JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Influence of Human Saliva on Odorant Concentrations. 2. Aldehydes, Alcohols, 3-Alkyl-2-methoxypyrazines, Methoxyphenols, and 3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone

ANDREA BUETTNER†

Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, D-85748 Garching, Germany

The influence of human whole saliva on selected alcohols, aldehydes, 3-alkyl-2-methoxypyrazines, and phenols in food-relevant concentrations was investigated. At pH 7.5−8 it was found that the alcohols, methoxyphenols, methoxypyrazines, and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone remained unmodified by saliva, whereas aldehydes were reduced to their corresponding alcohols. Generally, the processes were found to be dependent on the salivary activity of the panelists as well as on the concentration of the applied odorants. Reduction of the aldehydes did not occur after thermal treatment of the saliva. These investigations are aimed at finding an explanation for longer lasting aftertaste in humans, as it is induced by some odor-active compounds after the consumption of food materials.

KEYWORDS: Aftertaste; aftersmell

INTRODUCTION

In the first part of these investigations the presence of esterases and peroxidases in human saliva and their possible influence on odorant metabolisation was discussed (*1*). It was found that esters and thiols were degraded during incubation with saliva with different specificities according to their chemical structures. Furthermore, the reduction of benzaldehyde and cinnamaldehyde has been reported previously when odorant solutions were rinsed in the oral cavity and were analyzed after solvent extraction of the spitted-out material (*2*). The present study is aimed at the investigation of the influence of the salivary enzymatic activities on key food odorants from the substance classes of aldehydes, alcohols, methoxyphenols, 3-alkyl-2-methoxypyrazines, and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone in food relevant concentrations.

MATERIALS AND METHODS

Chemicals. The following pure odorants were obtained from the suppliers shown: ethyl butanoate, hexanal, hexanol, octanal, decanal, methional, methionol, guaiacol (2-methoxyphenol), 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolone), 3-isopropyl-2-methoxypyrazine, 3-isobutyl-2-methoxypyrazine, 3-*sec*-butyl-2-methoxypyrazine, 2-phenylethanthiol, phenylacetaldehyde, phenylethanol, and 2-furfurylthiol (Aldrich, Steinheim, Germany); 4-vinylguaiacol (2-methoxy-4-vinylphenol) (Lancaster, Mühlheim, Germany). The compounds were freshly distilled prior to analysis. Chemical and sensory purity was checked by gas chromatography−olfactometry (GC-O) as well as gas chromatography−mass spectrometry (GC-MS).

Preparation of the Aqueous Odorant Solutions. One percent stock solutions of the single odorants in absolute ethanol were freshly prepared and diluted with deodorized water prior to enzymatic analysis to obtain 500 mL of single aqueous solutions of each odorant (concentration = 100 and 1000 μg/L water, respectively).

Syntheses. The following labeled internal standards were synthesized according to the literature cited: [5,5-$^2H_2$]decanal (*3*), [2,2,2-$^2H_3$]ethyl butanoate (*4*), 2-[α-$^2H_2$]-2-furfuylthiol (*5*), [$^2H_3$]guaiacol (*6*), [3,3,4,4-$^2H_4$]hexanal (*6*), [3,3,4,4-$^2H_4$]hexanol (*6*), [$^{13}C_2$]-3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (*7*), 3-isopropyl-2-[$^2H_3$]methoxypyrazine (*8*), 3-isobutyl-2-[$^2H_3$]methoxypyrazine (*9*), [$^2H_6$]-3-mercapto-3-methyl-1-butanol (*10*), 3-([$^2H_3$]methylthio)-1-propanal (5), 3-([$^2H_3$]methylthio)-1-propanol (*11*), [5,5,6,6-$^2H_2$]nonanal (*12*), [3,3,4,4-$^2H_4$]octanal (*13*), phenyl[$^{13}C_2$]acetaldehyde and phenyl[$^{13}C_2$]ethanol (*14*), [1,1-$^2H_2$]-2-phenylethanthiol (*15*), 3-*sec*-butyl-2-[$^2H_3$]methoxypyrazine (*16*), and [$^2H_3$]vinylguaiacol (*17*).The concentrations of the labeled internal standards and the response factors (FID) were determined gas chromatographically using methyl octanoate as the internal standard as described recently (*3*). The calibration factors for the labeled compounds were calculated as reported recently (*18*). The mass traces as well as the calibration factors used for quantitation are given in **Table 1**.

Enzymatic Analysis. *Collection of Saliva and Enzyme Assay.* Mixed whole saliva (10 mL) was collected separately from four panelists 2 h after breakfast and thorough cleaning of the teeth and was used immediately for analysis. Panelists (two males and two females) were volunteers (nonsmokers) of the Technical University of Munich (22−30 years of age), exhibiting no known illnesses at the time of examination and with normal olfactory and gustatory function. Subjective aroma perception was normal in the past and at the time of examination. Before sampling, each panelist rinsed his/her mouth several times with tap water to avoid any contamination.

