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Intranasal Concentrations of Orally Administered Flavors

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Abstract

The odorants emanating from the oral cavity during eating and drinking reach the olfactory mucosa via the pharynx (retronasal olfaction). It is unclear which variables influence the perception of intraorally applied substances. The aim of the present study was to determine the temporal profiles of volatile odor concentrations at different locations in the nasal cavity during consumption of liquid and solid custard samples using proton transfer reaction mass spectrometry. Intranasal odor concentrations were measured at least twice in nine subjects (six female, three male) at four nasal positions during the consumption of liquid and solid custards. The low-viscosity custard was swallowed earlier than the more solid one. The compounds were found to reach the nose in different concentrations. Largest maximal amplitudes were measured in the nasopharynx, whereas lowest concentrations were found in the region of the olfactory cleft. In addition, different odorants reached the different regions in the nasal cavity in varying concentrations, indicated by a significant interaction between factors "position" and "compound." Furthermore, the compounds were found to reach the positions within the nasal cavity with different latencies. These results indicate that different volatile flavor compounds exhibit different temporal and spatial profiles in terms of their intranasal distribution.

Key words: food, retronasal olfaction, smell, swallow

Introduction

The sense of smell plays a dominant role in the perception of flavors. Substances from the oral cavity reach the olfactory mucosa via the pharynx (Halpern, 2004a,b). This is called "retronasal olfaction," in contrast to "orthonasal olfaction" in the case of smelling via the nostrils. The ability to perceive flavors is severely impaired in patients with anosmia (Heilmann *et al.*, 2002). However, this is often confused with a lacking sense of taste (Deems *et al.*, 1991).

Differences in the perception of orthonasal and retronasal olfactory perception are observed daily, as odor and flavor of some foods can be surprisingly different. For example, some cheeses have a strong and offensive smell; their flavor, however, can be mild and delicious. A number of factors contribute to this phenomenon (see below), but a part is still unclear. It is known that "flavor" is the result of an interplay of different senses, i.e., the olfactory system, the gustatory system, and the trigeminal system. These senses have been shown to interact (e.g., Cain and Murphy, 1980; Murphy and Cain, 1980; Livermore *et al.*, 1992; Davidson *et al.*, 1999; Hummel and Livermore, 2002; Livermore and Hummel, 2004). As different sensory channels are activated when chemical compounds reach the nasal cavity through the nostrils or through the nasopharynx from the mouth, it is conceivable that these interactions account for differences in perception of orthonasal and retronasal stimuli (Frasnelli *et al.*, 2004). However, they do not explain all the differences between ortho- and retronasal perception; even pure olfactory stimuli are perceived differently when applied orthonasally or retronasally (Heilmann and Hummel, 2004). One possible explanation may relate to the ideas of Mozell about a "chromatographic model" of olfaction (Mozell, 1970). This model suggests that the direction of odorant flow across the olfactory epithelium, from front to back, or the reverse, may change the perception of odorants. As phrased by Mozell (1970), "... the molecules of one substance, when allowed to do so, will migrate along a liquid or solid surface more rapidly and reach a given point in greater numbers per unit time than will the molecules of another substance." A physiological basis for this hypothetical effect may include the zonal organization of olfactory receptor neurons within the olfactory epithelium (Ressler *et al.*, 1993; Whitby-Logan *et al.*, 2004).

Apart from anatomical and physiological characteristics (Geary *et al.*, 2004; Zhao *et al.*, 2004), another aspect to take into consideration with retronasal olfaction is the interaction

between sensory perception of the flavor and texture of food products. Variables such as hardness, water-holding capacity, or microstructure of foods have been shown to affect the perception of flavor. The influence of the viscosity of food has not yet been elucidated very well. Modification of the viscosity often results in a significant change in perceived flavor (Clark, 1992; Carr *et al.*, 1996; van Ruth *et al.*, 2004). The explanation used to be that increased viscosity hinders the mixing process by which flavor molecules are brought from the interior of the sample to the surface (Morris, 1995). However, thickened solutions of similar viscosity do not induce the same flavor perception. Furthermore, some studies showed that although thickening of solutions affected flavor intensities, it did not result in a change in the in-nose–measured flavor concentration (Hollowood *et al.*, 2002). Moreover, one study claims that the texture of gels determines perception of volatile flavor intensity rather than in-nose flavor concentrations (Weel *et al.*, 2002).

