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Investigation of the Retronasal Perception of Strawberry Aroma Aftersmell Depending on Matrix Composition

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The development of strawberry aroma aftersmell after consumption in aqueous and milk model systems was followed. A recently developed sensory approach was used to seize the qualitative, quantitative, and temporal aspects of aroma profile development. The results obtained from sensory evaluation were then correlated to analytical data, obtained by means of intraoral aroma detection using the buccal odor screening system (BOSS). A clear correlation was found between the sensory persistence of single odor impressions and the intraoral detectability of odorants by BOSS. For the strawberry aroma mixture it could be shown that BOSS is capable of selecting those odorants of a complex aroma mixture that elicit a certain aftersmell impression developing after a certain period of time in the oral cavity. Also, the changes in in vivo odorant persistence depending on changes in food matrix composition could be directly followed by means of BOSS.

KEYWORDS: Buccal odor screening system; BOSS; aftersmell; SBSE; aroma profile development

INTRODUCTION

Aroma persistence is little understood at present. Some food aromas can be perceived for a considerable time after consumption, whereas others linger for just a short period. Most studies on retronasal aroma perception have been targeted at the temporal aspect of immediate aroma impressions during chewing and swallowing, and only a few investigations have dealt with aftersmell impressions after the food has been swallowed. It has been shown that due to the complexity of the oropharyngeal performances the aroma pulses during eating often differ considerably from those after swallowing (*1*−*4*). However, often both types of retronasal perceptions were studied as one sensation by means of time−intensity profiling. One attempt to gain insight into the persistence behavior of volatiles in vivo has been performed by breath analysis using APCI-MS (*5*). By measuring the ratio between the first and second breaths after swallowing, considerable differences in odor intensity decline were found depending on odorant structure. These differences in direct breath decline were assigned as reasons for odorant persistence. However, no sensory evaluations of persistence were related to the analytically determined persistence. On the basis of the experimental data, an empirical model was developed with hydrophobicity, volatility, ether linkage, and carbonyl count as key parameters.

Other studies described theories on the physiological sources for prolonged aroma delivery (*6*−*9*). Although release of odorants during eating can be regarded as a direct process from food material or saliva itself, the subsequent release of aromas after swallowing involves liberation from in vivo aroma depots delivering odorants to the exhalation breath. Aroma depots can be odorants that are adsorbed to oral and pharyngeal mucosa and coatings of odorant-loaded food matrices on oral and pharyngeal mucosa. Previously, adsorption of odorants to oral mucosa has been quantified indirectly by rinsing model solutions in the oral cavity using the spit-off odorant measurement (SOOM) (*6*−*8*). Furthermore, it has been used to elucidate matrix effects on odorant persistence *(10)*. It has also been shown that for aroma persistence degradations of odorants with saliva have to be taken into account (*6, 11, 12*). Generally, the release of adsorbed odorants from oral mucosa, as a key premise for their continued perception, has only been hypothesized. Later, modeling studies incorporated the idea of continuous release of odorants from the pharyngeal areas of the throat and aimed to describe breath-by-breath aroma profiles theoretically (*13*−*15*).

Regarding aroma depots, a coating effect on persistence has been recently observed with lower persistence of ethyl octanoate when consumed in water than in a lipid emulsion (*3*). Coating formation of the emulsion on pharyngeal mucosa with continuous odorant delivery in the throat has been discussed. Another aspect of matrix materials as persistence modulators is competition of matrix substances with mucosal adsorption. It has been recently shown that milk can retain odorants such as pyrazines from adsorption to oral mucosa (*10*). Sensory persistence was found to be considerably reduced.

Generally, precise sensory evaluation of aroma persistence is rare, mainly due to the fact that determination of a perception

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Table 1. Strawberry Aroma Composition and Final Odorant Concentrations in the Water and Milk Samples, Retronasal Odor Threshold Values (ROTV), Retronasal Odor Activity Values (ROAV), Molecular Weights (MW), and log *P* Values of the Single Constituents in the Samples

odorant	odor quality	concn (mg/L)	ROTV (μg/L)	ROAV	MW	log *P*[a]	RI[b] (DB-FFAP)
ethyl butanoate	fruity	36	0.1	360000	116	3.00	1028
ethyl 3-methylbutanoate	fruity	4	0.1	40000	130	3.52	1041
ethyl hexanoate	fruity	8	0.6	13333	144	4.04	1226
(*Z*)-3-hexenyl acetate	fruity	2	12.1	175	142	3.63	1328
methyl cinnamate	sweet	9.6	11	873	162	3.69	2056
styrallyl acetate	sweet	0.4	39	10	164	3.45	1680
benzyl acetate	sweet	0.8	37	22	150	3.15	1704
methyl anthranilate	sweet	0.4	1.5	267	151	1.52	2229
methyl dihydrojasmonate	sweet	2	28	71	226	4.08	2265
γ-decalactone	coconut-like	8	88	91	170	4.45	2137
hexanal	grassy	0.4	10.5	38	100	1.81	1072
(*Z*)-3-hexenol	grassy	6	30	200	100	1.46	1389
β-ionone	violet-like	0.4	0.1	4000	192	4.22	1933
4-HDF	caramel-like	2	30	67	128	–0.89	2031
vanillin	vanilla-like	2	30	67	152	1.08	2569

[a] log *P* values were calculated according to ref *25*. [b] Retention indices were calculated according to ref *26*.

duration and the exact end-point poses some difficulties. Recently, it has been shown that consumption of espresso coffee can elicit aroma sensations up to 30 min after consumption, whereas the aroma of Chardonnay wines lasted only a few minutes (*10*, *16*, *17*). Using a novel analytical approach, the buccal odor screening system (BOSS), it has been found that these impressions are directly correlated with the intraoral detectability of characteristic espresso and wine aroma compounds. The technique is based on a modified stir bar sorptive extraction (SBSE) system, comprising intraoral aroma extraction at defined times after food consumption under optimized in vivo sampling conditions followed by analysis via high-resolution gas chromatography−olfactometry (HRGC-O). Sensory retronasal aroma evaluation, with panelist interrogation at precisely defined time intervals, is then related to the analytical data. This methodology offers the possibility to screen intraorally even traces of key aroma compounds for their impact on prolonged aroma perception or to follow changes in aftersmell induced by variations in the food matrix composition (*10*, *15*, *16*). Using this technique, the aim of the present work was to characterize the retronasally perceived aftersmell of a model strawberry aroma with time, thereby varying the matrix system. Time-resolved sensory evaluation should be compared to the analytical data obtained by the BOSS approach.

