Aroma Comparisons of Traditional and Mild Yogurts: Headspace Gas Chromatography Quantification of Volatiles and Origin of α-Diketones

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A quick headspace GC method for quantification of volatiles was developed, involving only minor sample preparation. Yogurt flavor compounds could be quantified in the micrograms per kilogram to milligrams per kilogram range without any difficulty, despite the complex matrix. Volatiles of traditional acidic and mild, less acidic yogurts were compared, and important differences were found for acetaldehyde, 2,3-butanedione, and 2,3-pentanedione. Concentrations of 2,3-butanedione and 2,3-pentanedione increased 2−3-fold in mild, less acidic yogurts compared to traditional acidic ones. This is due to accumulation of the precursors of the diketones, 2-acetolactate and 2-acetohydroxybutyrate, during fermentation in mild, less acidic yogurt. These precursors are subsequently converted to the corresponding diketones during storage. On the contrary, acetaldehyde formation was reduced in the mild yogurt, due to growth differences between the lactic acid bacteria used for fermentation of the milk. The quantitative results presented in this study validate previous GC sniffing conclusions (Ott et al. *J. Agric. Food Chem.* **1997**, *45*, 850−858), showing that yogurt aroma is the superposition of impact flavor compounds generated by fermentation on milk compounds.

Keywords: *Quantification; yogurt; headspace GC; aroma; volatiles; milk; fermentation; acetaldehyde; 2,3-butanedione; 2,3-pentanedione; 2-acetolactate; 2-acetohydroxybutyrate*

INTRODUCTION

Yogurt is the product of milk fermented with homofermentative lactic acid bacteria such as *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. *L. bulgaricus* has been identified as being responsible for the production of aromatic compounds and for the sharp acidity of the final product. Consumers in western countries prefer mild, less acidic yogurts (Hunger, 1985; Kneifel, 1992; Eberhard et al., 1995). Therefore, lac minus mutants of *L. bulgaricus*, in which the β-galactosidase gene is inactive, were selected (Mollet and Delley, 1990). Yogurt prepared with these mutants is mild, less acidic, without postacidification during storage, but these products are less flavorful (Kneifel, 1992).

Yogurt flavor is due to a delicate equilibrium between volatiles already present in the milk and secondary metabolites synthesized by homofermentative lactic acid bacteria. In these bacteria, lactose is converted to lactic acid and small amounts of acetaldehyde, 2,3-butanedione, and 2,3-pentanedione, which belong to the impact flavor compounds of yogurt aroma. These compounds were quantitatively compared in milk fermented in the presence of wild-type *L. bulgaricus* (traditional acidic yogurt) or their lac minus (lac^-) mutants (mild, less acidic yogurt) using a quick headspace gas chromatography (GC) method.

The literature reports a number of methods to quantify volatiles in fermented milks. Static headspace (Ulberth, 1991) requires only a simple sample preparation, but its use is limited to very volatile compounds present in the milligrams per kilogram range. Steam distillation dilutes the volatiles, making reextraction necessary (Moio et al., 1993) and is, therefore, time intensive.

Steam distillation, as well as static headspace as it is used for yogurt analysis, involves a heating step that could degrade precursors of the diketones (2-acetolactate and 2-acetohydroxybutyrate) into 2,3-butanedione and 2,3-pentanedione and thus to an overestimation of these components (Jordan and Cogan, 1988; Veringa et al., 1984; Monnet et al., 1994). Fermented samples supposed to contain the precursors of the vicinal diketones need therefore a mild sampling technique.

Purge-and-trap headspace (P&T-HS) in combination with a standard addition method allowed the quantification of 32 compounds in fermented milk (Imhof and Bosset, 1994a). However, this method requires very strict experimental P&T conditions to ensure reproducible recoveries (Chaintreau, 1999). Other methods describe quantification of specific compounds, for example, vicinal diketones, either by steam distillation and subsequent derivatization (Walsh and Cogan, 1974; Jordan and Cogan, 1988) or by derivatization and subsequent HPLC or GC/MS analysis (Martineau et al., 1994; McCarthy, 1995). Detection limits for these methods are normally very low, but procedures are tedious and quantify only some specific compounds.