With regard to flavor–texture interactions, texture can affect the flavor of food products through physical or chemical interactions, resulting in a change of availability of flavor. Flavor compounds may be bound, entrapped, etc. to textural agents, which alters the thermodynamic properties of the system (Noble, 1996). Texture can also have an effect on the kinetic aspects of flavor release, by reducing flavor transport through the food product (van Ruth, 2002). The cross-modal interactions are most likely to occur at the perception level during neural processing (Nahon *et al.*, 1996). Thus, there are a large number of factors that determine whether, to what extent, and at what level flavor–texture interactions occur.

So, in order to understand the processes leading to retronasal olfaction, the respective influence of numerous variables needs to be understood. The aim of the present study was to determine the temporal profile of volatile flavor concentrations at different locations in the nasal cavity during consumption of liquid and solid custard samples by proton transfer reaction mass spectrometry (PTR-MS). By doing so, spatial and temporal differences in maximal concentrations of compounds with different physicochemical properties could be examined. In addition, the influence of viscosity on release of flavor compounds was evaluated. The following hypotheses have been put forward:

1. Different volatile flavor compounds exhibit different temporal profiles in terms of their intranasal distribution.
2. Release of volatile flavor compounds is dependent on the viscosity of the custard.

Materials and methods

Flavor

A commercial strawberry flavor mixture was obtained from Givaudan (Duebendorf, Switzerland). It was composed of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (furaneol; 5 mg/g), vanillin (5 mg/g), methyl cinnamate (24 mg/g), ethyl hexanoate (20 mg/g), ethyl butyrate (90 mg/g), benzyl acetate (2 mg/g), styrallyl acetate (1 mg/g), γ-decalactone (20 mg/g), methyl anthranilate (1 mg/g), ethyl iso-pentanoate (10 mg/g), hexanal (1 mg/g), *cis*-3-hexenyl acetate (5 mg/g), *cis*-3-hexenol (15 mg/g), methyl dihydrojasmonate (5 mg/g), and β-ionone (1 mg/g) in triacetin. Diacetyl (Sigma–Aldrich Chemie, Steinheim, Germany) was added to the strawberry flavor mixture (75 μl/ml flavor mixture) to allow evaluation of a hydrophilic compound. Carboxymethyl cellulose (CMC) was used to vary the firmness of the custards (C-5678; Sigma–Aldrich Chemie).

Subjects

A total of 10 subjects participated (three men, seven women). All were in excellent health. The nasal cavities of the subjects were examined by endoscopy.

Custard preparation

Two custards of different viscosities were prepared, namely, a liquid and a solid custard containing 0.1% and 1% CMC, respectively. The textural properties of the custards have been characterized previously (van Ruth *et al.*, 2004). This study showed significant differences between the custards in terms of rheological and sensory properties. For custard preparation, full-fat milk was heated to 60°C. Sucrose (Siucra; Irish Sugar Ltd, Carlow, Ireland) was added, and the mixture was stirred for 3 min. CMC was added in small increments to ensure that it was fully dispersed. To obtain the custard texture, the mixture was stirred again for 5 min. The custard was transferred to a 95°C water bath with continued stirring. When the custard reached a temperature of 90°C, heating continued for another 10 min. It was subsequently cooled down at room temperature for 15 min and further to 30°C by placing the bottle in cold water. A total of 40 g of the custard was placed in a 100-ml glass bottle, 14 μl of the strawberry flavor mixture was injected in the custard, and the bottle was sealed. The custard was stirred for another 5 min and stored at 4°C for 24 h prior to analysis. Three batches of custard were prepared per subject. Each batch of custard was evaluated at the four nasal positions. At least two batches were analyzed per nasal position.

Procedure

Intranasal flavor concentrations were measured at least in duplicate at four nasal positions during consumption of liquid and solid custards. A Teflon tube (polytetrafluoroethylene; KronLab, Sinsheim, Germany; inner diameter: 0.75 mm, outer diameter: 1.6 mm) was placed in the respective position under endoscopic control. To keep the tubing in place, it was attached to clips mounted on a frame similar to lensless glasses. The setup had one inlet for intranasal sampling and an orthogonal inlet. This setup allowed the

removal of air, without disturbing the assessor's breathing or eating pattern. After connecting the tubing to the PTR-MS, background was measured for 60 scans (1 scan lasted approximately 1.8 s). At scan 60, the subject put a spoon with 7 g of custard (20°C) into his/her mouth and chewed and swallowed freely. Subjects indicated their first swallow by raising their hand. Measurements continued until scan 120 (approximately 3.5 min). After that, the position of the tubing was again controlled by endoscopy.

