JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Timing of Intensity Perception of a Polar vs Nonpolar Aroma Compound in the Presence of Added Vegetable Fat in Milk

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Differences in timing of intensity perception of the retronasal aroma of a nonpolar (linalool) vs polar (diacetyl) compound when the matrix (milk) fat content was varied (0%, 1%, 5%, or 10% rapeseed oil) were studied using a time−intensity method. Aromas were also evaluated by orthonasal means and with static headspace gas chromatography (GC). With increasing fat content, linalool was considerably retained in the matrix, while the release of diacetyl was not affected. As little as 1% fat was sufficient to significantly reduce the volatility (GC results) of linalool and orthonasal, but not retronasal, intensity. No effect of fat was found on the rate of linalool release. The linalool perception of the sample containing the greatest amount of fat lasted a shorter time than that of the samples containing less fat; however, the decrease in intensity perception was steeper in lower fat samples. The observed temporal release of linalool partly challenges the often-repeated statement that reduction of fat results in a more rapid and shorter aroma release.

KEYWORDS: Aroma release; retronasal aroma; orthonasal aroma; fat

INTRODUCTION

Fat is believed to affect temporal aroma release, and the aroma of a reduced-fat product is claimed to be harsh and unbalanced and to persist for a shorter time than the aroma of a full-fat product (*1*). A schematic representation of time vs flavor intensity curves of full-fat and reduced-fat products is commonly shown in which the full-fat product has a delayed release curve with decreased intensity compared with that of the reduced-fat counterpart (e.g. *2*, *3*). Many studies have reported faster release rates of aromas when the matrix fat content has been decreased (e.g. *4*); however, some conflicting results exist (*5*, *6*). Some studies have showed shorter duration of aroma as the fat content is reduced (*5*, *7*), while other studies have reported the opposite (*1*, *6*). Brauss et al. (*4*) found with an instrumental method (measuring the volatiles from the nose with atmospheric pressure ionization mass spectrometry) that nonpolar flavor compounds were less persistent in low-fat samples; however, with the sensory time−intensity method they found no significant differences in the duration of flavor. Some studies have shown different temporal effects of fat, depending on the the polarity of aroma compounds (e.g., the polar compound vanillin is less persistent in low-fat samples, but there was no effect of fat on the persistence of the more nonpolar aroma compound limonene (*5*)). In studies using mixtures of flavor compounds it remains unclear which compounds are affected (*1*, *6*, *7*).

The aromas perceived orthonasally and retronasally are claimed to be different quantitatively and qualitatively (*8*). Retronasal aroma is affected by salivation, chewing, and temperature change occurring as food is placed in the mouth, and thus the sensation will be different from that obtained with orthonasal aroma. Voirol and Daget (*9*) found greater sensitivity/lower thresholds in the retronasal perception of vanillin and citral than in sniffing (orthonasally). Some studies have found higher suprathreshold intensities for retronasal aroma (*10*−*12*) and some for orthonasal aroma (*13*), while others have found no differences in ortho- and retronasal aroma intensities (*14*, *15*).

The aim of the present study was to examine the timing of retronasal perception of two aromas differing in polarity when the matrix fat content (added rapeseed oil in nonfat milk) was varied. Aromas were also evaluated orthonasally to study the differences in ortho- and retronasal intensity perception. In addition, the headspaces of samples in a static situation were instrumentally characterized by measuring the relative amounts of aromas in the gas phase with static headspace gas chromatography (GC). There was also additional interest in whether the methods of this study are capable of detecting the effects of fat on a low level (1%), as some instrumental studies have suggested significant effects of even very low levels of fat on aroma volatility (*16*−*18*).

MATERIALS AND METHODS

Materials. The matrix was commercial nonfat milk with added rapeseed oil (Kultasula, Raisio Group Ltd., Raisio, Finland) at levels of 0%, 1%, 5%, and 10% (v/v). According to the manufacturer (Valio Ltd, Helsinki, Finland) the inherent dairy fat content of the milk was 0.04−0.08%. The oil phase was first mixed with milk in a regular kitchen blender, and then the mixture was homogenized with a Rannie homogenizer (Model LAB, Rannie Ltd, Copenhagen, Denmark) at 100 bar to obtain a stable sample matrix. Homogenization was performed so that the entire matrix was forced through the homogenization needle three times to obtain a homogeneous matrix.