The enzyme assays were performed four times for each panelist at pH 7.5−8 (pH of saliva) according to the method given in ref *1*. To make sure that contamination of the samples with odorants originating from the saliva or from the water could be excluded, reference (without addition of saliva) and blank samples (without odorant) were run in exactly the same way as the enzyme assays.

Inhibition of Enzymatic Activity. The same experiments were performed after thermal treatment of the saliva samples in a closed

† Telephone 089-28914181; fax 089-28914183; e-mail Andrea.Buettner@Lrz.tum.de.

10.1021/jf020714o CCC: $22.00
Published on Web 10/19/2002

Table 1. Selected Ions and Calibration Factors Used for Quantitation by Stable Isotope Dilution Assays

odorant	ion (*m*/*z*)	internal standard	ion (*m*/*z*)	calibration factor
3-isopropyl-2-methoxypyrazine	105	3-isopropyl-2-[$^2H_3$]methoxypyrazine	108	1.00
3-isobutyl-2-methoxypyrazine	167	3-isobutyl-2-[$^2H_3$]methoxypyrazine	170	0.95
3-*sec*-butyl-2-methoxypyrazine	167	3-*sec*-butyl-2-[$^2H_3$]methoxypyrazine	170	1.00
sotolone	129	[$^{13}C_2$]-3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	131	1.00
guaiacol	125	[$^2H_3$]guaiacol	128	0.94
vinylguaiacol	151	[$^2H_3$]vinylguaiacol	154	1.00
hexanol	85	[3,3,4,4-$^2H_4$]hexanal	89–90	0.90
methionol	89	3-([$^2H_3$]methylthio)-1-propanol	92	0.75
phenylethanol	105	phenyl[$^{13}C_2$]ethanol	107	1.00
hexanal	101	[3,3,4,4-$^2H_4$]hexanal	104–106	0.73
octanal	111	[3,3,4,4-$^2H_4$]octanal	113–115	0.87
nonanal	143	[5,5,6,6-$^2H_2$]nonanal	147	0.87
decanal	157	[5,6-$^2H_2$]decanal	158–160	0.64
methional	105	3-([$^2H_3$]methylthio)-1-propanal	108	0.71
phenylacetaldehyde	121	phenyl[$^{13}C_2$]acetaldehyde	123	1.00
ethyl butanoate	117	[2,2,2-$^2H_3$]ethyl butanoate	120	1.00
2-phenylethanthiol	105	[1,1-$^2H_2$]-2-phenylethanthiol	107	0.95
2-furfurylthiol	115	2-[α-$^2H_2$]-2-furfuylthiol	117	1.00
3-mercapto-3-methyl-1-butanol	87	[$^2H_6$]-3-mercapto-3-methyl-1-butanol	92	1.00

Table 2. Important Odorants in Foods

odorant	odor quality[a]	orthonasal odor threshold in water[a] (μg/L)	concentration in food[b] (μg/kg)
3-isopropyl-2-methoxypyrazine	earthy	0.002	0.1–5 (bell pepper, coffee)
3-isobutyl-2-methoxypyrazine	earthy	0.005	10–100 (bell pepper, coffee)
3-*sec*-butyl-2-methoxypyrazine	earthy	0.002	0.1–10 (bell pepper, coffee)
sotolone	spicy	0.3	1–5 (chicken, beef, pork)
guaiacol	smoky	2.5	1–7000 (chicken, beef, coffee)
vinylguaiacol	clove-like	20	1–60000 (clementine, coffee)
hexanol	grassy	50	1500–2000 (white wine)
methionol	cooked potato	5	1000–2000 (beer)
2-phenylethanol	honey-like	1000	200–20000 (milk chocolate, olive oil, bread crumb, white wine)
hexanal	grassy	10.5	100–200 (orange juice)
octanal	citrus-like	8	10–100 (orange juice)
decanal	citrus-like, soapy	5	50–200 (orange juice)
methional	cooked potato	0.2	100–200 (coffee)
phenylacetaldehyde	honey-like	4	10–50 (milk chocolate)

[a] Data from refs *36* and *37*. [b] Data from refs *3*, *11*, *12*, *14*, *15*, and *38*–*48*.

vessel (100 °C, 10 min). The saliva was cooled to 37 °C and immediately applied for the enzyme assays as described above.

Quantitation of the Odorants by Stable Isotope Dilution Assays. The solution was then spiked with known amounts of the labeled internal standards, stirred for equilibration (20 min), and extracted with dichloromethane (three times, total volume = 200 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, then concentrated to a total volume of 200 μL, and subsequently analyzed by multidimensional GC-MS.

High-Resolution Gas Chromatography−Mass Spectrometry. The odorants were quantified by two-dimensional gas chromatography (TD-HRGC) using a Mega 2 gas chromatograph (Fisons Instruments, Mainz-Kastel, Germany) as the precolumn system in tandem with a Fisons GC 5160 as the main column system. MS analyses were performed with an ITD-800 (Fisons Instruments, Mainz-Kastel, Germany) 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, Folsom, CA) in combination with a 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*).