Intranasal analysis

In each subject, intranasal flavor concentrations were measured in four positions as follows: (1) in the nostril, (2) in front of the middle turbinate, (3) in the area of the olfactory cleft, and (4) in the nasopharynx (see Figure 1).

Proton transfer reaction–mass spectrometer

The air was drawn from the tubing into the heated transfer line and further to the PTR-MS at a rate of 100 ml/min, 15 ml of which was directed into the PTR-MS. Preliminary scans of the flavored custards as well of the individual flavor compounds (*m*/*z* 20–220) revealed that the masses *m*/*z* 87, 117, and 145 could be assigned exclusively to diacetyl, ethyl butyrate, and ethyl hexanoate, respectively. The other compounds were either below detection limits or had parent/major product ions in common. The samples were analyzed according to Lindinger (Lindinger *et al.*, 1998; Roberts *et al.*, 2004) employing a constant drift voltage of 600 V. Transmission of the ions through a quadrupole was considered according to the specification of the instrument. Spectra were background and transmission corrected. From the individual curves, maximum intensities (I_{max}), time to maximum intensity (t_{Imax}), and total amount [concentrations at single scans (ppmv) × volume screened in the respective time interval (ml)] were determined. In addition, the latency of the swallow was assessed ($t_{swallow}$), and data were corrected for it when applicable. The slope of the response was calculated as the ratio of maximum intensity (I_{max}) to latency of maximum intensity (t_{Imax}).

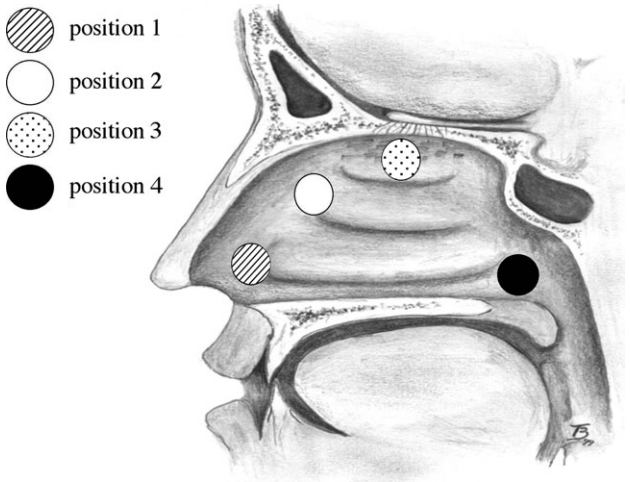

Figure 1 Schematic drawing of the positions of measurement within the nose.

The liquid and solid custards were analyzed on two different days for each subject. On one day, a single custard was evaluated at the four positions and at least in duplicate. Usually, two subjects were subjected to the analysis of one type of custard per day.

Statistical analysis

To normalize the data, I_{max} and total amount values were submitted to *z*-transformation. Data were analyzed by means of SPSS 12.0 for Windows (SPSS Inc., Chicago, IL). A repeated measures analysis of variance was computed [within-subject factors: custard (solid, liquid), intranasal recording position (1, 2, 3, 4), and compound (diacetyl, ethyl butyrate, ethyl hexanoate)]. *Post hoc* *t*-tests were applied where appropriate. The α level was set at 0.05.

Results

In 9 of 10 subjects, measurements could be performed with both custards at all positions; measurements could be repeated at least once. Grand means from these measurements are presented in Figure 2.

I_{max}

The amplitude of the maximal response was found to be dependent on the position of measurement. In the nasopharynx, largest responses were measured, whereas lowest concentrations were found in the region of the olfactory cleft ["position": $F(3,24) = 13.2$; $P < 0.001$]. The compounds were found to reach the nose in different concentrations ["compound": $F(2,16) = 154$; $P < 0.001$]. The average I_{max} of ethyl butyrate was 33 ppmv, whereas diacetyl and ethyl hexanoate reached, on average, 6.6 and 2.6 ppmv, respectively. In addition, a significant interaction between these factors was found [position × compound: $F(6,48) = 9.9$; $P = 0.001$], indicating that different chemical substances reach the different regions in the nasal cavity in varying concentrations. For example, the ratio diacetyl:ethyl hexanoate was 2.0 in the olfactory cleft, whereas this ratio was 3.6 in the nasopharynx (liquid custard; see Figure 3 for *post hoc* comparisons between positions).