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10.1021/jf0342066 CCC: $25.00
Published on Web 08/05/2003

Nonfat milk matrixes were flavored with either diacetyl (2,3-butanedione; Sigma Aldrich, Steinheim, Germany; purity >95%), or linalool (*dl*-3,7-dimethyl-3-hydroxy-1,6-octadiene; Sigma; purity >97%). These compounds were chosen on the basis of their very different polarities (log $P = -2.0$ for diacetyl and 4.0 for linalool (*19*)) and their appropiate sensory properties for the milk matrix. To provide a moderate aroma intensity, a concentration of 40 mg/kg for diacetyl was chosen on the basis of sensory pretests. The orthonasally isointensive concentration of linalool was determined in the 0% fat matrix to be 37.7 mg/kg, but this was rounded to 40 mg/kg. The isointensive concentration was determined as a part of the training procedure. The samples were kept refrigerated in tightly capped and sealed Erlenmeyer bottles. The samples were used within 2 days after preparation.

Headspace Gas Chromatography. To characterize the samples instrumentally, the relative amounts of volatile compounds in the headspace were measured with static headspace GC (Perkin-Elmer Autosystem YL Gas Chromatograph with a Perkin-Elmer Headspace Sampler HS40XL) using an NB54 (5% phenyl 1% vinyl methylpolysiloxane phase, Nordion Ltd) column (25 m × 32 μm) at 80 °C. Helium was used as the carrier gas (carrier gas pressure 8 psi). The compounds were detected with a flame-ionization detector at 250 °C.

For the GC headspace analysis 5 mL of the sample was placed in a 22 mL headspace vial 1 h prior to the measurements. Samples were equilibrated at 60 °C for 20 min, and the sampling time was 0.2 min. Each sample was analyzed in triplicate. The peak area was measured as a result.

Sensory Evaluation. Twelve female subjects (mean age 28.8 y, University staff) served as panelists. All the panelists had a normal sense of smell on the basis of the SOIT (Scandinavian Odor Identification Test (*20*); 11−16 correct identifications out of 16, mean 14.3).

The panelists participated in five training sessions and six evaluation sessions. Two of the actual evaluation sessions were orthonasal evaluations, and four were retronasal. The training sessions included familiarization of aromas and evaluation techniques used in the study and also determination of the isointensive concentration of linalool compared to 40 mg/kg diacetyl orthonasally in nonfat milk.

The 10 mL samples with random three-digit codes were presented in plastic cups (80 mL) covered with lids. The samples were equilibrated at least 1 h prior to the evaluations in the refrigerator and then at room temperature 15 min before the panelists arrived. With this procedure the temperature of samples was 17 ± 1 °C at the beginning of the sessions. Half of the panelists participated first in the orthonasal sessions and the other half in the retronasal (TI) sessions.

The orthonasal evaluations were done in 2 sessions; half of the panelists first evaluated all the linalool samples and the other half the diacetyl samples. The number of samples in a session was 8 (replicates of all matrixes with one aroma, all in randomized order). The panelists were asked to evaluate the intensity of diacetyl or linalool aromas by sniffing, using a scale from 1 to 9 (1 = no aroma, 9 = very strong aroma). The panelists were instructed to bring the sample cup close to their nose, open the lid, and sniff the sample properly but briefly in order not to spread the aroma in the evaluation booth. For the same reason the panelists were asked to close the lids carefully after sniffing. They were allowed to open each sample only once. After each sample, the panelists were instructed to take a short break and breathe freely before proceeding to the next sample.

A computerized TI method was used to evaluate the retronasal aroma (CSA Computerized Sensory Analysis System, Compusense Inc., Guelph, Canada, version 3.8). Retronasal evaluations were done in four sessions, with each replicate in a separate session. Five samples were evaluated in each session: all matrixes of one aroma and a replicate of the sample containing 10% fat, in randomized order. The additional replicate of the sample containing 10% fat was used to obtain information on the consistency of the panelists within a session. The task was too demanding for the panelists to evaluate all the replicates at one session as in an orthonasal evaluation. Prior to each session the panelists tasted the extreme matrixes (samples containg 0% and 10% added fat) in order to focus on the actual aroma compounds in the samples and ignore the aroma of the various matrixes.

