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Effect of Whey Protein on the In Vivo Release of Aldehydes

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Retention of aldehydes by whey proteins in solutions buffered at a range of pH values was studied under static and dynamic headspace conditions and in vivo in exhaled air. Static headspace measurements showed a clear increase in retention in the presence of whey proteins for aldehydes with longer carbon chains and for buffer solutions with higher pH values. For in vivo aldehyde release measurements, these effects were much less pronounced. The presence of saliva or the binding of aldehydes to the surface of the oral cavity was not responsible for this effect. This difference can be explained by the highly dynamic conditions of in vivo aroma release of liquid products, due to the relatively large flow of air during exhalation. After swallowing, a thin film of aldehyde solution remains in the pharynx; subsequent exhalation will release both the free aldehydes present in this film and those reversibly bound to the whey protein.

KEYWORDS: APCI-MS; reversible interaction; aroma retention; flavor release

INTRODUCTION

Aroma quality, together with texture, taste, and color, is a consumer driver for the overall liking and acceptance of a food product. Aroma compounds are perceived when they are released from a food product and reach the olfactory region in the nasal cavity, either before or during consumption (*1*). Measurement of real-time in vivo aroma release can be performed by new mass spectrometric methods that have been developed for this purpose based on atmospheric pressure chemical ionization−mass spectrometry (APCI-MS) (*2*, *3*) and proton transfer reaction−MS (PTR-MS) (*4*).

Aroma compounds can interact with other food ingredients, and these interactions are thought to influence the amount of aroma compound available for release. This might affect the overall perception of a product (*5*). Much research effort has been invested in protein−aroma interactions, especially in the interactions between aroma compounds and β-lactoglobulin. The subject of interactions between aroma compounds and food ingredients, and parameters affecting these interactions, has recently been reviewed (*5*). Many studies have revealed an increase of binding constants with increasing carbon chain length for the binding of small molecules as aldehydes, ketones, esters, and alcohols to β-lactoglobulin (*6*−*11*), strongly suggesting hydrophobic interactions, which have a reversible character (*8*). β-Lactoglobulin is reported to have two separate binding sites for hydrophobic ligands (*12*−*14*). While retinol (*12*) and fatty acids (*14*, *15*) are reported to bind in the central cavity of β-lactoglobulin, information about the exact binding side of other ligands is sometimes contradictory (*6*, *16*−*19*). A recent study of the binding sites of two aroma molecules, γ-decalactone and β-ionone, using nuclear magnetic resonance spectroscopy, demonstrated binding of the former compound into the central cavity and binding to a groove near the outer surface of the protein of the latter (*20*).

The studies mentioned above, describing the interactions between β-lactoglobulin and various ligands, give no information about the behavior of these interactions in the dynamic situation in the mouth during the consumption of a food. In an attempt to understand the dynamic conditions of food consumption, several mathematical models were developed (*21*, *22*). The model developed by Harrison and Hills (*22*) predicts dynamic volatile release from solutions containing aroma binding macromolecules. The model is based on first-order kinetics to describe the reversible binding between the aroma compound and the polymer and on the penetration theory of interfacial mass transfer to describe the aroma release across the liquid−gas interface. The latter has been shown to be the rate-limiting step for aroma release in most situations (*22*). Andriot and coworkers (*11*) followed the initial release of volatiles from protein solutions by static headspace measurements after different times of equilibration (15−2700 s). These experimental data were fitted to the model developed by Harrison and Hills (*22*), and in general, there was good agreement between experiment and

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theory (*11*). However, the model was not validated by in-mouth measurements. In addition, possible interactions between aroma compounds and saliva and the effect of mucous membranes of the oral, nasal, and pharyngeal cavities on aroma release were not taken into account.

These interactions between aroma compounds and saliva or mucus membranes have been investigated in other studies (*23*−*26*). Aldehydes and other aroma compounds can interact with salivary proteins, especially mucin, as shown by static headspace measurements (*24*, *25*) and by the SOOM technique (spit-off odorant measurements) (*23*). Losses in the oral cavity were observed for aldehydes and esters during 1 min of contact time (*23*). In addition, esters, thiols, and aldehydes were found to be subject to enzymatic conversion upon contact with saliva within a period of 10 min (*27*−*29*).

