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Comparison of Nosespace, Headspace, and Sensory Intensity Ratings for the Evaluation of Flavor Absorption by Fat

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The goal of this study was to better understand the correspondence between sensory perception and in-nose compound concentration. Five aroma compounds at three different concentrations increasing by factors of 4 were added to four matrixes (water, skim milk, 2.7% fat milk, and 3.8% fat milk). These were evaluated by nosespace analysis with detection by proton transfer reaction mass spectrometry (PTR-MS), using five panelists. These same panelists evaluated the perceived intensity of each compound in the matrixes at the three concentrations. PTR-MS quantification found that the percent released from an aqueous solution swallowed immediately was between 0.1 and 0.6%, depending on the compound. The nosespace and sensory results showed the expected effect of fat on release, where lipophilic compounds showed reductions in release as fat content increases. The effect is less than that observed in headspace studies. A general correlation between nosespace concentration and sensory intensity ratings was found. However, examples of perceptual masking were found where higher fat milks showed reductions in aroma compound intensity ratings, even if the nosespace concentrations were the same.

KEYWORDS: Proton transfer reaction mass spectrometry; quantification; milk; flavor release; partition coefficient; masking; correlation; lipid; SPME

INTRODUCTION

Since the introduction of gas chromatography (GC) olfactometry methods in the mid 1980s and the increasing sensitivity of mass spectrometers, one focus has been on the identification and quantification of many foods' odor active compounds (*1*). Thus, on the flavor chemistry side, we know which compounds are present above the odor threshold and can contribute to the overall flavor impression. Sensory analysis is often conducted on complex food products to evaluate an induced flavor profile change. However, the changes seen sensorially are sometimes difficult to explain by changes in flavor compound composition.

Studies are needed to bridge this gap between flavor chemistry of single molecules and sensory perception of complex flavorings. Several fields work toward this goal as they study the relationships between compound concentration and perceived intensity, mixture perception, and compound synergy and suppression, among others.

Nosespace analysis can aid in this understanding, as we can measure the actual compound concentration close to the olfactory receptors. This technique should give closer results to human perception than headspace analysis, as the human mouth is used to generate the flavor released, and the compounds pass over the same retronasal passage to the olfactory receptors in the nasal cavity. In one study seeking to correlate nosespace analysis to sensory perception (*2*), a linear relationship was seen between the in-nose carvone concentration and the maximum perceived intensity, which depended on the person. In gels where texture plays a role, the perception was better correlated to the rate of volatile release in-mouth than to the maximum in-nose volatile concentration (*3*). The higher variability of nosespace methods caused by the human input, as compared to instrumental methods, has also been noted (*4*, *5*).

The purpose of this paper was to use nosespace analysis and sensory intensity measurement to better understand the psychophysics of flavor perception. In addition, the effect of fat in absorbing aroma compounds has been shown to be significant using headspace analysis, especially for lipophilic compounds, and well-predicted using the oil−water partition coefficient of the compound and the fat level (*6*). We were interested to investigate this significance in-mouth. Finally, headspace measurements were taken to evaluate the correspondence between nosespace and headspace analyses. The technique used for nosespace analysis was proton transfer reaction mass spectrometry (PTR-MS). As with atmospheric pressure chemical ionization (*7*), this technique offers the ability to monitor the release of protonated volatile compounds in real time (*8*, *9*). Several applications with PTR-MS nosespace analysis have been reported with coffee (*10*) and banana (*11*).

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MATERIALS AND METHODS

Samples. Four matrixes were studied as follows: water, whole milk (3.8% g/100 mL fat), semiskim milk (2.7% g/100 mL fat), and skim milk (0.033% g/100 mL fat). Bottled water (Vittel) and commercial shelf-stable ultrahigh-temperature milk were purchased locally. Five aroma compounds were used at the following concentrations: β-damascenone (3.125, 12.5, and 50 mg L^{-1}), hexanal (1, 4, and 16 mg L^{-1}), ethyl butyrate (1, 4, and 16 mg L^{-1}), benzaldehyde (1, 4, and 16 mg L^{-1}), and 2,3-butanedione (10, 40, and 160 mg L^{-1}) (Firmenich SA, Genève, Switzerland for β-damascenone and Fluka Chemie GmbH, Buchs, Switzerland, for the others). Three different concentrations were chosen that differed by a factor of 4: a low, a middle, and a high concentration series. The range of concentrations was chosen so that they would give a detectable signal by nosespace analysis and also an acceptable range sensorially. The compounds were verified to be soluble in all matrixes at the concentrations used. For the nosespace studies, the five compounds were analyzed together for the low, middle, and high concentration series. For the sensory studies, each compound was analyzed separately. Compounds were placed in 500 mL of milk or water using a high precision Microman Pipet (Gilson Inc. Middleton, U.S.A.) directly at the concentration indicated. They were dissolved with manual shaking and ultrasonic bath for 10−20 min. Samples were analyzed at room temperature after 1 h on the day that they were prepared.