We previously developed a headspace cell and a method to quantify the vapor phase compounds (Chaintreau et al., 1995). This technique appeared to be accurate and did not require the use of standards for the calibration of the GC response. The method requires prior determination of air-to-liquid partition coefficients of each compound and is applicable only to ideal solutions. Therefore, we propose hereafter a simplified

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Table 1. Properties of the *S. thermophilus* and *L. delbrueckii* Subsp. *bulgaricus* Strains Used in This Study

bacterial strain		properties	abbrev
S. thermophilus	YS4, YS7	Lac $^+$, nonropy, slow acidifier	slow St
	YS33	Lac $^+$, nonropy, fast acidifier	fast St
L. bulgaricus	YL30	Lac $^+$, slightly ropy, fast acidifier	Lac$^+$ Lb
	LB52	Lac $^-$ mutant of YL30	
	LFi5	Lac $^+$, ropy, fast acidifier	
	LFi31	Lac $^-$ mutant of LFi5	
mixed cultures	YL30 + YS4, YS7		mix lac$^+$
	LB52 + YS4, YS7		mix lac$^-$

and quicker method based on calibration by standard addition. The headspace cell mentioned above was used to sample the gas phase.

In this work, limitations of the HS quantification procedure were first determined, and then the method was applied to the quantification of flavor compounds in traditional acidic and mild, less acidic yogurts.

EXPERIMENTAL PROCEDURES

Chemicals. Acetaldehyde, acetone, 2-butanone, dimethyl sulfide, ethanol, methanol, and 2,3-pentanedione were from Merck (Les Acacias, Switzerland); benzaldehyde, 2,3-butanedione, cyclohexanone, dimethyl disulfide, and DL-lactic acid (solution at 90%) were from Fluka (Buchs, Switzerland); and benzothiazole (>95%), 2-heptanone (98%), 3,4-hexanedione (95%), methyl 2-hydroxy-2-methyl-3-oxobutyrate (98%), 2-pentanone, and 3-penten-2-one (90%) were from Aldrich (Buchs, Switzerland). They were of analytical grade if not otherwise stated.

2-Acetolactate was synthesized from methyl-2-hydroxy-2-methyl-3-oxobutyrate according to Krampitz's method (Krampitz, 1948). The compound was stored at −20 °C prior to use. At room temperature, it slowly degrades to 2,3-butanedione. To calculate the proportion of this degradation during S&T-HS (procedure 1), the following method was applied: 2-Acetolactate (3.3 mg) was dissolved in 100 mL of either acidified milk or phosphate buffer (0.1 M, pH 7.5) and incubated for 60 and 90 min at 30 °C. The amount of degraded 2-acetolactate was calculated as the quantity of 2,3-butanedione formed after a given incubation time. The kinetics was fitted to the equation $x = x_0\ e^{-\alpha t}$ (x = concentration of 2-acetolactate, x_0 = initial concentration, α = reaction rate) using "Table Curve-2D" (Jansen Scientific, Erkrath, Germany).

Preparation of Fermented or Acidified Milk. All microorganisms were from the Nestlé culture collection (NCC): *Streptococcus thermophilus*, YS4 (NCC 2284), YS7 (NCC 2302), and YS33 (NCC 2442); *Lactobacillus delbrueckii* subsp. *bulgaricus*, YL30 (NCC 576), LB52 (NCC 499), LFi5 (NCC 556), and LFi31 (NCC 15) (Table 1).

Cow's milk (UHT, 3.9% fat) was inoculated either with a single strain or with mixed strains. In the latter case the two *S. thermophilus* strains YS4 and YS7 (inoculum 0.5% each) were always used in combination with one strain of *L. bulgaricus* (inoculum 2%): either YL30 or LFi5 to produce traditional acidic yogurt; and their lac minus mutants LB52 or LFi31, respectively, to produce mild, less acidic yogurts. Single strains were fermented at 41 °C for 8−9 h, whereas mixed strains were fermented at 41 °C for 4 h to reach similar final pH values. All samples were stored in the dark, in hermetically closed 150 mL glass jars at 4 °C for 1 day and 1, 2, and 4 weeks before analysis.