In a recent study, Linforth et al. (*26*) investigated the effect of factors such as absorption to epithelia of the mouth, nose, and pharynx and dilution by saliva on aroma release through the nose. It was demonstrated that the mass transfer from aqueous solution in the mouth into exhaled air was a major factor affecting the actual released amount of aroma. However, up to now, the effect of the in-mouth conditions on the interactions between aroma compounds and food proteins has not been studied.

Therefore, the aim of the present work was to reveal the relevance for aroma release of interactions between food proteins and aroma compounds under in-mouth conditions by measuring and comparing the interactions between aldehydes and whey proteins under static and dynamic headspace conditions and under in-mouth conditions during consumption. During preparation of this paper, we became aware of comparable experiments performed by Le Guen and Vreeker and presented at the 10th Weurman symposium (2002).

MATERIALS AND METHODS

Materials. Butanal was obtained from Fluka Chemie (Buchs, Switzerland). Hexanal, octanal, and nonanal were obtained from Merck (Darmstadt, Germany). The purity of all aldehydes was higher than 98% (v/v). Buffers were prepared with citric acid (pH 3, 5, and 6), NaH_2PO_4 (pH 7), and glycine (pH 9). The pH was set with NaOH or HCl. Demineralized water was used. MCT (medium chain triglycerides) oil was provided by Quest International (Naarden, The Netherlands). Whey protein isolate (Bipro, JE 153-9-420) was obtained from Davisco Foods International Inc. (Le Sueur, MN). Specifications were as follows: pH 7.23; nonprotein nitrogen, 0.17%; ash, 1.8%; lactose, 0.34%; calcium, 0.13%; fat, 0.57%; total protein, 93.39% ($N \times 6.38$); α-lactalbumin, 12.6%; bovine serum albumin, 3.2%; immunoglobulin G, 5.2%; β-lactoglobulin A, 33.2%; and β-lactoglobulin B, 37.1%.

Artificial saliva was prepared in demineralized water according to van Ruth et al. (*25*) and consisted of $NaHCO_3$ (5.2 g/L), K_2HPO_4 (1.04 g/L), NaCl (0.88 g/L), KCl (0.24 g/L), $CaCl_2 \cdot 2H_2O$ (0.44 g/L), and 2.16 g/L porcine stomach mucin (Sigma, Steinheim, Germany). No α-amylase was added.

Human saliva was a mixture of the saliva donated by 30 nonsmoking individuals, who had not eaten for at least 1 h prior to donation. After it was mixed, the human saliva was stored in small portions at −20 °C. The mucin concentration in mixed human saliva was determined by fluorimetric measurement of O-glycosylated reducing oligosaccharides, according to the method of Crowther and Wetmore (*30*). The protein concentration in mixed human saliva was measured as total Kjeldahl-N. A conversion factor of 6.25 was used.

Preparation of Solutions. The solutions used in this study consisted of 50 mM buffer, containing 5 ppm of each aldehyde, with and without 3% (w/v) whey protein isolate. The final ionic strengths of all solutions were standardized to 0.272 by addition of NaCl. Aldehydes were first dissolved in ethanol (100% v/v). This introduced 0.2% (w/v) ethanol into the final solutions. To dissolve the whey protein, the solutions were stirred for at least 3 h.

Table 1. Amount of Saliva Formed (± Standard Deviations) during 3 s of Rinsing of 15 mL of Aldehyde Solution (Buffered at a Range of pH Values) and pH Values after Expectoration[a]

buffer	set pH	saliva formed (g)	pH after expectoration
citric acid–NaOH	3.00	1.7 ± 0.5	2.93 ± 0.02
citric acid–NaOH	5.00	1.1 ± 0.3	5.07 ± 0.02
citric acid–NaOH	6.00	0.7 ± 0.2	6.23 ± 0.01
$NaHPO_4$–NaOH	7.00	0.9 ± 0.2	7.03 ± 0.00
glycine–HCl	9.00	0.8 ± 0.3	8.88 ± 0.02

[a] All values are averaged over five people, who assessed the samples in triplicate.

To test the effect of fat present in the protein isolate, 0.017% (w/v) of MCT oil was added to the aroma solutions of pH 3 and 7, described above. The oil/aroma solution was stirred for 3 h. Whey protein isolate (35 ppm) was used as an emulsifier. No creaming was observed.