Nosespace Analysis. The air exhaled through the nose was collected and combined into one larger tube of 7 mm inner diameter, which is open to the laboratory. In our experiments, the majority of the breath air is released into the laboratory air. Only 80 mL/min of the breath air was drawn up for analysis into a heated stainless steel tubing of 0.53 mm inner diameter. The tube was inactivated with an inner quartz coating ("silcosteel" tube from RESTEK, Bad Homburg, Germany). These 80 mL/min were split into two fractions: 14 mL/min was introduced for analysis into the drift tube of the PTR-MS (model FDT, IONICON Analytik GesmbH, Innsbruck, Austria), and the remainder was released through a flow controller and membrane pump into the laboratory air. Tubings were heated to 70 °C (60 °C for the drifttube) to prevent condensations.

The PTR-MS technique has been discussed in several review papers (*8*, *9*). Briefly, it combined a soft, sensitive, and efficient mode of chemical ionization, adapted to the analysis of trace volatile organic compounds (VOCs), with a quadrupole mass filter. The gas to be analyzed was continuously introduced into the chemical ionization cell (drift tube) and ionized by proton transfer from H_3O^+. The protonated VOCs are extracted by a small electrical field from the drift tube and mass-analyzed by a quadrupole mass spectrometer. The specific aspect of the chemical ionization scheme in PTR-MS is that the generation of the primary H_3O^+ ions and the chemical ionization process, VOC + $H_3O^+ \rightarrow$ VOC−H^+ + H_2O, are spatially and temporally separated and can therefore be individually optimized. A time resolution of 0.5 s was used, which corresponds to 100 ms dwell time per compound.

One interesting calculation made was the absolute amount of aroma compounds released in the mouth during eating. PTR-MS quantifies the amount reaching the instrument in nL L^{-1} using the following equation (*8*):

$$\frac{\Delta m_A}{\Delta t} = \frac{M_A \times F \times P_{amb} \times \mathrm{Int}_{A^*H^+} \times \mathrm{Trans}_{H_3O^+}}{\mathrm{Int}_{H_3O^+} \times N \times k \times t_{drift} \times P_{drift} \times \mathrm{Trans}_{A^*H^+}}$$

where $\Delta m_A/\Delta t$ is the quantity of compound (A) released per minute (μg/min); $\mathrm{Int}_{A^*H^+}$ is the = count per second corresponding to protonated compound (A^*H^+); $\mathrm{Int}_{H_3O^+}$ is the count per second of the primary ion H_3O^+; $\mathrm{Trans}_{H_3O^+}$ is the transmission of the MS at the mass of H_3O^+; $\mathrm{Trans}_{A^*H^+}$ is the transmission of the MS at the mass of protonated compound (A^*H^+); P_{drift} is the pressure in the drift tube (bar); k is the reaction rate constant (2×10^{-9} cm^3/s, estimated value, ref *8*; t_{drift} is the reaction time (105 μs); M_A is the molecular mass of A (g/mol); F is the breath flow (sccm); P_{amb} is the ambient pressure (bar); and N is Avogadro's number 6.0220×10^{23} (mol^{-1}).

The calculation for absolute amount released in nosespace analysis takes into account a value of the breath exhalation flow rate, 10 L/min, based on simple laboratory estimations with the panelists using the same breathing regulation. The previously published air volume flow rate through the external nares was 6 L/min (*12*). The values are directly proportional to the flow rate so values can be adjusted accordingly.

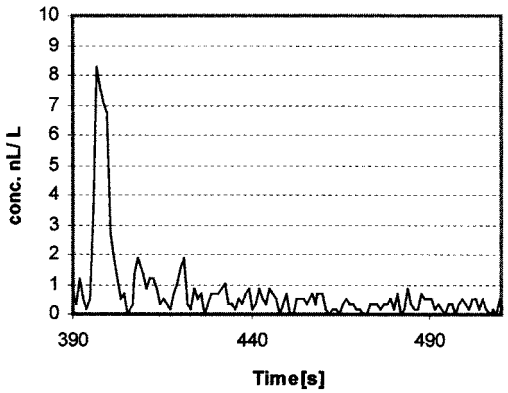

Figure 1. Example of nosespace analysis output showing the large exhalation peak of ion *m/z* 107 for benzaldehyde immediately after swallowing, followed by breathing where little additional benzaldehyde is released.