For preparation of acidified milk, 10 g of skimmed milk powder (Berneralpen Milchgesellschaft, Konolfingen, Switzerland) was dissolved in 100 mL of distilled water. One gram of DL-lactic acid (Fluka) was added under vigorous stirring (final pH 4.25).

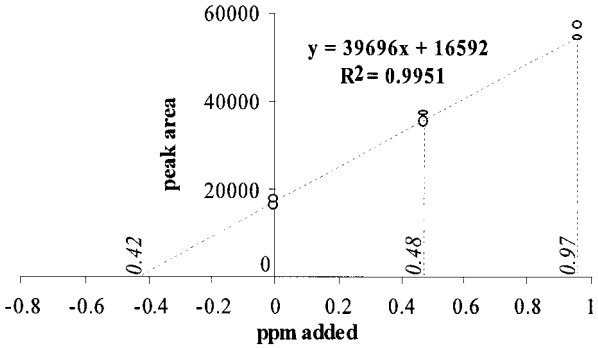

Figure 1. Calibration curve of dimethyl disulfide by standard addition method and extrapolation to a peak area of zero (procedure 1).

D and L-Lactate Quantification. D-lactate and L-lactate were determined at 340 nm by an end-point enzymic method, which is based upon the oxidation of lactate to pyruvate with concomitant transformation of NAD+ to NADH by D- or L-specific LDH, in 100 mM Tris-HCl pH 8, 2 mM EDTA, 3% hydrazine, and 1 mM NAD+.

Preparation of Standard Solutions. The compounds were weighed into a 1000 mL flask containing 100 mL of methanol as cosolvent. Compounds were added with a volumetric syringe (Hamilton AG, Bonaduz, Switzerland), which was accurately weighed before and after delivery. The syringe, as well as acetaldehyde and dimethyl sulfide, was cooled to 4 °C before use. Dimethyl sulfide was diluted in distilled water (Fontavapor 260, Büchi AG, Flawil, Switzerland) by a factor of 100 prior to use. After addition of all compounds, the volume of the solution was adjusted to 1000 mL with distilled water. Standard compounds were added at concentrations similar to those of target compounds (Imhof and Bosset, 1994a). Final concentrations of the different compounds were as follows: acetaldehyde, 450 mg/L; dimethyl sulfide, 2.5 mg/L; acetone, 110 mg/L; 2-butanone, 30 mg/L; ethanol, 100 mg/L; 2-pentanone, 30 mg/L; 2,3-butanedione, 150 mg/L; 2,3-pentanedione, 40 mg/L; 2-heptanone, 40 mg/L; benzaldehyde, 50 mg/L; benzothiazole, 300 mg/L. All standard solutions were prepared daily.

Quantification Procedure. *Quantification of 2-Acetolactate and 2-Acetohydroxybutyrate.* Both compounds are nonvolatile and were, therefore, first transformed into their corresponding vicinal diketones according to the modified method of Postel and Meier (1981). Fifty grams of yogurt was incubated in a 150 mL glass jar for 1 h in a water bath at 80 °C under strong magnetic stirring. Contents of 2-acetolactate and 2-acetohydroxy butyrate were calculated from the differences in vicinal dione content before and after heating, that is, before and after degradation of the precursors into their corresponding diketones.

Sample Preparation (Prior to Quantification Procedures 1 and 2). To 100 g of fermented milk was added 0, 1, or 2 mL of the standard solution containing all analytes, and volumes were corrected by addition of 2, 1, or 0 mL of distilled water, respectively. Samples were homogenized by vigorous shaking for 1 min. All samples were stored for 3 h at 4 °C prior to analysis.

Static and Trapped Headspace GC (Procedure 1). An aliquot of 25 g of fermented milk (if necessary, spiked with standards) was introduced into the sample space of the headspace cell. Volatiles of the vapor phase in equilibrium with the sample were collected on Tenax traps and injected into the GC column using a thermal desorber according to a reported procedure [procedure 1c of Ott et al. (1997)] using the same column and elution conditions.

