5 g L^{-1}), d-glucose (0.01 g L^{-1}), urea (0.13 g L^{-1}), uric acid (0.03 g L^{-1}), cyclo-AMP (0.5 g L^{-1}), K_2HPO_4 (0.68 g L^{-1}), KCl (0.94 g L^{-1}), $CaCl_2$ (0.16 g L^{-1}), NaCl (0.75 g L^{-1}), and $NaHCO_3$ (1.09 g L^{-1}) as reported by Jenkins (*11*). Urea and D-glucose were purchased from E. Merck, Darmstadt, Germany. The other constituents were supplied by Sigma-Aldrich, Steinheim, Germany. Saliva was freshly prepared before experiments were started.

Design of the Apparatus. The construction scheme of the apparatus is shown in **Figure 1**. It consists of two main parts, a 5 L glass reactor and a self-constructed, temperature-controlled hood. On top of the reactor a glass lid (LAT, Garbsen, Germany) provided six unions for several connections of glass tubes and measurement tools. The hood ensured headspace sample capturing and storage at constant temperature (50 °C). The reactor and the lid were joined using a special connection device (Rettberg Reaction Vessel Accessories, Göttingen, Germany) and sealed with a Teflon-coated silicon O-ring. The temperature of the double-jacketed reactor was controlled by a heating immersion circulator (Julabo MV-4, Seelbach, Germany). The inner surface of the reactor was baffled in order to reduce vortex formation during stirring and to increase shear forces in the bulk phase. At the bottom of the reactor an automatic outlet valve (AV2, stainless steel; BSG MG34N, Ingelfingen, Germany) provided quick removal of the bulk phase fromthe reactor. In the center of the reactor lid a stirrer shaft was placed equipped with a six-star impeller for mixing the bulk phase. Two optional screwed blades for the adjustment of the Reynolds number (Re) in the headspace can be added. Sealing was provided by a conventional dynamic seal for stirrers (Hans W. Schmidt, Mainz, Germany). Shear rates were controlled by a stirrer (IKA Eurostar Power control-visc, Staufen, Germany), which also provided on-line torque values of the bulk phase.

Table 1. Composition of the Flavor Mix, Final Concentrations in the Bulk Phase, and Concentrations Applied in Nonalcoholic Beverages

flavor	portion in the flavor model (%)	final concn (mg L^{-1})	concn applied in nonalcoholic beverages[a] (mg L^{-1})
diacetyl	2.47	0.41	21
isobutyl acetate	0.06	0.010	13
ethyl 2-methylbutyrate	0.05	0.009	23
(*Z*)-3-hexenyl acetate	1.97	0.32	4
2,3-dimethylpyrazine	7.40	1.21	10
(*Z*)-3-hexenol	4.98	0.81	9
2-isobutylthiazole	4.23	0.69	1
furfuryl acetate	4.99	0.82	13
linalool	4.92	0.81	6
2-pentylpyridine	7.40	1.21	1[b]
D-carvone	7.38	1.21	41
β-damascenone	29.51	4.83	2
γ-nonalactone	24.63	4.03	13
initial total flavor	100.00	16.37	

[a] Maximum concentration (*27*). [b] Baked goods.

One union of the lid was used to join a tube for synthetic saliva addition using a gear wheel pump (Heidolph PD5130/ZP2, Schwabach, Germany). Temperature monitoring of the headspace and the bulk phase was possible using two conventional Pt100 thermoelements installed on top of the reactor. Air flow in the mouth was mimicked by a precisely controlled flow of pressurized air through the reactor. One inlet tube and one outlet tube (glass, i.d. = 1 cm), both regulated by automatic valves (AV1, stainless steel; AV3, Teflon; BSG MG12N/TG38T, Indelfingen, Germany), were connected to the lid. The flow of the carrier gas was regulated by an automatic mass flow controller (MFC; MKS 1179A, Munich, Germany). Purification of the air was achieved using a filter (Alltech 14633, Deerfield, IL) filled with activated charcoal. Sealed connections between unions and tubes as well as thermoelements were ensured using plastic screw caps with a centric drill hole and silicon rubber sealing rings coated with Teflon (Schott, Mainz, Germany).

