Altering the Fat Content Affects Flavor Release in a Model Yogurt System

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Flavored yogurts differing in fat content were eaten, and the release of flavor volatiles was measured by monitoring the volatile composition of air from the nose in real time by atmospheric pressure ionization mass spectrometry. Low-fat yogurts (0.2%) were found to release volatiles more quickly and at higher intensity but with less persistence than yogurts containing fat at 3.5 and 10% fat. Yogurts with increasing fat content had higher viscosity and lower relative particle size. Lipophilic compounds were more affected by fat for maximum volatile intensity, but not time-to-maximum intensity or persistence. Sensory assessment of the yogurts found significant differences in intensity and speed of onset of flavor, but not overall length of perception.

Keywords: *API-MS; low-fat foods; log P; flavor; yogurt; volatile*

INTRODUCTION

The importance of fat in the perception of food has been reviewed extensively (Drewnowski, 1992; Forss, 1969). Fat plays a key role in modifying the physical properties of food, including mouthfeel, appearance (gloss, color, opacity), structure (texture, consistency, melting profile), heat transfer, and nonsensory effects (satiety). Fat is also important as a flavor precursor, flavor carrier, and flavor release modulator. It has been reported to influence qualitative, quantitative, and temporal perception of flavor in products (Tuorila et al., 1995; Plug and Haring, 1994).

The problems of poor textural properties of low-fat foods have been resolved with some success. However, producing low-fat foods with flavor similar to that of their high-fat equivalents has proved to be somewhat more difficult (Hatchwell, 1994). The other components of the yogurt (protein, carbohydrate, etc.) may adsorb and bind with flavor chemicals (Franzen and Kinsella, 1974; Stampanoni et al., 1996) but cannot act as solvents. Hence, removal of fat creates a whole range of quality changes, including changes in the rate and concentration at which food flavor molecules are released during consumption.

The typical British diet derives ~41% of daily calories from fat, whereas it is the Government's aim to reduce intake to 35% by the year 2000 (Department of Health, 1992), and American nutritional guidelines recommend reducing fat consumption to 30% (Food and Nutrition Board, 1989). An excessive intake of fat, and especially saturated fat, in the diet can result in various diseases. By far the most important of these is cardiovascular disease [both the atherosclerotic and thrombogenic components (Hu et al., 1997)], although high saturated fat intake also plays a role in the etiology of many other diseases.

Although the population is now generally aware of the consequences of a diet rich in saturated fats, consumers are still not willing to reduce fat intake, a low-fat diet being perceived as being difficult to adhere to (Harnack et al., 1997). It has also been shown that, whereas consumers may be aware of the health benefits of a low-fat diet, poor flavor quality may be one factor hindering the acceptance of low-fat foods (Giese, 1994; Mela, 1995). The continued improvement of the taste of low-fat alternatives is clearly a long-term aim of the food industry and may benefit the population by helping to reduce the overall fat intake.

Fat is an important flavor solvent, and flavor compounds would be expected to partition themselves between the fat, water, and air phases in yogurt. However, flavor compounds differ in their hydrophobicity and, hence, their partitioning between the phases. The distribution of hydrophilic compounds between the food and headspace is independent of fat content, whereas increasing the fat content would significantly decrease the concentration of hydrophobic compounds in the headspace. Although this effect has been hypothesized (de Roos, 1997), there are few quantitative data in the literature.

The work by Delahunty et al. (1996) on low- and high-fat cheeses showed that hydrophobic volatiles will preferentially partition into the fat phase and that this may slow their volatilization during eating. Van Boekel and Lindsay (1992) also demonstrated that changes in fat content will affect the partitioning of compounds depending on their lipophilicity, and hence the balance of compounds between high- and low-fat cheeses will differ. Therefore, by changing the fat content of foods, one would expect changes in volatile composition, volatile intensity, and rate of release (Plug and Haring, 1993). However, although the in-vivo experiments de-

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Table 1. Formulation Used in Preparing Yogurts

	fat content		
	0.2%	3.5%	10%
skimmed milk powder (g)	6	6	3
Domovictus 300 (g)	2	0	0
sucrose (g)	10	10	10
skimmed milk (0.2% fat) (g)	82	0	0
full-fat milk (4.2% fat) (g)	0	84	66
full-fat cream (37% fat) (g)	0	0	21
total weight (g)	100	100	100

scribed by Delahunty et al. (1996) showed compositional changes, it was not possible to comment on changes in rates of release.