RESULTS

The odor qualities, the orthonasal odor thresholds in water, and an overview of concentration ranges in foods of the investigated compounds are given in **Table 2**. Care was taken, as already stated in the Introduction, to perform all experiments in food relevant concentrations.

3-Alkyl-2-methoxypyrazines, Methoxyphenols, and Sotolone. After 1, 5, and 10 min of incubation with saliva at pH 7.5−8 no degradation of 3-isopropyl-2-methoxypyrazine, 3-isobutyl-2-methoxypyrazine, 3-*sec*-butyl-2-methoxypyrazine, guaiacol, vinylguaiacol, and sotolone was observable for all panelists (data not shown), indicating that no significant enzymatic or chemical reaction takes place between these odorants and enzymes or other salivary constituents. That means 100% of the initial concentrations of the odorants were present in the model solutions after incubation.

Alcohols. No enzymatic or chemical degradation of hexanol, methionol, and phenylethanol could be observed during an incubation with saliva of 1, 5, and 10 min, respectively (data not shown).

Aldehydes. In contrast to this, an effect of incubation with saliva was found for the aldehydes. In **Figure 1** the decrease of hexanal during incubation is shown for two different initial concentrations, 100 and 1000 μg/L of water, respectively. In the higher concentration, hexanal was reduced by only ~10% in comparison to the initial concentration after 10 min, whereas at lower concentration the reduction was more significant at ~20%. Moreover, the decrease of hexanal was related to the formation of hexanol (**Figure 2**), indicating that reduction of hexanal to hexanol is the obvious reaction occurring with saliva. Methional was found to follow the same reaction, being reduced

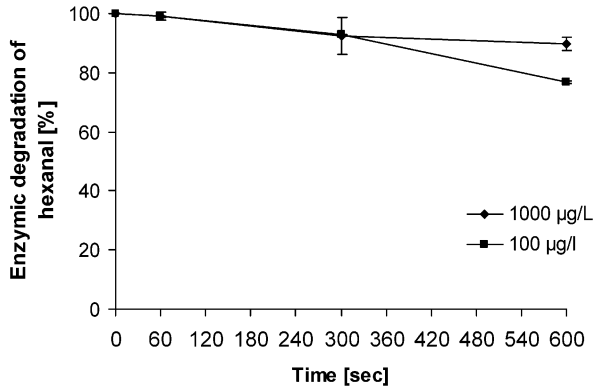

Figure 1. Remaining quantities of hexanal after treatment with saliva, as dependent on the initial odorant's concentration (100 and 1000 μg/L, respectively). Values are the means of four replicates; error bars show the standard deviations.

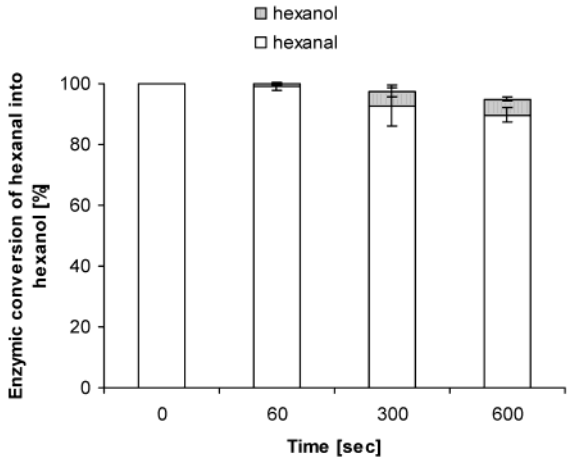

Figure 2. Conversion of hexanal to hexanol during incubation with saliva. Initial concentration of hexanal = 100 μg/L. Values are the means of four replicates; error bars show the standard deviations.

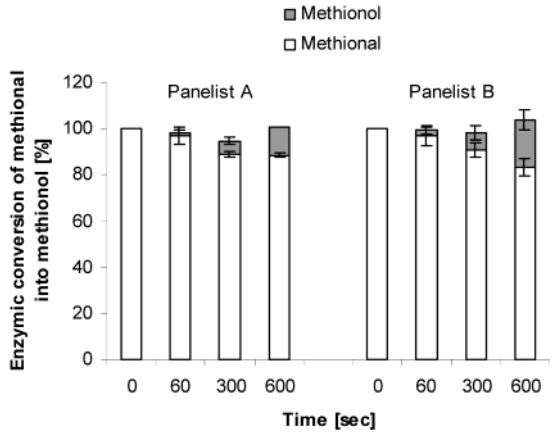

Figure 3. Conversion of methional to methionol in the presence of saliva (two female panelists). Initial concentration of methional = 100 μg/L. Values are the means of four replicates; error bars show the standard deviations.

to methionol to about the same extent as hexanal (**Figure 3**). Generally, variations between saliva samples and panelists were found to be very low.