The incubation hood consisted of a steel frame on which heat-resistant polyacrylate walls were fixed. The front and one side wall were transparent and designed as doors. A microprocessor-controlled ventilation device (Uniequip, Martinsried/Munich, Germany) assured exact temperature control of the hood. Approximately 50 °C was chosen to avoid condensation of volatiles onto the glass surface during the process of capturing and storage of headspace volatiles. In the hood four cascades of high-precision syringes (Poulten-Graf Fortuna 500 mL, Wertheim, Germany) provided a storage volume of 4 times 2 L of headspace. Each cascade including the corresponding inlet and outlet valves (**Figure 1**) was mounted on a separate drawer for easy installation and removal. The algorithm of the whole capturing and evacuation process was directed by a set of automatic valves (AV4−12, Teflon; BSG TG38T, Indelfingen, Germany) made of Teflon to prevent flavor adsorption and migration. Headspace samples were gently displaced from the syringes through corresponding Tenax traps (Gerstel desorption tubes-Tenax TA 60/80, Mühlheim an der Ruhr, Germany) by a vacuum pump (Ilmvac MP052Z, Ilmenau, Germany). The flow rate was controlled by a flow meter (Platon, Kledering, Austria) and manually adjusted using hand-operated PTFE valves (Rettberg, Göttingen, Germany).

The reactor, the valves, the high-precision syringes, and the Tenax trap holders were connected through glass tubes. Where necessary,

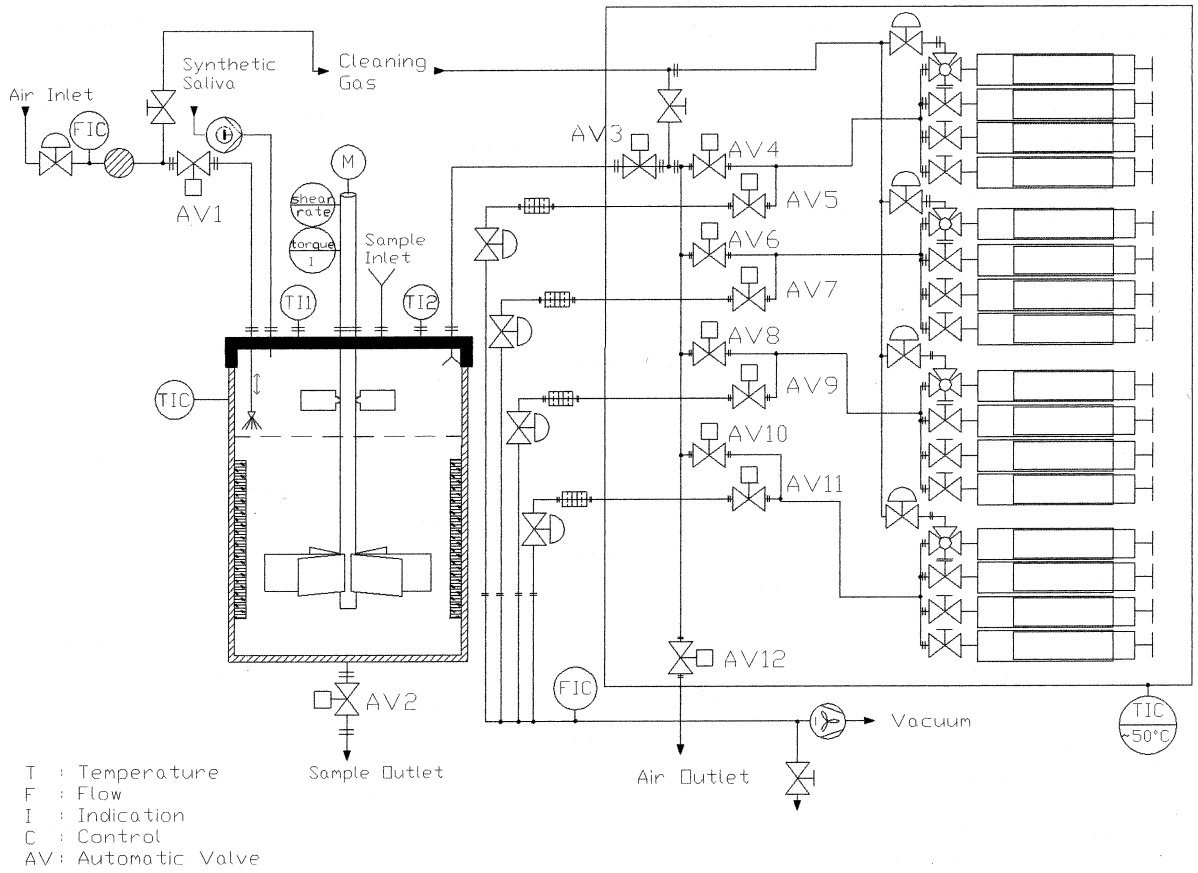

Figure 1. Apparatus for kinetic flavor release analysis from liquid food matrices in real time.

single glass parts were linked with plastic screw caps with a centric drill hole and silicone rubber sealing rings coated with Teflon (Schott).