t_{Imax}

Depending on the type of custard, maximal responses were reached with a different latency ["custard": $F(1,8) = 19.4$; $P = 0.002$]. This was mainly due to different latencies between uptake and swallowing ($t_{swallow}$) which was 4.9 and 6.3 s for the liquid and solid custards, respectively [custard: $F(1,7) = 7.6$; $P = 0.028$]. Thus, after administration of the liquid custard, maximal concentration was reached, on average, after

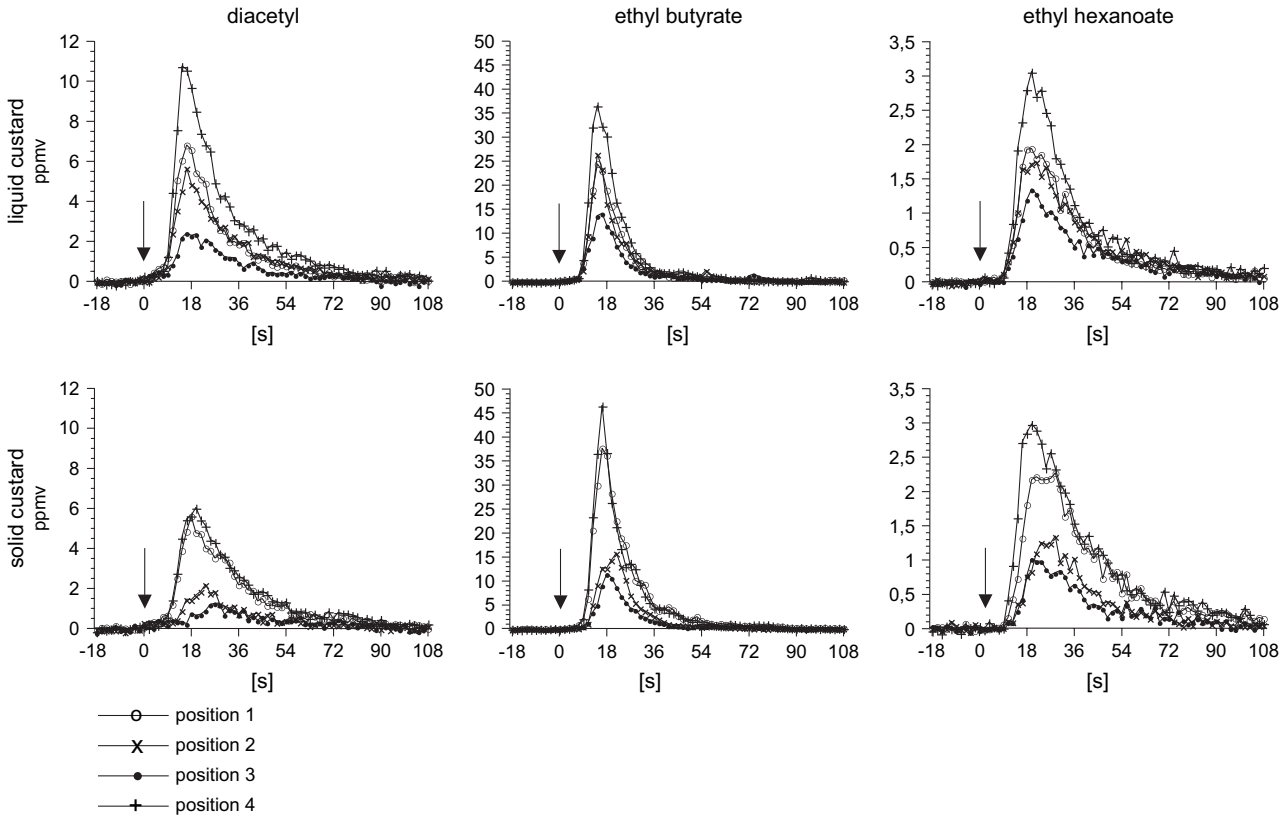

Figure 2 Grand means (n = 9) of intranasal concentrations of three flavor compounds after administration within custard of different viscosities at four different positions (position 1: at the nostril; position 2: in front of the middle turbinate; position 3: in the area of the olfactory cleft; position 4: in the nasopharynx). Custard intake took place at time point 0 as indicated by the arrow.