McNulty and Karel (1973) have shown that the more hydrophobic the compound, the greater the effect of oil on release of *n*-alkanals. However, in the examination of the behavior of compounds, it is important to in some way quantify the physicochemical characteristics. One well-accepted measure of the polarity of compounds are log *P* values. Log *P* describes the partitioning of the compound under investigation between the solvents water and octanol, in a two-phase system. This measure has been used in determining partitioning of pharmaceuticals in the body (Rekker and Mannhold, 1992) and is of equal relevance in flavor delivery systems.

Our objective was to quantify the effect of fat on volatile release in terms of intensity (I_{max}), speed of onset of maximum intensity (T_{max}), and persistence by measuring the release of volatiles on the breath in real time (nose space). The sensory significance of these changes in release patterns was determined using a trained sensory panel. Different compounds would be expected to act in different ways when the fat content is changed.

MATERIALS AND METHODS

Choice of Flavor Compound. In choosing the compounds for addition to the yogurt, the following characteristics were considered: lipophilicity [quantified in terms of log *P* (Rekker and Mannhold, 1992; Suzuki and Kudo, 1990)], degree of breakdown during the fermentation process, and distinction of flavor (for ease of identification by panelists). The compounds used in the initial experiments and their log *P* values were *trans*-2-hexenyl acetate (2.13), anethole (4-propenylanisole) (3.45), and terpinolene (1-isopropenyl-4-methylcyclohex-3-ene) (4.44). Ethanol (log $P = -0.19$) was used as a solvent for the flavor compounds. All compounds were obtained from Firmenich SA (Geneva, Switzerland). It had previously been determined that these compounds are not broken down by the fermentation process (data not shown). Appropriate concentrations of the chosen volatiles in the yogurt were determined by brief sensory evaluation (i.e., the panelists could identify with ease the identity of the flavor of the compound) and instrumental testing (i.e., a reasonable signal-to-noise ratio, in excess of 100 in the case of the API-MS).

Preparation of Yogurt Samples. Yogurts were prepared with fat contents of 0.2, 3.5, and 10% fat by weight. Skimmed milk, whole milk, and full-fat cream were used to obtain the required fat contents, using a formulation provided by Firmenich SA (Table 1). Milk, skimmed milk powder (SMP), and Domovictus 300 (skimmed milk solids replacer, Domo Food Ingredients, PZ Beilen, The Netherlands), if necessary, were mixed together and left overnight at 4 °C to hydrate. The sucrose content was maintained constant at 10% (w/w) because sucrose can enhance perceived fattiness and the effect on flavor release can be considerable (Nawar, 1971; Tuorila et al., 1993). After mixing, the ingredients were pasteurized at 85−90 °C and then cooled quickly in ice to 45 °C. The flavoring (dispersed in ethanol and at a final concentration of 80 mL/200 mL of yogurt, i.e., 400 ppmv) and culture were then mixed in, and the mixture was left for 15 min before being poured into pots and left to ferment for 3 h at 45 °C. After this time, the pots were placed in a refrigerator (4 °C) for 3 days to mature. Once mature, the yogurts were used immediately, to reduce the effect of sensory and chemical changes that occur with excessive maturation (Laye et al., 1993).

Table 2. Instrumental Results of Yogurt Headspace[a]

	hexenyl acetate	anethol	terpinolene
pot surface	1721	55	368
SD	32	2	21
pot interior	1758	56	377
SD	22	3	17

[a] Samples taken from surface and interior of fermentation pots, postfermentation. Peak height ($\times 10^{-3}$).

This method produces a set yogurt and contrasts with stirred yogurt, for which the flavoring is added after fermentation and is stirred into yogurt. It is believed that the set yogurt system allows better dispersal of the flavoring, and samples taken from different parts of the pot were found to have the same flavor content, as measured by headspace sampling (Table 2).