In the retronasal sessions the panelists took the entire 10 mL sample into their mouth and simultaneously pressed the "start" button on the computer screen. They evaluated the aroma of the sample by moving the cursor along the vertical scale (1 = no aroma, 9 = very strong aroma). The panelists made smooth mouth movements while the sample was in their mouth and swallowed after 10 s as the evaluation program prompted them to do. After swallowing, they continued evaluating the aroma intensity while keeping their mouth closed and breathing through their nose. As was observed during the practice sessions, tongue movements after swallowing made the aroma stronger (and the aroma differences more apparent); therefore, these were included in the protocol. The total evaluation time per sample was 90 s. To clean their mouth between samples, panelists ate crackers and drank nonfat milk and before proceeding to the next sample rinsed their mouth carefully with tap water.

Many TI studies have used expectoration in the evaluation procedure (e.g. *5*, *6*). Several studies have shown that sensory responses obtained either by expectoration or swallowing of the samples correlate well (*21*−*23*). However, in a recent study by Buettner et al. (*24*) it was seen that over 90% of the aroma was detected (exhaled odorant measurement technique) immediately after swallowing of the sample (in "swallowing breath"), while prior to swallowing only a very small proportion of the volatiles reached the epithelium in the nose; the velum-tongue border effectively limited access to the nasal cavity. In our own pretests the aroma intensity was also rated higher when the samples were swallowed compared with the samples that were expectorated. This was considered favorable with respect to detection of aroma differences among the samples.

The term aroma as used in this study refers to retronasal aroma in TI measurements and to orthonasal aroma in orthonasal evaluations.

Data Analysis. The parameters calculated from the TI data included time to maximum (TMax), maximum intensity (IMax), duration (Dur), area under the curve (AUC), increase angle (IAng), increase area (IArea), decrease angle (DAng), and decrease area (DArea). The three-way multivariate analysis of variance (GLM procedure) was used to assess the main effects and interactions of fat content, panelists, and replication on the TI parameters. With regard to the orthonasal results a three-way analysis of variance (GLM procedure) was used to assess the main effects and interactions of fat content, panelists, and replication on perceived intensity of aromas. Multiple comparisons of means were performed with Tukey's test (5% level of significance). Paired *t* tests were performed to compare means obtained for hidden reference (10% fat sample) in the TI sessions. To evaluate the interrelationships of the various parameters calculated from the TI data, principal component analysis (PCA) was performed on the average results obtained for linalool (correlation matrix, no rotation).

Table 1. Relative Amounts of Aromas in the Headspace of Different Milk Samples

	headspace area ($N = 3$)	
fat %	linalool	diacetyl
0	47409 (10.2)[a]	33259 (2.8)
1	25086 (13.3)	31668 (2.0)
5	9204 (14.4)	26725 (2.6)
10	4861 (11.8)	23394 (0.4)

[a] The standard deviation (%) is given in parentheses.

RESULTS

Static Headspace Gas Chromatography. The relative amounts of aromas in the headspace of samples are summarized in **Table 1**. The standard deviations for diacetyl were satisfactory, but for linalool they were generally above 10%. No reason could be determined for the poor repeatability of the linalool measurements; however, despite the lack of repeatability, the differences among the linalool samples were pronounced.

Intensity Values of Orthonasal and Retronasal (IMax) Evaluation. The effect of fat on aroma release was pronounced. Linalool, a very nonpolar compound, was considerably retained in the matrix as the fat content was increased (main effect of

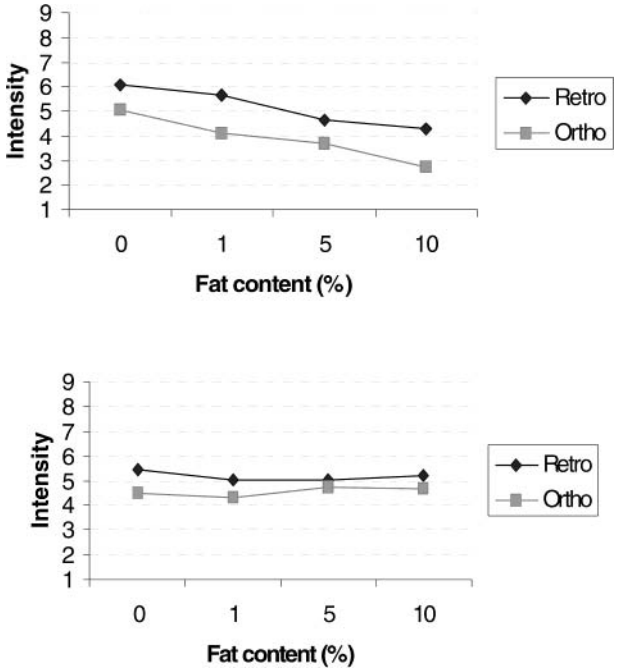

Figure 1. Mean intensity values of orthonasal evaluation and mean IMax values of retronasal evaluation for linalool (a, top) and diacetyl (b, bottom) in different matrixes ($N = 24$).