MATERIALS AND METHODS

Chemicals. The following odorants were obtained from the suppliers shown: ethyl butanoate, ethyl 2-methylpropanoate, ethyl 3-methylbutanoate, ethyl hexanoate, butane-2,3-dione, pentane-2,3-dione, 2-furfurylthiol, hexanal, (*Z*)-3-hexenol, 2,5-dimethyl-4-hydroxy-2(5*H*)-furanone (4-HDF), 3-isobutyl-2-methoxypyrazine, 2-ethyl-3,5-dimethylpyrazine, 3-(methylthio)propanal, 3-methylbutanal, 2-/3-methylbutanoic acid, (*E*,*E*)-2,4-nonadienal, (*E*)-2-nonenal, octanal, and γ-decalactone (Aldrich, Steinheim, Germany); acetic acid, butanoic acid, 2-methoxyphenol, and vanillin (Merck, Darmstadt, Germany); β-ionone (Roth, Karlsruhe, Germany); (*E*/*Z*)-2,6-nonadienal (Alfa Products, Karlsruhe, Germany); 1-octen-3-one, 4-vinyl-2-methoxyphenol, and (*Z*)-3-hexenyl acetate (Lancaster, Mühlheim, Germany); methyl cinnamate, styrallyl acetate, benzyl acetate, and methyl dihydrojasmonate (Givaudan, Dübendorf, Switzerland); methyl anthranilate (Merck). (*E*)-β-Damascenone was a gift from Symrise (Holzminden, Germany). *trans*-4,5-Epoxy-(*E*)-2-decenal was synthesized according to the method of ref *18*. The compounds were freshly distilled prior to analysis. Chemical and sensory purity was checked by high-resolution gaschromatography−olfactometry (HRGC-O) and high-resolution gas chromatography−mass spectrometry (HRGC-MS).

Strawberry Aroma. The strawberry aroma, as used in COST 921 "Food Matrices: Structural Organisation and Impact on Flavour Release and Perception", was kindly provided by Givaudan. The composition is given in **Table 1**.

Poly(dimethylsiloxane) (PDMS)-Coated Stir Bars. Commercially available Twister-SBSE bars (10 mm long, 0.5 mm PDMS film thickness; Gerstel GmbH, Mühlheim a/d Ruhr, Germany) were used. Prior to analysis, the bars were subjected to a conditioning procedure according to the suppliers recommendations: the stir bars were soaked in 100% acetonitrile for at least 2 days and then conditioned at 300 °C for 4 h.

Each bar was screened for odorants ("background"; 15) and then directly applied for analysis. Stir bars were used for a single experiment, then reconditioned and screened for background again. Experiments were performed with at least three different bars to take into account SBSE bar variation.

Encapsulation of the SBSE Bars. For intraoral application, adapted glass capsules (15 mm length for 10 mm bars, i.d. = 5 mm) were designed (*15*) and sealed with a glass stopper. To allow unhindered penetration of air and saliva, the capsules were regularly perforated with pores (1−2 mm diameter) with a distance of ~3 mm between pores.

Preparation of Aqueous Odorant Model Solutions. One percent stock solutions of the single odorants in absolute ethanol were freshly prepared and diluted with deodorized water prior to analysis to obtain 500 mL of single aqueous solutions of each odorant (concentration = 100 and 1000 μg/L of water, respectively). From these, the respective concentrations of the odorants for determination of their retronasal odor threshold values (ROTV) at 2-fold concentrations steps or at the concentrations given in **Table 1** were prepared by further dilution with deodorized water.

Preparation of Strawberry Aroma Samples. The aroma stock solution was diluted with pure EtOH. From this aroma solution 100 μL was pipetted into 100 mL of water or milk (3.5% fat, UHT) to obtain the final odorant concentrations as given in **Table 1**.

Panelists. Panelists were nonpregnant volunteers (nonsmokers) of the Technical University of Munich, with no known illnesses at the time of examination and normal olfactory and gustatory function. The panelists had a normal salivary flow and excellent oral hygiene. Ten assessors (five males, five females) were recruited and trained in preceding weekly training sessions in recognizing orthonasally and retronasally about 150 selected odorants at different odorant concentrations according to their odor qualities. Participation in these sessions was at least for one year prior to participation in the actual sensory experiments.

Intraoral Sampling of Odorants. Intraoral analyses were performed 2 h after breakfast and thorough cleaning of the teeth and oral cavity with a commercial toothpaste (5 min) and with a commercial alcohol-free, low-aromatized, and antimicrobial mouthwash. Prior to oral

application of the sample, the oral cavities of the panelists were screened for odorants ("blank", see Discussion).

Then, 25 mL of the respective sample was taken into the oral cavity, kept for 10 s with closed lips and closed velum, rinsed carefully within the oral cavity, and then expectorated. After expectoration, panelists were allowed to behave in a completely free manner according to their usual habits, for example, to swallow or talk. Only consumption of other foods was prohibited. At defined time intervals (2-fold increase) after expectoration (15, 30, 60 s, etc.), an extraction capsule containing one SBSE bar was placed in the oral cavity ("time dilution" approach, cf. ref 15). The lips and velum were kept closed, and the capsule was moved carefully within the oral cavity, thereby avoiding swallowing actions. After 5 min of equilibration, the capsule was removed from the oral cavity and the SBSE bar was removed with tweezers, dipped into deodorized water, briefly dried with lint-free tissue, and immediately placed into the thermodesorption unit.

SBSE Thermodesorptive Sample Application. Thermodesorption of the samples was performed by means of a TDS-2 thermodesorption system (Gerstel GmbH) in combination with a CIS-4 PTV injector (Gerstel GmbH) for cryofocusing the analytes prior to transfer onto the analytical column. The following sampling parameters were used: splitless thermal desorption was performed by programming the TDS-2 from 40 to 240 °C (5 min) with a rate of 60 °C/min, cryofocusing with liquid nitrogen at −100 °C, and injection with a ramp of 12 °C/s from −100 to 240 °C (5 min).

