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Temporal Aroma Delivery from Milk Systems Containing 0−5% Added Fat, Observed by Free Choice Profiling, Time Intensity, and Atmospheric Pressure Chemical Ionization−Mass Spectrometry Techniques

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A temporal aroma delivery from milk systems containing 0, 0.5, or 5% added fat and flavored with seven-component strawberry flavoring and linalool was observed by free choice profiling (FCP), time intensity (TI), and atmospheric pressure chemical ionization−mass spectrometry (APCI-MS) techniques. A suppressing effect of fat on the volatility of the relatively nonpolar compound linalool was observed by all methods, but only slight evidence (with the TI method) of the effect of fat on the overall strawberry (based on more polar compounds) intensity was found. With the TI method, the strawberry aroma of the fattiest sample lingered the longest, but no temporal differences were found in the release of linalool. The APCI-MS results showed no effect of fat on the temporal release of ethyl butyrate (mainly responsible for the strawberry note), but linalool of the sample containing 5% fat was found to be the most persistent. However, the effect on linalool was observed using a slightly different sampling technique than in the TI. Overall, FCP, TI, and APCI-MS showed parallel results for the effect of fat on the intensity of aroma, but temporal release data only partly supported the theory that fat slows down the release of aroma compounds and their perception.

KEYWORDS: Aroma; time intensity method; APCI-MS; fat

INTRODUCTION

The suppressing effect of fat on the volatility of some lipophilic aroma compounds and thus their perceived intensity has been well-documented since early studies in the 1970s (*1*, *2*). The effect of fat on the temporal characteristics of the release of aroma compounds has not been studied as equally and extensively, and there is less consensus. The reduction of fat in a food product is often claimed to lead to a harsh, unbalanced aroma with shorter duration as compared to the full fat product (*3*). These temporal effects of fat seem to be difficult to prove, especially with sensory methods (*4*). Brauss et al. (*5*, *9*) measured the release of volatiles in the nose with atmospheric pressure chemical ionization−mass spectrometry (APCI-MS) and found that added lipophilic aroma compounds from low-fat yogurt and cookie samples were less persistent as compared to those from regular fat samples. However, the sensory time intensity (TI) method, used as a reference method in the study with yogurts, showed no significant differences in the duration of flavor in the low- and regular-fat samples (*5*). These conflicting sensory results were suggested to be due to adaptation or to the fact that the suprathreshold concentrations of two out of three aroma compounds were so high that changes in concentration may have been difficult to perceive.

In our previous TI study (*6*), we found that the perception of the relatively lipophilic compound, linalool, lasted for a shorter time from the sample containing the greatest amount of fat (fat levels 0−10% studied); yet, the decrease in perceived intensity perception was more rapid in lower fat samples. Other studies support either a shorter (*7*, *9*) or a longer (*3*, *8*, *10*) duration of aroma in low-fat samples as compared to samples containing more fat. The rate of aroma compound release/perception has been found to be either quicker (*5*) in low-fat products as compared to full-fat counterparts, or no difference in the rate of release has been observed (*6*, *7*, *10*). Yet, direct comparisons are complicated by the great variety in methods and designs of the studies.

The effect of fat on aromas depends on the physicochemical characteristics (especially lipophility) of the aroma compound. For example, Chung et al. (*11*) found faster release rates for stale (hexanal) and cherry (benzaldehyde) flavors but slower rates for vanillin flavor, a more hydrophilic compound than the former two, with a decreasing matrix fat content. In our previous study, fat had no effect on the intensity/volatility or on the temporal release characteristics of the relatively lipophobic

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compound diacetyl but affected all of those parameters in the case of a more lipophilic linalool (*6*). In studies in which aroma mixtures have been used, it is not possible to identify the compounds affected by fat (*3*, *8*, *10*).

In the present study, the temporal retronasal of the release of aroma compounds in strawberry−linalool-flavored milk systems with varying fat levels was examined. In an earlier study (*6*), we used a simple aroma system (two aromas) while in the present study a more complex flavor mixture (a total of eight aroma compounds added) was studied using sensory and instrumental measurement techniques. The hypotheses tested were that as the amount of fat in the matrix increases, (i) the perceived flavor quality changes, as compared to the aroma in the nonfat sample for which the aroma mixture was designed, due to the differing effect of fat on the intensities of aroma compounds with different polarities, and (ii) the perceived flavor is even more imbalanced or further changed due to the effect of fat on the temporal release of lipophilic compounds. In our earlier study, we found partially contradictory results for this latter hypothesis. A fast aroma compound release from nonfat matrices has been assumed in the literature but not convincingly supported by temporal release studies; thus, the aim in the present work was to study the phenomenon in more detail.

The approach was to use free choice profiling (FCP) to roughly assess the quality of flavor during the temporal release of aromas, using two time points ("first impression" and "after taste"). Using attributes chosen based on FCP, the changes in temporal release were then quantified with the TI method. The perceived aroma release was related to the actual aroma compound release using the APCI-MS technique.