10.1 scans. As one scan lasted approximately 1.8 s, this corresponds to 18.2 s. The respective value was 12.7 scans (22.9 s) for the solid custard. When corrected for individual swallow latencies, there was no significant effect of custard detectable on $t_{I\max}$. These results imply that $t_{I\max}$ was related to t_{swallow}. However, even after correction for swallow latencies, the compounds were found to reach the nasal cavity with different latencies [compound: $F(2,16) = 6.3$;

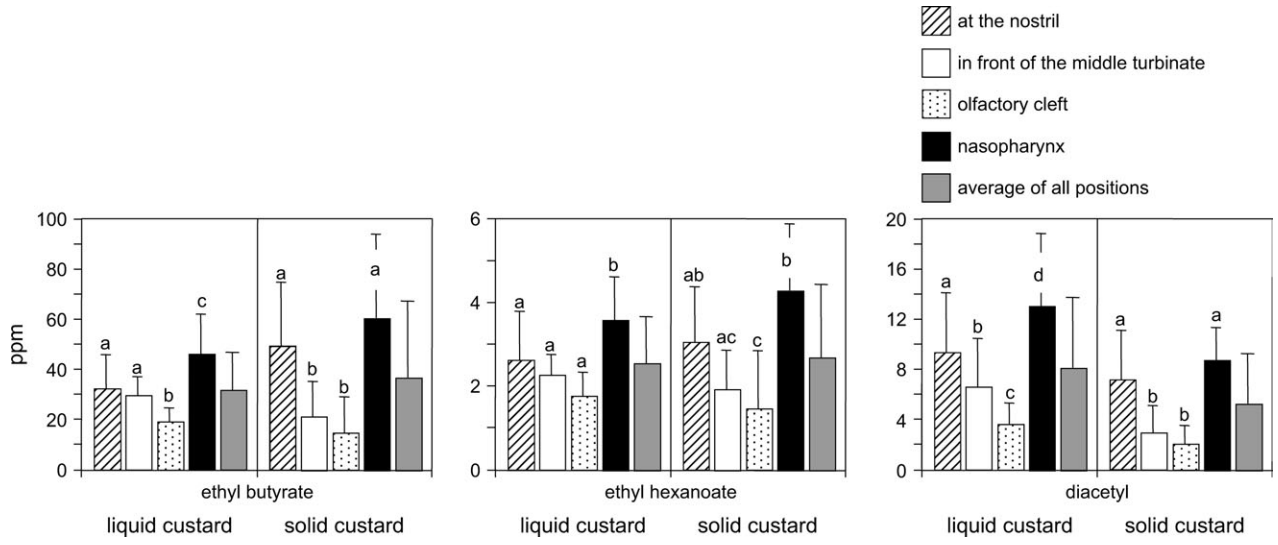

Figure 3 Maximum intranasal concentrations of three flavor compounds after administration with custards of different viscosities measured at four different positions (means, standard deviation). Significant differences in *post hoc* comparisons between positions are indicated by different letters ($P < 0.05$).

$P = 0.022$). The maxima of ethyl butyrate, diacetyl, and ethyl hexanoate were reached after scan 10 (18 s), scan 11.5 (20.7 s), and scan 12.8 (23.8 s), respectively. When not corrected for $t_{swallow}$, there was an interaction between the two factors custard and compound [custard × compound: $F(2,16) = 4.81$; $P = 0.029$], indicating that different chemical compounds are released from the custard depending on its viscosity. For example, the difference of t_{Imax} between liquid and solid custards for diacetyl was 4.2 scans [7.6 s; liquid custard: peak at scan 9.3 (16.7 s); solid custard peak at scan 13.5 (24.3 s)]. This difference was 1.1 scans (1.9 s) for ethyl butyrate (see Figure 4 for *post hoc* comparisons between compounds).