Five panelists participated in the study. They were introduced to the nosespace technique through previous studies or by training sessions beforehand. The panelists' breathing was regulated following a light on/light off timer, with a new breath every 11 s (5 s inspiration, 6 s expiration). A standardized method for tasting was used where 10 mL samples were swallowed around every 2 min. Just before exhaling, the samples were consumed and were swallowed immediately. Two minutes was chosen between samples because after this time, aroma compounds persistent in the breath returned to the baseline level. Although the sample order was randomized, five replicates of each sample were analyzed one after the other. The protonated mass ions (or a fragment for hexanal) were monitored simultaneously with the following *m/z*: 2,3-butanedione (87), benzaldehyde (107), β-damascenone (191), hexanal (83), and ethyl butyrate (117). Blank milk analyses for each milk type without aroma compounds were also run to verify that the ions followed were not present at substantial quantities in the milks. **Figure 1** shows an example of the aroma compound release when drinking a milk sample. The peak maximum of release from the first exhalation was used as the value for nosespace release. This also corresponds with the finding that the odor transfer to the nose is released mainly with the swallowing event (*13*).

Perceived Intensity Determination. The same five panelists participated in both the nosespace analysis and the perceived intensity determination, although the two analyses were conducted separately. For each compound, panelists tasted 12 samples (3 concentrations × 4 matrixes), presented in a randomized order and labeled with random three digit codes. The samples were presented in 10 mL quantities, and the panelists were not given any particular instructions about how to drink them. After a 30 min break, the panelists returned to analyze the duplicate 12 samples in a randomized order with different codes. The panelists were experienced tasters yet were not specifically trained with these samples. The nosespace analysis was conducted first; thus, the panelists were familiarized with the samples through this analysis. The panelists were asked to rate the perceived intensity of the added flavor compound, even with the sample's background of milk flavor. They marked their perceived intensity on a line, which was anchored at either end by the words weak and strong. Before the analysis, the weak and strong references were given and were also available throughout the tasting session. The weak reference was the lowest concentration in whole milk, as this sample was perceived the weakest in preliminary tastings with all compounds. The strong reference was the highest concentration in water, as this sample was perceived as the strongest in preliminary tastings with all compounds. These reference assignments were also based on the headspace analysis. Intensity values are assigned as line scale values 0−100.

Headspace Analysis by Solid Phase Microextraction (SPME). The samples analyzed by SPME were the lowest concentration series in all

Table 1. Compound Physical Properties and In-Nose Release Quantification

compd	compd lipophilicity (log k_w value)[a]	air–water partition coefficient at 30 °C using method from *23* [c]	% released in nosespace from aqueous solution[b]
2,3-butanedione	−0.3	0.0011 (0.00021)	0.13 (0.06)
benzaldehyde	1.02	0.0019	0.12 (0.04)
ethyl butyrate	1.44	0.052 (at 37 °C) (0.022)	0.55 (0.45)
hexanal	2.13	0.011	0.23 (0.17)
β-damascenone	2.79	0.0037 (0.0019)	0.07 (0.05)

[a] Higher values indicate higher lipophilicity; the method in ref *24* was used. [b] Quantification using eq 1. Aqueous solution of the highest concentration was used; average of 25 samples (5 replicates × 5 panelists). Standard deviation is in parentheses. [c] Standard deviation is in parentheses when available.

matrixes, and the concentrations were verified to be in the linear quantification range. A 800 μL amount of the flavored sample was placed in a 2 mL vial, in three vials for triplicate analysis. A balanced sample order was used where the first replicate of each milk type was analyzed, then the second, and last the third. A minimum time of 2 h was determined for equilibration. The release of each compound in water was used as a benchmark for the release of that compound in the emulsion. The peak area of each compound in milk (H_M) was expressed relative to the peak area of each compound in water (H_w) using the formula, $(H_M/H_w) \times 100$. The temperature for preparation, equilibration, and analysis was 25 °C. After equilibration, the headspace of the samples was sampled using a Varian SPME 8200cx autosampler (Walnut Creek, CA) and a HP 6890 gas chromatograph (Avondale, PA) equipped with a 5973 MS detector. A SPME fiber was inserted into the headspace and allowed to equilibrate for 1 min exactly. This time was chosen so that the extraction would be from the headspace and not from the sample, thus resembling a traditional static headspace analysis (*14*). For further experiments and data about this 1 min headspace absorption, see ref *14*. The fiber used was poly(dimethylsiloxane)/divinylbenzene with 65 μm thickness. It was placed into the injection port of the gas chromatograph for 5 min at 250 °C containing a 0.75 mm ID liner. During the first 3 min of desorption, the purge was off and the last 2 min with purge on further cleaned the fiber. Full desorption of the fiber was confirmed. GC separation with flame ionization detection was used for quantification of the aroma compounds (DBWAX, J&W, 30 m; 0.25 mm ID, 0.25 μm film, 1 mL/min constant flow). Blank milk analyses for each milk type without aroma compounds were also run to verify that the compounds followed were not present at substantial quantities in the milks. The average coefficient of variation for the triplicate analyses was 8% for 2,3-butanedione, 4% for benzaldehyde, 5% for ethyl butyrate, 6% for hexanal, and 9% for β-damascenone.