Measurement of Aroma Release by APCI-MS. Aroma concentrations in the breath of panelists as well as in the mouth model system were monitored by on-line sampling of part of the exhaled air by the MS-Nose, an APCI gas phase analyzer attached to a VG Quattro II mass spectrometer (Micromass UK Ltd., Manchester, U.K.). The compounds were ionized by a 3.0 kV discharge. Source and probe temperatures were 80 °C. Butanal, hexanal, octanal, nonanal, and acetone were analyzed in selected ion mode (0.08 s dwell on each ion), at *m*/*z* values of 73.0, 101.0, 129.0, 143.0, and 58.8, respectively. The cone voltages used were 19 V for acetone and 21 V for the aldehydes. Acetone was measured as an indicator of the panelists' breathing pattern.

Measurement of In Vivo Aroma Release in Exhaled Air. Aroma release measurements in exhaled air were conducted according to a strict protocol, developed for liquid samples (*31*) for which the panelists were trained. Panelists were considered to be sufficiently trained when their averaged relative standard deviation for all samples of a training session did not exceed 15%. In addition to this protocol, every measurement was preceded by rinsing the mouth with water followed by 30 s of drying the oral cavity by means of a hair dryer blowing unheated air. This procedure was followed in order to standardize the amount of saliva present in the mouth during the measurements. The in vivo sampling protocol started with taking 15 mL of solution in the mouth. This was followed immediately by nasal exhalation through the MS-Nose sampling unit, without any movement of the mouth for 3 s, indicated by a timer. In this way, the beginning of the measurement was marked in the acetone signal. Three seconds of inhalation followed, while every second a chewing movement was made. Then, the entire sample was swallowed, followed by an exhalation of 3 s. The panelists continued to breathe in and out for another 20 s, with 3 s per breath and a chewing movement every second. The panelists breathed in and out through a tube, from which continuously 80 mL/min of air was sampled directly into the APCI-MS source. The area of the peak in the release signal corresponding to the first exhalation after swallowing was used as a measure of aroma release. Two panelists assessed all samples in five replicates.

Mouth Model Measurements. A mouth model system, developed by Van Ruth and co-workers (*32*), was used for dynamic headspace measurements. A volume of 2 mL of solution was "chewed" by a plunger, making rotating and up and down movements, both at 1.25 Hz. The system was kept at 37 °C. The release of aldehydes from the mouth model was monitored for 4 min. Either human saliva, artificial saliva, or demineralized water was added to the samples in amounts proportional to the real-life situation. To determine this, the average saliva production of five persons who rinsed their mouth in triplicate with 15 mL of all of the buffer solutions (**Table 1**) was measured. The saliva production was determined by weighing the expectorates. From inside the mouth model system, above the plunger and liquid−gas interface, 3 mL/min of air was sampled continuously into the APCI-MS source. The maximum release signal was taken as a measure of dynamic aroma release. All samples were assessed in triplicate.

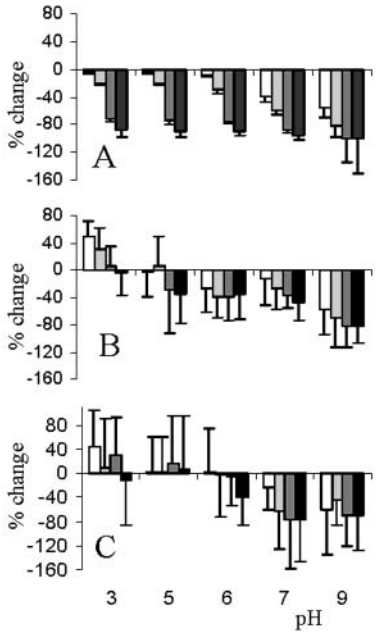

Figure 1. Relative change (%) in headspace concentrations (**A**) and in vivo aroma release of two panelists (**B** and **C**) of 5 ppm butanal (white bars), hexanal (light gray bars), octanal (dark gray bars), and nonanal (black bars) due to the presence of 3% whey protein at various pH values. Error bars represent standard deviations.

Spit-Out Experiments. Two panelists rinsed 15 mL of an aroma solution in the mouth in accordance with the protocol used during in vivo release measurements. Now, instead of swallowing the sample, it was spit out. Immediately after spitting it out, freshly secreted saliva was collected from the same panelist and added to 15 mL of nonrinsed aroma solution, in the same amount as the averaged increase in weight of the corresponding spit-out sample. All samples were directly frozen (−20 °C) and defrosted only 20 min prior to analysis by static headspace gas chromatography (HS-GC). For this purpose, the sample tray was coupled to a cooling water bath (−5 °C). All samples were prepared and analyzed in triplicate.