fat, $F(3;33) = 20.4$, $p < 0.001$ for IMax in the TI method and $F(3;33) = 18.3$, $p < 0.001$ in orthonasal evaluation). The release of the more polar compound diacetyl was not affected by the fat content of the matrix (main effect of fat, $F(3;33) = 0.85$, $p = 0.47$ in TI and $F(3;33) = 0.42$, $p = 0.74$ in orthonasal evaluation). The results of the orthonasal evaluation and the mean IMax values obtained with the TI method are shown in **Figure 1**. Although the aromas were determined to be isointensive in the matrix containing 0% fat in the practice sessions, in actual evaluations the linalool aroma intensity was rated to be slightly higher than the diacetyl aroma. This may be partly due to the fact that the determined isointensive concentration of linalool was rounded off to a slightly higher level for practical reasons in sample preparation.

Temporal Retronasal Aroma Release (TI Method). The individual TI curves were averaged across intensities at fixed times (each second) (Figure 2). As expected, the individual variation was large, and therefore the curves were also normalized along the time and intensity axes on the basis of the method proposed by Overbosch et al. (*25*) (shown in the upper right corners of parts a and b of **Figure 2**). The differences among samples were expected to be more clear as the variation due to the panelists' different styles of using the scale were extracted. However, this did not appear to be the case, and raw data were used in further analyses.

The temporal aspects of the aroma release can be examined in the TI parameters TMax, Dur, IAng, and DAng and in the area-related parameters (**Figure 3**). In the linalool samples, the fat content of the matrix had no effect on the TMax value (main effect of fat, $F(3;33) = 0.5$, $p = 0.700$). The Dur value was

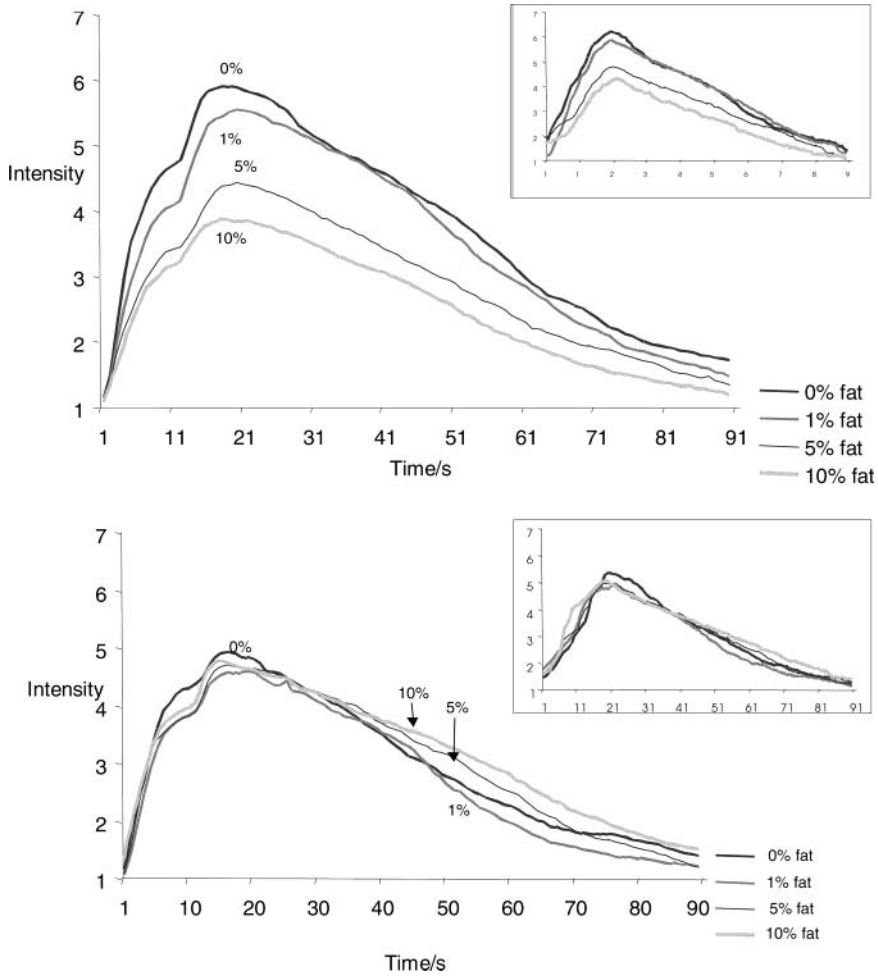

Figure 2. Mean time intensity curves for linalool (a, top) and diacetyl (b, bottom) in different matrixes ($N = 24$, except for 10% sample $N = 48$). The normalized data curves are shown in the upper right corners.