MATERIALS AND METHODS

The samples were strawberry−linalool-flavored milks with three different fat levels. The base matrix was commercial nonfat UHT milk with added UHT cream (10% fat). The added fat levels were 0, 0.5, or 5% (w/w). The fat levels represented a nonfat matrix, a very low-fat matrix, and a matrix that according to our hypothesis, would make a clear difference in the release of aroma compound characteristics as compared to the fat-free matrix. The inherent dairy fat content of the milk was 0.04−0.08% (according to the manufacturer Valio Ltd., Helsinki, Finland). The composition of the UHT milk and UHT cream, other than fat, was fairly similar (protein, 3.3 g/100 g in the milk and 3.2 g/100 g in the cream; carbohydrates, 4.9 g/100 g in the milk and 4.5 g/100 g in the cream; less than 1 g of lactose in both). The cream and milk were mixed thoroughly using a manual kitchen blender. The matrices were aromatized with a seven component strawberry flavoring (Givaudan, Barneveld, Netherlands) and linalool (DL-3,7-dimethyl-3-hydroxy-1,6-octadiene; Sigma Aldrich, Steinheim, Germany; purity >97%). The amount of strawberry flavoring, 2 g/kg matrix, was determined in pretests to represent a moderate strawberry flavor in a nonfat matrix. The solvent in the flavoring was propylene glycol. The aromatized matrices contained 20 mg/kg ethyl butyrate, 10 mg/kg ethyl isobutyrate, 20 mg/kg ethyl methyl phenylglycidate, 20 mg/kg *cis*-3-hexenol, 0.5 mg/kg γ-undecalactone, 1 mg/kg butandione, and 4 mg/kg butyric acid, in addition to 35 mg/kg linalool. The amount of linalool was determined in pretests to yield a moderate bergamot flavor in the nonfat matrix. Linalool was added to the flavoring system to represent a very nonpolar compound. In pretests, it did not interfere with the strawberry flavor but contributed a "green" note to the strawberry milk. The samples were kept refrigerated in tightly capped and sealed glass bottles. The storage time was at minimum 24 h and at maximum 48 h.

Methods. *Sensory Evaluations: Overall View.* Included in the sensory evaluations were the FCP and TI methods. All assessors (N = 12, nine females and three males, mean age 29.5 years) were staff of the University of Helsinki and had previous experience in sensory evaluation. Most were familiar with the TI method and with milk-based samples. All assessors had a normal sense of smell based on the SOIT (Scandinavian odor identification test, *12*). Samples (10 mL) with random three-digit codes were presented in plastic cups (80 mL) covered with lids. Prior to the evaluations, samples were equilibrated at least 1 h in the refrigerator and then kept at room temperature for 1 h before the evaluations. Thus, they were served at room temperature. Assessors used noseclips while taking the sample into their mouth to prevent orthonasal aroma perception, as the interest of this study was the retronasal aroma. Immediately after the sample was placed in the mouth, the noseclips were removed. Red lights were used to mask slight color differences among the samples. The assessors were instructed to rinse their mouths with tap water and to eat crackers between samples to clean their mouths.

FCP. FCP was done in a total of four sessions, two of which were practice sessions and the remaining two actual evaluation sessions. Assessors familiarized themselves with the samples (the blank matrices and the aromatized samples) and described the flavor of the samples (first impression and after taste) using their own vocabulary. The first impression was evaluated after keeping the sample in the mouth for a short time (up to 5 s) while making smooth mouth movements, and the after taste was evaluated after swallowing the sample. In the second practice session, assessors evaluated the intensities of the attributes generated in the first session (first impression and after taste). After the second practice session, the attributes were individually discussed with each assessor. Assessors were given an opportunity for a third practice session in case they felt that they needed extra practice with the attributes they had chosen, and one assessor used this opportunity.

Actual FCP evaluations were done in two sessions mainly. Assessors who had generated only two attributes had only one evaluation session. In each session, two attributes, individual for each assessor, were evaluated in duplicate samples, and thus, a total of 12 samples were evaluated in one session (three fat levels × two attributes × two replications). If the assessor had three attributes, only one attribute was evaluated in the latter session. Each assessor put the noseclip on, took the whole 10 mL sample in his or her mouth, closed his or her mouth, and removed the noseclip. The sample was kept in the mouth for a short time (up to 5 s) while making smooth mouth movements, and afterward, each assessor evaluated the first impression of the intensity of one of the attributes he or she had chosen on a 10 cm line scale anchored no aroma−strong aroma. Then, the assessor swallowed the sample, waited for a short time (typically up to 5 s), and evaluated the intensity of the chosen attribute in the after taste.

Fat suppressing the volatility of linalool, the intensity, and the quality of flavor should vary based on fat content. A temporal effect of fat on aroma compound release should appear as an interaction between time point and fat level.

TI. TI evaluations consisted of four sessions, two of which were practice sessions. The rated attributes were "overall strawberry flavor" and "bergamot flavor", which were chosen based on the FCP results, as all assessors agreed that they were the predominant and clearly recognizable attributes in the samples. Most of the assessors were familiar with the computerized TI method (CSA, Computerized Sensory Analysis System, Compusense Inc., Guelph, Canada, version 3.8); those with no experience were given extra practice. During the evaluations, one attribute was measured at a time and each sample was evaluated twice (a total of six samples/session). Each assessor put the noseclip on, placed the sample in his or her mouth, removed the clip, and simultaneously started evaluating the intensity of the attribute on a vertical TI scale (bottom, no aroma; top, strong aroma) making smooth mouth movements until instructed to swallow the sample (after 10 s). During the smooth mouth movements, the assessors were allowed to breathe through their nostrils but not to open their mouths. After they swallowed, the assessors were instructed to continue evaluating the aroma intensity while keeping their mouths closed, breathing through their nostrils, and keeping their tongues still. The total evaluation time was 90 s.