Investigation of homologous aliphatic aldehydes in the presence of saliva showed that the degradation after 10 min varied from about 30−50% for the single compounds (**Figure 4**). Slightly higher decreases were observed for the less polar

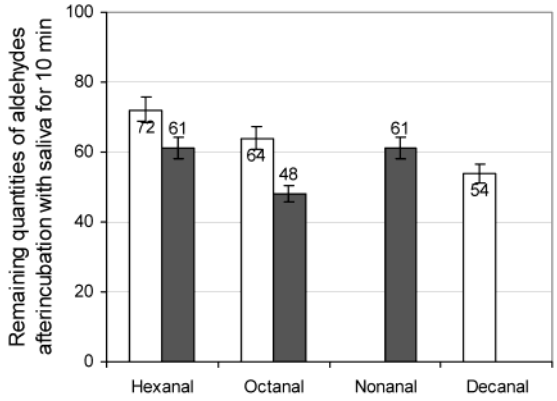

Figure 4. Remaining quantities of homologous aldehydes after treatment with saliva for 10 min (one panelist); comparison between two different sampling days (day 1, open bars; day 2, shaded bars). Values are the means of four replicates; error bars show the standard deviations. Initial concentration of the aldehydes = 100 μg/L.

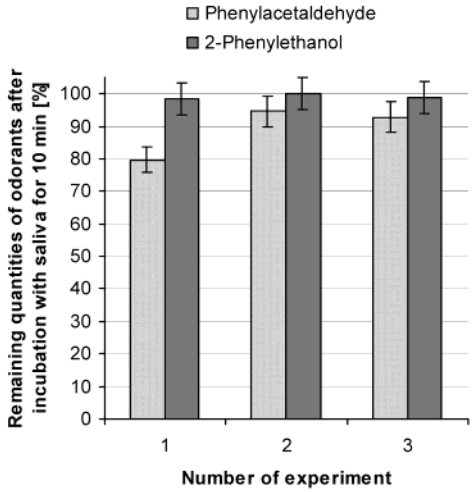

Figure 5. Remaining quantities of phenylacetaldehyde and phenylethanol after incubation with saliva for 10 min on three different days (experiments 1–3; one panelist). Initial concentration of phenylacetaldehyde and 2-phenylethanol = 100 μg/L.

even-chained aldehydes. However, this tendency was not valid for nonanal (uneven carbon atom number), which was slightly less degraded as compared to octanal.

Treatment of phenylacetaldehyde also resulted in a decrease of the odorant (whereas phenylethanol remained unmodified, as discussed before) (**Figure 5**). The experiment was repeated three times with three different samples of saliva from one panelist at three different days (experiments 1, 2, and 3). It was found that the enzymatic degradation of phenylacetaldehyde could vary by ~5−20% from one day to the other, indicating that reductive salivary activity for one panelist is not fully consistent. As for the other aldehydes, a conversion of phenylacetaldehyde into phenylethanol was found (data not shown). On the other hand, for all investigated aldehydes no distinct oxidation to the corresponding acids was observed. However, the possibility of a slight acid formation cannot be excluded, because all examined acids (butanoic, hexanoic, octanoic, and phenylacetic acid) were found to some extent in human untreated saliva, making the determination of additional trace amounts very difficult.

After thermal treatment of the saliva (100 °C, 10 min), no degradation of the aldehydes was observable, resulting in a 100% recovery of the odorants after incubation with saliva.

DISCUSSION

3-Alkyl-2-Methoxypyrazines, Methoxyphenols, Sotolone, and Alcohols. The finding that no degradation of the compounds was observable at pH 7.5−8 does not exclude the possibility that some of the compounds might be decomposed at other pH values. For example, it is possible that the phenols might be oxidized at a different pH as an oxidizing capacity of saliva has already been shown for thiols (*1*, *20*). However, the buffering capacity of saliva has been reported to be very high, so the relevance of such reactions at higher or lower pH has to be checked carefully when one is focusing on degradations in the oral cavity during or after food consumption.

Aldehydes. As already stated, evidence has been previously found for the reduction of benzaldehyde and cinnamaldehyde to their corresponding alcohols (*2*). This observation is in full agreement with the present investigation. Generally, the two major metabolic pathways for aldehydes in human beings are oxidation to carboxylic acids and reduction to the corresponding alcohols, with the first being catalyzed by NAD-linked alcohol dehydrogenases and by NADP-linked aldehyde reductases (*21*). The presence of enzymes with such hydrogen-transferring properties has been reported numerous times in saliva and salivary glands. In 1968, a series of dehydrogenases, such as lactate dehydrogenase, malate dehydrogenase, succinate dehydrogenase and many more, were reported to be present in saliva, but were said to be of bacterial origin (*22*). Later, salivary lactate dehydrogenase was studied in detail, for example, the enzymatic activity and isoenzyme pattern thereof (*23*, *24*). Also, the polymorphism and substrate specificity, as well as the induction of human salivary aldehyde dehydrogenases, for example, by ingestion of coffee and broccoli, has been investigated (*25*−*29*). Generally, alcohol dehydrogenase, aldehyde reductase, and aldehyde dehydrogenase activities are found in many tissues, each accepting a great variety of aldehydes as substrates (*21*). Interestingly, in the present study no significant evidence of an oxidative metabolism of the investigated aldehydes was found, but the reductive pathway seemed to be dominating. On the other hand, an ability of salivary enzymes to reduce carbonyl-containing odorants has, to my knowledge, not yet been reported. However, a general reducing potency of saliva has been reported previously but has not been attributed to certain salivary systems (*30*). Furthermore, the capability of saliva to reduce aldehydes has not been discussed in this context.