Statistical Analysis of Results. Analysis of variance ($\alpha \leq 0.05$) was used to determine the existence of significant differences among samples combined with a multiple comparison test (Fisher's LSD, $\alpha \leq 0.05$) to determine which samples were significantly different from the others. To have similar variance among the nosespace samples, a ln transformation of the values was performed prior to the statistical tests.

RESULTS AND DISCUSSION

Nosespace Release. Table 1 shows the amount released for the different compounds from the highest concentration sample in water. This liquid sample was swallowed immediately, as one would consume a beverage. These amounts ranged from 0.1 to 0.6% of the amount in the sample. This indicates that most of the volatile compounds that are present in the sample were either swallowed, absorbed by the mouth and nose membranes, or degraded. Of these options, the first probably has the largest effect. This has interesting consequences for flavoring food, if we know that most of the volatile flavoring added is not available to the receptors in the nasal cavity. There is a large potential reserve of flavoring that could still be released in mouth, rather than swallowed. Using the approach of quantifying the amount in spitted-off juice after 1 min of mastication, volatile compounds were still present in the solutions at levels of 60−90%, thus not released into the mouth air (*15*). The authors also found that more lipophilic compounds showed higher "losses" in the mouth. In our study, compound lipophilicity did not correlate with losses. Volatility in water is the parameter that correlated with compound losses. The compounds with the lowest volatilities in water showed the lowest % released: 2,3-butanedione, benzaldehyde, and β-damascenone. The compound with the highest volatility in water showed the highest % released: ethyl butyrate. In another approach of exhaled odorant measurement where the released volatile compounds exhaled from the nose were trapped on Tenax and quantified (*12*), the release for ethyl butyrate from an aqueous solution held in the mouth for 1 min and then swallowed was 0.2%. This compares similarly with our data.

Table 2. Nosespace Release of Compounds (nL L^{-1}) in Four Different Matrixes and with Three Different Concentrations of VOCs (See Materials and Methods Section; Each Differing by a Factor of 4)[a]

	low VOC	medium VOC	high VOC
	β-damascenone		
water	0.98 b	3.5 d	11.6 f
skim milk	0.65 a	2.3 c	8.7 e
semiskim milk	not detected	1.3 b	3.5 d
whole milk	not detected	0.93 b	3.3 d
	hexanal		
water	6.3 a	33.0 b	131 d
skim milk	6.3 a	28.5 b	110 cd
semiskim milk	5.4 a	33.4 b	114 cd
whole milk	5.9 a	26.1 b	81 c
	ethyl butyrate		
water	7.7 a	42.1 c	158 e
skim milk	9.0 a	33.8 bc	159 e
semiskim milk	7.7 a	45.3 bc	120 de
whole milk	8.3 a	29.5 b	81 d
	benzaldehyde		
water	3.1 b	16.1 d	65.7 f
skim milk	3.4 b	12.1 c	55.8 ef
semiskim milk	2.3 ab	11.7 c	46.2 e
whole milk	1.9 a	9.8 c	46.5 e
	2,3-butanedione		
water	22.5 a	98.0 b	398 c
skim milk	23.3 a	83.7 b	330 c
semiskim milk	21.1 a	102 b	319 c
whole milk	19.7 a	81.1 b	396 c

[a] Each value is the average of 25 measurements (5 panelists × 5 replications). Statistically significant differences by compound group are noted by different letters.

Table 2 shows the values for the nosespace release from the samples, that varied in compound concentration and matrix. Some individual differences were observed and a complete discussion of the intra- and interpersonal variability seen in this study can be found in ref *5*.

Table 1 shows the lipophilicity values of the compounds. As expected, on the basis of its low lipophilicity, 2,3-butanedione did not show any statistically significant differences in nosespace released depending on the fat content of the milk. This agrees with previous results using headspace analysis (*6*).