The whole process of measurement was Labview 5.1 software (National Instruments, Munich, Germany) assisted, allowing control of all automatic valves, the stirrer, the gas flow, and the pumps. After a time algorithm was programmed on a personal computer, the software, via a special interface, provided reproducible activation of the electronic parts and data acquisition over time of the bulk phase and headspace temperatures, the gas flow rate, torque values, and the saliva flow rate.

All parts of the apparatus are commercially available.

Glass Treatment. All glass parts starting from the outlet of the reactor to the polymer traps including the high-precision syringes were silanized using a 10% (v/v) dichlorodimethylsilane (DCDMS) in toluene solution. Prior to silanization, glass surfaces were treated with 25% HCl overnight to clean the surface from metal atoms adsorbed on the glass surface. After the glass parts had been thoroughly rinsed with distilled water and methanol and dried below 100 °C, the 24 h silanization process started. Therefore, all glass parts were put into the DCDMS solution. Next, the parts were rinsed carefully with toluene and methanol and dried for another 24 h at 100 °C. Chemicals of analytical grade were all from Sigma-Aldrich, Seelze, Germany.

Instrumentation. *Gas Chromatography−Thermodesorption−Flame Ionization Detection (GC-TDS-FID).* Thermodesorption of the Tenax traps was carried out using a thermal desorption device (Gerstel TDS2, Mühlheim an der Ruhr, Germany) mounted on an HP 6890 GC (Agilent Technologies, Palo Alto, CA) equipped with a temperature-programmable vaporization inlet (Gerstel CIS 4 PTV). The PTV inlet incorporated a Tenax-packed liner (Gerstel glass liners−Tenax TA) and was cooled by liquid nitrogen. Analytical conditions were as follows: thermal desorption, 30−260 °C at 60 °C min^{-1} and held for 8 min; splitless mode; 50 mL min^{-1} desorption gas flow (N_2); PTV, 1 °C (cryofocusing temperature) to 260 °C at 12 °C min^{-1} and 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); carrier gas flow, 52 cm s^{-1} hydrogen; oven temperature, 40 °C (held for 1.5 min) to 130 °C at 4 °C min^{-1} to 180 °C at 8 °C min^{-1} to 250 °C at 25 °C min^{-1} and held for 10 min; detection, FID; 250 °C. Chromatograms were evaluated using HP ChemStation software (Agilent Technologies).

Table 2. Operating Conditions of the Novel Apparatus

condition	setting
temperature	37 °C
air flow	9.6 L min^{-1}
stirrer speed	450 rpm
saliva flow	175 mL min^{-1}
ratio bulk phase/headspace	4.88
vol of air samples	1.6 L $(10\ s)^{-1}$
no. of air samples	3 per 30 s

Gas Chromatography−Mass Spectrometry (GC-MS). Purity of flavor compounds was analyzed by means of GC-MS. On-column injection was performed using hexane solutions containing single flavor compounds. Instruments used were a GC 8060 (Fisons Instruments) equipped with a cool on-column injection port combined with a mass detector MD 800 (Fisons Instruments). Analytical conditions were as follows: injection, 0.5 μL cool on-column; column, 30 m × 0.32 mm i.d. × 0.25 μm DB-Wax (J&W Scientific); carrier gas, helium, 38 cm s^{-1}; oven temperature, 40 °C (held for 3 min) to 230 °C at 5 °C min^{-1} and held for 10 min; detection, electron impact mode; ionization voltage, 70 eV; continuous scanning from *m*/*z* 33 to 300; scan time, 0.95 s; interscan delay, 0.05 s.

Flavor Release Experiments. Operating conditions of the apparatus are given in **Table 2**. After full assembly of the apparatus, all automatic valves were in the closed position. Using a water bath at 37 °C the reactor temperature was adjusted. Next, the automation program designed by the software was initialized. The time table of operations is given in **Table 3**. When the air temperature in the reactor reached 34 °C, 1 g of the flavor stock solution containing 0.1 g of total flavor was dissolved in 5 L of water at 26 °C under slight stirring. This gave flavor concentrations of the single compounds in the range of micrograms to milligrams per kilogram (**Table 1**) typically present in

Table 3. Operation (down)/Time (acros) Table of the Automatic Software Program[a]

start	3 s	13 s	23 s	33 s	34 s
AV1+	Av2–	AV10–	AV8–	AV6–	AV12–
AV2+	AV3+	AV8+	AV6+	AV12+	AV3–
stirrer[b]+	AV10+			stirrer–	
saliva pump[c]+				saliva pump–	
MFC[d]+				MFC–	

[a] +, activated/open; –, deactivated/closed. [b] 450 rpm. [c] 175 mL min^{-1}. [d] 9.6 L min^{-1}.

nonalcoholic beverages (*27*). The solution was introduced into the reactor using a funnel inserted through a port of the reactor lid to fill it completely. This port was sealed again right after bulk phase addition, and the automatic process was started (**Table 3**).