Measurement of Volatile Release. A Platform quadrupole mass spectrometer (Micromass, Altrincham, U.K.) operating in the atmospheric pressure ionization (API) positive ion mode was fitted with a custom-built air-sampling interface (Linforth and Taylor, 1997). The API-MS system produces molecular ions predominantly and discriminates solely on the basis of ion mass/charge ratio. Positional isomers, stereoisomers, or fragments of different ions with identical molecular weights cannot be differentiated. Therefore, care was taken to select compounds with molecular weights that did not coincide with those of compounds naturally present in yogurt. In selected ion mode (SIM), the Micromass Platform software allows a different cone voltage to be used with each ion monitored; thus, the ionization conditions can be optimized for each analyte. The cone voltage was adjusted to give maximum sensitivity for the MH^+ ion. The compounds and the cone voltages (volts) were as follows: anethole, 23; terpinolene, 23; *trans*-2-hexenyl acetate (hexenyl acetate), 27.

For the eating experiments, one trained subject ate one spoonful of the yogurt sample (5 g) while resting one nostril at one end of a plastic tube (12 mm × 50 mm). The tidal flow of air from the nostril passed back and forth through the tube. Part of this airstream was continuously sampled into the API source (30 mL/min) through a capillary tube (0.53 mm i.d.), inserted through the wall of the plastic tube at right angles to the direction of flow (Linforth and Taylor, 1997). Breath volatile concentrations were expressed as peak heights, and five replications were performed for each experiment.

Sensory Analysis. Ten trained panelists performed sensory analysis of the samples simultaneously with instrumental measurements of breath concentrations of volatiles. Samples were marked with randomized numbers and presented in a random sequence. Panelists were neither told what flavor to expect nor what parameters were being changed. A questionnaire was used, which asked the panelists to mark the yogurts in terms of the following criteria: time to first perception of flavor (T_0), time to maximum intensity (T_{max}), maximum intensity (I_{max}) relative to other samples (i.e., among the three fat contents), time for overall perception (T_{end}), and how long the maximum intensity lasted (T_{plat}). A discrete scale from 1 to 5 was used, and panelists marked each variable independently. A consequence of such analysis is that although comparisons can be made between yogurts of differing fat contents, one cannot compare different variables. An example of this may be found in Table 3, where the value for T_{max} is apparently higher than that for T_{plat} and T_{end}. This does not imply that T_{max} occurred later than T_{plat} or T_{end} (an impossibility), but merely that it received a higher score, at that fat content, relative to the other variables. Statistical analysis of the data was performed by Variance Analysis (two factors, product and subject).

Table 3. Sensory Results of Eating of Yogurts by Panelists[a]

	fat content		
	0.2%	3.5%	10%
terpinolene			
T_0	1.8	1.7	2.3
I_{max}	3.5*	3.3*	2.7*
T_{max}	2.4	2.1	2.7
T_{plat}	2.5	2.2	2.8
T_{end}	3.4	3.1	3.4
anethole			
T_0	1.7	2.1	2.4
I_{max}	3.1	3.4	2.9
T_{max}	2.4	2.3	2.6
T_{plat}	2.1	2.8	2.6
T_{end}	2.5	3.0	3.0
hexenyl acetate			
T_0	1.2*	1.9*	2.2*
I_{max}	3.8**	3.4**	2.5**
T_{max}	1.7*	2.3*	2.7*
T_{plat}	2.5	2.2	2.3
T_{end}	3.2	2.8	2.6

[a] Arbitrary units, scale 1−5 (*, **, significant at 95 and 99% significance levels, respectively).

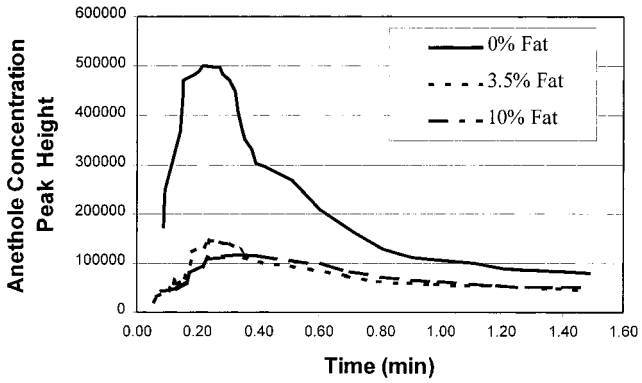

Figure 1. Release of anethole from various fat content yogurts, absolute data. Each curve is based on five replicate samples.

