

Determination and Origin of the Aroma Impact Compounds of Yogurt Flavor

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Yogurt exhibits a delicate and low intense flavor which requires mild sample isolation techniques and sensitive identification means. This paper describes the application of two new flavor separation techniques: combined static and dynamic headspace, and preparative simultaneous distillation–extraction under vacuum. Of the 91 components identified by mass spectrometry, 21 were found to have a major impact on the aroma using a new method for the treatment of GC–sniffing data. One of these compounds, 1-nonen-3-one, was identified by spectral means for the first time in a flavor. Its odor detection threshold of 8 pg/kg appears to be one of the lowest among known flavor compounds. On the basis of the peak areas of yogurt and milk aromagrams, some compounds seem to be already present in milk, others appear to be generated by the strains.

Keywords: *Yogurt aroma; headspace; GC–olfactometry; impact-compound; composition*

INTRODUCTION

Since the 1950s, yogurt volatiles have given rise to many publications (for review, see Maarse, 1994), and more than 60 flavor compounds have been identified so far. Its consumption has become very popular since the 1960s due to the delicate flavor associated with its particular texture and the claimed health benefits of lactic acid bacteria for humans.

Headspace and simultaneous distillation–extraction (SDE) have been commonly used for the analysis of its flavor. Because of the low intensity of yogurt odor, the sample is often heated to increase the volatility of the flavorings (Görner et al., 1968; Hild, 1979; Ulberth, 1991; Kang et al., 1988). This can alter the composition of this sensitive aroma, and a mild sampling technique must be applied. Using a purge-and-trap sampling at a lower temperature, Laye and Imhof respectively identified 23 and 33 compounds (Laye et al., 1993; Imhof and Bosset, 1994; Imhof et al., 1994a,b). Among 19 papers listed in the TNO compilation, none has tried to determine the key volatiles responsible for the typical yogurt aroma. Recently, Imhof suggested 2,3-butanedione, 2,3-pentanedione, dimethyl sulfide, and benzaldehyde to be the most potent ingredients (Imhof et al., 1995). However, this selection was indirect, as it was based on odor unit values deduced from the author's quantitations and from literature thresholds.

Hyphenated gas chromatography–olfactometric techniques allow the direct determination of impact odorants in foods. Most commonly applied methods are Charm analysis (Acree et al., 1984) and aroma extract dilution analysis (AEDA) (Ullrich and Grosch, 1987). Both methods are based on GC–olfactometry of an aroma extract which is diluted until no odor is detected at the sniffing port. More recently Grosch's group sampled the headspace surrounding the food: its composition better represents the smell that is perceived by the consumer (Semmelroch and Grosch, 1995).

Since the vapor phase odor is first perceived when opening a yogurt pot, its quality will influence the consumer's preference. Because the retronasal percep-

tion is also sensitive to the headspace odor released by the yogurt, we decided to focus on the headspace and to determine the main odor contributors by direct measurement of their individual impact.

As critical evaluation of dilution sniffing methods pointed to reproducibility problems (Abbott et al., 1993), we recently developed another approach. Only one dilution level was used, but GC–olfactometry was repeated several times. Aromagrams of individuals were then "averaged" to get rid of previously mentioned reproducibility problems. Resulting aromagrams allow odor profile comparisons as peak intensities are related to the frequencies of odor detection: "NIF" and "SNIF" (respectively "Nasal Impact Frequency" and "Surface of Nasal Impact Frequency; Ott et al., 1996). A detailed paper will report this headspace/GC/sniffing method (Pollien et al., in press).

MATERIALS AND METHODS

Yogurt Preparation. Pasteurized full fat milk from a local retailer (Orlait SA, Lausanne, Switzerland) was fortified with 2.5% of skimmed milk powder and heat treated at 98 °C for 15 min, immediately cooled to about 35 °C in a water bath and inoculated with a starter culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. The prepared solution was filled in sterile pots of 150 mL, hermetically sealed, and fermented at 41 °C during 5 h (until pH 4.2 was reached). Immediately after termination of fermentation the yogurt samples were cooled to 4 °C and stored during 2 weeks at this temperature prior to analysis.