Creation of 0.85 L of headspace ($n = 6$, CV = 0.6%, data not shown) was done by opening valve 2 and simultaneous introduction of air through the MFC and valve 1 at a flow of 9.6 L min^{-1}. Valve 2 was closed after 3 s and both valves 3 and 10 were opened to being on-line sampling of the headspace. Simultaneously, saliva addition into the reactor was activated at a flow of 175 mL min^{-1}. After 10 s of sampling, the first cascade was filled with 1.6 L of headspace and valve 10 was closed. At the same time valve 8 was opened to start the next 10 s filling of the second cascade. After closing valve 8 and simultaneously opening valve 6, the last sample was captured in the third cascade within 10 s, ending with the closing of valve 6. Now, real time sampling was finished and, simultaneously, air and saliva flow were stopped and valve 12 was opened to prevent overpressure.

The evacuation process of the cascades was achieved off-line by means of a vacuum pump. After adjustment of the gas flow using manual PTFE valves, the automatic valves 7, 9, and 11 were opened. In agreement with earlier studies (*5*, *28*), the flow rate through the corresponding polymer trap for flavor accumulation ranged from 60 to 80 mL min^{-1}. Using two traps in series it was shown that no breakthrough of flavors occurred. After the evacuation process, the Tenax traps were sealed with Swagelok end caps (Gerstel). Consecutively, traps were analyzed using GC-TDS-FID. Chromatograms showed no degradation products due to the thermodesorption process. Second analysis of thermodesorbed traps proved complete desorption of the flavor molecules from the Tenax material. Volatiles were quantified independently by external calibration of each flavor compound. The regression line consisted of four triplicate concentration points. Regression analysis was done in Excel, and R^2 values ranging from 0.9979 to 1 showed good correlation between compound concentration and FID peak area. Flavor release experiments from water were replicated four times.

Statistical Analysis. Analysis of variance (ANOVA) was performed on release data. Duncan's multiple range (DMR) test was carried out to determine significant differences among released flavor quantities of the 10 s time intervals. A significance level of $p = 0.05$ was applied.

RESULTS AND DISCUSSION

Most artificial mouth model devices provide temperature control, shear force input control, and saliva addition. The high degree of automation of the software-controlled apparatus offers additional features, particularly, quantification of real time flavor release in different liquid food matrices.

Creation of Headspace. The reactor of the apparatus is filled completely before the measurement is started. Then, within 3 s a defined headspace volume of 0.85 L is created. As there were no data available in the literature, our own experiments suggested a ratio between headspace and bulk phase of 0.85 to 4.15 L, as ~80 mL of water was contained in the mouth, whereas an estimated headspace volume of ~20 mL was still coexisting (data not shown, *29*). Headspace creation just prior to sampling start ("starting from zero") avoids untimely flavor release into the headspace. If the reactor would first have to be filled with a sample volume of 4.15 L, premature equilibration of volatiles into the headspace would occur, and the true kinetics of release in the first few seconds would not be measurable (see, for example, refs *19* and *21*).

Sampling of Headspace. After headspace creation, the sampling of dynamic headspace is started using a defined flow of pressurized air. Flavors are captured on-line in optionally up to four cascades of high-precision syringes at either different or identical time intervals. This volume displacement principle allows almost isobaric sampling. During the measurements described in this paper three cascades were used in 10 s time intervals resulting in a flavor release study of up to 30 s. A flow rate of 4.8 L per 30 s was chosen to fill each cascade on-line with 1.6 L of headspace during simulated liquid consumption. As the flow is constant, it has to be clearly pointed out that this protocol does not reflect the real situation in the mouth during the consumption of liquids. First, 30 s of ongoing consumption of liquids is rather untypical, unless a person is very thirsty. Second, constant exchange of air between the mouth cavity and the nasopharynx was shown not to occur when liquids are being drunk (*30*). Any in vitro approach is not and will never be able to supplant the mouth cavity with its complex physiology: for example, the process of oropharyngeal deglutition activates 26 muscle groups within a very short period of time (*30*). On the other hand, the low variability from experiment to experiment and the high sensitivity of the apparatus can produce high-quality dynamic release data. An idealized situation was assumed: a displacement of 5−20 mL volumes of air (*31*, *32*), the so-called "swallow-breath", out of the mouth cavity per deglutition, ~10 deglutitions per 30 s, and a mouth volume of 100 mL (*29*). Accordingly, the flow of air through the vessel had to be 2.5−10 L per 30 s to fit mouth conditions (cf. **Table 4**). Thus, a flow rate of 9.6 L min^{-1} was chosen for the simulation of a constant drinking process. A usable volume of each cascade from 100 to 2000 mL allowed for adjustment for different flow rates.