Rating of Odorants Using the BOSS. Detectability of the odorants was based on their sensory properties, which means first and foremost on their odor intensities. Only those substances that were perceived by HRGC-O after SBSE thermoserption were rated as detectable by BOSS. Detection by HRGC-MS or HRGC-FID was not taken into account as these parameters do not necessarily correlate with the sensory impact of the respective compound.

HRGC-O. Application of the samples was performed as described above (SBSE Thermodesorptive Sample Application). The odorants were screened in parallel by five panelists by sniffing the effluent after either one- or two-dimensional gas chromatography. Sniffing analysis was repeated five times by each panelist. All detected odorants were identified by comparison with reference substances based on the following criteria: retention index (RI) on three stationary phases of different polarities (FFAP, SE-54; OV-1701), mass spectra obtained by MS (EI) and MS (CI), and odor quality as well as odor intensity perceived at the sniffing port.

The one- or two-dimensional gas chromatography system (TD/HRGC) consisted of a Mega 2 gas chromatograph (Fisons Instruments, Mainz-Kastel, Germany) for the precolumn system in tandem with a Fisons GC 5160 as the main column system. The following fused silica columns were used: DB-FFAP (30 m × 0.32 mm i.d., 0.25 μm FD, J&W Scientific, Folsom, CA) and/or DB-5 (SE-54; 30 m × 0.32 mm i.d., 0.25 μm FD, J&W Scientific). The gas chromatographic conditions were the same as described previously (*19*).

HRGC-MS. The odorants were analyzed by two-dimensional gas chromatography (TD/HRGC) as described above. MS analyses were performed with an ITD-800 (Fisons Instruments) running in the CI mode with methanol as the reagent gas. The following fused silica columns were used: DB-FFAP (30 m × 0.32 mm i.d., 0.25 μm FD, J&W Scientific) in combination with a DB-5 (SE-54; 30 m × 0.32 mm i.d., 0.25 μm FD, J&W Scientific). The HRGC and MS conditions were the same as described previously (*18*).

Sensory Evaluation. Sensory analyses were performed in a sensory panel room at 21 ± 1 °C at three different sessions. Samples, containing the single compounds or strawberry aroma, were freshly prepared, stirred for 30 min, and immediately presented to the sensory panel for retronasal evaluation in covered glass vessels (capacity = 45 mL, 25 mL samples). The whole sample was taken into the mouth, kept for 10 s with closed lips and closed velum, rinsed carefully within the oral cavity, and then expectorated (*16*). At defined time intervals (2-fold increase, "time dilution" approach, cf. ref *15*) after expectoration, the intensity of the overall retronasal aroma perception as well as of single predefined odor qualities was rated by the panelists by deliberately opening the velum−tongue border exactly at these times. Panelists were always asked to score odor intensities from 0.0 (not perceivable) to 3.0 (very intense). The averaged results from three different sessions were plotted in spider web diagrams. Values obtained differed by not more than 10%.

For comparative evaluation of the strawberry aroma in water and milk, respectively, the water sample was first evaluated; then, after a 15 min break and rinsing of the oral cavity with tap water, evaluation of the milk sample was performed. Panelists were asked to score odor qualities and overall intensities at given times as described above. Then, panelists were asked to rate the overall difference between both samples from 0.0 to 3.0. Also, they were asked for a hedonic rating on a seven-point scale from 0.0 (very unpleasant) to 3.0 (very pleasant). During the hedonic rating, panelists could describe in a free, unstandardized manner the key differences between the water and the milk sample and to give reasons for possible differences in the hedonic rating.

Determination of Retronasal Odor Threshold Values (ROTV). Conditions for sensory analysis were the same as described above. Determination of retronasal odor thresholds in a triangular test according to the "forced choice" approach and statistical treatment of the data was performed according to ref *20* (cf. **Table 1**). Panelists were presented 25 mL of the respective samples together with two blanks each. Retronasal evaluation of the samples was performed either by deliberately opening the velum−tongue border or by swallowing.

RESULTS AND DISCUSSION

Sensory Evaluation. *Single Odorants.* Recently, single aroma compounds in aqueous solutions were evaluated with regard to the initial retronasally perceived intensity when introduced into the mouth (*21*). Their retronasal sensory persistence was profiled, following the "time dilution" approach (*15*). It was found that the initially perceived intensity does not allow any correlation or prediction of the further persistence of the odorant in mouth. That means that odorants which elicit a very high initial aroma intensity can persist for only a very short time interval after swallowing and vice versa. For example, the initial sensory intensity of (*Z*)-3-hexenyl acetate was reported with the highest value of 3 (very intense), and the total retronasal persistence did not exceed 2 min (**Figure 1a**,**b**, diagrams on left side). 4-HDF was perceived with only low initial intensity (1.25), but persisted for up to 8 min. This observation has been made before but has not been seized by analytical measures such as temporal determinations of persistence using the time dilution approach. With this tool, the striking discrepancies in retronasal odorant perception depending on chemical structure become more obvious.

When the initial sensory intensities upon sample introduction into the oral cavity as well as the total duration of retronasal sensory persistence of the single odorants are compared with the respective retronasal odor activity values (ROAV) or log *P* values (water−octanol partition coefficients; **Figures 1** and **2**), it can be clearly seen that neither ROAV nor log *P* follow a simple correlation and, therefore, do not offer the possibility of any direct prediction of both sensory parameters. For example, ethyl butanoate with the by far highest ROAV of 360 000 was intraorally perceivable with a relatively high initial intensity but persisted for only 2 min, whereas 4-HDF with medium initial intensity and a comparatively low ROAV of 67 was sensorially detected even after 8 min. Therefore, apart from concentration and aroma intensity, factors such as polarity, volatility, and stability in the presence of salivary constituents can be regarded as additional factors involved in this phenomenon. According to previous investigations, it might be, for example, assumed that compounds such as ethyl butanoate are degraded by salivary constituents, so that they do not persist for a long time (*11*, *12*). On the other hand, 4-HDF is not subjected to salivary modifications as shown previously. Also, it has to be taken into account that odor intensity does not follow a straight correlation