For these reasons, the factors inducing salivary reduction of the investigated aldehydes cannot be confirmed at present. On the other hand, the possibility that the observed decreases of the aldehydes are also partially induced by a formation of condensation products such as Schiff bases (e.g., with salivary proteins) or other chemical reactions cannot be excluded. However, because the degradation did not occur after heating of the saliva, and because of the velocity of aldehyde decrease and of the salivary parameters (pH 7.5−8), it is unlikely that these reactions play an important role besides the reduction process.

Generally, it is interesting to note that the relative turnover (as related to the initial concentration) depends strongly on the concentration range of the odorant applied. This effect has already been observed previously for the degradation of furfurylthiol (FFT), where ∼80% of a 100 μg/L solution of FFT was degraded after 10 min but only 30% was modified when a 1000 μg/L solution was applied (*1*). Therefore, it can be assumed that the observed reactions should lead to a considerably higher turnover for lower concentrations of the odorants. This effect has to be regarded as highly relevant, especially for key food odorants that are often present at very low concentrations in foods (lower ppb to ppt range).

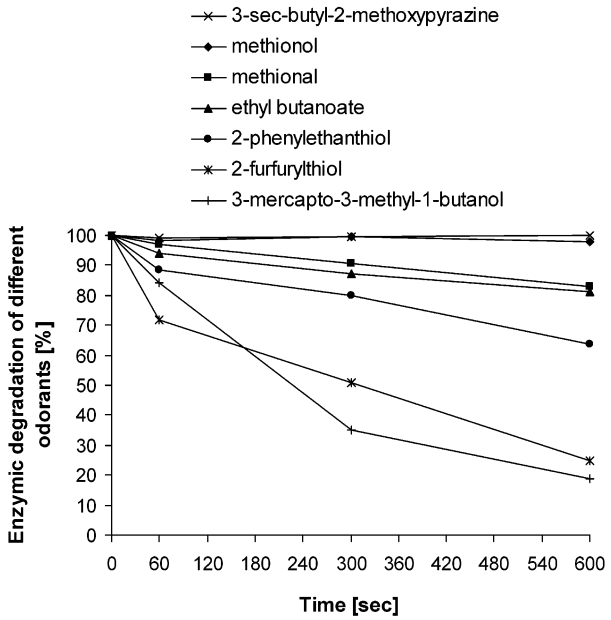

Figure 6. Enzymatic degradation of selected odorants during incubation with saliva for different time intervals (60, 300, and 600 s). Initial concentration of the odorants = 100 μg/L.

On the other hand, it has to be stated that the turnover rates found in the present investigation for odorant solutions are unlikely to be the same as one would find for odorants which are adsorbed to oral mucosa. Under in vivo conditions odorants are continuously exposed to a constant flow of freshly produced saliva so that the turnover might be considerably higher. Also, when the observed degradations are caused by, for example, an NADP-linked aldehyde reductase, the reaction rate in vitro might be decreased by depletion of the reducing agent during incubation. This effect might lead to an underestimation of the reaction rate in vivo.

Comparison between Odorants. The target of the current and previous (*1*) study was to find an explanation for longer persistence (several minutes or more) of some key food odorants in the oral cavity after the consumption of foods as it was also observed recently by sensory experiments (*31*). In **Figure 6**, the decreases of selected odorants from different substance classes (3-alkyl-2-methoxypyrazines, alcohols, aldehydes, esters, and thiols) are displayed for comparison (data from ref *1*). The odorants being the most degraded by far after incubation with saliva are the thiols, with remaining quantities between 10 and 70% after an incubation for 10 min, whereas methional and ethyl butanoate were reduced by only ∼20% and methionol and the pyrazines remained unmodified. This observation corroborates the idea that the extent of degradation might significantly influence the duration of retronasal perceptability of these compounds. Those odorants that are highly adsorbed to oral mucosa and not degraded by salivary constituents should be the compounds which induce long-lasting "aftersmell". Generally, the relatively slight degradation of esters and aldehydes does not rule out the possibility that other members of these groups react more readily with salivary constituents. Also, relative degradation should be more distinct for lower concentrations as already discussed.