Benzaldehyde showed a significant decrease at all concentrations (36% decrease, averaged over three concentrations) in release from whole milk, as compared to water. Skim milk and semiskim milk showed slight decreases from water, seen for

the two higher concentrations. In general, similar results were obtained at all three concentrations. On the basis of the low lipophilicity value of benzaldehyde, the expected slight effect of fat content was seen. There appears to be a small interaction with skim milk (average 10% reduction from water). This interaction was also seen in the sensory intensity ratings. Indeed, previous studies showed the presence of noncovalent binding complexes between benzaldehyde and β-lactoglobulin (*16*).

Ethyl butyrate also showed a significant decrease in release from whole milk, as compared to water at the two higher concentrations (30 and 49% decrease, with increasing concentrations, respectively). Because of higher variability in the results, differences between release in water and release in skim or semiskim milks were not statistically significant. A moderately lipophilic compound, decreases in nosespace measured release upon increasing fat content can be seen in the highest concentration series.

The only statistically significant results with hexanal are that whole milk showed a 39% decrease over water for the highest concentration series. As with ethyl butyrate, the effects with whole milk were greater at higher concentrations.

β-Damascenone is the most lipophilic compound studied and thus had the largest effects based on fat content. The lowest concentration fell beneath the instrument's limit of detection for the 2.7 and 3.8% fat milks. The two highest concentrations showed similar results. Release in water was the highest followed by release in skim milk (30% decrease), release in 2.7% fat milk (67% decrease), and release in 3.8% fat milk (73% decrease). Concentration was studied as a variable. We found the same effects at different concentrations (benzaldehyde, β-damascenone) or greater influences of fat at higher concentrations (ethyl butyrate, hexanal).

Compound Perceived Intensity. A typical stimulus response curve for odorant compounds contains several regions: up to a certain concentration (the threshold), no odor is perceived (region I). At concentrations above threshold and until a saturation point, there is a region where the perceived intensity increases as a function of concentration (region II). At the saturation point, the perceived intensity no longer increases (or increases more slowly) with increasing concentration (region III). Multiple models have been proposed to describe the stimulus response curve for odorants (*17*−*19*): Fechner, Weber, Stevens, Biedler, and Chaserette. This study only looked at three different concentrations, thus not enough points to adequately test any of these models. The concentrations used were different by a factor of 4, which is more than enough for the concentrations to be perceived as different.

Table 3 gives the values for perceived intensity as a function of concentration and matrix for each compound. In general, the differences between the milk types remained at different concentrations, except for ethyl butyrate, which showed high variability. For each compound, we could be in a different region of the stimulus response curve. Some of the points were in region I, below threshold. Most of the other points were likely in region II. Not enough concentrations were analyzed to determine if the intensity plateau was reached. Some panelists perceived a change in odor quality as the odor concentration increased for 2,3-butanedione, which made the judgment of perceived intensity with the references not valid. For this reason, the data for 2,3-butanedione will not be interpreted further.

With the exception of benzaldehyde, the other compounds show a very similar intensity perception when in water and in skim milk. Slight decreases in nosespace release (from water) were observed with skim milk for β-damascenone and benzaldehyde, but this degree of difference was more perceived sensorially for benzaldehyde.

Table 3. Perceived Intensity of Compounds[a] (Line Scale Value, 0–100) in Four Different Matrixes and with Three Different Concentrations of VOCs (See Materials and Methods Section; Each Differing by a Factor of 4)[b]

	low VOC	medium VOC	high VOC
	β-damascenone (LSD = 18)		
water	34	52	92
skim milk	32	59	84
semiskim milk	5	18	48
whole milk	6	6	24
	hexanal (LSD = 18)		
water	29	52	84
skim milk	31	49	86
semiskim milk	5	38	65
whole milk	6	28	48
	ethyl butyrate (LSD = 22)		
water	17	28	77
skim milk	10	26	79
semiskim milk	5	51	56
whole milk	8	37	45
	benzaldehyde (LSD = 20)		
water	5	49	86
skim milk	6	33	73
semiskim milk	2	14	43
whole milk	4	13	44

[a] The intensity assessment of 2,3-butanedione was not valid due to perceived differences in odor quality upon changing concentration. [b] Each value is the average of 10 measurements (5 panelists × 2 replications), and the LSD is shown for each compound, noting statistically significant differences.

The effects of fat were seen with all analyses, to different extents. Sensorially, the three most lipophilic compounds were ranked in the expected order: 3.8% fat milk < 2.7% fat milk < skim milk = water. This order was found for the perceptible concentrations (except ethyl butyrate midconcentration). The nosespace results also showed this effect of fat, most clearly for the most lipophilic β-damascenone.