Headspace Sampling. *Dynamic Headspace/GC/MS (Procedure 1a, Used for MS Identification).* A 10 g yogurt sample was dispersed into 5 mL of a saturated salt solution and filled into the sparger of a purge-and-trap system (Tekmar Co., Cincinnati, OH). Samples were purged with ultrapure nitrogen at a flow rate of 40–60 mL/min during a time period varying from 5 to 99 min. Volatiles were thermally desorbed from Tenax (180 °C, 4 min), cryofocused, and injected into an HP 5995 GC/MS (Hewlett–Packard, Avondale, PA) equipped with a FFAP (Supelco Inc., Bellefonte, PA) column, 30 m length, 0.25 mm i.d., 0.25 µm phase thickness. Helium was used as carrier gas at a 0.65 mL/min flow. The column was kept at 20 °C for 1.0 min, increased at a 4 °C/min rate from 20 to 200 °C, and maintained for 1 min at 200 °C. The mass spectra were acquired with a source temperature of 220 °C, under a 70 eV ionization potential. Mass spectra and retention indices of the compounds detected were compared with those in our user-generated libraries.

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Linear retention indices were determined (Van den Dool and Kratz, 1963) after injection of a *n*-alkane series (C5–C17), under the same conditions.

Headspace Traps/GC/MS (Procedure 1b, Used for MS Identification). 100 g of yogurt and 16 g of distilled water were poured into a 3-neck vessel of 500 mL volume, thermostatted to 30 °C, and rapidly stirred with a magnetic bar. Volatiles were purged with an ultrapure helium flow of 30 mL/min and trapped on Tenax tubes for 60 min. Volatiles were thermally desorbed from Tenax (250 °C, 5 min), using a TD-2 thermal desorber (Scientific Instruments Services Inc., Ringoes, NJ) at 250 °C. They were cryofocused and injected into an HP 5890 GC (Hewlett-Packard) equipped with a DB-Wax column (J&W Scientific, Folsom, CA), (30 m length, 0.25 mm i.d., 0.25 μ m phase thickness). Helium was used as carrier gas at a 0.65 mL/min flow. The column was kept at 20 °C for 5 min, increased at a 4 °C/min rate to 200 °C, and maintained for 10 min. The column was directly coupled with the EI source of a HP 5971 mass spectrometer operating in the same conditions as mentioned above, in scan or in SIM mode.

Criteria for identification of compounds were the same as previously mentioned.

Headspace Cell/GC/Sniffing or FID (Procedure 1c; Chaintreau et al., 1995). This procedure was used for all sniffing experiments and to test the repeatability of the headspace/GC method. An aliquot of 25 g of yogurt was placed into the sample space of the headspace cell and equilibrated during 2 h at 30 °C in a water bath. The headspace of the cell (160 mL) was then passed through the trap containing 250 mg of Tenax with a flow of 40 mL/min.

Volatiles were thermally desorbed from Tenax (300 °C, 15 min), using an ATD400 thermal desorber (Perkin-Elmer Corp., Norwalk, CT). They were refocused on a internal cold Tenax trap (–30 °C) and desorbed at 300 °C for 3 min into an HP 5890 GC (Hewlett-Packard) equipped with a DB-Wax column (J&W Scientific) (60 m length, 0.53 mm i.d., 1.00 μ m phase thickness). Helium was used as carrier gas at a 10.6 mL/min flow. The column was kept at 20 °C for 5 min, increased at a 4 °C/min rate to 200 °C, and maintained for 10 min. The column outlet was connected either to a FID or to a sniffing port (Brechtbühler SA, Plan-les-Ouates, Switzerland) equipped with a humidified air make-up.

After each sampling, the cell was cleaned in a vacuum oven at 50 °C under 100 mbar for at least 1 h. Tenax sampling tubes were cleaned before use by heating for 1 h to 300 °C under a helium flow (50 mL/min).

Vacuum Transfer (Procedure 2; Bemelmans, 1979). 100 g of lyophilized yogurt was extracted in a Soxhlet apparatus during 30 h with 700 mL of freshly distilled diethyl ether. The extract was separated from fat by high-vacuum transfer under 10^{–3} mbar and trapped at liquid nitrogen temperature. Condensates were concentrated to 3 mL on a Vigreux column. The concentrate was extracted 3 times with 1 mL of a 0.5 M Na₂CO₃ solution and washed 3 times with 1 mL of saturated NaCl solution. The ether extract was dried over Na₂SO₄ and concentrated with a microdistillation device (Bemelmans) to 100 mL. The aqueous fractions were acidified with 1 N HCl and extracted 3 times with 10 mL of distilled diethyl ether. The ethereal extract was washed with 3 \times 10 mL of saturated NaCl solution and dried over Na₂SO₄. The extract was concentrated to 100 μ L with a microdistillation device.