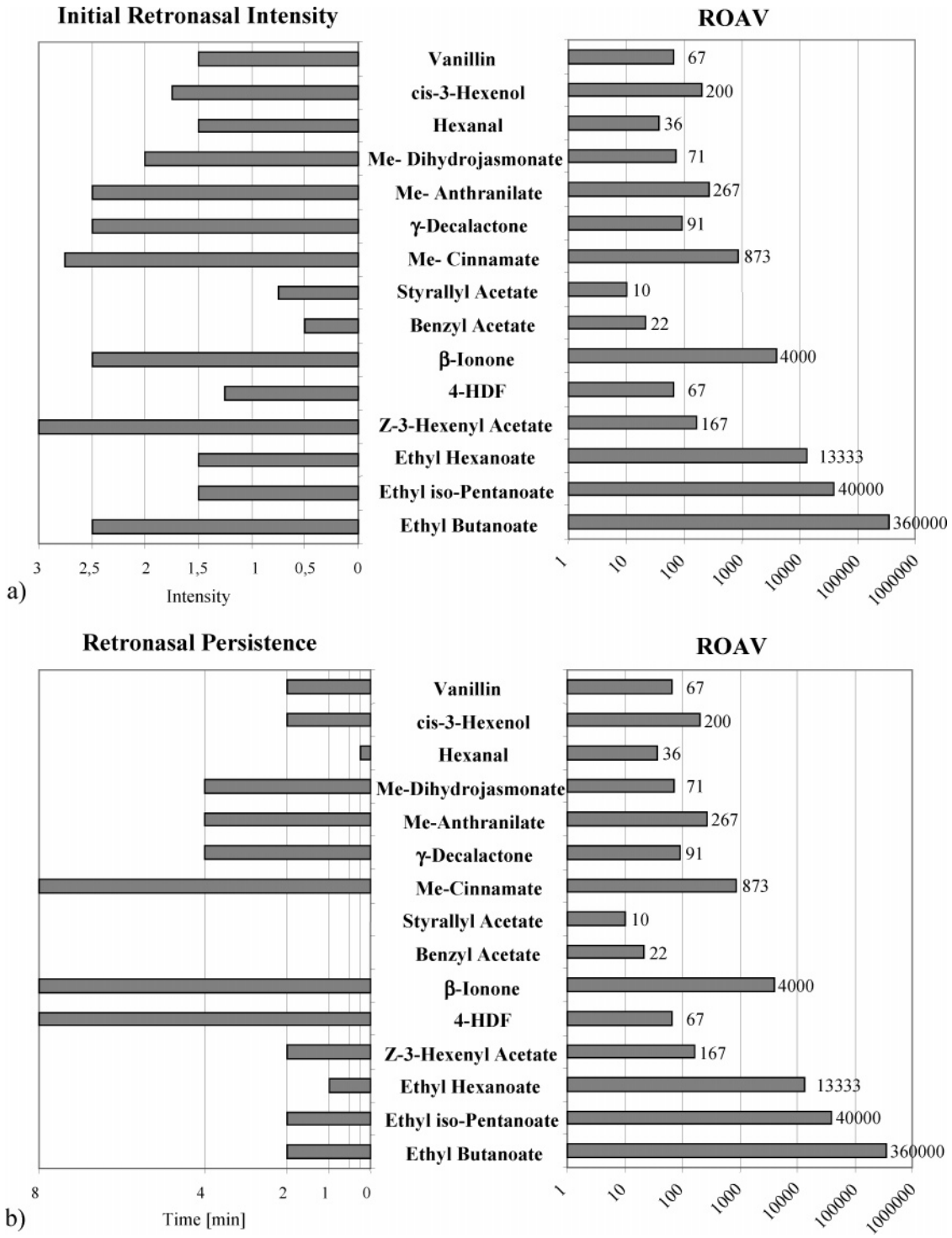

Figure 1. Comparison between (**a**) initial retronasal aroma intensity and (**b**) time-resolved (15, 30, 60, 120, 240, 480 s) retronasal sensory persistence of single aqueous strawberry aroma compounds (left) versus the respective retronasal odor activity values in water (ROAV, right).

with increase of odor quantity but that it should be subjected to psychophysical phenomena as described by Stevens's law (*22*). This in turn means that the immediate retronasal aroma detection at retronasal odor threshold value (ROTV) level is in no respect comparable to long-time persistence, intensity, and detectability of odorants by means of BOSS at suprathreshold levels. However, it still has to be regarded as striking that there is obviously so little relationship between ROAV and log *P* values and retronasal sensory persistence.

Strawberry Aroma Mixture. On the basis of these findings, all odorants were then evaluated together in a strawberry aroma mixture, both in aqueous and in milk model solutions. In preceding sensory evaluations, the odor qualities citrusy, buttery, sweaty, vanilla-like, grassy, caramel-like, peach-like, flowery, and fruity were selected as descriptors.

The intensities of these odor qualities as well as the overall odor intensities were rated comparatively, both in the aromatized water and in milk samples following the time dilution approach (spider web graphs in **Figure 3**). In parallel, the overall aroma intensities were rated upon sample introduction into the oral cavity by opening the velum−tongue border as described in ref *15* (cf. small bar diagrams in **Figure 3**). It becomes evident