Correlation of Perceived Intensity with Nosespace Release. **Figure 2** shows the average panel correlation results. Each individual panelist's graph was also examined in order to look for trends. Some caution should be taken when interpreting this figure as the order of products presentation and the complexity of the products were different in the nosespace study and in the perceived intensity study. Product presentation order can influence the sensory results but has no influence on the nosespace results. The two analyses were performed at different times so there could also be differences in tasting technique. However, a general correlation was observed as increasing concentrations of nosespace release were found with increasing sensory intensity ratings. **Figure 2** also shows the details of the points (milk type and concentration) making up the correlation. One can note that the compound in water with the highest concentration (the uppercase A) has usually the highest rating. Also, the compounds in 2.7 and 3.8% fat milks at the lowest concentrations (lowercase c and d) have usually the lowest ratings. One can then look at the correlation as the concentration increases with each milk by following the same letter, for example, a, a, and A.

The nosespace technique measures the actual compound concentration that comes out of the nose after passing through the nasal cavity. However, perceived intensity quantitation also comprises the brain's central processing and assessment of compound intensity. This intensity determination, even though

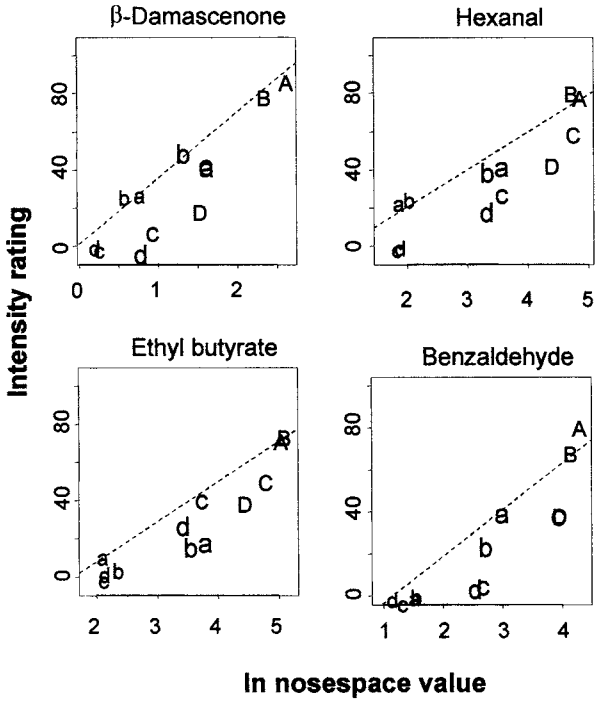

Figure 2. Correlation of the average panel sensory intensity ratings (**Table 3**) with nosespace concentration (**Table 2**). Letters a–d correspond to water, skim milk, 2.7% fat milk, and 3.8% fat milk, respectively. Small lowercase letters a–d are the low concentrations. Large lowercase letters a–d are the middle concentrations. Uppercase letters A–D are the high concentrations.

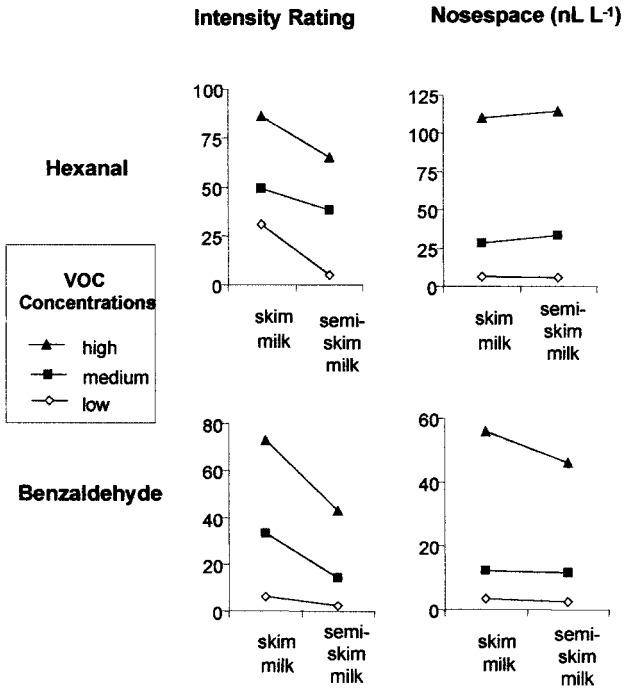

Figure 3. Examples of lower perceived intensity ratings in 2.7% fat milk than skim milk yet little nosespace difference, showing similar effects at all concentrations.