Static HS-GC Measurements. The equilibrium headspace aroma concentrations of aldehydes (4 mL of solution in 10 mL of headspace vials) were determined by GC. To this end, 1.0 mL of headspace was injected splitless on the column after 20 min of incubation at 30 °C. A GC-8000 top gas chromatograph (CE Instruments, Milan, Italy) was equipped with a CP-SIL 5 CB low-bleed column (50 m × 0.32 mm; film thickness, 1.2 μm; Varian Chrompack, Bergen op Zoom, The Netherlands) and a flame ionization detector. The oven temperature was initially 40 °C for 2 min, then increased by 25 °C/min to 250 °C, and was kept at 250 °C for 10 min. Inlet and detector temperatures were 250 and 270 °C, respectively. The headspace concentrations were expressed as peak areas in arbitrary units. All samples were prepared and analyzed in triplicate.

RESULTS AND DISCUSSION

Aldehyde−Whey Protein Interactions under Static Headspace and In Vivo Conditions. Interactions between whey protein and a homologous series of aldehydes were studied using both HS-GC and in vivo analysis. At the concentrations of whey protein isolate and aldehyde mixture used, aldehyde molecules and β-lactoglobulin monomer units were present in a ratio of approximately 1:6. The interactions were studied over a range of pH values. The static headspace results are shown in **Figure 1A**. This figure shows the relative change in static headspace aldehyde concentration due to the presence of 3% (w/v) whey protein. An increasing retention of the aldehydes is found with increasing length of the aldehyde carbon chain. This effect has been observed previously for aldehydes and other aliphatic compounds, and a hydrophobic interaction between protein and aldehyde has been proposed (*6*−*10*). Also, a higher retention at higher pH is found; this has also been reported previously (*8*, *33*, *34*). At a higher pH of the medium, the structure of β-lactoglobulin is more flexible, allowing a better accessibility of ligands to hydrophobic binding sites (*35*).

The static headspace concentration was reduced for all aldehydes (30−50%) in the solution of pH 9, irrespective of the presence of whey protein. This reduction could be caused by the formation of aldol condensation products from two or more aldehydes, a reaction well-known to occur at high pH (*36*). The calculation of retention due to presence of whey protein (as displayed in **Figure 1**) at pH 9 was based on the reduced headspace concentrations.

The whey protein isolate contained 0.57% (w/v) fat. This means that 0.017% (w/v) fat was present in the solutions of 3% (w/v) whey protein isolate. This hydrophobic phase might additionally affect the liquid−air partitioning of the aldehydes. This was tested with an aqueous emulsion of 0.017% MCT oil. A significant decrease in static headspace concentration was found for octanal (7 and 9%) and nonanal (25 and 27%) at pH 3 and 7, respectively (no further data shown). The presence of whey protein isolate (including the fat fraction), however, caused a decrease of 87 and 96%, respectively, for these two compounds (at pH 7). Therefore, as compared to the effect of protein, the fat content of the whey protein isolate was considered to play only a minor role in the aldehyde retention.

The results of the in vivo aroma release measurements in exhaled air for two panelists show (**Figure 1B**,**C**) a pattern that clearly differs from the static headspace data. In most cases, there is still retention of the aldehydes by the protein at pH values of 5−9. However, in contrast to the static headspace conditions, there is a much weaker effect of carbon chain length of the aldehydes on the extent of retention. At pH 3, there even seems to be an increase of release for all aldehydes, when whey protein is added. On the basis of these experiments, it is clear that large differences exist between measurements in exhaled air and static headspace. The aldehyde−protein interactions seem to be less significant under dynamic in vivo conditions than under static headspace conditions. The averaged relative standard deviations of our trained panelists were below 15% in earlier investigations (*31*). In the current study, higher values (20−40%) were obtained, which was probably due to the influence of the very bad taste and smell of the solutions studied.

Subsequently, three possible hypotheses were studied to explain the observed differences in aroma−protein interactions between static headspace and exhaled air. Two possible explanations, the effect of saliva and the effect of mouth mucosa and epithelium, concern factors that are present under mouth conditions and absent under static headspace conditions. The third explanation deals with the difference that exists between the physical conditions under which the aroma release takes place under static headspace and the in-mouth conditions.