In comparison to the flow rate used in this work the set gas flow rates given in the literature using either nitrogen or helium differ to a different extent from the assumed reality (**Table 4**). A valid parameter to characterize the flow of fluids is the Reynolds number. The definitions of Re for tubes and stirred tanks are given in eqs 1 and 2, respectively.

$$\mathrm{Re} = wd/\nu \quad (1)$$

$$\mathrm{Re} = nd_2^2/\nu \quad (2)$$

w is the mean velocity of the fluid, d is the diameter of the tube or the hydraulic diameter, n is the stirrer speed, d_2 is the diameter of the impeller, and ν is the kinematic viscosity of the fluid. Studying the influence of Re on dynamic flavor release in vitro, Marin et al. (*33*) found the laminar and turbulent character of Re to be important for compounds with low partition coefficients between air and water (K_{aw}) for systems at equilibrium. No influence of Re was shown for compounds with high K_{aw}. Both Re and K_{aw} influenced the dynamic flavor release of compounds with medium K_{aw}. A Re of ~500 describing laminar flow in the mouth cavity was estimated by Overbosch et al. (*34*). Accordingly, a Re of the same range should exist during the sampling process using the presented apparatus. Applying eq 1 produces a Re of ~100 (**Table 4**) characterizing laminar conditions. Although Marin et al. (*33*) found the laminar character of gas flow only to be less important for the influence on dynamic flavor release, further studies on the effect of different laminar flow rates may be important.

Table 4. Characteristics of in Vivo and in Vitro Approaches to the Study of Flavor Release in the Mouth and under Mouth Conditions

method	real time measurements	reproducibility: mean CV for replicates (%)	sensitivity: minimum concn in the bulk phase	ratio "mouth" vol/gas flow rate[a]	Re[b] in the headspace	ratio "mouth" vol/saliva flow	shear rate introduction (s^{-1})
			In Vivo				
assumed mouth conditions	yes			0.66–2[c]	~500[d]	30–100[c,e]	10–1000[f]
ref *3*, breath by breath, 1988	yes	50[g]	120 mg kg^{-1}	0.66–2	~500	30–100	10–1000
ref *12*, TI, 1996	yes	29[h]	10 g kg^{-1} [i]	0.66–2	~500	30–100	10–1000
ref *18*, breath by breath, 2001	yes	32[h]	14 mg kg^{-1} [k]	0.66–2	~500	30–100	10–1000
			In Vitro				
ref *23*, 1986	no	–[l]	450 mg L^{-1}	0.03	~3.5	6[m]	yes
ref *22*, 1995	yes	<8[g]	1 μl L^{-1}	0	0[n]	8[m]	yes
ref *21*,1995	no	<5[o]	0.5 mg L^{-1}	0.83	~15.2	2.5–15[m]	332
ref *24*,1996	no	19[g]	10 mg L^{-1}	4.16	~0.9	–	10–200
ref *25*, 1999	no	<1[g]	50 μl L^{-1}	–[p]	–	–	yes
ref *35*, 2000	no	25	–[q]	0.7	~3.8	8.75–70[m]	yes
new apparatus	yes	<8[g]	9 μg L^{-1}	1.04	~100	30–100	150

[a] Assumption, as given in the text. [b] Calculated from eq 1. [c] Reference *29*. [d] Reference *34*. [e] Reference *36*. [f] Reference *38*. [g] Liquid matrix. [h] All kind of foods. [i] Total concentration of banana flavor composition. [k] Reference *27*. [l] Not given. [m] Saliva addition prior to experiments. [n] Static headspace. [o] All kinds of foods, but with low tear resistance. [p] Reactor volume not given. [q] Natural product (French beans, bell peppers), 60 min of sampling time.