Total amount

The total amount of compounds measured was significantly different at the varying positions [position: $F(3,24) = 9.4$; $P = 0.001$]. Highest amounts were reached in the nasopharynx, lowest in the olfactory cleft. Depending on the viscosity of the custard, the total amount of compounds was different [custard: $F(1,8) = 12.5$; $P = 0.006$). On average, after the solid custard (210 nl), the total amount of compound was only 66% of the amount reached with the liquid custard (315 nl). The fact that the compounds were released in different concentrations was reflected by a significant effect of compound [$F(2,16) = 103$; $P < 0.001$). Largest total amounts were reached for ethyl butyrate (490 nl) and lowest for ethyl hexanoate (85 nl). In addition, there were interactions between these factors: the measured volume of the single compound was dependent on the position [position × compound: $F(6,48) = 6.6$; $P = 0.004$]. This indicates that different compounds show different distribution patterns within the nasal cavity. For example, the ratio ethyl hexanoate:diacetyl was 22:37 (59%) in the olfactory cleft (liquid custard). In the nasopharynx, this ratio was 43:136 (32%). Finally, single compounds were found to be released differently depending on the custards' viscosity [custard × compound: $F(2,16) = 18.8$; $P = 0.001$] (for *post hoc* comparisons between positions, see Figure 5).

Slope

The buildup of the response was found to be dependent on a number of variables. Different compounds were found to have different slopes [compound: $F(2,16) = 77$; $P < 0.001$]; steepest slopes were found for ethyl butyrate (5.4 ppmv/s) and the smoothest for ethyl hexanoate (0.3 ppmv/s). In addition, compounds were found not to peak with the same speed at the different positions [position: $F(3,24) = 11,3$; $P = 0.001$]; on average, the slope was largest in the nasopharynx (4.6 ppmv/s) and smallest in the olfactory cleft (1.3 ppmv/s). In addition, there was an interaction between these two factors [position × compound: $F(6,48) = 8.8$; $P = 0.002$]. For example, the slope was 4.8 times steeper for diacetyl than for ethyl hexanoate at the nostril, whereas it was 2.3 times higher in the olfactory cleft (liquid custard).

Discussion

The present study showed that different volatile flavor compounds have different temporal intranasal profiles when custards are consumed.

Firstly, a significant effect was found for the factor compound. Ethyl butyrate reached overall higher intranasal concentrations than the other two compounds. This was expected, as the compounds' concentrations were not the same in the custards. However, the interaction between the factors compound and position on I_{max} indicated that the compounds did not distribute homogenously within the nasal cavity. A possible explanation for this may be in the different absorption rates at the mucosa due to different hydrophobicities.

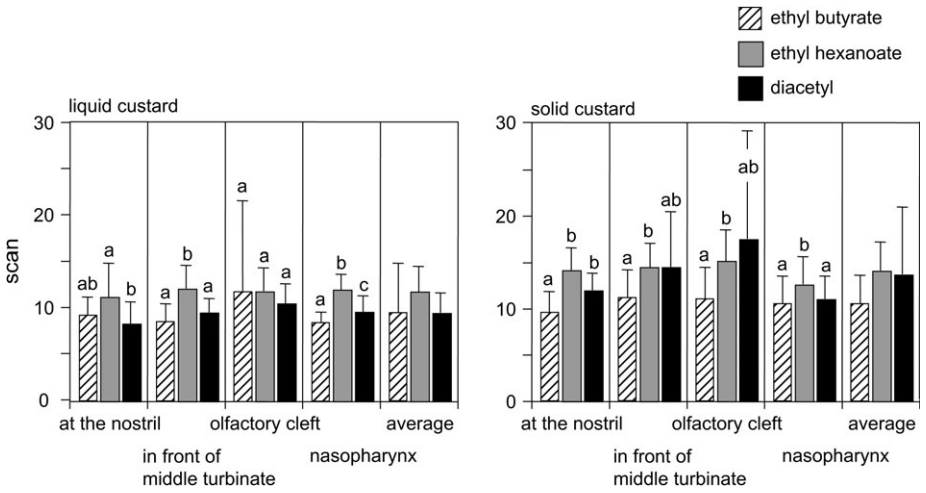

Figure 4 Scan number at which maximum intranasal concentrations were reached for the three flavor compounds after administration with custards of different viscosities measured at four different positions (means, standard deviation). Significant differences in *post hoc* comparisons between compounds are indicated by different letters ($P < 0.05$).