Analytical procedure 1 was tested with four model compounds that do not (or only in trace amounts) occur in fermented milk: dimethyl disulfide, 3,4-hexanedione, 3-penten-2-one, and cyclohexanone. Standard solutions were prepared as described above and added to a fermented milk sample (YS4/YS7, incubation for fermentation: 0.5% of each starter,

Table 2. Amount of Standard Compounds Added to a Fermented Milk Sample and Standard Deviation, Coefficient of Variation (CV), and Recovery for Triplicates ($n = 3$)

compound	amount added (mg/kg)	amount found[a,b] (mg/kg)	lower limit[c] (mg/kg)	upper limit[c] (mg/kg)	SD (mg/kg)	CV (%)	recovery (%)
dimethyl disulfide	0.48	0.42[a]	0.32[a]	0.51[a]	0.01	2.38	87.50
3,4-hexanedione	0.83	0.91[b]	0.78[b]	1.04[b]	0.05	5.49	109.64
3-penten-2-one	0.79	0.90[a]	0.77[a]	1.03[a]	0.04	4.44	113.92
cyclohexanone	0.72	0.76[a]	0.63[a]	0.89[a]	0.04	5.26	105.56

[a,b] Average of eight[b] or nine[a] measurements. [c] Upper and lower limits for each compound were calculated as the intercept of the 95% confidence curves of the regression line with the abscissa.

8.5 h of fermentation at 41 °C) to give a final concentration of 0.4−0.9 mg/kg of each compound in the sample (Table 2).

Concentration of the different compounds was determined by extrapolating the straight line formed by the three concentration points to a *y*-value of zero (Figure 1).

Quantification by Static Headspace GC (Procedure 2). Acetaldehyde and ethanol were quantified by static headspace using a Carlo Erba GC 8130-MFC800 coupled to an automatic headspace sampler Carlo Erba HS 800 (both CE Instruments, Milano, Italy). An aliquot of 5 g of sample was weighed into a 10 mL headspace vial and capped with a Teflon seal (both Brechbühler SA, Plan-les-Ouates, Switzerland). The sample was equilibrated at 80 °C for 15 min. Headspace (1 mL) was injected onto the GC column using a split ratio of 20. The syringe temperature was kept at 80 °C. Volatiles were separated on a DB-Wax column (J&W Scientific, Folsom, CA), 30 m length, 0.32 mm i.d., 0.5 μm phase thickness. Helium was used as carrier gas at 2 mL/min. Septum purge was set at 1 mL/min. The column was kept at 50 °C for 6 min, and then the temperature was increased to 80 °C at 10 °C/min and kept during 2 min at this temperature. The column outlet was connected to an FID detector set at 200 °C.

After each sampling, the syringe was cleaned at 80 °C for 10 min. Chromatograms were integrated using Turbochrom software (Perkin-Elmer, San Jose, CA).

RESULTS AND DISCUSSION

Validation of Quantification. As a verification step, the method was tested with a model mixture of compounds that were found to be absent or present in only trace amounts in our samples and which possess structures and volatilities similar to those of the target compounds (Imhof and Bosset, 1994a). Four compounds fitting these requirements were selected and added in known concentrations to the fermented milk sample. Afterward, their quantities were determined in triplicate according to procedure 1. Calibration curves were calculated by least-squares regression from these nine measurements. All four model compounds produced standard curves with high coefficients of determination ($R^2 \geq 0.995$). Extrapolation of the calibration straight line to a zero area of the target peak gave the concentration of the added compound originally present in the fermented milk (Figure 1). Coefficients of variation of the concentrations were calculated to be between 2.4 and 5.5%. When this standard addition procedure was applied to determine the concentration in fermented milk containing a known added amount of a target volatile, all values fell within the limits of the 95% confidence interval (lower and upper limits of Table 2) calculated from all nine points. Taking into account the complexity of the matrix, these satisfying results show the applicability of this quick method to the quantification of real yogurt volatiles.

Quantification of acetaldehyde and ethanol using Tenax traps was not satisfactory. These compounds produced poor standard curves because of their insufficient retention on Tenax (Maier and Fieber, 1988).

Table 3. Degradation of 2-Acetolactic Acid during 30 min at 30 °C in the HS Cell

medium	pH	rate (h^{-1})	R^2	degradation (%)
acidified milk	4.25	0.052	0.996	2.4
aqueous phosphate buffer	7.5	0.045	0.980	2.7

Therefore, quantification of acetaldehyde and ethanol was performed by static headspace using a modified method after Wilkins et al. (1986).