Influence of Saliva on Aldehyde−Whey Protein Interactions. Preliminary HS-GC measurements of hexanal solutions that had been rinsed in the mouth showed a decrease of hexanal headspace concentration (25−90% depending on the test person, during hours of waiting time in the GC sample tray at ambient temperature). The decrease of aldehydes was accompanied by an increase of the same magnitude in hexanol headspace concentration, suggesting an enzymatic conversion. Moreover, a positive correlation was found between concentration of alcohol formed and waiting time in the GC sample tray before

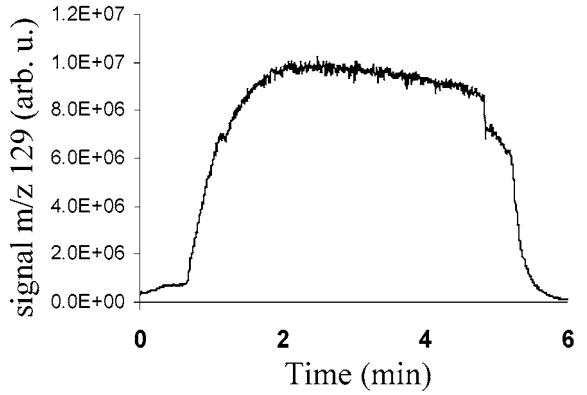

Figure 2. Raw data example of release of octanal from a 5 ppm solution in the mouth model.

analysis (results not shown). Conversion of aroma compounds by saliva within 10 min has been described previously for esters and thiols by Buettner (*28*). Hussein et al. (*27*) reported a reduction of benzaldehyde and cinnamaldehyde to their corresponding alcohols within 5 min, when solutions containing these compounds were rinsed in the mouth. The reduction of simple aldehydes to corresponding alcohols upon 10 min of incubation with saliva was demonstrated recently (*29*). Because we were interested in a shorter contact time between aldehyde−protein solutions and saliva, application of a mouth model directly coupled to APCI-MS seemed to be useful to exclude possible effects of longer contact times. In the mouth model used in this study, it is possible to add saliva just before the start of the real time dynamic headspace measurement of the release of aldehydes into the headspace. In **Figure 2**, an example of a dynamic headspace measurement in the mouth model is shown for octanal. As the plunger starts plunging and rotating, a steep increase in release is observed. After 1 min, a steady state is reached. In this situation, the rate of aldehyde release into the headspace is equal to the rate at which the aldehydes are sampled into the MS. After 3 min, a decrease in aldehyde release becomes visible, due to slow exhaustion of the aldehydes in the solution. In the 4 min during which the release of aldehydes from the mouth model was monitored, no differences were observed between samples with and samples without saliva.

The amount of water, human saliva, or artificial saliva to be added to the mouth model was determined by measuring the increased weight of spit-out buffered aldehyde solutions after rinsing these solutions in the mouth for 3 s. The amount of saliva produced varied slightly with pH (see **Table 1**). Solutions with lower pH provoke a stronger (more sour) taste sensation, and this is known to cause higher saliva secretion rates (*37*).

The pooled human saliva was analyzed for its total protein and mucin protein content, which were 4 and 1 g/L, respectively. This means that the pool of saliva contained 3 g/L of other proteins such as α-amylase, proline-rich proteins, immunoglobulin, lysozyme, staterin, histatin, lactoferrin, and many other small protein fractions (*38*), which are not present in our artificial saliva. An advantage of using human saliva is that the effect of these fractions can be taken into account as well. However, the addition of artificial or pooled human saliva had no effect on the aldehyde−whey protein interactions, as shown in **Figure 3A−C**, which represent aldehyde−whey protein interactions in the mouth model upon addition of water, artificial saliva, and human saliva, respectively. Moreover, no effect of addition of either artificial or human saliva on the dynamic headspace concentration of any aldehyde in the mouth model was found, irrespective of the presence of whey protein.

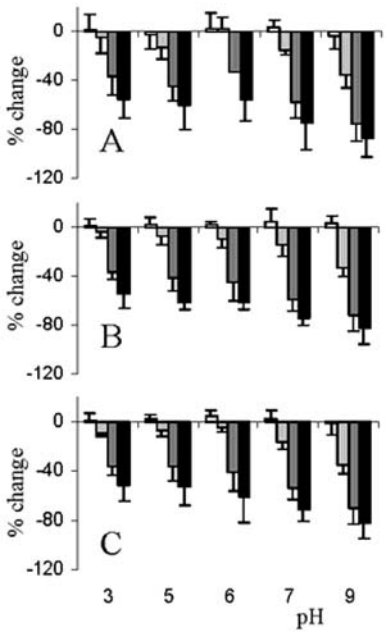

Figure 3. Relative change (%) in mouth model release signal with addition of water (**A**), artificial saliva (**B**), and human saliva (**C**) of 5 ppm butanal (white bars), hexanal (light gray bars), octanal (dark gray bars), and nonanal (black bars) due to the presence of 3% whey protein at various pH values. Error bars represent standard deviations.