Measurement of Particle Size. Particle size was measured using a Mastersizer (Malvern Instruments, Malvern, U.K.). A 5 g sample was introduced into the circulating water system, and particle size was measured by laser diffraction. This was performed for all fat contents and all compounds, to ascertain their effects.

Measurement of Viscosity. Viscosity was measured using an RS 150 Rheostress (Haake Instruments, Crawley, U.K.), using a 5 g sample. It was defined by the stress required to reach a shear rate of 21 s^{-1}, expressed as pascals per second.

RESULTS AND DISCUSSION

Instrumental Analysis. Breath-by-breath release curves were obtained for each volatile, allowing comparison of the key volatile release characteristics, maximum intensity (I_{max}), time to maximum intensity (T_{max}), and persistence.

The release of anethole from yogurts of various fat contents is shown in Figure 1 (absolute data) and Figure 2 (normalized data). There was a large difference in I_{max} measured by the API-MS, the low-fat yogurt having a 4-fold higher I_{max} than the medium- and high-fat yogurts (Figure 1). The difference in I_{max} between the yogurts containing 3.5 and 10% fat was not significant. The normalized data in Figure 2 show further detail not apparent with the absolute data. There were two main effects: The low-fat yogurt appeared to reach I_{max} first,

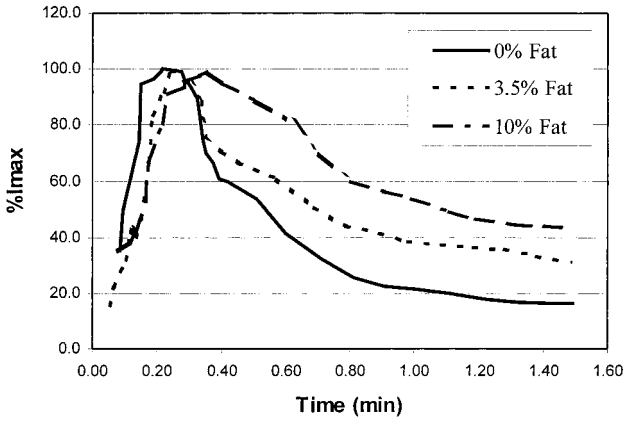

Figure 2. Release of anethole from various fat content yogurts, normalized data. Each curve is based on five replicate samples.

Table 4. Instrumental Volatile Release Data[a]

	fat content		
	0.2%	3.5%	10%
I_{max}			
anethole	500[a]	150[b]	120[b]
terpinolene	305[a]	205[a]	85[b]
hexenyl acetate	2955[a]	1700[ab]	1160[b]
ethanol	1040	1310	1230
T_{max}			
anethole	0.22[a]	0.32[ab]	0.33[b]
terpinolene	0.17[a]	0.21[ab]	0.25[b]
hexenyl acetate	0.19	0.20	0.23
ethanol	0.23	0.24	0.31

[a] I_{max} (peak height × 10^{-3}) and T_{max} (minutes) of yogurts of different fat contents. Data in a row with the same superscript are not significantly different.

and the breath volatile concentration decreased more rapidly after I_{max} relative to the yogurts containing more fat. This suggested that 3.5% fat was sufficient to act as a reservoir for the volatile, and any increase in fat had less effect on release than would be expected.

When the range of compounds were compared, lipophilic flavor compounds (anethole, terpinolene, and hexenyl acetate) had a significantly higher I_{max} at 0.2% fat, whereas ethanol, a hydrophilic compound, was not affected by fat content (Table 4).

As the fat content was increased, T_{max} was significantly delayed for terpinolene and anethole (Table 4). One explanation is that fat acts as a volatile reservoir and slows the release of volatiles into the nose space. The lipophilicity of hexenyl acetate, however, is lower than for the other two compounds, and this may explain why, although there was a trend of increasing T_{max} with an increase in fat content, the effect was not statistically significant.