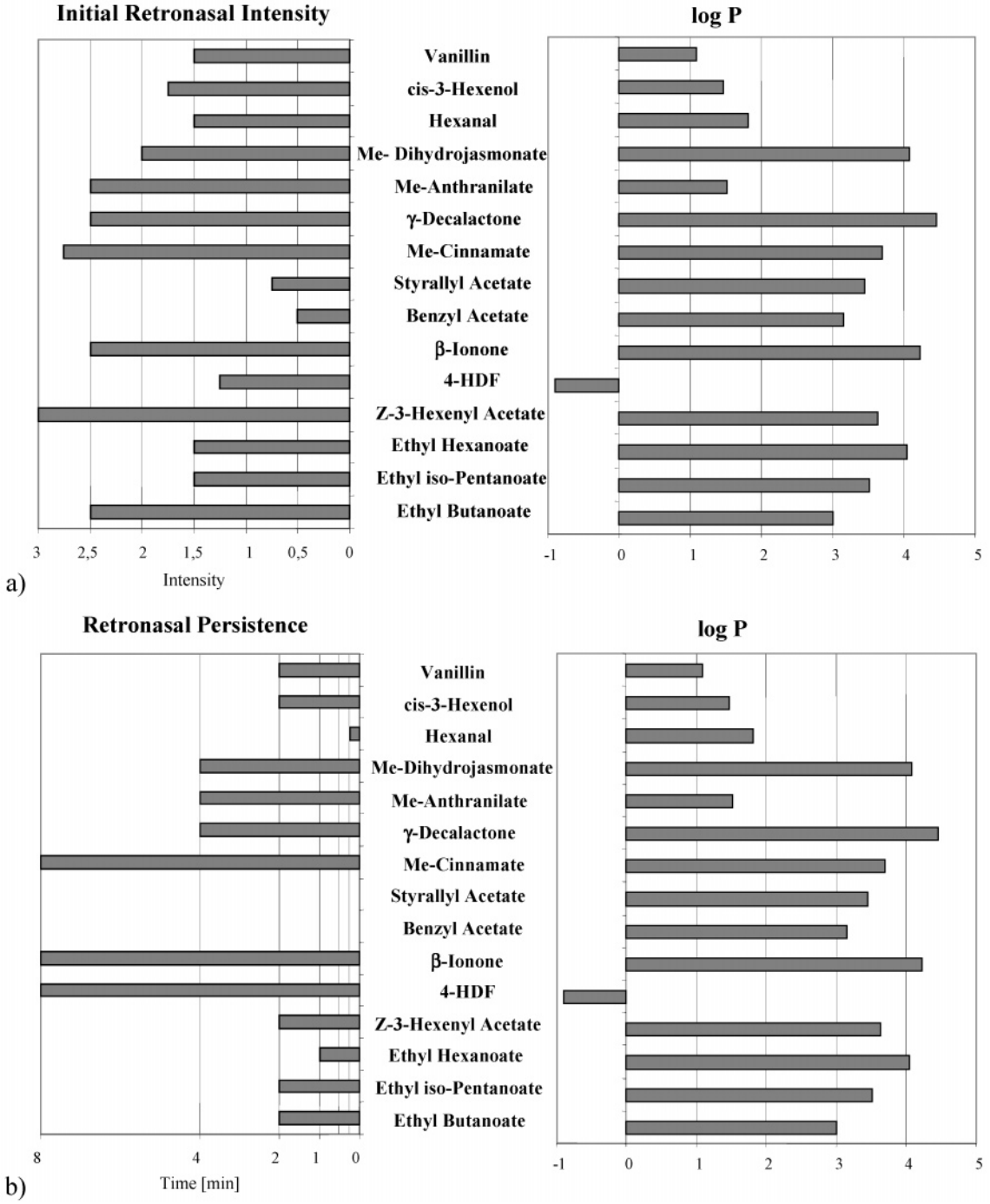

Figure 2. Comparison between (**a**) initial retronasal aroma intensity and (**b**) time-resolved (15, 30, 60, 120, 240, 480 s) retronasal sensory persistence of single aqueous strawberry aroma compounds (left) versus their respective log *P* values (right).

that the water sample elicits generally a considerably higher aroma intensity than the milk sample from the beginning until the end of evaluation. The overall aroma intensity of the water sample at the beginning of the evaluation was rated at 2.25 as significantly more intense than that of the milk sample (1.5). Regarding single odor attributes, mainly the flowery, fruity, and caramel- and peach-like notes were perceivable with significantly higher intensities in the water sample compared to the milk sample at 30 s (**Figure 3a**). This discrepancy was not so distinct for the grassy and citrusy impressions, which were generally rated as much less intense in both samples. Characteristic milk notes such as buttery and sweaty were more intense in the milk sample. This can be expected as the milk adds certain amounts of odorants such as pentane-2,3-dione and butanoic or methylbutanoic acid to the mixture (*23*). However, no quantitation of these compounds was performed in the present study as the focus was on the changes in the strawberry aroma related odor qualities. From the changes in both aroma profiles with time, it is evident that the fruity, flowery, and peach- and caramel-like notes persist much longer and with higher intensities in the water sample (up to 2 and 4 min, respectively). In milk, they were perceivable for only 1 min and with lower intensities. On the other hand, the buttery and sweaty notes persisted in the milk samples for up to 4 min but were merely undetectable in the water samples as one would expect. The only aroma quality persisting for up to 8 min with low aroma intensity (0.5) was the flowery impression of the water sample, whereas no more perception occurred for the milk sample (not