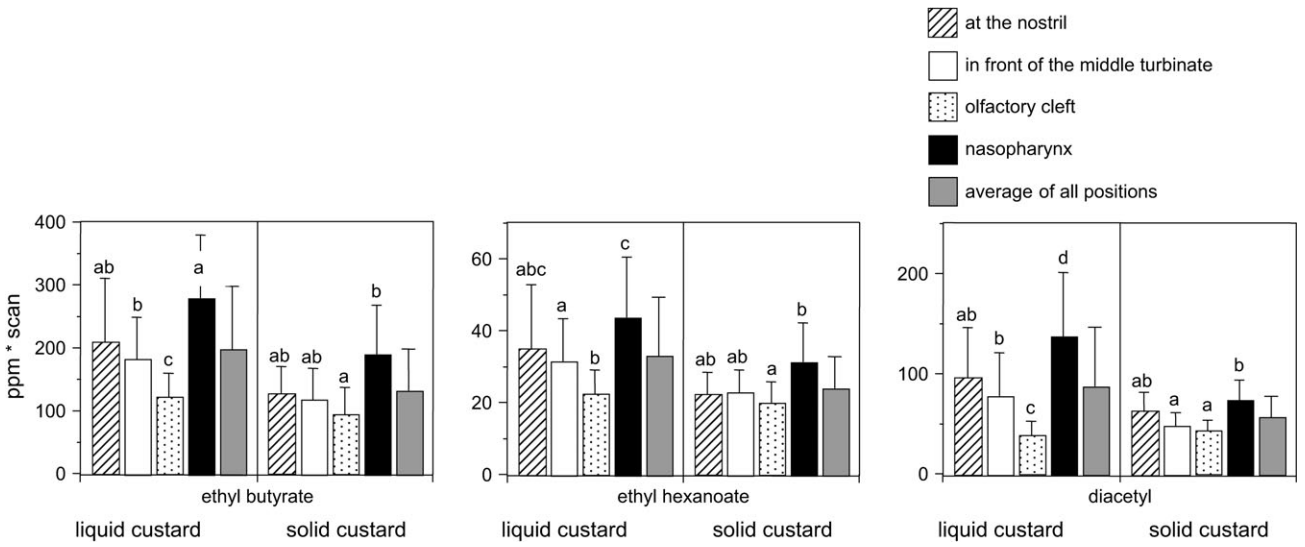

Figure 5 Total amount of the three flavor compounds after administration with custards of different viscosities measured at four different positions (means, standard deviation). Significant differences in *post hoc* comparisons between positions are indicated by different letters ($P < 0.05$).

The three compounds differ in hydrophobicity, with ethyl hexanoate being highly hydrophobic, followed by ethyl butyrate. Diacetyl is considered a hydrophilic compound; it has a log octanol/water partition coefficient of 0.80 (Lide, 1998). In order to relate differing distribution patterns within the nose to their hydrophility, one needs to look at the concentrations of the single compounds at the various positions. The data of the present study show that for the most hydrophilic substance, diacetyl, in the olfactory cleft, only 25% of its value in the nasopharynx could be measured. In contrast, in the olfactory cleft, 31% and 40% of the nasopharynx values were measured for ethyl butyrate and ethyl hexanoate, respectively.

It has been shown in the last few years that the human olfactory mucosa is not restricted to the olfactory cleft. In contrast, specific olfactory responses could be recorded from a region near to the anterior middle turbinate insertion. In addition, olfactory tissue could be detected histologically and immuncytochemically in some biopsy samples from that region (Leopold *et al.*, 2000). In another study, the olfactory mucosa was found in biopsy samples not only from the olfactory cleft but also from the ventroposterior middle turbinate (Feron *et al.*, 1998; Nibu *et al.*, 1999). Thus, the olfactory mucosa seems to be distributed over a larger area than in the olfactory cleft exclusively. It has to be remembered that in the present study concentrations were measured at different positions within the whole nasal cavity, i.e., partly at positions where no olfactory mucosa is present. However, the *post hoc* comparisons show that in some measures variables like I_{max} and total amount are significantly different between positions where it is highly likely that olfactory mucosa is present (Feron *et al.*, 1998; Nibu *et al.*, 1999; Leopold *et al.*, 2000).

Considering these findings, data from the present study could sustain the chromatographic theory of Mozell (1970), and together with the known zonal distribution of olfactory neurons in the olfactory mucosa (Ressler *et al.*, 1993; Whitby-Logan *et al.*, 2004), it may contribute to the differential perception of odors applied through the orthonasal and retronasal routes (Heilmann and Hummel, 2004). In this scenario, different odorants would not reach the olfactory mucosa at the same time, but "might spread in different spatial and temporal patterns along the mucosa in accordance with their diffusion rates and mucus solubilities" (Mozell, 1970). Therefore, the "earlier" odorants could occupy receptors and block them for later arriving ones.