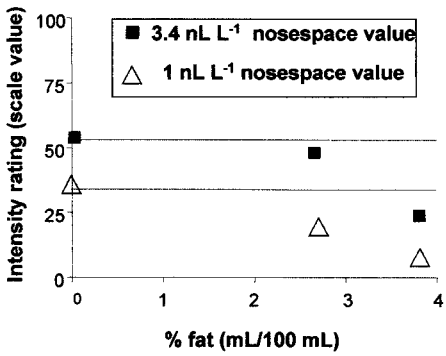

Figure 4. Influence of fat (creamy note) on the perceived intensities of β-damascenone. The graph shows select samples from skim milk, 2.7% fat milk, and 3.8% fat milk of different compound concentrations that had the same nosespace concentration.

carried out on one compound at a time, can be influenced by other factors. One factor that appears in our data is the perceptual flavor masking in the higher fat milks. There are several examples in which the absolute nosespace concentration does not change; yet, the assessed compound intensity decreases when comparing skim milk to the higher fat milks. One hypothesis is that the creamy note of the higher fat milks is responsible, as the panelists described the higher fat milks as being more creamy than skim milk. **Figure 3** shows that for hexanal and benzaldehyde, the nosespace concentrations were similar in skim milk and 2.7% fat milk. However, the rated intensities in 2.7% fat milk were less than in skim milk. This is a type of masking that may be present in aroma compound mixtures (*20*). In another example with β-damascenone (**Figure 4**), samples that had the same nosespace concentrations had lower perceived intensity ratings as the fat content increased. This could also be due to the inherent flavor note such as creaminess in the 2.7 and 3.8% fat samples that causes a perceived decrease in the intensity of β-damascenone.

There are also examples where the nosespace concentrations are slightly different; yet, no difference is perceived in intensity. This is the case for β-damascenone in water and skim milk where a 30% reduction in nosespace was seen at all concentrations but this reduction was not systematically present in perceived intensity. This result was expected due the compressive nature of olfaction where normally larger differences in compound concentration are needed to result in sensorially perceived effects (*17*).

Comparison of Headspace and Nosespace Results. The purpose of this section is now to compare the nosespace results with a method that is frequently used in flavor release studies: headspace analysis. Previous studies based on static headspace results (*6*) verified a partition coefficient-based model with experimental results, showing that the headspace concentration could be predicted based on the knowledge of the fat content and the oil−water partition coefficient of the compound. Especially for highly lipophilic compounds, the headspace concentration markedly decreased with increasing fat content. The nosespace results here still showed the effect of fat but to a lower extent than in headspace studies. **Figure 5** shows these results superimposed on a previous graph determined with headspace measured release (*6*). Interestingly, the absorption of β-damascenone by the fat phase appears to be greater as seen by headspace analysis than by nosespace analysis.

This same effect was seen when repeating the headspace analysis with these exact samples. Except for β-damascenone, the skim milk results were rather similar in both analyses. However, overall, the reduction in headspace values due to increasing fat content was greater than in nosespace analysis. **Figure 6** shows that indeed for more lipophilic compounds, β-damascenone and hexanal, the headspace measurement results in a greater estimate of fat absorption. Also, the more polar benzaldehyde and 2,3-butanedione also showed slightly greater fat absorption with headspace analysis. Ethyl butyrate, the

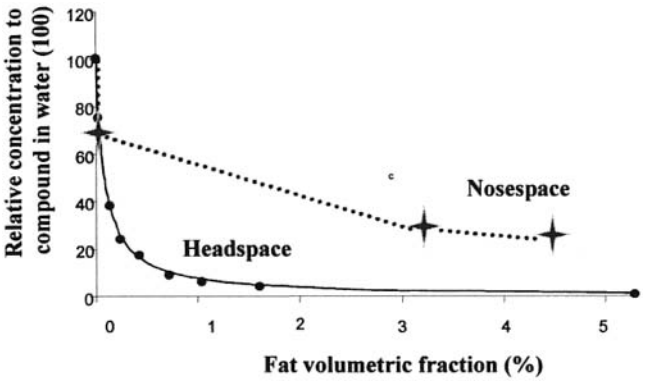

Figure 5. Comparison of results with headspace analysis (*6*) and nosespace analysis for β-damascenone.

compound with the highest volatility in water, showed similar results with both analyses.

These types of results have also been previously seen (*21*) where greater reductions in headspace concentrations were observed than in nosespace analysis for ethyl octanoate, ethyl hexanoate, and ethyl butyrate, using a 2% lipid emulsion. They calculated that the amount in the breath is 25, 5, and 2 times, respectively, higher than that expected on the basis of headspace analysis. Also corresponding to our results, the more lipophilic compounds showed a greater method-dependent difference.