In previous investigations it has been shown that odorants are adsorbed to a considerable extent to the oral mucosa (*32*−*34*). For instance, reduced amounts of 3-alkyl-2-methoxypyra-

zines, esters, alcohols, and aldehydes were determined in spit-out odorant solutions after the oral cavity had been rinsed, with only ~20−30% recovery for the investigated esters and aldehydes and a 60−70% recovery for methionol and the 3-alkyl-2-methoxypyrazines. As removal of odorants via the airways has been excluded and enzymatic degradation (only for the esters and aldehydes) does not occur to this extent, adsorptive effects (or even resorption) can be regarded as the only explanation for this observation.

Interesting in this context are recent findings of Andy Taylor and his group, who observed a very high persistence of dimethylpyrazine and two alcohols (linalool and α-terpineol) in the breath of six panelists after consumption of aqueous odorant solutions, whereas esters such as ethyl butanoate and aldehydes were observable only for a very short time interval in the exhaled breath (*35*). The authors described a correlation of these differences in persistence and physicochemical parameters of the compounds such as hydrophobicity and vapor pressure. The mentioned investigations focused on time intervals of a maximum of 1 min as the compounds were not detectable in the exhaled air after this period of time by using APCI-MS. However, the present investigation indicates that differences in prolonged persistence (not only 1 min but significantly longer) seem to be not only influenced by physicochemical parameters but can also be, at least partially, induced by metabolic activity of the saliva inducing hydrolysis of esters (e.g., ethyl butanoate and ethyl hexanoate), reduction of aldehydes (e.g., benzaldehyde and hexanal), and oxidation of thiols (*1*, *20*). Pyrazines such as 2-ethyl-3-methylpyrazine and dimethylpyrazine and alcohols (e.g., linalool and α-terpineol) would then remain unmodified and would, therefore, persist for the longest time as long as the compounds are not removed by saliva. On the other hand, differences in persistence, as observed for (presumably) enzymatically unmodified compounds such as cymene and anethole, could, indeed, be induced by differences in polarity, resulting in a different adsorptive behavior to oral mucosa. Generally, it can be assumed that some of the compounds studied by APCI-MS were also perceivable for >1 min but were not detectable thereafter due to relatively low amounts being exhaled through the nose.

Up to now, the formation of only less odorous substances was observed during incubation with saliva. However, a possible formation of even more odor active compounds from odorless (maybe nonvolatile) or only weak odorants can, from the present study, not be excluded and needs further investigation.

Sensory Relevance. Generally, the sensory relevance of the observed reactions has to be investigated carefully, as each degradation of an odorant can lead at the same time to the formation of odoriferous reaction products, for example, formation of the corresponding alcohols from aldehydes, liberation of butanoic acid from ethyl butanoate, or formation of disulfides during thiol degradation. Therefore, sensory analyses have been undertaken with a sensory panel trained specifically for this purpose, bearing in mind the impact of each reaction product under the respective odorant concentrations applied (*31*).

Conclusions. Reductive degradation of aldehydes was shown to occur in the presence of saliva, whereas alcohols, methoxyphenols, methoxypyrazines, and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone remained unmodified. Generally, differences observed between sampling days indicate varying salivary activity for humans according to their physiological status. The extent of metabolization of thiols, esters, aldehydes, alcohols, and pyrazines was then compared to their in-mouth behavior as studied previously by application of the SOOM technique. These results are a new possibility to explain differences in the persistence of odorous molecules in the oral cavity after consumption of food materials.

ACKNOWLEDGMENT

I thank Prof. P. Schieberle for support of my scientific work and advice and A. Friedrich and P. Gumbrecht for excellent technical assistance.