Release of Aroma Compounds Measured Using APCI-MS. The release of aroma compounds was measured in the headspace of samples and in the nosespace during consumption of samples using the APCI-MS (MS-Nose, Micromass, Manchester, United Kingdom). Sample air (headspace/breath) was drawn into the ionization source through a heated deactivated fused silica tubing (1 m × 0.53 mm i.d.). Compounds

entering the source were ionized by a 4 kV positive ion corona pin discharge, and the ions formed were introduced into the high vacuum region mass spectrometer, where they were separated and detected according to their *m*/*z* ratio (*13*). The molecules measured were *cis*-3-hexenol, ethyl butyrate (/iso-ethyl butyrate), and linalool. In the positive ion mode APCI-MS, *cis*-3-hexenol yielded the principal ion *m*/*z* 83, ethyl butyrate (/iso-ethyl butyrate) *m*/*z* 117, and linalool *m*/*z* 137. The other aroma compounds present in the flavoring were not measured due to the sensitivity problems. For example, ethyl methyl phenylglysidate and undecalactone may have some contribution to the perceived strawberry aroma, but they could not be quantified here as the amounts of these compounds in flavoring were too low in regard to their relatively low volatility. The measured gas phase aroma concentrations were calibrated by comparison against known standards, which were injected (1.5 μL/min via microsyringe using a syringe pump) at the beginning of each analysis (*13*).

Headspace Measurements (In Vitro). Five replicates of each sample matrix (40 mL in 100 mL Schott bottles, Fisher Scientific, fitted with stoppered lids) were allowed to equilibrate at room temperature and then measured by sampling the headspace into the MS-Nose by removing the headspace stopper and inserting the heated transfer line directly into the headspace. The sampling rate was 4.7 mL/min. The mass spectrometer was operating in the selected ion mode, monitoring *m*/*z* 83 (*cis*-3-hexenol), *m*/*z* 117 (ethyl butyrate/iso-ethyl butyrate), and *m*/*z* 137 (linalool).

Nosespace Measurements (In Vivo). The release of aroma compounds in the nosespace of panelists was measured with two different sampling protocols. Panelists ($N = 5$) either swallowed the sample (10 mL, five replicates) immediately after taking it into their mouths or followed the same sampling protocol as in the sensory TI evaluation (i.e., kept the sample 15 s in the mouth making smooth mouth movements before swallowing). In either case, panelists breathed in, sipped the whole sample (only opening the sample cup immediately before sipping), closed their mouths, swallowed (right away/after 15 s), exhaled, and continued to breathe normally while resting their nose on the MS-Nose nasal sampling tube. The sampling rate was 27.8 mL/min in the 15 s protocol and 29.2 mL/min in the straight swallowing protocol. Exhaled air was monitored for *m*/*z* 83 (*cis*-3-hexenol), *m*/*z* 117 (ethyl butyrate/iso-ethyl butyrate), and *m*/*z* 137 (linalool). All samples of one sampling protocol were done on the same day. After each sample, panelists rinsed their mouths with bottled water (at room temperature), ate crackers, and rested for at least 90 s.

Statistical Analysis. To analyze the FCP results, the generalized procrustes analysis was used (Senstools 2.3.28, OP&P Product Research BV, Utrecht, The Netherlands). Data from each replication were treated separately to determine the repeatability of the evaluations. The three-way univariate analysis of variance (the GLM procedure) was used to study the effects of the timing of the evaluation (the first impression or after taste), fat content, the panelists, and interactions. Subsequently Student's *t*-tests (paired) were used to assess the significant differences in the values obtained for the first impression and after taste.

The parameters calculated from the TI data included time to maximum (T_{max}), maximum intensity (I_{max}), duration (Dur), area under curve (AUC), increase angle (IAng), increase area (IArea), decrease angle (DAng), and decrease area (DArea). The three-way multivariate analysis of variance (the GLM procedure) was used to assess the main effects and interactions of fat content, panelists, and replication on the TI parameters.

The differences among matrices in the APCI-MS headspace results were tested with Student's *t*-test (paired). The parameters calculated from the APCI-MS nosespace data included time to maximum (T_{max}), maximum intensity (I_{max}), and time to 75, 50, or 25% of maximum intensity on the descending part of the curve (T75%, T50%, and T25%). Student's *t*-test (paired) was used to assess the significances of the differences in the values obtained for different matrices.

RESULTS

FCP. The strawberry and linalool notes were predominant in the samples, and assessors commented that it was difficult to generate other descriptors. Thus, assessors typically had only 2−4 attributes to evaluate. As compared to most FCP studies in which a considerable number of attributes were elicited (e.g., *14*−*16*), the number of attributes was low. This was not considered a problem as the FCP data were needed (i) to obtain "raw" temporal information on the release of aroma compounds by using two time points, (ii) to find relevant attributes for TI measurements, and (iii) to practice and familiarize the assessors with the samples before the TI method. GPA group average plots with s correlation to attributes (dimension 1 vs dimension 2, **Figure 1a** for the first impression and **Figure 1b** for the after taste) suggested that the samples were separated based on the fat content of the matrix. The attributes related to linalool were accumulated near the samples containing less fat, and the attribute strawberry was in most cases located near the 5% fat samples. This indicated that linalool was more related to samples containing 0% added fat and the strawberry flavor was more related to the fattier samples. The replicate samples were fairly similarly evaluated indicating good repeatability. Dimension 1 explained 62.6% (first impression)/71.7% (after taste), and dimension 2 explained 23.5% (first impression)/14.8% (after taste) of the variance. The residual variances per assessor were generally low, <2%.