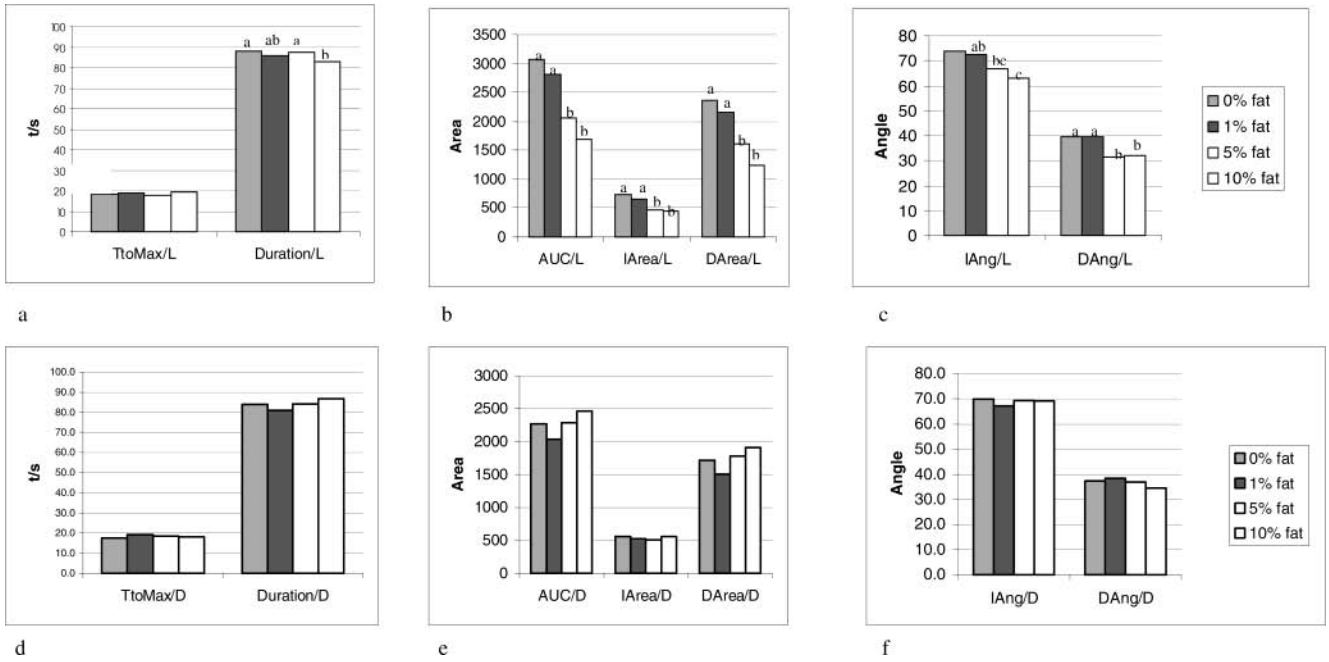

Figure 3. Average parameters calculated from time intensity data for linalool (a–c) and for diacetyl (d–f) ($N = 24$ except for 10% sample $N = 48$). Bars marked with same letter (or no letter) are not significantly different; $p = 0.05$.

affected by the fat content of the matrix (main effect of fat, $F(3;33) = 3.7$, $p = 0.021$). The duration of linalool aroma was shortest in the sample containing 10% fat; the sample containing 1% fat did not differ significantly from it, but samples with no fat or 5% fat did (**Figure 3a**). It must be noted however, that as the intensity perception did not revert to zero level in the time course of evaluation for some assessors, the term Dur has to be interpreted cautiously. The area-related parameters of linalool (**Figure 3b**) were affected by the fat content of the matrix (main effect of fat, $F(3;33) = 40.0$, $p < 0.001$ for AUC, $F(3;33) = 7.9$, $p < 0.001$ for IArea, and $F(3;33) = 39.3$, $p < 0.001$ for DArea). Increased fat content was related to decreased area parameters; generally two subgroups were observed, one with the nonfat and 1% fat samples and another with the other two fat-containing samples. The parameters IAng and DAng both were affected by the fat content of the matrix (main effect of fat, $F(3;33) = 8.6$, $p < 0.001$ for IAng and $F(3;33) = 12.7$, $p < 0.001$ for DAng). None of these parameters varied significantly among the diacetyl samples (**Figure 3d**−**f**).