In contrast to these results, Van Ruth et al. (*25*) and Friel et al. (*24*) reported a decrease in static headspace aldehyde concentration upon addition of artificial saliva. They both used pig gastric mucin, at concentrations of 2.16 and 2 g/L, respectively. The interaction between aldehydes and artificial saliva was tested without any dilution. In our study, artificial or human saliva was added to the mouth model in the same weight ratio (saliva:aldehyde solution) as human saliva was formed under in vivo conditions (**Table 1**), which resulted in a more than 10-fold lower mucin concentration as compared to Van Ruth et al. (*25*) and Friel et al. (*24*). This might be the reason that no effect of saliva was found in our experiments. In an additional experiment, the mucin concentration was increased by a factor 10. A decrease in dynamic headspace aldehyde concentration was found (data not shown). These results confirm the importance of choosing a representative mucin concentration. The magnitude of retention was comparable to the results of Van Ruth et al. (*25*). Friel et al. (*24*) reported a higher retention for aldehydes. However, it is difficult to compare the results in detail, because of variation in experimental conditions used.

Effect of Mucosa and Epithelial Tissue on Aldehyde−Whey Protein Interactions. As an excretion product, saliva can be obtained easily by expectoration and tested in vitro. However, the effect of the inside surface of the mouth is more difficult to study since isolation of this material is less simple. Nevertheless, the effect of the mouth coating could be studied by comparing headspace concentrations above aldehyde solutions that were rinsed in the mouth with those that were not. To account for the effect of saliva, the mouth was dried in advance. Furthermore, freshly formed saliva, sampled immediately after expectoration, was added to the nonrinsed sample in the same amount as the saliva, which was secreted into the rinsed sample during rinsing in the mouth. Because the saliva was collected directly afterward, its composition closely resembles that of the saliva secreted during the actual rinsing. Therefore, both the rinsed and the nonrinsed samples are assumed to have had a similar interaction with saliva. Two

Table 2. Static Headspace Concentrations (GC Area, Arbitrary Units, Divided by 10^5) of Aldehyde Solutions at Various pH Values, Before and After Rinsing the Solution in the Mouth[a]

		without protein		with protein	
	pH	before	after	before	after
butanal	3	8.1	7.5	8.3	7.1
	5	8.0	7.6	7.1	6.8
	6	8.1	7.6	6.7	6.3
	7	8.1	7.5	5.1	5.0
	9	5.3	5.2	2.1	2.2
hexanal	3	13.6	12.5	11.3	10.4
	5	13.8	12.8	11.1	10.4
	6	13.8	12.8	9.3	8.7
	7	14.1	12.6	6.3	6.1
	9	9.4	9.2	1.8	1.9
octanal	3	11.4	10.4	4.7	4.3
	5	13.0	11.6	4.3	4.2
	6	13.0	12.0	3.7	3.7
	7	12.9	11.8	2.2	2.2
	9	6.4	6.2	0.2	0.3
nonanal	3	11.3	10.2	4.7	4.4
	5	12.5	11.5	4.5	4.4
	6	13.0	11.4	3.9	3.8
	7	12.9	11.6	2.3	2.4
	9	7.6	7.1	0.3	0.3

[a] Data were collected in triplicate by two panelists.

differences remained between rinsed and nonrinsed solutions: the contact of the rinsed sample with the coating of the mouth and a minimal loss of aroma molecules due to a short exposure to the airflow during chewing. The first factor is the subject of this experiment, and the latter factor will be ignored because the actual released amount during 3 s of rinsing is extremely low. This was concluded from an additional experiment. In vivo aroma release measurements of 15 mL aqueous solutions containing 10 ppm hexanal were performed using the same protocol. The total released amount of hexanal in exhaled air was quantified and determined to be less than 1%. Buettner and Schieberle (*23*) also showed for aldehyde solutions that the release during the first 5 s of mastication was minimal by using SOOM.