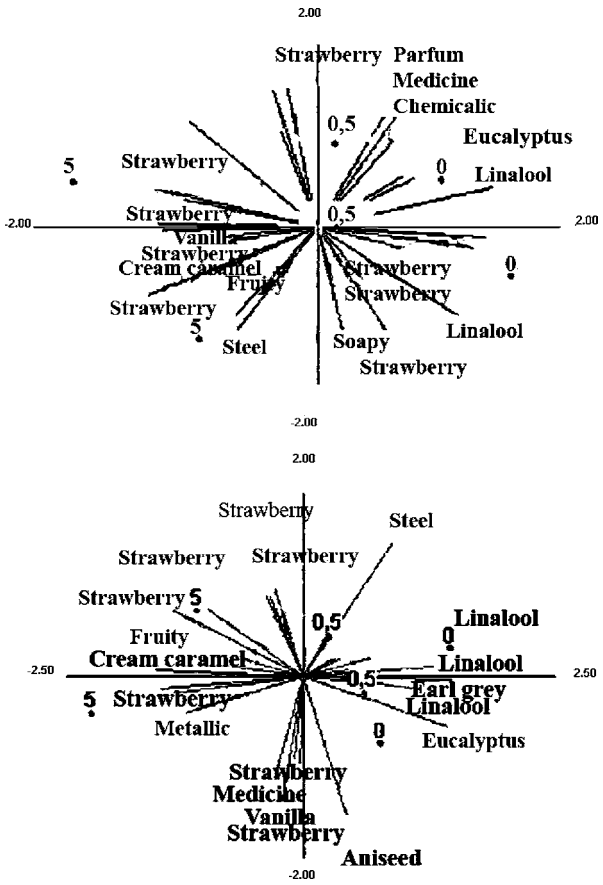

Figure 1. GPA group average plots with correlation to the attributes: (**a**) for "first impression" and (**b**) for "after taste". Dimension 1 explained 62.6% (first impression)/71.7% (after taste), and dimension 2 explained 23.5% (first impression)/14.8% (after taste) of the variance (symbols: 0, sample with 0% added fat; 0.5, sample with 0.5% added fat; 5, sample with 5% added fat; some of the overlapping terms are removed).

All assessors used the attribute strawberry, and consequently, the intensity of the strawberry flavor was evaluated in the TI. Other attributes included bergamot, linalool, Earl grey tea, steel, medicine-like, pungent, eucalyptus, aniseed, perfume-like, chemical, and soapy, all related to the flavor of linalool. On the basis

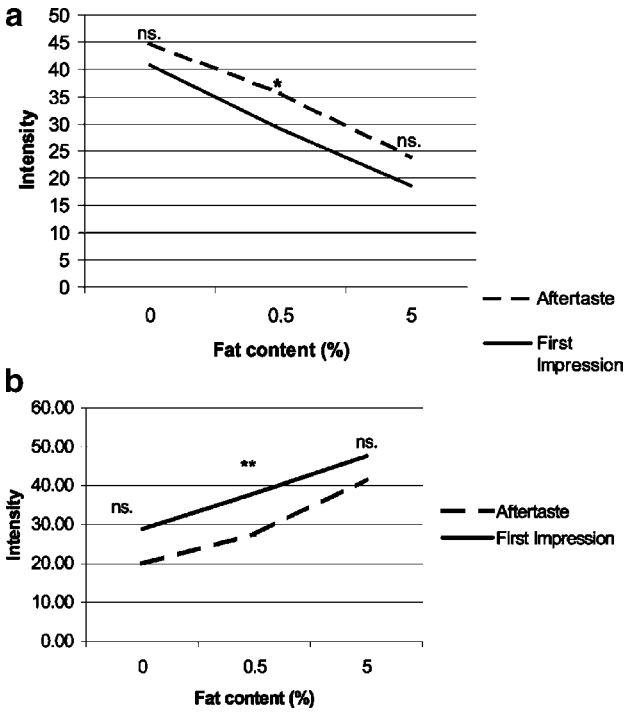

Figure 2. Means over all linalool-related attributes (**a**) and strawberry flavor (**b**) in FCP.