Production of Yogurt Impact Flavor Compounds and Evolution of Their Concentration during Storage. Milk was fermented with different lactic acid bacteria as single or mixed strains according to Table 1. The two types of mixed strain fermentations gave traditional acidic yogurt (lac^+) and mild, less acidic yogurt (lac^-).

The different impact flavor compounds were then quantified as described above, 1 day postfermentation and after 1, 2, and 4 weeks of storage at 4 °C.

Acetaldehyde, one of the impact flavor compounds of yogurt (Ott et al., 1997), is produced during milk fermentation by *L. bulgaricus* as single strain (lac^+ Lb) and also in mixed cultures with its *S. thermophilus* partners (mix lac^+). At 1 day postfermentation, ∼15 mg/kg was measured (Figure 2). The amount of acetaldehyde reached only 5 mg/kg in mild, less acidic yogurt fermented with mixed cultures in the presence of *L. bulgaricus* lac minus (mix lac^-). Slow and fast strains of *S. thermophilus* also produced ∼5 mg/kg of acetaldehyde at 1 day postfermentation (Figure 2).

Production of acetaldehyde is related to the growth potential of the lactic acid bacteria used for fermentation of the milk. During storage the concentration of acetaldehyde decreased in the presence of lactobacilli, whereas it increased with a fast strain of *S. thermophilus*.

L. bulgaricus, a rapid acidifier, produced the largest amount of acetaldehyde, whereas its lac minus mutant, in a mixed milk fermentation, did not contribute to the production of acetaldehyde. The lac minus mutants of *L. bulgaricus* do not grow in mixed cultures in milk. Their metabolic activity, measured by the production of D-lactate (not produced by *S. thermophilus*), corresponds to $\sim 1/50$ of the wild-type activity. This low metabolic activity does not seem to be sufficient to contribute significantly to the production of acetaldehyde.

During storage the amount of acetaldehyde decreased to <10 mg/kg in milk fermented in the presence of *L. bulgaricus* (Figure 2). During that time the amount of ethanol in the yogurt stayed ∼2−3 mg/kg (data not shown), which was the amount found in milk. Therefore, alcohol dehydrogenase does not seem to be involved in the reduction of acetaldehyde concentration. However, with a fast-growing *S. thermophilus* that postacidifies, like *L. bulgaricus*, an increase in acetaldehyde concentration upon storage was observed (Figure 2). The