All of these findings tell us that the physiology of swallowing liquid samples from the mouth, the dilution with saliva, the transfer of the compound from the sample to the air, the transport of the volatile compound through the retronasal route to the nasal cavity, and the final exhalation through the nostrils are not adequately simulated in static headspace analysis. Indeed, values for nosespace were found to be significantly different from values for mouthspace (*22*), showing that the retronasal transport is important. Several explanations can be postulated. Linforth et al. (*22*) postulated an effect due to the different mass transfer rates in the two systems, as it affects volatile delivery from the solution to the gas phase. This is elaborately explained with nosespace, headspace, and mouthspace data (*22*). Certainly the situation in the mouth is not "static". One additional effect could be that the skin membranes in the mouth have some lipophilic properties and can also act as a reservoir for lipophilic compounds. Thus, water in the mouth is not a completely aqueous system as there is a potential for interaction with these membranes. However, compounds in water in a deactivated glass vial for headspace analysis are a completely aqueous system and would have a higher release than in a mouth containing some lipophilic membranes. As the release is expressed relative to the compounds in water, this could account for some differences. Finally, another hypothesis for the lower fat absorption effect in nosespace analysis is the importance of dilution with saliva that occurs in the throat where liquid sample coats the throat lining. This dilution could mean that the effective fat concentration at the point of release is less than in headspace analysis.

Overall Comparison. Three methods were used to compare the flavor perception or flavor release of the same products. The sensory intensity measurement gave results that corresponded generally to the nosespace methods. However, evidence of perceptual masking was found, probably due to the creamy note in higher fat milks. Also, some differences found by nosespace analysis were not large enough to be perceived sensorially. The headspace method overestimated the flavor compound absorption by fat, as compared to the nosespace method. All methods found that the lipophilic compounds were released to a lower degree as fat content increased. Similar sensory results were found at different concentrations, whereas in nosespace, some compounds showed greater effects of fat at higher concen-

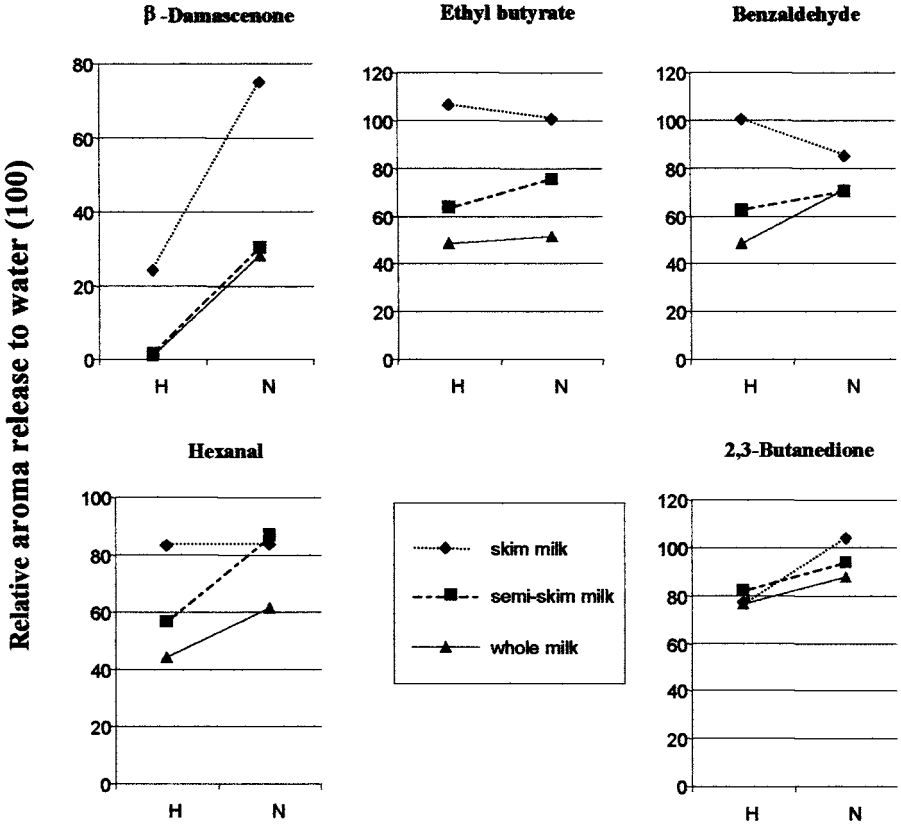

Figure 6. Comparison of aroma release results obtained from headspace analysis (H) and nosespace analysis (N).

trations. An interesting quantitative aspect was found; in the aqueous samples, a maximum value of 0.6% of the contained aroma compounds was detected in nosespace analysis, probably indicating a large amount being swallowed. Compounds with lower air−water partition coefficients were quantitatively released to a lower extent. It is promising that correspondences between different methods can be obtained in simplified flavored systems, with hope that the findings will lead us to a greater understanding of more complex flavored products.

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