There was a significant effect of compound on the latency of the maximal response. Ethyl butyrate had the maximal response at an average of 18.0 s after intake of the custard. The latencies of the maximal response for diacetyl and ethyl hexanoate were 20.5 and 23.0 s, respectively. In other words, the maximal concentration of diacetyl was reached 2.5 s after ethyl butyrate peaked; the maximum of ethyl hexanoate was measured 5.0 s after the ethyl butyrate peak. The reason for this phenomenon remains unclear. However, it is possible, that the latency of the peak response is related to the concentration of the compound. A flavor—in most cases a mixture of chemical substances—reaching the olfactory mucosa is thought to create a specific pattern of activation in the olfactory bulb (Buck and Axel, 1991; Mori *et al.*, 1999). Although slow when compared to vision or audition, the olfactory system processes information data in the range of hundreds of milliseconds. For example, odorant transduction may take 150 ms (Duchamp-Viret *et al.*, 2000; Knecht and Hummel, 2004), olfactomotor reflexes change 150–250 ms after stimulation (Johnson *et al.*, 2003), and olfactory event-related

potentials occur 300 ms after odor administration (Kobal and Hummel, 1988). Early components occur even earlier, and they also exhibit some variance (Kobal and Hummel, 1988). As they reached their respective peaks at different time points, in the present study, the proportion between odorants changed over the range of seconds. In the present study, in the beginning, mainly ethyl butyrate was measured. However, after its peak, its concentration decreased, whereas the concentration of the other compounds still increased. This indicates that in the case of retronasal olfaction, the picture evoked in the brain by a flavor changes over time. This is in spite of a relatively constant perception of a food odor, e.g., when eating a ham sandwich. It may be hypothesized that individual odors perceived within the context of eating this sandwich may trigger the entire ''gestalt'' of the sandwich flavor once this individual odor has been learned to be associated with the food. The temporal spacing of the concentration of different odors at the level of the olfactory epithelium may also relate to the capacity of the olfactory system to integrate odorous information over a longer period of time (Frasnelli *et al.*, 2005; Lorig *et al.*, 2005).

For all compounds, lowest concentrations were found in the region of the olfactory cleft. Only a small portion of flavor compounds reaching the nasal cavity also arrive at the olfactory cleft and, thus, at the olfactory receptors. The olfactory system seems to require only minute amounts of odorants in order to be activated and to be highly sensitive. This is in agreement with orthonasal olfaction, where it was shown that only a minor portion of the inhaled air reaches the olfactory cleft, whereas the largest part of the airstream flows through the lower portions of the nose (Keyhani *et al.*, 1995). However, some characteristics of orthonasal and retronasal smelling differ significantly. For example, lower thresholds after orthonasal stimulus presentation when compared to retronasal presentation were found in subjects when stimuli were presented independent of breathing (Heilmann and Hummel, 2004). In addition, the means to increase the airflow of odorous molecules to the olfactory cleft are different for orthonasal and retronasal smelling. When smelling orthonasally, this is possible through sniffing, a mechanism which is only partly under conscious control (Johnson *et al.*, 2003). Sniffing amplifies olfactory acuity by two ways. Firstly, the total amount of odorous substances in the nasal cavity is increased, and secondly, the probability of turbulent flow in the nose is increased (Churchill *et al.*, 2004), which, again, would lead to a higher number of odorous molecules reaching the olfactory mucosa. For retronasal olfaction, it has been shown that volatile compounds have access to the nasal cavity only during certain periods of the eating process, e.g., during swallowing (Buettner *et al.*, 2001). So, mouth movements were found to significantly increase odor intensity ratings when compared to a ''no mouth movement'' condition (Burdach and Doty, 1987). Based on the techniques presented in the present study, future investigations will focus on comparison of defined ortho- and retronasal stimulations on the level of measuring intranasal concentrations.

In the present study, no significant effect of the custard's viscosity could be detected on the latency of the maximal response when responses were corrected for swallow latencies. This underlines the fact that more liquid custards are swallowed quicker when compared to more solid ones (Aprea *et al.*, 2005). However, the overall release of compounds was dependent on the custard's viscosity, with the liquid custard leading to higher total amount when compared to the more solid one.

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