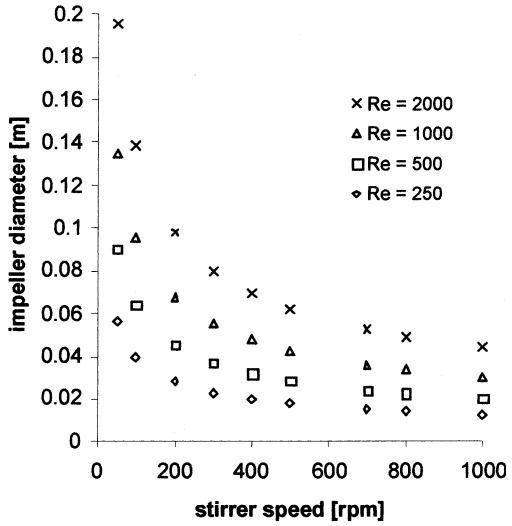

Figure 2. Correlation between impeller diameter and stirrer speed for the optimization of fixed Reynolds numbers (Re) in the headspace above food matrices in the reactor.

Therefore, the shaft of the stirrer can optionally be equipped with blades of various sizes and shapes for further Re adjustment. Re optimum can be adjusted using eq 2 to a set Re of 400, calculated by the difference of the in-mouth Re and the Re contributed by the air flow. The correlation of blade size versus stirrer speed to produce defined values of Re is given in **Figure 2**.

Addition of Artificial Saliva. Saliva is continuously added by means of a pump. This is closer to eating conditions than adding the total volume before the start of the experiment (*21*−*23*, *35*). The average flow of saliva of adults during the day is ~0.25−1.0 mL min^{-1} without stimulation and ~1.0−3.5 mL min^{-1} with stimulation (*36*). Considering an average mouth volume of 100 mL (*29*), the saliva flow in the device should reach 50−175 mL min^{-1} to ensure real mouth conditions during eating (cf. **Table 4**).

Addition of Shear Force. Shear force input is supplied by a stirrer equipped with a six-star impeller in adaptation of a work of Rao and Cooley (*37*). Using the same type of impeller with slightly different blade size, they estimated the shear rate as

$$\gamma_e = k_s n \qquad (3)$$

where γ_e is the effective shear rate of the impeller, n is the rotational speed (rps), and k_s is a proportionality constant empirically found as ~20. In the experiments described here a stirrer speed of 450 rpm was used, resulting in a shear rate input of 150 s^{-1}. Shama and Sherman (*38*) reported that shear rates during food consumption in the mouth ranged from 10 to >1000 s^{-1}. A further function of the stirrer is the measurement of torque values of the liquid bulk phase, creating data on sample viscosity and viscosity changes with time.

Table 5. Flavor Quantities (micrograms) Released from Water within 10 s Time Intervals[a]

flavor	0–10 s	10–20 s	20–30 s
diacetyl	0.280a	0.279a	0.259a
isobutyl acetate	0.267a	0.286a	0.281a
ethyl 2-methylbutyrate	0.418a	0.437a	0.422a
(*Z*)-3-hexenyl acetate	7.341a	7.754a	7.750a
2,3-dimethylpyrazine	0.277a	0.326b	0.300ab
(*Z*)-3-hexenol	0.850a	0.915a	0.882a
2-isobutylthiazole	5.188a	5.579a	5.571a
furfuryl acetate	1.030a	1.073a	1.044a
linalool	2.611a	2.946a	2.899a
2-pentylpyridine	5.587a	7.034b	6.515b
D-carvone	2.221a	2.571a	2.485a
β-damascenone	22.050a	30.599b	27.182ab
γ-nonalactone	0.163a	0.253b	0.259b

[a] Values with different letters within a row are significantly different, ANOVA and DMR test ($p < 0.05$).

Dynamic Flavor Release from Water. First experiments using the apparatus showed that the flavor release of 9 of 13 volatiles was constant during measurement time. Consequently, the released quantities of these compounds within the 10 s time intervals showed no significant differences according to ANOVA at $p < 0.05$ (**Table 5**). Therefore, within 3 s of headspace creation, flavor release must have been very fast, and

volatile transfer from the aqueous to the gas phase reached a constant velocity representing a dynamic partition coefficient. In agreement with this, small compounds were reported to be able to almost reach equilibrium with aqueous systems within a few seconds (*31*, *39*). Molecules with good water solubility, in this study 2,3-dimethylpyrazine, and volatiles possessing low volatility, such as β-damascenone, γ-nonalactone, and 2-pentylpyridine, showed a different behavior (**Table 5**). These molecules were less released within the first 10 s time interval (DMR, $p < 0.05$), showing that the process of mass transfer did not reach a steady state within the time of headspace creation in these cases. However, similar release rates for the second and third time intervals indicated a constant velocity of volatile transfer within the first 10 s of sampling of the compounds. This was especially true for γ-nonalactone and 2-pentylpyridine, as these compounds showed also significantly different release quantities during the third time interval. This difference was insignificant for β-damascenone and 2,3-dimethylpyrazine, but consistently higher release rates were obtained during the last time interval (**Table 5**). The summed flavor release from water in the course of the first 30 s correlated linearly with time for each compound measured. Linear regression coefficients calculated from the accumulated 10 s time interval measurement points ranged from 0.9911 to 0.9999. Using the slope of the regression equations, the released quantity of each flavor in the reactor can be directly calculated for times up to 30 s, respectively.