LITERATURE CITED

(1) Buettner, A. The influence of human salivary enzymes on odorant concentration changes occurring in vivo. 1. Esters and thiols. *J. Agric. Food Chem*. **2002**, *50*, 3283−3289.
(2) Hussein, M. M.; Kachikian, R.; Pidel, A. R. Analysis for flavor residuals in the mouth by gas chromatography. *J. Food Sci*. **1983**, *48*, 1884−1885.
(3) Buettner, A.; Schieberle, P. Evaluation of aroma differences between hand-squeezed juices from Valencia late and Navel oranges by quantitation of key odorants and reconstitution experiments. *J. Agric. Food Chem.* **2001**, *49*, 2387−2394.
(4) Schieberle, P.; Hofmann, T. Evaluation of the character impact odorants in fresh strawberry juice by quantitative measurements and sensory studies on model mixtures. *J. Agric. Food Chem*. **1997**, *45*, 227−232.
(5) Sen, A.; Grosch, W. Synthesis of six deuterated sulfur containing odorants to be used as internal standards in quantification assays. *Z. Lebensm. Unters. Forsch*. **1991**, *192*, 541−547.
(6) Guth, H.; Grosch, W. Odorants of extrusion products of oat meal—changes during storage. *Z. Lebensm. Unters. Forsch.* **1993**, *196*, 22−28.
(7) Blank, I.; Schieberle, P.; Grosch, W. Quantification of the flavor compound 5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone by stable isotope dilution assay. In *Progress in Flavour Precursor Studies*; Schreier, P., Winterhalter, P., Eds.; Allured Publishing: Carol Stream, IL, 1993: pp 103−109.
(8) Semmelroch, P. Untersuchungen zur Objektivierung des Unterschieds im Aroma von Arabica- und Robusta-Kaffee. Ph.D. Thesis, Technical University, Munich, Germany, 1995.
(9) Semmelroch, P.; Grosch, W. Studies on character impact odorants of coffee brews. *J. Agric. Food Chem*. **1996**, *44*, 537−543.
(10) Masanetz, C.; Blank, I.; Grosch, W. Synthesis of [$^2H_6$]-3-mercapto-3-methylbutyl formate to be used as internal standard in quantification assays. *Flavour Fragrance J*. **1995**, *10*, 9−14.
(11) Komarek, D.; Schieberle, P. *J. Agric. Food Chem.* **2002**, to be submitted for publication.
(12) Kerscher, R. Species-specific aroma differences in processed meat from beef and pork. Ph.D. Thesis, Technical University of Munich, 2000.
(13) Blekas, G.; Guth, H. Evaluation and quantification of potent odorants of Greek virgin olive oils. *Dev. Food Sci*. **1995**, *37A*, 419−427.
(14) Pfnür, P. Untersuchungen zum Aroma von Schokolade. Ph.D. Thesis, Technical University, Munich, Germany, 1998.
(15) Schieberle. P. Odour-active compounds in moderately roasted sesame. *Food Chem.* **1996**, *55*, 145−152.
(16) Preininger, M. Identifizierung typischer Geruchsstoffe von Emmentaler Käse durch chemisch-instrumentelle und sensorische Analyse. Ph.D. Thesis, Technical University, Munich, Germany, 1995.
(17) Semmelroch, P.; Laskawy, G.; Blank, I.; Grosch, W. Determination of potent odourants in roasted coffee by stable isotope dilution assays. *Flavour Fragrance J*. **1995**, *10*, 1−7.
(18) Sen, A.; Laskawy, G.; Schieberle, P.; Grosch, W. Quantitative determination of β-damascenone in foods using a stable isotope dilution assay. *J. Agric. Food Chem*. **1991**, *39*, 757−759.
(19) Buettner, A.; Schieberle, P. Evaluation of key aroma compounds in hand-squeezed grapefruit juice (*Citrus paradisi* Macfayden) by quantitation and flavor reconstitution experiments. *J. Agric. Food Chem*. **2001**, *49*, 1358−1363.