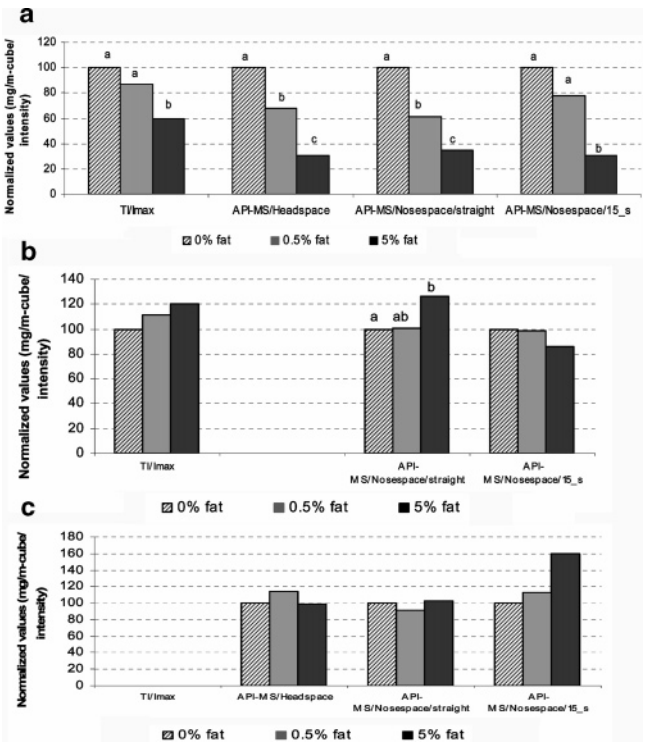

Figure 3. Relative average maximum intensities (TI)/maximum head/nosespace (APCI-MS) concentrations for (**a**) bergamot/linalool, (**b**) strawberry/ethyl butyrate, and (**c**) 3-*cis*-hexenol. The values are calculated as relative values as compared to values obtained for the 0% sample. The data for APCI-MS headspace/ethyl butyrate are not shown as they were not reliable.

of discussions with the assessors, the intensity of the bergamot flavor was an attribute chosen to be evaluated in the TI. The means over the linalool-related attributes and the strawberry attribute were calculated to examine whether any temporal effect was evident (**Figure 2a** for linalool and **Figure 2b** for strawberry). Linalool was more strongly perceived in the after taste than in the first impression; however, this trend was significant only in the 0.5% fat samples. The opposite was found in the case of strawberry; higher average values were observed for the first impression than for the after taste. This trend was likewise significant only in the 0.5% fat-containing samples. There was no indication that an increase in fat content caused slower release of nonpolar compounds (e.g., linalool) as the after taste parameter did not change significantly [$F(2, 110) = 0.007$, $p = 0.993$ for the interaction of the fat content of the sample and the timing of the evaluation]. Similarly, no interaction of the fat content and timing was found in the strawberry evaluations [$F(2, 138) = 0.242$, $p = 0.785$]. Some other attributes appeared (candy-like, vanilla, and cream caramel), but no general agreement of these was evident, and they were not included in further studies.

TI. The perceived intensity of linalool (bergamot flavor) was greatly reduced as the matrix fat content was increased [linalool relative I_{max} values in **Figure 3a**, $F(2, 22) = 8.72$, $p < 0.002$ for main effect of fat]. Fat did not significantly affect the strawberry flavor, although strawberry tended to be slightly more intense with an increase in the fat content of the matrix [strawberry flavor relative I_{max} values in **Figure 3b**, $F(2, 22) = 2.37$, $p = 0.117$)].

The timing of the linalool perception was not greatly affected by the fat content of the matrix (see the normalized data in **Figure 4a**). Some significant effects on timing-related TI parameters were found [$F(2, 22) = 9.87$, $p < 0.001$ for AUC; $F(2, 22) = 4.94$, $p < 0.017$ for IArea; $F(2, 22) = 2.69$, $p < 0.09$ for DAngle; and $F(2, 22) = 8.90$, $p < 0.001$ for DArea, TI parameters in **Figure 5a**]. These parameters appear to reflect significant differences in I_{max} values, rather than true timing effects. The duration of linalool perception was not significantly affected, but there was a slight trend of a shorter duration of perception in samples containing 5% fat as compared to the less fatty samples (**Figure 5a**). The timing of overall strawberry aroma was more affected by the matrix fat content than the linalool aroma. Normalized data (**Figure 4b**) indicate that maximum intensity perception is delayed in samples containing 5% fat as compared to samples containing less fat [$F(2, 22) = 6.92$, $p < 0.005$ for T_{max}]. However, DAng was the greatest in the sample containing 5% fat; thus, there were no differences in the duration of the strawberry aroma (TI parameters in **Figure 5b**).

The assessors were a significant source of variance for most of the parameters, as expected in sensory methods. Replication was a significant source of variation only in one case (IArea of the stawberry flavor attribute). Some interactions were significant, but none of these were relevant to the substance but more related to the high variations among the assessors.

APCI-MS/Static Equilibrium Headspace. Headspace results clearly showed the differing effect of fat on the volatility of nonpolar and more polar compounds. The relative concentration of linalool (a nonpolar compound) in the headspace was very significantly reduced as the fat content of the matrix increased while the relative concentration of *cis*-3-hexenol (a more polar compound) in the headspace concentration was not affected by the fat content (**Figure 3a**,**c**). The headspace results for ethyl butyrate are not reliable as the concentration in the headspace was too high as compared to the calibrations (and due to that the standard deviations were high) (**Figure 3b**). For both linalool and *cis*-3-hexenol, the standard deviations were very acceptable (<2%, $N = 5$).