The static headspace concentrations of aldehydes, averaged over two panelists (whose values closely agreed), which were either rinsed in the mouth or not, are given in **Table 2**. It can be seen that in both the presence and the absence of whey protein, the differences between rinsed and nonrinsed samples are small. The overall averaged decrease due to rinsing is 7% (ANOVA, $p = 0.0002$). Furthermore, the retention effect of whey protein did not differ between rinsed and nonrinsed samples (ANOVA, $p = 0.65$). The effect of whey protein on aldehyde retention and the aldehyde release itself were not influenced by the contact with the oral cavity.

Because the surface of the oral cavity causes only a small retention of aldehydes during a short rinsing time, it is reasonable to assume that the surfaces of the pharynx and nasal cavity will not cause large aldehyde retention. Linforth and Taylor reported similar results (*39*). In their investigation of the persistence in the breath of a range of aroma compounds, a relatively low value was found for aldehydes.

In the protocol used in this study, neither saliva nor mouth coating can account for the large difference in interactions between aldehydes and whey protein under mouth conditions as compared to static headspace conditions. Apparently, the difference is caused by another factor.

Effect of In-Mouth Conditions on Aldehyde−Whey Protein Interactions. An important difference between the in vivo and the static headspace measurements is the way the measurements are performed. Static headspace data are the result of an equilibrium headspace concentration, while in vivo aroma release data are obtained from a highly dynamic nonequilibrium release during a short time interval. Linforth et al. (*26*) have shown that the mass transfer from aqueous solutions in the mouth into exhaled air was a major factor affecting the actual released amount of aroma compounds.

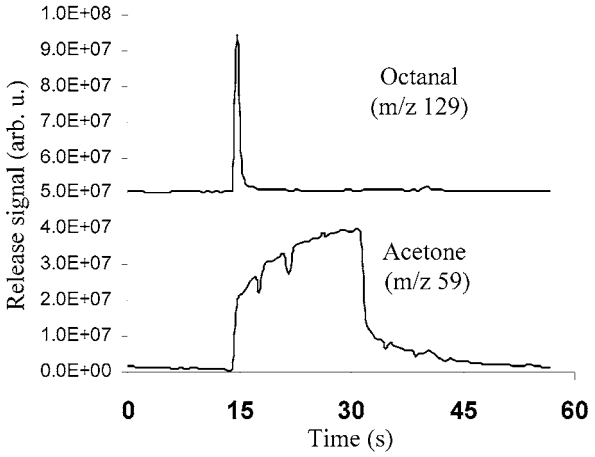

Figure 4. Raw data example of in vivo release of octanal during 15 s of continuous exhalation, directly after swallowing 15 mL of a 5 ppm octanal solution. Acetone is displayed as a breath indicator.

To fully understand the results of the present study, a closer look into the protocol used for measurement of in vivo aroma release is necessary. The sample is swallowed, immediately followed by an exhalation. After swallowing, most of the sample disappears via the throat into the esophagus. However, a small fraction remains as a thin film, coating the inner wall of the pharynx. This process was visualized by videofluroscopy and real time magnetic resonance imaging (*40*). The aroma compounds present in this thin film are exposed to a relatively large air flow (1.5−2.0 L/min) during the subsequent exhalation. To illustrate this effect, a panelist swallowed 15 mL of 5 ppm octanal solution and exhaled continuously for 15 s afterward. The release of octanal and acetone as breath indicator is shown in **Figure 4**. Octanal is released in a sharp peak at the beginning of the exhalation. Apparently, the octanal present in the thin film coating the pharynx is exhausted quickly. In this respect, the in vivo release process clearly differs from the release during HS-GC and mouth model measurements. Static HS-GC measurements involve the analysis of a small part of the equilibrated headspace air. In the mouth model system, a relatively large volume of aroma solution is exposed to a relatively small airflow, as compared to the in vivo situation. Our hypothesis for the in vivo aroma release process is that not only will all free aldehydes in the film be released into the exhaled air but also all of the aldehydes will be reversibly bound to the whey protein. This would explain why the presence of whey protein does not cause retention of aldehydes under in vivo conditions. The results also agree with the model of Harrison and Hills (*22*), where fast partitioning between bound and unbound states of aroma compounds plays an important role in the similar release of aroma compounds from samples with and without aroma binding macromolecules during initial stages of release. More experiments are currently being conducted in our laboratory to test this hypothesis.

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