(20) Buettner, A. Prolonged retronasal aroma perception—a phenomenon influenced by physiological factors and food matrix composition. In *Proceedings of the 10th Weurman Flavour Research Symposium*; 2002, in press.
(21) Sladek, N. E.; Manthey, C. L.; Maki, P. A.; Zhang, Z.; Landkamer, G. J. Xenobiotik oxidation catalyzed by aldehyde dehydrogenases. *Drug Metab. Rev.* **1989**, *20*, 697−720.
(22) Ferguson, D. B. Dehydrogenase enzymes in human saliva. *Arch. Oral Biol*. **1968**, *13*, 583−588.
(23) Weinstein, E.; Khurana, H.; Mandel, I. D. Lactate dehydrogenase isoenzymes in human saliva. *Arch. Oral Biol*. **1971**, *16*, 157−160.
(24) Liu, T. Z.; Qawasmeh, A.-R. I.; Mazbar, R. A. A new variant of lactate dehydrogenase isoenzyme in human saliva. *Enzyme* **1984**, *32*, 232−234.
(25) Harada, S.; Muramatsu, T.; Agarwal, D. P.; Goedde, H. W. Polymorphism of aldehyde dehydrogenase in human saliva. *Prog. Clin. Biol. Res*. **1989**, *290*, 133−139.
(26) Dyck, L. E. Polymorphism of a class 3 aldehyde dehydrogenase present in human saliva and in hair roots. *Alcoholism: Clin. Exper. Res*. **1995**, *19*, 420−426.
(27) Sreerama, L.; Hedge, M. W.; Sladek, N. E. Identification of a class 3 aldehyde dehydrogenase in human saliva and increased levels of this enzyme, glutathione *S*-transferases, and DT-Diaphorase in the saliva of subjects who continually ingest large quantities of coffee or broccoli. *Clin. Cancer Res*. **1995**, *1*, 1153−1163.
(28) Wierzchowski, J.; Wroczynski, P.; Laszuk, K.; Interewicz, E. Fluorimetric detection of aldehyde dehydrogenase activity in human blood, saliva, and organ biopsies and kinetic differentiation between class I and class III isozymes. *Anal. Biochem*. **1997**, *245*, 69−78.
(29) Wroczynski, P.; Wierzchowski, J. Aromatic aldehydes as fluorogenic indicators for human aldehyde dehydrogenases and oxidases: substrate and isozyme specificity. *Analyst* **2000**, *125*, 511−516.
(30) Kohen, R.; Tirosh, O.; Kopolovich, K. The reductive capacity index of saliva obtained from donors of various ages. *Exp. Gerontol*. **1992**, *27*, 161−168.
(31) Buettner, A. 2002, in preparation.
(32) Buettner, A.; Schieberle, P. Changes in the concentrations of key food odorants induced by mastication. In *Flavor Release*; Roberts, D. D., Taylor, A. J., Eds.; ACS Symposium Series 763; American Chemical Society: Washington, DC, 2000; pp 87−98.
(33) Buettner, A.; Beer, A.; Hannig, C., Settles, M.; Schieberle, P. Quantitation of the in-mouth release of heteroatomic odorants. In *Flavor Chemistry of Heteroatomic Compounds*; Reineccius, T., Reineccius, G., Eds.; ACS Symposium Series; American Chemical Society: Washington, DC, 2002; in press.
(34) Buettner, A.; Beer, A.; Hannig, C.; Settles, M. Observation of the swallowing process by application of videofluoroscopy and real-time magnetic resonance imaging—consequences for aroma perception. *Chem. Senses* **2001**, *26*, 1211−1219.
(35) Linforth, R.; Taylor, A. J. Persistence of volatile compounds in the breath after their consumption in aqueous solutions. *J. Agric. Food Chem*. **2000**, *48*, 5419−5423.
(36) Rychlik, M.; Schieberle, P.; Grosch, W. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants*; Deutsche Forschungsanstalt fuer Lebensmittelchemie: Munich, Germany, 1998.
(37) Guth, H.; Grosch, W. Identification of the Character Impact Odorants of Stewed Beef Juice by Instrumental Analyses and Sensory Studies. *J. Agric. Food Chem*. **1994**, *42*, 2862−2866.
(38) Murray, K. E.; Whitfield, F. B. The occurrence of 3-alkyl-2-methoxypyrazines in raw vegetables. *J. Sci. Food Agric*. **1975**, *26*, 973−986.
(39) Czerny, M. Untersuchungen ueber den Beitrag von Carbonylverbindungen, Pyrazinen und Furanonen zum Aroma von Arabica-Kaffee. Ph.D. Thesis, Technical University, Munich, Germany, 1999.
(40) Czerny, M.; Grosch, W. Potent odorants of raw arabica coffee. Their changes during roasting. *J. Agric. Food Chem*. **2000**, *48*, 868−872.
(41) Kerler, J.; Grosch, W. Character impact odorants of boiled chicken: changes during refrigerated storage and reheating. *Z. Lebensm. Unters. Forsch*. **1997**, *205*, 232−238.
(42) Schieberle, P.; Mestres, M.; Buettner, A. Characterization of aroma compounds in fresh and processed mandarin oranges. In *Freshness and Shelf Life of Foods*; Weenen, H., Cadwallader, K., Eds.; ACS Symposium Series; American Chemical Society: Washington, DC, 2002, in press.
(43) Mayer, F.; Czerny, M.; Grosch, W. Influence of provenance and roast degree on the composition of potent odorants in Arabica coffees. *Z. Lebensm. Unters, Forsch. A: Food Res. Technol*. **1999**, *209* (3−4), 242−250.
(44) Guth, H. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem*. **1997**, *45*, 3027−3032.
(45) Blekas, G.; Guth, H.; Grosch, W. Changes in the levels of olive oil odorants during ripening of the fruits. In *Trends in Flavour Research*; Maarse, H., van der Heij, D. G., Eds.; Elsevier Science: Amsterdam, The Netherlands, 1994; pp 499−502.
(46) Gassenmeier, K.; Schieberle, P. Potent aromatic compounds in the crumb of wheat bread (French-type)—influence of preferments and studies on the formation of key odorants during dough processing. *Z. Lebensm. Unters. Forsch*. **1995**, *201*, 241−248.
(47) Schieberle, P. Studies on the flavour of roasted white sesame seeds. In *Progress in Flavour Precursor Studies*; Schreier, P., Winterhalter, P., Eds.; Allured Publishing: Carol Stream, IL, 1993; pp 343−360.
(48) Mayer, F. Untersuchungen über das Aroma von Arabica Kaffee: Einfluss von Provenienz und Röstgrad auf Phenol- und Schwefel-Verbindungen und (*E*)-*β*-Damascenon. Ph.D. Thesis, Technical University, Munich, Germany, 2000.

Received for review June 27, 2002. Revised manuscript received September 9, 2002. Accepted September 10, 2002. This work was supported by the Deutsche Forschungsgemeinschaft (Grant Bu1351/1) and by the Deutsche Forschungsanstalt fuer Lebensmittelchemie.

JF020714O