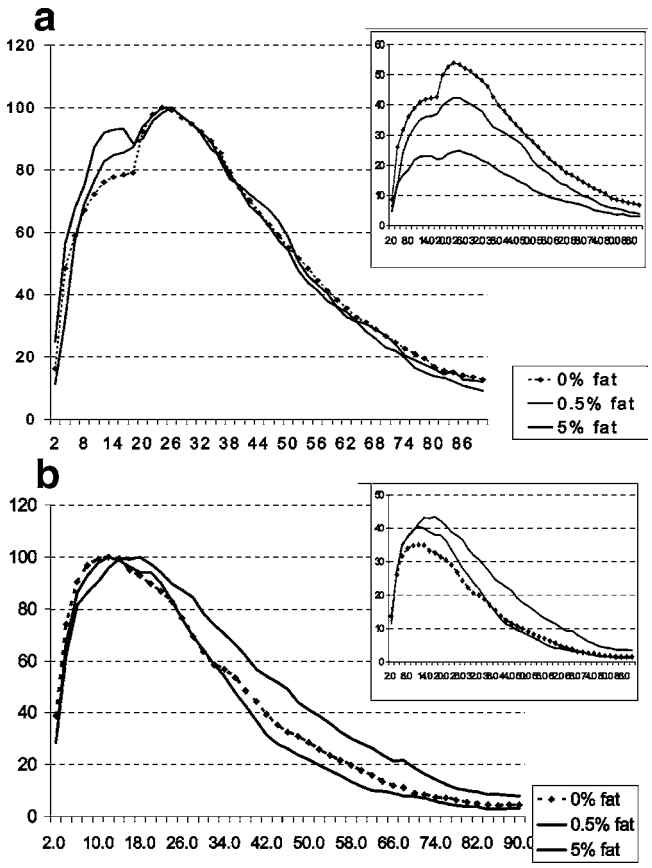

Figure 4. Normalized TI data for (**a**) linalool and (**b**) strawberry flavors. The values are normalized so that maximum intensity is given the value of 100%, and the others are calculated as proportions of that value. Raw data curves are shown in the upper right corners.

APCI-MS/Nosespace. *15 s Protocol.* As in the case of the sensory and headspace results, the average in-nose concentrations showed the effect of fat on the volatility of compounds with different polarities; the concentration of linalool in the nosespace was greatly reduced in the 5% fat sample as compared to the samples with less fat (**Figure 3a** for the relative maximum concentration). The concentrations of ethyl butyrate were more similar (**Figure 3b**). In the case of *cis*-3-hexenol, the concentration in the nosespace was the greatest in the 5% fat sample (**Figure 3c**). The normalized intensity values for linalool show that T_{max} seems to be slightly delayed in the 0% fat sample as compared to the others, but the descending part of the curves is variable and there is no obvious difference (**Figure 6b**).

Straight Swallowing. A clear effect of fat on the nosespace concentrations of linalool was also seen with the straight swallowing procedure (**Figure 3a**−**c**). The concentration of linalool was reduced as the fat content was increased. The in-nose concentration of ethyl butyrate was significantly higher in the sample containing 0% fat as compared to the sample containing 5% fat. No obvious effects of fat were observed on the in-nose concentration of 3-*cis*-hexenol.

The time to maximum concentration was not affected significantly in the case of any of the aromas. However, here again, the standard deviations were considerable. The other time-related parameters of linalool release were affected by the fat content; linalool of the 0% fat sample persisted for a shorter time than the other samples. The differences shown here are clearer than with the 15 s protocol. The normalized concentration values for linalool are shown in **Figure 6a**.

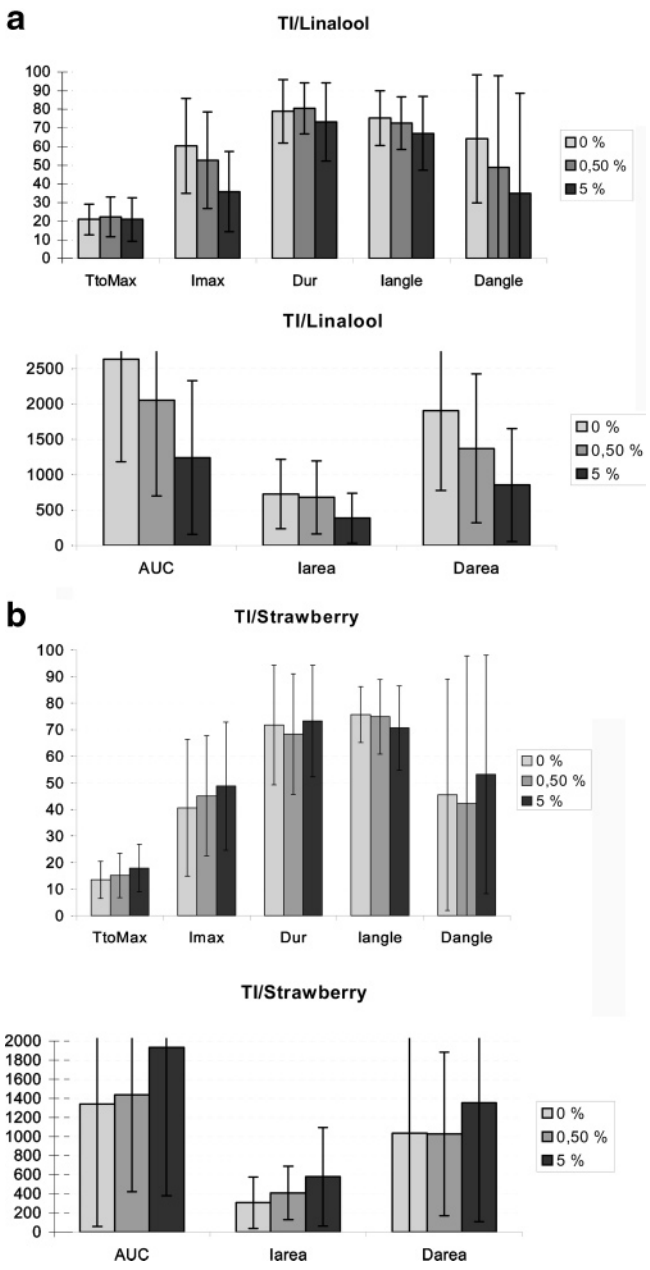

Figure 5. Average values for the TI parameters for (**a**) linalool/bergamot and (**b**) overall strawberry.