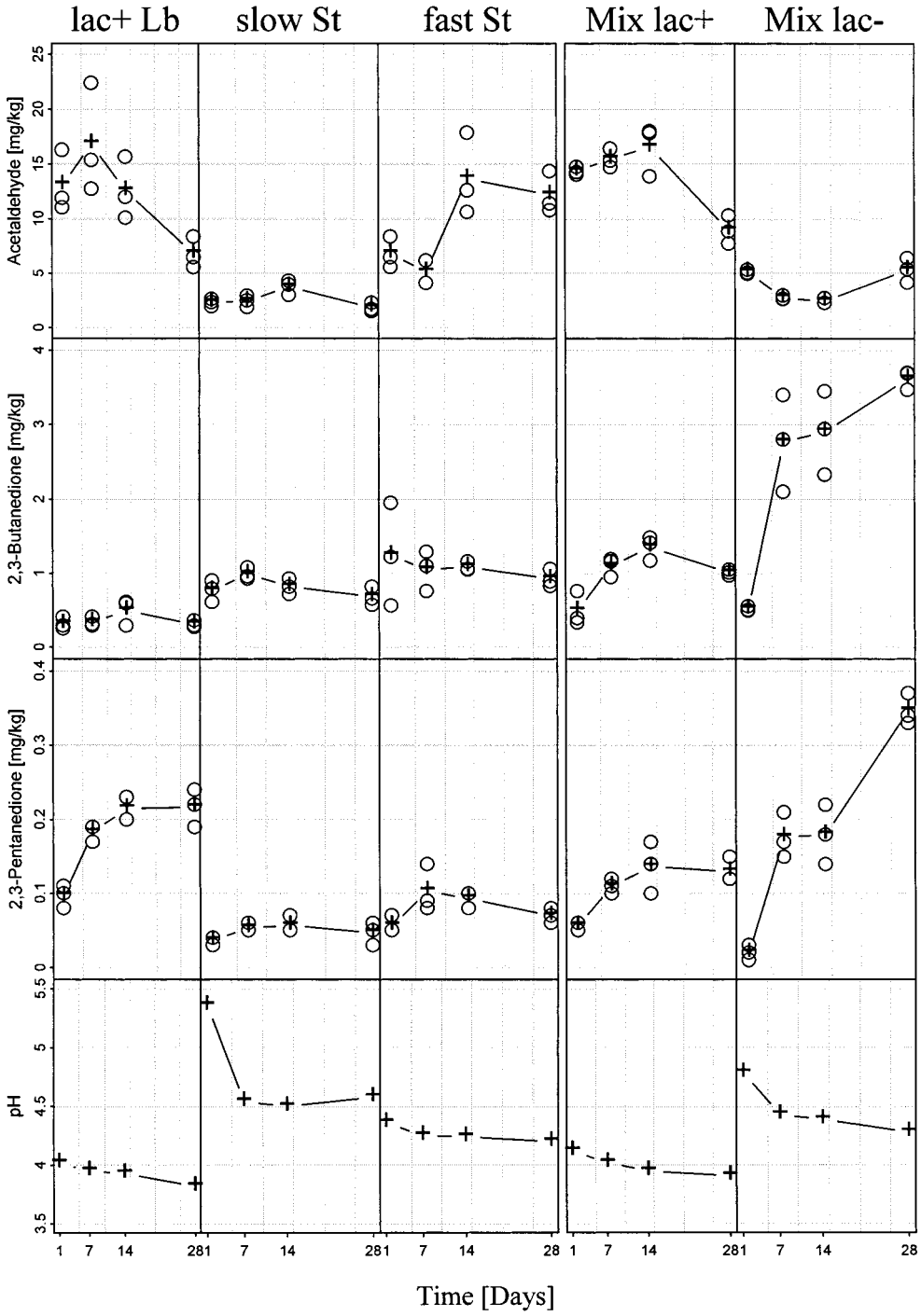

Figure 2. Evolution of pH and concentration of yogurt impact flavor compounds during storage at 4 °C. Fermentation was done with single and mixed strains according to Table 1. All measurements were done in triplicate (○), and the mean was determined (+).

metabolism of acetaldehyde by lactic acid bacteria will be published separately (Ott et al., 1999b).

2,3-Butanedione and 2,3-pentanedione are also impact flavor compounds of yogurt. Around 1 and 0.1 mg/kg were produced, respectively, during fermentation by the lactic acid bacteria used in this study (Figure 2). During storage at 4 °C the concentration of both diketones increased slightly in yogurts fermented with mixed cultures in the presence of the wild type of *L. bulgaricus* (mix lac$^+$) and increased almost 10 times in the presence of its lac minus mutant (mix lac$^-$, Figure 2). These results are in agreement with those of Imhof and Bosset (1994b), except that they observed a decrease of diketone concentration in yogurt fermented with the wild type of *L. bulgaricus*.

Similar results were obtained with another strain of *L. bulgaricus* (LFi5) and its lac minus mutant (LFi31) for all three impact flavor compounds. These compounds were not detected in milk.

Precursors of Diketones. Apparently, the increase in concentration of the diketones could be attributed to the basal metabolic activity of lactic acid bacteria at 4 °C (Figure 2). However, during fermentation, diketones are known to be produced by chemical decarboxylation of their precursors, 2-acetolactate and 2-acetohydroxybutyrate (Ramos et al., 1994) (Figure 3). Those two

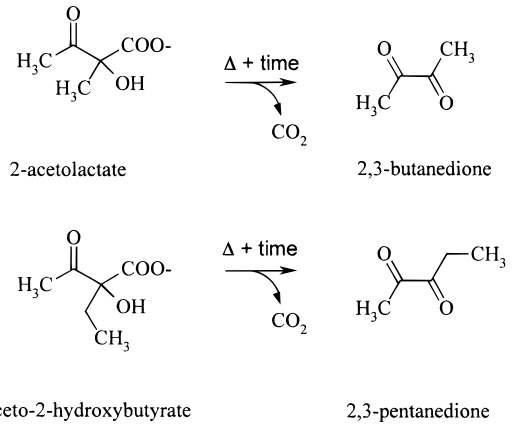

Figure 3. Oxidative decarboxylation of 2-acetolactate and 2-acetohydroxybutyrate.