Method Performance. The relative results obtained with different methods (IMax in the case of TI) were examined (**Figure 4**), although it is emphasized that, as the methods are very different (SHS-GC and orthonasal evaluations measure aroma in a static situation, and TI is a dynamic method), the comparison is only suggestive. The relative values were calculated such that the response for a nonfat sample was given a value of 100% and the other values were then calculated in proportion to it. The response of the GC method appeared to be the most sensitive to the effect of fat on the linalool release compared with the two sensory methods, which responded similarly. In the case of diacetyl release, there was a slight effect of fat content of the matrix detected by GC, which was not observed by either of the sensory methods.

In the TI method the consistency of the panelists was confirmed by replicating the sample containing 10% fat in each session (hidden reference). Consequently, each panelist evaluated this sample four times. The means and standard deviations of this sample for all TI parameters were calculated individually. The main criterion being the standard deviation of the IMax,

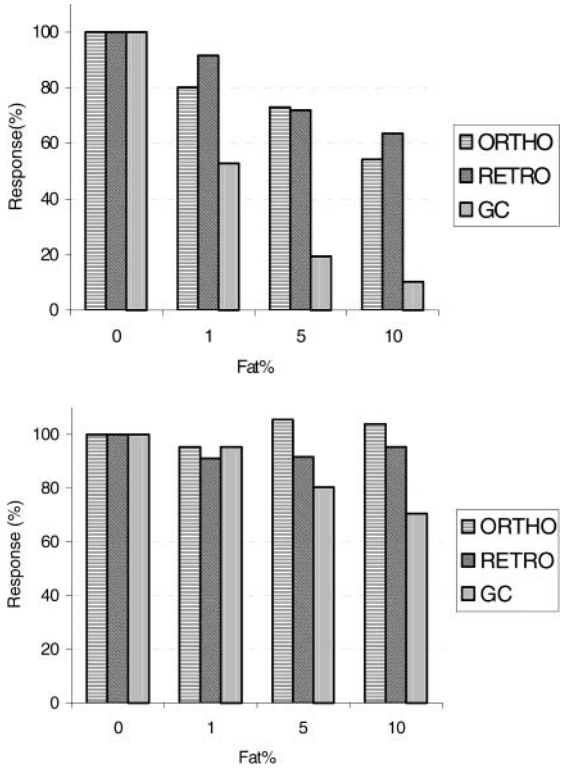

Figure 4. Comparison of the relative results (mean intensity for orthonasal method, mean IMax for retronasal method, and mean peak area for GC method) obtained with different methods for linalool (a, top) and for diacetyl (b, bottom). Relative values were calculated as a proportion of the result for the nonfat sample.

two panelists were excluded, and the means and standard deviations for each parameter were recalculated. However, since there was no significant improvement in the standard deviations after the removal of the two panelists, data on all the panelists were included in the results. The means of IMax, Dur, and TMax obtained for the two samples containing 10% fat (hidden reference and the actual sample) were compared using paired

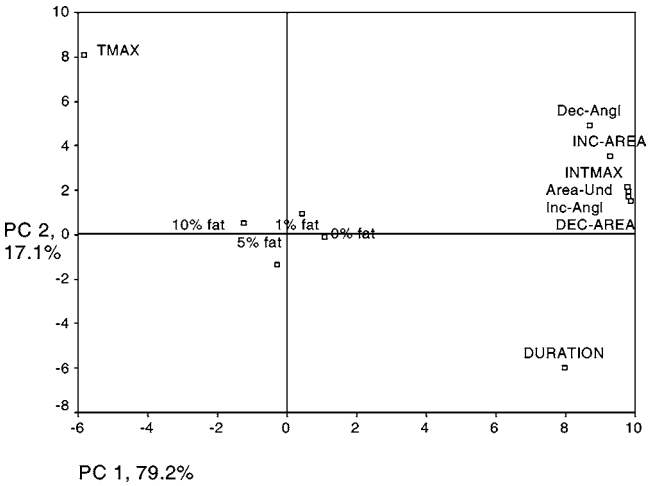

Figure 5. PCA biplot of linalool samples and TI parameters (abbreviations in text).

t tests (separately in each session). Since there were no intrasession differences in any of these parameters in either session, all four ratings were used in the analysis of the 10% fat sample.