Flavor Release Profiles from Water. In vivo release profiles of foods that stay in the mouth for >30 s and of which only parts of the bolus are swallowed periodically, for example, soft mint-flavored sweets (*40*) or chewing gum (*18*), showed, after an exponential rise, a constant release of flavor, because the matrices constituted a depot of volatiles. Similar results were obtained for the release of butanone from water, but a slight decline of butanone release over time showed a steady exhaustion of the 1 mL water sample in the mouth (*3*). The release profiles generated by the apparatus differ from MS-nose profiles by the missing exponential character of the initial release phase. The authors consider the flavor release from the aqueous to the gas phase during creation of headspace within the first 3 s to be comparable with the in-mouth release process during ingestion of liquids, which typically lasts ~2−3 s (*41*) while the mouth cavity is completely closed off from the airways (*30*). A constant velocity of mass transfer for a majority of volatiles can be assumed during this process. However, this constant release is initially disturbed in vivo when volatiles are passing the retronasal route. Results of Linforth and Taylor (*42*) and Linforth et al. (*32*) are useful to explain the initial character of the release profile. Linforth et al. showed that the swallow breath concentration of flavors measured by APCI-MS via the nose is reduced due to dilution by exhaled air (*32*). Additionally, volatiles are absorbed into the nasal mucosa and afterward gradually desorbed depending on the physicochemical character of the compounds (*42*). Thus, the in vivo system needs a specific time for absorption and desorption processes to finally result in a dynamic equilibrium state at which constant release rates are exhaled through the mouth. The whole process is even more complex, because after food material is swallowed, a film containing saliva and residuals of the food with volatiles will be formed at the oral and pharyngeal mucosa (*30*), contributing to the desorption and absorption processes. Furthermore, compounds that will be less released from liquids within initial ingestion also contribute to the exponential character of the initial release profile (**Table 5**). Obviously, these complex conditions cannot be simulated using any apparatus, and a comparison of in vitro and MS-nose data is not useful. On the other hand, technical approaches can provide insight into the in-mouth flavor release. As the results of the apparatus showed no absorption of flavors during release experiments, the device appears to be useful for the study of single, separated variables affecting retronasal release such as synthetic saliva and simulated oral mucosa.

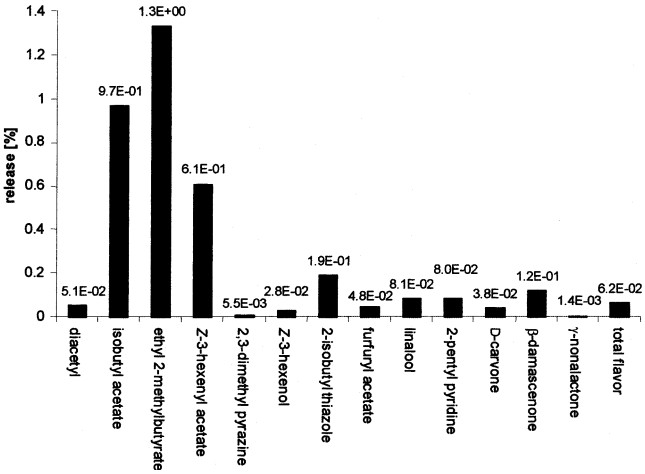

Figure 3. Comparison of flavor compounds from different chemical classes: total flavor release from water after 30 s. Release is expressed relative to the initial concentration of each compound.

Table 6. Coefficients of Variation for Flavor Release Experiments from Water Using the Novel Apparatus (Coefficients Were Calculated from Four Replicates)

flavor	10 s CV (%)	20 s CV (%)	30 s CV (%)
diacetyl	7.1	6.6	7.3
isobutyl acetate	8.3	6.4	6.5
ethyl 2-methylbutyrate	6.8	5.8	6.5
(*Z*)-3-hexenyl acetate	8.2	5.2	6.5
2,3-dimethylpyrazine	8.1	4.3	6.2
(*Z*)-3-hexenol	8.1	5.0	7.3
2-isobutylthiazole	7.9	5.5	7.0
furfuryl acetate	8.8	5.0	8.8
linalool	9.0	6.4	7.4
2-pentylpyridine	5.5	7.1	6.8
D-carvone	9.1	6.1	6.7
β-damascenone	12.7	12.6	12.8
γ-nonalactone	6.4	2.0	6.1