Table 4. Concentration of the Precursors 2-Acetolactate and 2-Acetohydroxybutyrate and Their Corresponding Diketones, 2,3-Butanedione and 2,3-Pentanedione, in Yogurt Fermented with Mixed Strains According to Table 1 after Storage for 1 and 28 Days at 4 °C

	mix lac$^+$		mix lac$^-$	
compound	1 day (mg/kg)	28 days (mg/kg)	1 day (mg/kg)	28 days (mg/kg)
2-acetolactate	0.10 [0.07][a]	nd[b]	1.92 [1.25]	nd
2,3-butanedione	0.76	1.01	1.58	3.14
2-acetohydroxybutyrate	0.06 [0.04]	nd	0.17 [0.12]	nd
2,3-pentanedione	0.06	0.13	0.09	0.17

[a] Numbers in brackets are potential amounts of diketones formed from the corresponding precursors taking into account the loss of CO_2. [b] nd, not detected.

compounds are heat unstable and are converted to their corresponding diketones in the presence of oxygen. Monnet et al. (1994) found that during incubation at 85 °C for 40 min, using a static headspace method at pH 4, 100% of 2-acetolactate was converted to 2,3-butanedione and acetoin. Therefore, the concentration of 2,3-butanedione is often overestimated in dairy products (Veringa et al., 1984; Monnet et al., 1994; Richelieu et al., 1997). As 2-acetohydroxybutyrate has a homologous structure (Figure 3), a similar degradation can be assumed.

Contrary to most of the published yogurt analyses, which involve a heating step for sufficient vaporization of the analytes, the present method uses equilibration of the headspace with the matrix close to room temperature. Monnet et al. (1994) reported that higher temperatures and acidic pH values increase the decarboxylation rate of 2-acetolactate into 2,3-butanedione, according to a first-order kinetics. To take into account this reaction during S&T-HS (procedure 1, 30 °C; 30 min), its rate was evaluated by quantifying known quantities of 2-acetolactate added to acidified milk (pH 4.25) or to buffered water (pH 7.5). Reaction rates were found to be 0.052 and 0.045 h^{-1}, respectively. These values agree well with Monnet's observations. Consequently, 2-acetolactate degradation during S&T-HS sampling was ignored as it accounted for <3% of the initial concentration (Table 3).

The precursors of both diketones accumulate in larger amounts in yogurt prepared with the lac minus mutant of *L. bulgaricus* (LB52) than with the wild type (YL30) (Table 4). Accumulation of 2-acetolactate has been reported for *Lactococcus lactis* and *Leuconostoc mesentoroides* (Stadhouders, 1974; Jönsson and Petterson, 1977; Veringa et al., 1984; Monnet et al., 1994). After 28 days at 4 °C, the precursors were entirely converted to their corresponding diketones. The final concentration of the latter after storage accounts for the total of the diketones and their precursors after fermentation (Table 4). This observation implies that negligible amounts of precursors are synthesized during the storage period.

A detailed investigation of the metabolic pathway in the strain, leading to the vicinal diketone formation, will be published elsewhere (Ott et al., 1999c).

Comparison of Volatile Compounds Found in Milk and Fermented Samples. We have already shown that some impact flavor compounds of yogurt are already present in cow's milk and that others are synthesized during fermentation (Ott et al., 1997). The results described in this paper suggest that mainly six odorants appear during fermentation or are present in considerably increased amounts in yogurt compared to milk. The present quantification of acetaldehyde, 2,3-butanedione, and 2,3-pentanedione confirms results of the GC olfactometry (Table 5). Methional, (2*E*)-nonenal, and 2-methyltetrahydrothiophene-3-one could not be quantified, as the amount was below the detection limit of the FID.