The relationship between polarity of compounds and their behavior in a complex fat/water/air phase system has been reviewed extensively (Overbosch et al., 1991; Archer et al., 1994). To assess how the release of a compound was affected by the fat level in yogurt, the T_{max} and I_{max} values (Table 4) for high-fat and low-fat yogurts were converted into ratios (calculated as $T_{max}^{high-fat}/T_{max}^{low-fat}$ and $I_{max}^{low-fat}/I_{max}^{high-fat}$) (Figure 3). Although T_{max} increased for all compounds between the high- and low-fat yogurts, the increase was independent of the log P values. In contrast, I_{max} was significantly higher for low-fat yogurt, and it was found that more highly lipophilic compounds (higher log P value) were

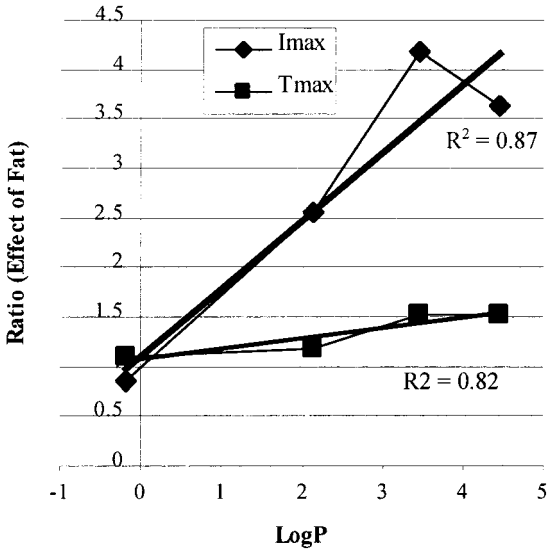

Figure 3. Effect of fat on T_{max} and I_{max}, relative to log *P*. Ratio indicates relative change in the variable as a result of increase in the fat content of yogurt samples (calculated as $T_{max}^{high-fat}/T_{max}^{low-fat}$ and $I_{max}^{low-fat}/I_{max}^{high-fat}$).

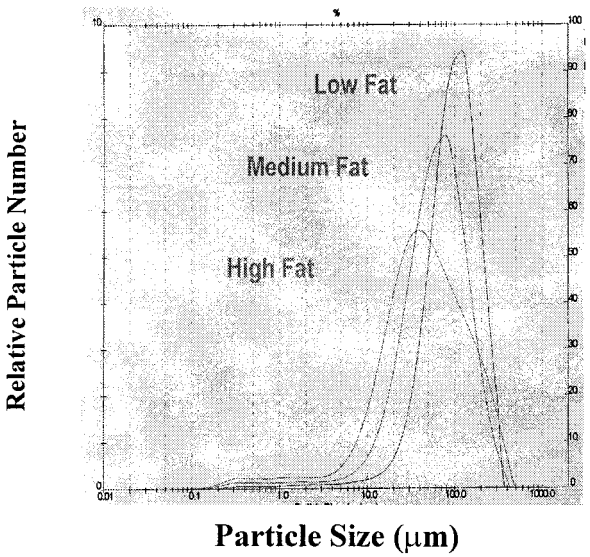

Figure 4. Distribution of particle size of various fat content yogurts.

more affected by changes in the fat content of the yogurts. One explanation is that a lipophilic compound will reside preferentially in the fat phase and will be less available for release into the nose space. A high fat content will encourage this, either through the flavor reservoir effect or by changing the emulsion structure. No trend for the effect on persistence, in terms of time for signal to decline to 50% of I_{max}, was found (data not shown).

Sensory Analysis. Although the fat content influenced the release of volatiles during eating, these differences were not always reflected in the sensory analyses (Table 3). Significant differences among the fat levels were observed for T_0, I_{max}, and T_{max} for hexenyl acetate and for I_{max} for terpinolene. However, the sensory data in Table 3 show clear trends, with low-fat yogurts being more quickly and strongly perceived than high-fat yogurts. There would appear to be no relationship between fat content and T_{plat} and T_{end}. This suggests that panelists were able to identify some differences in I_{max} and, in the case of hexenyl acetate, T_{max}, that were shown instrumentally (although the trend for instrumental T_{max} for hexenyl acetate was not significant). Although panelists were unable to differentiate, in most cases, for T_{max} between medium- and low-fat yogurts, they consistently judged the high-fat yogurts to have later T_{max} values compared to the other samples.