Direct comparison of relative flavor release, defined as the amount released within the first 30 s divided by the initial amount in the bulk phase, is shown in **Figure 3**. The relative release ranges from 0.0014% for γ-nonalactone to 1.33% for ethyl 2-methylbutyrate, showing that, depending on the type of molecule, the relative release rates differed by a factor of ~1000. The total flavor release with 0.062% after 30 s was very low. Hence, it appears that most of the flavor in foods in fact is swallowed before it becomes perceivable. Especially, isobutyl acetate, ethyl 2-methylbutyrate, and (*Z*)-3-hexenyl acetate were quickly released from water. The opposite was true for γ-nonalactone and 2,3-dimethylpyrazine: despite high concentrations in the flavor mix (**Table 1**), only little release was measured.

Reproducibility and Sensitivity of Release Measurements. The entire method of measurement reported in this paper including sample preparation, release, sampling, trapping, thermodesorption, and GC analysis showed good sensitivity [nanograms $(10\ s)^{-1}$] and reproducibility. **Table 6** shows the CV for each compound used ranging from 2.0 to 12.8% for the

three points of measurement. Although low flavor concentrations, typical of real foods (**Table 1**), were used, 92% of all CVs were <9.1%. β-Damascenone, as an exception, showed a CV >10%. This is explained by inhomogeneous distribution in water due to its high log *P* value. However, the concentrations of all odorants were at infinite dilution level, at which interactions are not likely to occur (*43*, *44*).

In Vitro versus in Vivo Measurements. For the assessment of flavor profiles of foods one can never forego a person's sensorial impression. Nevertheless, by simulating and controlling mouth conditions and eating parameters, such as mouth movement and shear rate, oral air flow, composition and flow of saliva, and mouth temperature, in vitro measurements can contribute to the knowledge of retronasal flavor release. Recently, the correlation between retronasal flavor release measured in vitro and in vivo using imitation cheese and chocolate as well as real foods (orange juice, banana, and cookies) was successfully verified (*17*). These results showed that the RAS developed by Roberts and Acree (*21*) was able to simulate retronasal flavor release. As shown in **Table 4**, the characteristics of this device are in good agreement with real mouth conditions. Temperature control, shear rate input, and gas flow through the blender, characterized by Re, approximate reality. However, within initial experiments using the RAS the first point of measurement was after 2.5 min (*21*). Combining the equipment with an APCI-MS resulted in on-line release data, but according to the authors "the study of temporal dimension of release" was not possible (*17*).

The presented apparatus follows the temporal dimension of flavor release from liquid food, as measurements are carried out in a kinetical rather than a thermodynamical manner. Hence, by avoiding premature release of aroma compounds using the "from zero start" technique, analysis of flavor release in real time is possible via on-line sampling of dynamic headspace and applying the idealized situation of continuous drinking and exchange of swallow breath between the oral and nasal cavities. Obviously, its dimensions do not fit the real world, but the size of the apparatus allows the analysis of realistic flavor concentrations in the food matrix down to micrograms per liter (**Table 1**) or even lower (data not shown). Therefore, in comparison to other in vitro approaches, its sensitivity is considerably increased (**Table 4**). The ratio between reactor volume and air flow describes mouth conditions producing a Re of ~100 (**Table 4**), which is optionally adjustable. Optimization of this parameter is possible using optional screwed blades for the mixing of headspace above the food matrix. Instead of preparing a presolution of the food matrix and the saliva prior to the start of the experiments, continuous addition of of saliva during the release experiments further improves mouth characteristics.

Simulation of the oral mucosa will be considered in future studies, as its influence seems to be of considerable significance for the retronasal flavor release (*42*, *45*). As the apparatus was shown to produce real time flavor release profiles, it is now important to verify these results with human in-mouth data. Furthermore, future application of the apparatus to study the influence of physicochemical properties of liquid food matrices and flavor molecules on the mechanism of dynamic flavor release under idealized mouth conditions will contribute to the insight into retronasal and in-mouth flavor release, ultimately utilizing the advantages of in vitro approaches.

ABBREVIATIONS USED

CV, coefficient of variation; RAS, retronasal aroma simulator; AV, automatic valve; Re, Reynolds number; MFC, mass flow controller; DCDMS, dichlorodimethylsilane; ANOVA, analysis of variance; DMR test, Duncan's multiple range test.

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