A significant effect of the panelist on every TI parameter was observed. A main effect of the panelist was observed also in the linalool results of the orthonasal method ($F(11;33) = 3.1$, $p = 0.006$). These effects were expected. Replication had only few main effects (linalool: $F(1;33) = 6.7$, $p = 0.014$ for IMax and $F(1;33) = 5.9$, $p = 0.020$ for DAng) and no interactions in the TI method, and the panel as a whole was considered to give reproducible evaluations. In the orthonasal method, replication had the main effect (linalool, $F(1;33) = 11.7$, $p = 0.002$; diacetyl, $F(1;33) = 6.1$, $p = 0.019$), but this was not considered fatal; however, it indicates that the orthonasal results are somewhat unstable.

PCA was performed to examine the interrelationships of the various parameters calculated from the linalool TI data (biplot in **Figure 5**). The first two components accounted for 96.3% of the total variance. Samples were generally loaded on the first principal component (PC). In the PCA plot it was clearly seen that many of the calculated parameters correlate strongly, e.g. IMax, AUC, IArea, DArea, IAng, and DAng. The first PC was heavily loaded with these parameters and accounted for 79.2% of the total variance. The first PC clearly separated the samples based on fat content and, thus, aroma release. The second PC, which accounted for 17.1% of total variance, was positively loaded with the parameter TMax and negatively with the parameter Dur, suggesting that these two parameters are related to the samples on the basis of factors other than the remaining parameters. However, in the case of TMax the significance of this finding is low, as there were no differences among samples in this parameter, and in the case of Dur the interpretation has to be cautious, as the intensity perception did not revert to zero level for some assessors in the time course of evaluation. Further interpretation of the second PC remains unclear, and it may also contain noise.

DISCUSSION

When considering the observed effects of fat on the intensities obtained orthonasally and the IMax values obtained retronasally, the results were as expected, on the basis of the literature (e.g. *26*−*28*), and also well in line with our earlier results (*29*). The aromas were chosen on the basis of their very different polarities to demonstrate the effect of fat on the volatility of nonpolar compounds. Some previous instrumental studies have shown that as little as 1% fat in the matrix or even less retains aroma compounds considerably (*16*−*18*). This effect is strongly dependent on the lipophility of the aroma compound. On the basis of previous studies, linalool, a very nonpolar compound, should be strongly retained by even small amounts of fat. Our instrumental results showed that adding 1% fat to the matrix resulted in an approximately 50% decrease in the headspace concentration of linalool. However, our sensory results on the effect of 1% fat were not as convincing. Although the sample containing 1% fat was differentiated in the orthonasal evaluation, the difference was not as clear as in the GC results. Our TI results showed that there was considerable change in the amount of retained aroma between the 1% and 5% fat-containing matrixes; the fat-free and 1% fat-containing matrixes formed their own subgroup (IMax and area-related parameters). Our sensory results do not strongly support the hypothesis that 1% fat in the matrix is enough to retain aromas considerably. The GC method appeared to be more sensitive to change in volatile concentration than the sensory methods. This was also seen in a study of the effects of matrix on the aromas of menthone and isoamyl acetate (*30*). In fact, a small decrease in diacetyl was observed in our GC results along with the increased fat content, which is not observed with sensory methods. However, as the dynamics of different methods used are very different, the observed differences are quite expected. The differences observed in the results between instrumental and sensory measurements may have also been partly due to the different temperatures used; due to sensitivity problems the GC samples were equilibrated at 60 °C. Another factor may be that the concentrations of aromas were chosen to be of moderate intensity and thus considerably above the odor threshold. At such intensity levels, the panelists, who operate according to psychophysical laws and not on equal distances (*31*), may have found it difficult to distinguish between the samples.

The retronasal IMax values were in general slightly higher than the corresponding orthonasal intensities, a finding supported by some earlier studies (*10*−*12*). In contrast, Kuo et al. (*13*) reported higher intensities for orthonasal perception than for retronasal. Despite the slight differences in levels observed, the differences in the samples were obvious and similar in both methods.