DISCUSSION

Effect of Fat on the Intensity/Volatility of Aroma Compounds. The flavors of the samples with different fat levels were clearly separated with FCP, both by first impression and after taste, and the effect of fat on the volatility/intensity of aroma compounds was seen in the TI and APCI-MS results. With an increasing fat content, the perceived maximum intensity of linalool was greatly reduced, and the maximum intensity of the strawberry flavor was slightly increased. APCI-MS measurements (both in vitro and in vivo) showed that linalool was significantly retained in the matrix as the fat content increased. The concentration of ethyl butyrate in the nosespace was found to be greatest in the sample containing 5% fat when using the straight swallowing technique but not when using the 15 s sampling protocol. The opposite effect was found for the concentration of 3-*cis*-hexenol. No effect of fat was seen in the headspace concentrations of 3-*cis*-hexenol. As a whole, these results are well in line with reports in the literature as linalool

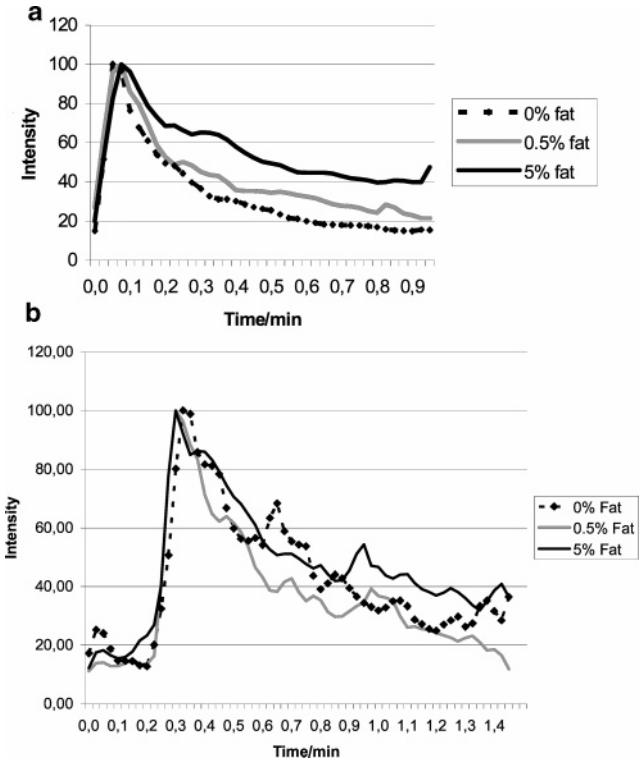

Figure 6. Normalized average nosespace concentrations of linalool for (**a**) straight swallowing and (**b**) the 15 s protocol measured with APCI-MS. The values are normalized so that the maximum concentration is given the value of 100%, and the others are calculated as proportions of that value.

is a relatively nonpolar compound and the two other compounds are less lipophilic. The minor effects of fat content on the less lipophilic compounds were well-expected as these compounds, more readily soluble in the water phase, are not likely to be strongly affected by small changes (5% change at maximum) in the fat content of the matrix.

Amount of Fat Required for Significant Suppression of Aroma Compounds. Instrumental studies on the effects of lipids on aroma volatility show that very low fat levels can exert a significant effect. Roberts et al. (*17*) found, using solid phase microextraction and the gas chromatography−mass spectrometry (GC-MS) method, that as little as 0.02% milk fat in the matrix reduced the headspace concentration of a relatively lipophilic aroma compound (limonene) by 50% as compared to water. Seuvre et al. (*18*) showed a significant retention (84% retained in the matrix) of 2-nonanone in the presence of 0.2% fat as compared to an aqueous matrix using headspace-GC. In our previous study (*6*), 1% fat in the milk matrix was sufficient to significantly reduce the volatility (measured using static headspace GC) and orthonasal intensity of linalool, but we failed to show an effect on retronasal intensity with the TI method. In the present study, 0.5% fat was enough to significantly reduce the headspace/nosespace concentrations of linalool in APCI-MS measurements. Although there was a parallel trend in the TI results, it was not statistically significant. However, this smaller suppression effect found in sensory results as compared to instrumental results is consistent with the psychophysical evidence suggesting a logarithmic or exponential rather than linear relationship between stimulus concentration and human response. It must be noted that the inherent fat content in the milks used in our study was 0.04−0.08%, which might have been able to retain some amount of linalool; thus, the effect of fat might have been more dramatic if the base level had been exactly 0%.