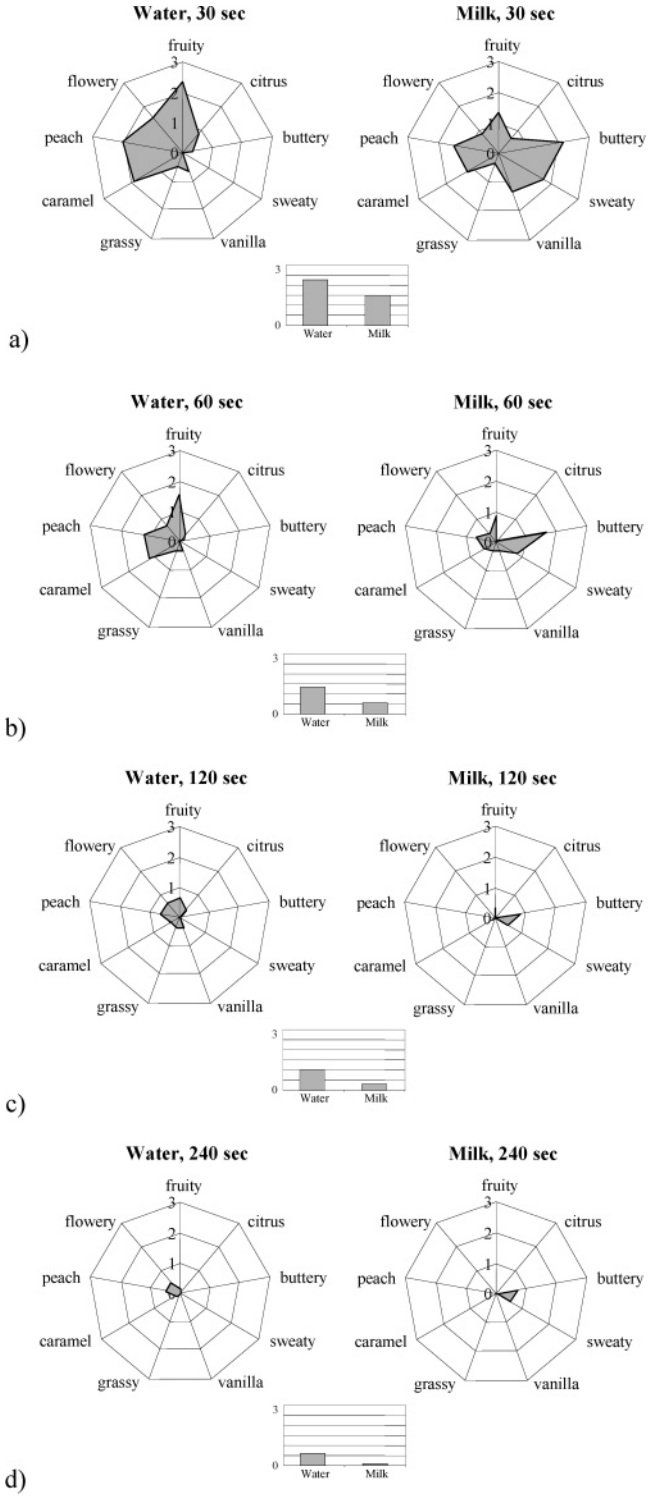

Figure 3. Time-resolved retronasal evaluation of the intensities of odor attributes and their overall odor intensities (middle graph) after intraoral application and expectoration of strawberry aroma samples in water and milk, respectively.

displayed as spider web diagram). For better comparison of the total perception durations of the odor qualities, see **Figure 4**. Also, the persistence of the grassy and the citrusy notes was higher in the water sample (2 min) than in milk (30 s and 1 min, respectively). Interestingly, the vanilla note was at first (30 s) rated as more perceivable in the milk sample but persisted then longer (2 min) in the water sample as compared to the milk sample (1 min). The reasons for this might be that the dominant sweet, flowery, and fruity impressions at the beginning

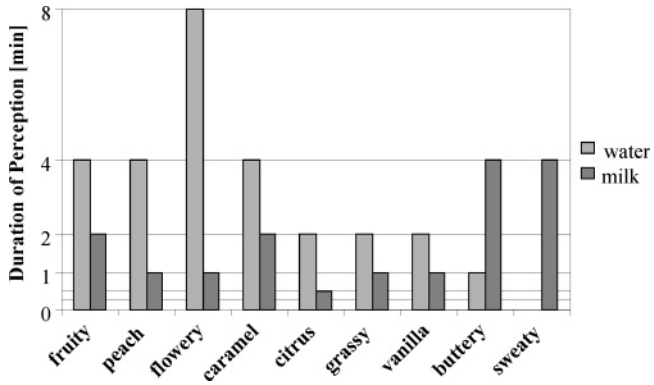

Figure 4. Retronasal sensory persistence of odor attributes after intraoral application and expectoration of strawberry aroma samples in water and milk, respectively.

lead to an overestimation of the vanilla note. The minor additional amounts originating from the milk (>100-fold lower than in the strawberry aroma; *24*) are not expected to play any significant sensory role in the higher intensity rating.

Generally, the difference between the overall aroma impressions of both samples (water and milk) was rated at 2.8 as very high.

Hedonic rating of both samples showed a significant difference. Although the strawberry-flavored milk sample was rated by all panelists unanimously as mainly pleasant (2.5), well-balanced, and only slightly artificial, the aqueous strawberry sample was mainly said to be not very pleasant (1.2), with an overly intense and very sweet−artificial overall aroma. Panelists described the initial aroma of the aqueous sample right after application as medium pleasant and medium balanced due to the additional fruity impressions, but reported then a shift to an unbalanced aroma with an especially artificial, flowery, persistent note that developed just after several minutes and which was mainly perceivable in the time interval between 4 and 8 min. This negatively rated aroma impression was not perceived in the milk sample.

In conclusion, milk obviously prevented the odorants from being liberated to the same extent as from water by changing the partition behavior from the aqueous to the gaseous and mucosal phases.

BOSS Analysis. Previously, continuous aroma delivery from the oral cavity has been shown to play an important role in longlasting retronasal aroma perception (*15*, *20*). By means of BOSS analysis the oral cavities of panelists have been screened at certain time intervals after the application/consumption of foods or aroma models. Thereby, even trace amounts of highly odor-active compounds under in vivo conditions were detected and characterized in terms of aftersmell development.

SBSE Bar. Previous investigations showed that no noteworthy migration of organic or inorganic constituents from the SBSE bars into saliva takes place during intraoral extraction (*20*).

Determination of "Background". After conditioning of the SBSE bars, the bars were screened by HRGC-O for remaining traces of odorant (*15*), which were set as background.

Blank Samples from Oral Cavity. Screening of the untreated oral cavities of the participants by means of SBSE/HRGC-O revealed always a weak detection of eight odor-active substances (*15*). These were also recorded as background.

Single Odorants. Correlation of retronasal sensory persistence of single odorants to in vivo BOSS/HRGC-O detection clearly showed that the intraoral BOSS/HRGC-O detectability was highly related to the total sensory persistence of single odorants as perceived by the panelists (*20*; cf. **Table 2**).

Table 2. Retronasal Persistence after Rinsing of Single Odorants in Aqueous Model Solutions in the Oral Cavity versus Time-Resolved BOSS Analysis of the Strawberry Aroma Compounds in Single Solutions and in the Strawberry Aroma Mixtrue, Respectively

odorant	retronasal odorant persistence of single odorants[a] (min)	time-resolved BOSS detection[a] (min)	
		single odorants	strawberry mixture
ethyl butanoate	2	2	2
ethyl 3-methylbutanoate	2	2	2
ethyl hexanoate	1	1	1
(*Z*)-3-hexenyl acetate	1	2	1
methyl cinnamate	8	8	8
styrallyl acetate	0	0	0
benzyl acetate	0	0	0
methyl anthranilate	8	4	8
methyl dihydrojasmonate	1	2	1
γ-decalactone	4	4	4
hexanal	1	0.5	1
(*Z*)-3-hexenol	2	2	2
β-ionone	8	8	8
4-HDF	8	8	8
vanillin	4	2	4

[a] Odorants were evaluated at fixed time intervals (0.25, 0.5, 1, 2, 4, and 8 min; 2-fold increase according to the time dilution approach as described in ref *15*). Latest detection is given as time value in minutes. Detection at 0 min means that the odorant was detectable, either sensorially or by BOSS, only right after expectoration of the sample. Data are the mean of five panelists (two replicates each); detection by BOSS and sensory analysis did not differ by more than one time dilution step.