From milk to yogurt, SNIF values (areas of GC olfactometry peaks) of benzothiazole increased while that of dimethyl sulfide decreased, by less than the least significant difference (Ott et al., 1997). It was necessary

Table 5. Comparison of Absolute Amounts of Different Volatiles and Impact Flavor Compounds in Milk and Yogurt (Mix lac$^+$) with SNIF Values

	milk			yogurt			
compound	this work[a] (mg/kg)	SNIF[l]	lit. (mg/kg)	this work (mg/kg)	mix lac$^+$ [b] (mg/kg)	SNIF[l]	lit. (mg/kg)
acetaldehyde	nd[c]	0	0.013−0.016[d]	1.8−16.8	16.57	7006	0.7−15.9[e]
acetone	2.7	nd	38.89,[f] 0.8−1.2[e]	2.3−4.2	4.03	nd	0.3−1.6[e]
dimethyl sulfide	0.027	5269	0.025[g]	0.013−0.048	0.022	3969	0.003,[h] 0.044−0.070[i]
ethanol	1.3	nd	0.77[f]	1.2−5.1	1.95	nd	1.4−4.1[e]
2-butanone	0.20	nd	0.08[g]	0.11−0.69	0.31	nd	0−7,[j] 0.085−0.106[i]
2-pentanone	0.060	nd	0.003,[g] 0.007−0.026[d]	0.024−0.066	0.054	nd	0.011[h]
2,3-butanedione	nd	3069	0.006[g]	0.31−3.62	1.35	8561	0−3,[j] 1.0−17.3[e]
2,3-pentanedione	nd	nd	0.063[g]	0.02−0.27	0.13	3549	1.5−4.5[i]
2-heptanone	0.14	nd	0.003,[g] 0.01−0.1[k]	0.08−0.16	0.13	nd	0.009−0.028,[i] 0.005[h]
benzaldehyde	0.030	nd	0.032[g]	0.027−0.128		nd	0.040−0.067[i]
benzothiazole	0.38	1779	0.001−0.010[k]	0.13−1.10	0.47	3486	

[a] Milk fortified with 2.5% skimmed milk powder and heat-treated at 98 °C for 15 min. [b] Same yogurt with indicated strains used for determination of key aroma compound by sniffing. [c] nd, not detected. [d] Wong and Patton, 1962. [e] Hild, 1979. [f] Imhof et al., 1995. [g] Imhof and Bosset, 1994a. [h] Badings et al., 1985. [j] Imhof et al., 1994b. [j] Gyosheva, 1982. [k] Badings, 1991. [l] Ott et al., 1997.

to quantify the compounds to confirm these trends (Table 5). These findings support the ability of the GC SNIF method of performing quantitative aromagram comparisons: differences of SNIF values of a given odorant between two samples correspond to differences of its concentrations (Pollien et al., 1997).

Concentration results reported in this work compare well with those found in the literature (Table 5). Only benzothiazole was significantly higher here than in the published data. This could, however, be because the milk was heat-treated before fermentation or because milk powder was added. Levels of benzothiazole reported in the literature for UHT milk were 10 times higher than in pasteurized milk (Badings, 1991), indicating an influence of heat treatment on its formation.

CONCLUSIONS

This quick S&T headspace method was found to be reproducible and accurate for quantification of volatiles present at milligrams per kilogram down to micrograms per kilogram levels in dairy products. It uses a simple procedure of standard addition. Very volatile compounds, such as dimethyl sulfide, 2-pentanone, and benzaldehyde could be quantified at micrograms per kilogram levels. As S&T-HS does not comprise heating of the samples, thermal degradation was negligible, especially for 2-acetolactate.

In mild yogurts produced with β-galactosidase negative strains of *L. bulgaricus*, 2−3 times less acetaldehyde was found compared to the traditional acidic products obtained with wild-type strains. The contents of 2,3-butanedione and 2,3-pentanedione increased in the mild products during storage. They reached ∼2−3 times the level found in the acidic products. This resulted from accumulation of 2-acetolactate and 2-acetohydroxybutyrate during fermentation and their subsequent decarboxylation during storage. Differences in these three key compounds of yogurt aroma might be responsible for flavor differences found for these two classes of yogurts (Ott et al., 1999a).

These quantitative results confirm the validity of the results obtained by the GC SNIF method. This technique provides quantitative comparisons of olfactometric peaks based on detection frequencies by a panel (Pollien et al., 1997). This work also confirms that yogurt aroma is the superposition of milk odorants on those produced by fermentation (Ott et al., 1997).

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