Effects of Fat on Temporal Release of Aroma Compounds and Their Perception. Part of the changes in the overall aroma that were obvious in FCP results could have been due to the effect of fat on temporal aroma compound release. However, in FCP, there was no indication of changes in the temporal release of linalool, as no interaction between the time point and the fat level was found. In line with the hypothesis (a slower release from fattier samples), the after taste in the 5% sample should have been rated higher as compared to the first impression than in the less fat-containing sample. Neither were changes in the temporal release of strawberry flavor in FCP proved, as no interaction was found between the time point and the fat levels.

With other techniques, some temporal differences were found. The TI results showed that the perception of strawberry flavor was delayed in samples containing the most fat. No relevant temporal changes in linalool release as a function of matrix fat content were found in TI; the significant effects of fat on AUC, IArea, and IAngle reflect the effect on intensities more than true timing effects. These TI findings are not in line with the hypothesis, as fat was expected to affect more strongly the temporal release of the very lipophilic compound linalool than ethyl butyrate (mainly responsible for the sensory strawberry). However, the hypothesized temporal effect is far from explained as the evidence is conflicting. Some have found temporal effects for fat, but the findings might be contraindicative (3, *6*, *8*, *10* vs *5*, *9*), and some have found no significant effects (the sensory results in *5*), partly due to different experimental conditions applied in different studies.

The two sampling protocols applied yielded different APCI-MS results on temporal release for the sample containing 0% added fat. There were no temporal differences among linalool samples when using the 15 s protocol; however, when using the straight swallowing technique, the linalool release varied with the fat content of the samples. Linalool release of the sample containing 0% fat persisted for a shorter time as compared to other samples, and the decrease in linalool concentration was much steeper in samples containing less fat than in the sample containing 5% fat. No significant temporal differences were observed for ethyl butyrate. This is in line with the hypothesis that the more hydrophilic compounds are hardly affected by the fat content of the matrix, especially at this low range used in the present study.

When using the straight swallowing technique in APCI-MS measurements, the results were clearer and the differences found were more significant. The length of the time the food is in the mouth is very likely to affect the changes that food is subject to in the mouth (*19*). The 15 s protocol is more prone to the physiological differences among assessors; the amount of aroma compounds retained in their mucosa is different, and there are individual differences in the dilutions of the sample or in the interactions with the saliva (depending on the amount and composition of the saliva), which will affect the release of aroma compounds. In addition, the smooth mouth movements (instructed to assessors while keeping the sample in their mouth) might be different among different individuals, and this might affect the release of aroma compounds. The 15 s sampling protocol was hypothesized to give distinct differences among the samples as the aroma compounds have more time to release,

but physiological factors seemed to mask the differences. In fact, the straight swallowing technique may be closer to the natural consumption of a liquid sample and thus perhaps more relevant (excluding wine tasting, etc.).

Instrumental vs Sensory Methods. The fact that it seemed to be easier to find significant differences in the release of aroma compounds using the instrumental (the straight swallowing technique) than with the sensory methods partly reflects the difficulty of the TI method. Despite the training, the variation among the assessors was great (the assessors had main effects in most of the attributes and many of the interactions). The differences in temporal aroma release patterns are difficult to measure with sensory methods (*4*). The question remains whether they are even possible to measure as clearly as with instrumental methods in a context in which the intensities released are very different. For humans, the temporal differences might be masked by more obvious intensity differences. To obtain a clearer picture of pure temporal release, samples with an isointensive aroma system might be worth studying, although it would require tailoring of isointensive samples for each assessor separately.

In comparisons between instrumental and sensory data of this study, it must be noted that due to sensitivity problems only four (linalool, ethyl butyrate + ethyl isobutyrate, and *cis*-3-hexenol) out of eight compounds of the flavoring mixture were measured using the APCI-MS technique. Of those compounds not measured, especially, ethyl methyl phenylglysidate and undecalactone may have affected the strawberry flavor attribute to some extent based on their sensory characteristics (fruity, sweet, etc.) and this may have a slight interfering effect. However, this was not considered crucial as ethyl butyrate was the main compound producing the strawberry note to the samples.

Comparisons between instrumental and sensory data are in general somewhat complex. The human perception of aroma can hardly be fully described by instrumentally measuring the release of aroma compounds. The different outcomes may partly be due to cross-modal interaction in flavor perception affecting sensory but not the instrumental data. Some impressive examples of cross-modal interaction in flavor perception exist (*4*, *20*, *21*). In addition to psychophysical findings, neuroimaging and neurophysiological studies provide similar evidence suggesting cross-modal interactions of taste and smell that are required to evoke flavor sensation (*22*, *23*). In addition to the aroma compounds, fat itself may act as a stimulus. The type of interaction could be a texture−aroma interaction but also an aroma−aroma interaction. The latter is based on increasing speculation on the taste/olfactory component in fat, although it has widely been considered to be identified by its textural properties (*24*). Neurophysiological studies by Rolls (*23*) support the perception of fat based on both texture and taste/odor. The perception of fats may depend on textural, olfactory, nociceptive, thermal, and gustatory modalities (*25*). The clearest evidence of cross-modal interaction in our sensory results would have been the perception of linalool decreasing less as a function of the fat content of the matrix as was expected based on instrumental results. It would have implied that the response to the increased fat content was added to the response for the aroma. However, this was not the case, as the perception of linalool was clearly reduced along with the increasing fat content of the matrix. However, the increasing knowledge of cross/multimodal interactions will hopefully aid in further understanding of the release of aroma compounds and their perceptions (including the temporal and other effects of fat on food aroma).

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