Strawberry Aroma Mixture. In the following, the strawberry aroma was administered to the panelists as a mixture, both in aqueous and in milk models, in exactly the same way as it has been done for sensory analysis. After rinsing in the oral cavities and expectoration, BOSS analysis was performed as described above.

BOSS/HRGC-FID Analysis. Extraction of the odorants by means of BOSS from the oral cavities (15 s after expectoration) and analysis after thermodesorption from the SBSE system via HRGC-FID resulted in considerably different chromatograms, with all of the detected odorants being significantly reduced in intensities for the milk sample (cf. **Figure 5**). Some compounds were even not detectable as peaks any more. Generally, not all compounds were detectable by HRGC-FID in both samples, such as β-ionone, whereas others gave dominant peaks. The matrix effect on the presence of odorants and, moreover, the liberation into the aqueous and gaseous phase already becomes obvious from these results. However, detection via HRGC-FID does not allow any correlation to sensory impact, for example, a highly potent compound such as β-ionone is not detected despite its clear retronasal sensory impact (cf. detection of single compounds by sensory analysis and by BOSS/HRGC-O). On the other hand, some compounds, such as styrallyl acetate and benzyl acetate, elicited a pronounced peak, although their sensory impact was low. Therefore, evaluation and rating of detectability were performed in the following by BOSS in combination with HRGC-O as done for the single compounds and according to refs *15*, *20*.

BOSS/HRGC-O. Time-resolved BOSS/HRGC-O analysis of the aqueous strawberry aroma mixture, according to the procedure described above for single aqueous odorant solutions, resulted in very similar detection time intervals compared to the results obtained for single odorants (cf. **Table 2**). This indicates that for the investigated aroma mixture, no significant interactions or changes in adsorption behavior to oral mucosa occurred and that the odorants showed the same persistence as if singly administered.

In **Figure 6**, the results from BOSS analysis of the aqueous strawberry aroma sample are displayed in comparison to the milk sample. It can be seen that BOSS screening of the oral cavity for potent odorants after exposure to the aromatized water and milk sample, respectively, led to the detection of most of the strawberry aroma constituents at the start point of analysis (15 s). Only styrallyl acetate and benzyl acetate were not detectable 15 s after expectoration of the solutions (not shown). However, detection was positive at an earlier stage of intraoral release (immediately after swallowing, data not shown). This is in agreement with the fact that both compounds exceeded their ROTV level (cf. **Table 1**). Also, this correlates with the finding that both odorants were sensorially detectable right at the beginning of the evaluation (introduction into oral cavity) but with very low intensities (0.5 and 0.75, respectively) when singly administered, but did not persist after expectoration (no more retronasal detection 15 s after expectoration).

Generally, the grassy compounds hexanal and (*Z*)-3-hexenol, as well as the fruity compounds ethyl butanoate, ethyl isopentanoate, ethyl hexanoate, (*Z*)-3-hexenyl acetate, and methyl dihydrojasmonate, were detectable by BOSS only for a relatively short period of time (up to 2 min) after water sample application, whereas the sweet and flowery compounds β-ionone, methyl cinnamate, methyl anthranilate, and the coconut-like δ-decalactone were detectable after even 4 and 8 min, respectively. Also, vanillin and 4-HDF yielded this long period of detection.

Compared to the water sample, all odorants were decreased in BOSS detectability by one or two time dilution steps for the milk sample (cf. **Figure 6**). For methyl dihydrojasmonate and hexanal, there was no more detection obtained at even the 15 s time dilution step. That means that the oral release, most probably due to higher aroma−matrix interactions, and, therefore, the persistence of the odorants within the oral cavity were significantly reduced.

Correlation of Sensory Impressions of Strawberry Aroma to BOSS Analysis. Generally, the aroma impressions and the development of an aroma profile with time of a complex aroma mixture are often difficult to correlate with its single constituents and their odor attributes as additive and suppressive effects need to be taken into account. Furthermore, it has been reported in the literature that even the odor quality of a binary odorant mixture can be completely divergent from the original singular aroma impressions and cannot be predicted at present. For example, a certain mixture of the catty smelling 4-mercapto-4-methylpentan-2-one together with citrusy impressions elicits grapefruit-like aroma quality (*18*).

Despite these facts, our findings indicate that the sensorially perceived changes in aroma profile after consumption of aqueous and milk strawberry aroma samples (**Figure 4**) show extensive parallels to the analytical data. The aqueous model shows a highly correlated fit between the BOSS detection (cf. **Figure 6**, light gray bars) of the grassy compounds (up to 2 min), the fruity (up to 2 min), and most of the flowery substances (8 min) and their corresponding duration of sensory perception (2, 4, and 8 min, respectively; cf. **Figure 4**, light gray bars). Only the sensory persistence of the vanilla and caramel notes was rated up to 2 and 4 min, respectively, whereas analytical detection yielded 4 and 8 min. However, the other odor impressions such as peach, flowery, and fruity might have covered to some extent the vanilla and caramel sensory impressions. It has to be taken into account that all of these