To gain information on the effects of fat on the temporal perception of nonpolar aromas, the effects of fat on the parameters TMax, Dur, AUC, IAng, IArea, DAng, and DArea in the linalool samples were analyzed. There were no differences in TMax among the various samples, which does not support the suggestion that the aroma of a product containing less fat is released more quickly than that of a product containing higher levels of fat (*32*). Guinard et al. (*6*) likewise failed to observe quicker release of garlic (but did in the case of pepper) in salad dressings upon fat reduction (0%, 6.75%, and 13.5% fat levels studied). They suggested that the garlic flavor was possibly retained in the mouth and that the panelists were perceiving it even before the sample was placed in the mouth. It must be noted that they used resoleum garlic oil, which contains a mixture of aroma compounds; thus, the results are not as easily interpretable regarding which compound had been affected by the fat content as in our study with single-aroma compounds. TMax was likewise not affected by the fat content of the matrix (0%, 10%, and 50% fat levels studied) in the case of the nonpolar compound limonene in an oil-in-water emulsion (*5*). However, Brauss et al. (*4*), using sensory and instrumental

methods, observed a quicker release of nonpolar compounds in a low-fat sample compared with samples containing higher levels of fat.

The IAng and DAng values were larger in the linalool samples containing less fat (nonfat and 1% fat) than in those containing higher levels. This may suggest quicker and shorter release of aroma, but on more careful examination of the results it is clear that this occurred because the IMax values of samples containg less fat are larger than the IMax values of the other samples. The area-related parameters (AUC, IArea, DArea) reflect the same phenomena; the areas of the two fattier samples are smaller due to the smaller IMax values, rather than temporal factors.

In addition to the more rapid release, the aroma of a product containing less fat has been suggested to persist for a shorter time than that of a product containing normal levels of fat (e.g. *2*, *3*). In contrast, the perception of linalool in the sample containing the greatest amount of fat (10%) lasted the shortest time in our study. However, it must be noted that the decrease was steeper in lower fat samples than in samples containing more fat. The fat content of the matrix had no effect on the duration of diacetyl perception. Guinard et al. (*6*) also found conflicting results; in their study of salad dressings, the total duration of garlic and pepper flavors increased with the removal of fat. Mialon & Ebeler (*5*) found no significant differences in duration of limonene flavor among matrixes with different amounts of fat (0%, 10%, or 50%), although there was a trend toward decreasing duration as the lipid concentration increased. In the case of the polar compound vanillin, the perception tended to last longer with increasing amounts of fat in the matrix, which was suggested to be due to an increase in the mole fraction of the vanillin in the aqueous phase. Guinard et al. (*6*) offered greater viscosity of samples containing more fat as an explanation for the shorter duration of flavors; the flavor was not totally released from the viscous samples during the time the sample was in the mouth. In addition to fat content, the viscosity of the matrix affects aroma release, because the diffusivity in a viscous matrix is smaller than in a less viscous matrix. The milk samples used in the present study may have slightly different viscosities. However, they were all very fluid and kept in the mouth for a reasonable time (10 s) before swallowing; thus, viscosity differences appear to play a minor role in these samples. In a study (*33*) with a fairly similar sample matrix (skim milk with varying levels of sunflower oil), it was stated that the oil exhibits only little change in viscosity, although the highest level of oil in that study was as high as 18%.

It must be noted, when examining the results of the TI parameter Dur in our study, that the evaluation time was restricted to 90 s and that not all the evaluations reverted to zero intensity during that time period. If the evaluation time had not been restricted, the differences may have been clearer. However, it was found in pretests that the panelists were more comfortable with a restricted time. On examination of the present results, it must be considered that we used only a very simple aroma model system (two separate compounds) and the results are not directly applicable to real food systems that contain perhaps hundreds of volatile compounds.

In conclusion, we suggest that as little as 1% fat in the matrix significantly reduced the headspace concentration of linalool in milk. However, the reduced intensity of linalool was not as pronounced with sensory methods. With the TI method the reduction in linalool intensity was significant only after addition of 5% fat. The matrix fat content had only a minor effect on the volatility of the very polar compound diacetyl. As the matrix fat content was increased, the aroma of linalool persisted for a shorter time than in the nonfat sample. No differences were observed in the rate of linalool release (time to maximum intensity) in matrixes containing different amounts of fat. These effects (on duration and time to maximum intensity) were not expected since, with increasing amounts of fat, a longer duration and slower release were hypothesized,on the basis of the literature. However, it must be noted that the decrease in intensity was steeper in low-fat samples than in fattier samples.

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Received for review February 28, 2003. Revised manuscript received July 3, 2003. Accepted July 6, 2003.

JF0342066