Panelists' difficulty in identifying differences between low- and high-fat samples for anethole and terpinolene may be explained in terms of odor thresholds (OT). The OT for the compounds were anethole, 44 ppbv; terpinolene, 37 ppbv; and hexenyl acetate, 1.9 ppmv (Devos et al., 1990). The concentration of the compounds in the yogurts was constant, at 400 ppmv. If the concentration in the nose space is much in excess of the OT, which is likely to be the case for anethole and terpinolene, then the panelist may find it difficult to distinguish between samples differing in concentration. However, if the concentration in the nose space is relatively closer to the OT (i.e., hexenyl acetate), then the panelist may find it much easier to distinguish between concentrations, because the ability to sense changes in intensity may be more acute, around that threshold point.

However, panelists were not able to identify the significant differences in persistence (quantified as T_{plat} and T_{end}) between high- and low-fat yogurts. This may be explained by adaptation to the volatile by panelists. If a volatile was more highly persistent due to the presence of fat, the effect may not be detected due to adaptation to that compound over the eating time course. Panelists also commented that the anethole flavor was highly persistent for all three samples. The considerable sensory carry-over from one sample to another, although anecdotal, may be responsible for panelists not being able to distinguish between the large instrumental differences in I_{max}.

The onset and intensity of flavor (T_{max} and I_{max}) are more important in perceiving differences due to changes in fat content in the consumption of yogurts. Persistence may be important in determining the quality of other foods (e.g., wine). It was not perceived as an important factor in this experiment.

Particle Size. A range of particle sizes was obtained for each of the compounds and fat contents. Particle size was found to be consistent within a fat content yogurt and was unaffected by the compounds used. However, particle size did vary according to fat content, mode particle size decreasing from 103 mm in low-fat to 35 mm in high-fat yogurts. Figure 4 shows a typical relative particle size distribution. The small particles that appear to dominate the high-fat yogurts may represent the many small fat droplets present, whereas the larger particles in medium- and low-fat yogurts may be protein aggregates. The many small fat particles in the high-fat samples would have a large surface area and may act as flavor reservoirs as described earlier. This would have significant effects on flavor release and may be partially responsible for the differences observed.

Viscosity. Viscosity was found to vary according to fat content (1.54 Pa·s for low fat, 2.29 Pa·s for medium fat, and 4.06 Pa·s for high fat). Panelists perceived the yogurt with the highest fat content to have the highest viscosity. Yogurt texture may have important effects on the release of volatiles from yogurt and may be especially important for persistence. A highly viscous yogurt

will be more difficult to remove from the oral cavity by mouth and tongue movements and may therefore be present in the oral cavity for a longer time, resulting in persistence. A viscous texture may also reduce the "spread" of the sample in the mouth. This would reduce the overall surface area of the sample and could be partially responsible for the lower I_{max} values observed. Future experiments could be directed at altering the texture of yogurts using emulsifiers and gelling agents (pectin, gelatin) while maintaining a constant fat content. Certainly there are many low-fat yogurts currently on the market that are advertised as being "thick and creamy" and have been developed to mimic the textural properties of the high-fat product.

Conclusions. Measurements by API-MS showed that hydrophobic flavor compounds had a higher I_{max} and a lower T_{max} in the absence of fat, but the presence of medium fat (3.5%) had effects similar to high (10%) fat. Flavor release was more persistent in high-fat yogurts. Panelists were able to identify only the differences in I_{max} due to fat content for two of the compounds and differences in timing of release for *trans*-2-hexenyl acetate. This may be explained in terms of odor threshold. Differences in particle size and viscosity may also affect flavor release.

Fat content has a profound effect on flavor release, and the extent of this effect is determined to some extent by the physical chemistry of the compound concerned. Because different compounds are affected differently, the physical chemistry of flavor molecules should be considered when formulations designed to accommodate changes in fat content are created.

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