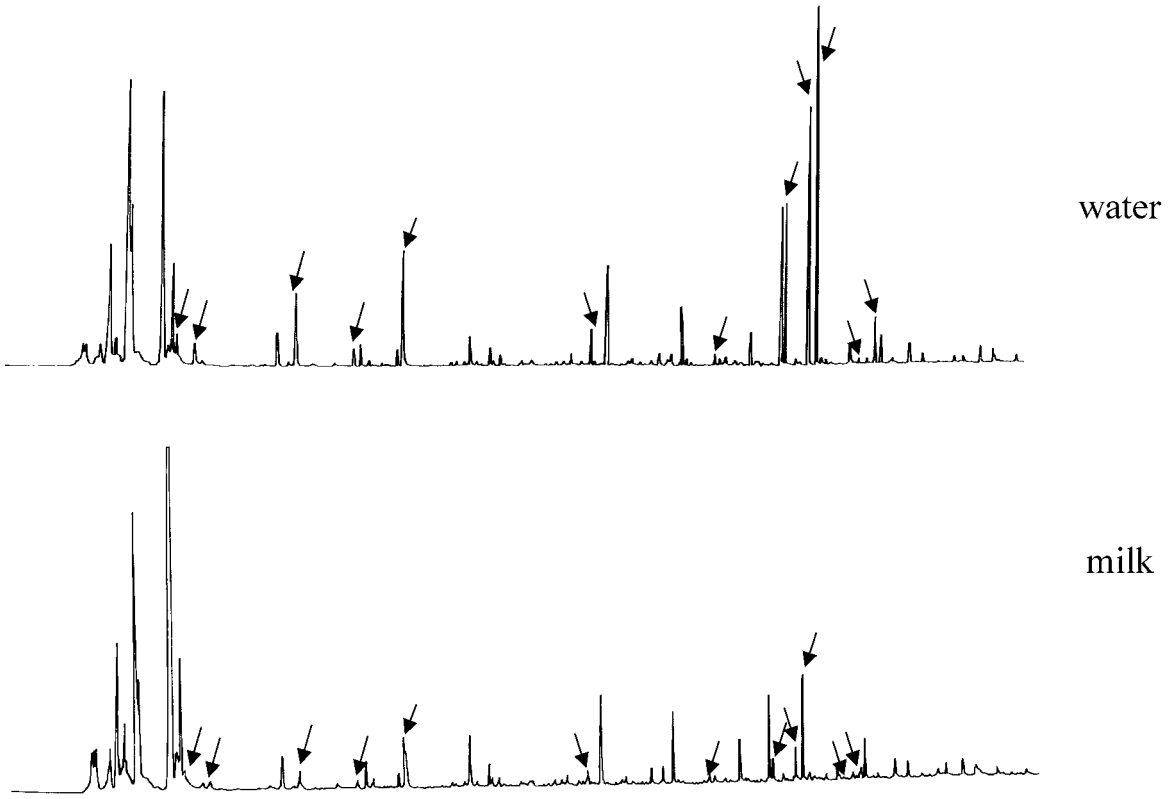

Figure 5. Representative HRGC-FID chromatograms of BOSS samples obtained from one panelist after application of the aqueous strawberry aroma model and the milk strawberry aroma model, respectively. Intraoral BOSS extraction was performed for both samples 15 s after expectoration of the sample, using the same freshly conditioned SBSE stir bar under exactly the same analytical conditions.

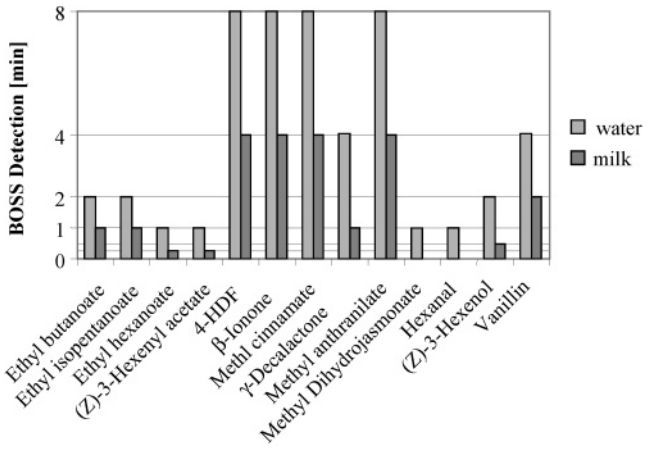

Figure 6. Time-resolved (15, 30, 60, 120, 240 s) intraoral odorant detection by BOSS after expectoration of strawberry aroma samples in water and milk, respectively.

aroma descriptors are very much related in their overall aroma character so that differentiation among them might cause some difficulties.

The significantly decreased intensities of the odor qualities in the strawberry-flavored milk sample (cf. **Figures 3** and **4**) agreed with the detection of the odorants of the strawberry aroma mixture after oral application by BOSS (cf. **Figure 6**, dark gray bars). However, sensory perception of the vanilla and caramel notes was reduced by one time dilution step compared to the analytical data, whereas the analytical detection of β-ionone, methyl cinnamate, γ-decalactone, and methyl anthranilate of 4 min was not fully mirrored by the sensory persistence of the fruity (2 min), peach-like (1 min), and flowery impressions (1 min). It has to be taken into account that in the milk samples the milk-related odor notes buttery and sweaty were perceived additionally and with considerable persistence (4 min) so that additive or suppressive effects need to be considered.

Even if direct correlation of selected aroma descriptors with intraoral single aroma constituents of a complex aroma mixture is limited due to interaction phenomena, the trend of matrix interaction could clearly be traced. This demonstrates the applicability of BOSS as an intraoral screening system for a complex matrix system.

Simulation of Negative Aftersmell Impression. As described above (sensory evaluation), panelists reported the development of an unpleasant flowery−artificial odor note after application of the aqueous strawberry aroma in the time interval between 4 and 8 min when all other descriptors had already vanished. It was assumed that, according to the results obtained by BOSS analysis, only 4 of the original 15 compounds of the mixture (4-HDF, β-ionone, methyl cinnamate, and methyl anthranilate) might be involved in this negative impression. To prove this, an aqueous aroma mixture containing these four compounds in the concentrations given in **Table 1** was evaluated by the panelists in a triangular test versus the complete aqueous strawberry aroma model. Panelists were asked to clip their noses during introduction of the samples, rinsing, and expectoration and for 8 min after expectoration. This means they were not able to perceive the initial aroma impressions of either the full aroma model or the limited one. Only 8 min after expectoration were they allowed to open their noseclips and to evaluate the retronasal aroma impressions of both samples. At this stage, panelists were not able to discriminate in triangular testing between the full model and the four-component mixture. This means that the negative aroma impression could be sufficiently simulated by the four-component mixture only. It might be that not only does this mixture elicit the reported off-aftersmell but

that of these four compounds even a more limited number (maybe even only one dominant compound) could be responsible for the negative impression. However, this has not been investigated in the present study and would need to be elucidated by further sensory evaluations involving omission experiments.

Generally, these results give evidence of the capability of the BOSS together with time-resolved sensory evaluation of being a useful and quick approach to screen intraorally even trace odorants at precisely defined time intervals for their contribution in aftersmell perception of complex aroma mixtures.

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