Vacuum SDE (Procedure 3 for MS Identification of the Most Diluted Impact Components). Simultaneous distillation extraction under vacuum was used as this technique dramatically reduces the formation of thermal artefacts (Maignal et al., 1992). 2 kg of yogurt was dispersed into 2 L of previously degassed water and saturated with salt. The dispersion was SDE extracted during 7 h in a preparative apparatus under 40 mbar, using continuous sample feeding according to a reported procedure (Chaintreau et al., 1995). Freshly distilled butyl ethyl ether (5 mL) (Fluka AG, Buchs Switzerland) was used as the solvent. Operating temperatures were 39, 15, and 9.5 °C, in the sample flask, in the sample reservoir, and in the solvent flask, respectively. The resulting organic extract (ca. 1 mL) was directly used for GC–MS without prior concentration.

Gas Chromatography–Tandem Mass Spectrometry (GC/MS/MS). Mass spectra were acquired with a Finnigan MAT TSQ-700 mass spectrometer (San Jose, CA) interfaced with an HP-5890 gas chromatograph. The column was a DB-Wax column (J&W Scientific), 30 m length, 0.32 mm i.d., 0.25 μ m phase thickness. Helium was used as carrier gas at a pressure of 10 psi. The column was kept at 60 °C for 1 min, increased at a 15 °C/min rate to 240 °C, and maintained for 2 min. Positive or negative chemical ionization with ammonia as reagent gas were used to generate [M + NH₄]⁺ ions or [M – H][–] ions, respectively, analysed by collision-induced dissociation experiments. A collision energy of 6 eV in the laboratory frame was used, with argon as collision gas set to 1 mTorr.

Sniffing Procedure (Ott, 1996). Eight people experienced in GC–olfactometry were selected. Sniffing of the chromatogram was divided into two parts of about 25 min. Each person participated in the sniffing of both parts of the chromatogram, but during two distinct sessions to be alert.

Eluting aroma relevant flavourings were recorded by pressing a button during the whole sensory impression. The square signal was recorded by an HP Pascal workstation. In addition, odor descriptors were registered on tape for every peak.

The eight individual aromagrams of a given sample were summed to one chromatogram with home-made software, yielding the averaged aromagram. Linear retention indices were calculated as mentioned. Compounds identified by GC/MS were reinjected into the GC/sniffing system to check their retention indices. Their odor quality was compared when necessary.

Synthesis of 1-Nonen-3-one. 1-Nonen-3-one was obtained by oxidation of the corresponding alcohol by pyridinium chlorochromate (Corey and Suggs, 1975) and purified by preparative HPLC. MS and ¹H and ¹³C NMR spectra were in agreement with published data (Nakahira et al., 1992).

Odor Threshold. The nasal perception threshold of 1-nonen-3-one was measured using a triangle presentation. A series of three samples in weighing jars of 65 mL volume containing 35 mL of liquid was presented to a group of 21 trained panelists. One sample contained the stimulus dissolved in mineral water (Vittel), the other two samples were mineral water only. For each session, five different groups with increasing concentrations of the stimulus were presented and the assessors had to indicate which sample was different from the two others. All trials were performed at room temperature (21 °C).

The glass vessels were washed before use with a 2% aqueous solution of RBS (Fluka AG) then rinsed with deionized and afterward with distilled water. All glass material was stored in a vacuum oven at 50 °C and 100 mbar at least overnight prior to use.

The equation expressing the probit as a function of the concentration logarithm was adjusted to the experimental data using the curve-fitting software "TableCurve 2D" for Windows (Jandel Scientific, Erkrath, Germany).

DISCUSSION

Headspace Sampling. Adsorption of volatiles on the glass wall of a syringe has been shown to occur (Buttery et al., 1969). Therefore we preferred the vapor phase sampling, which has a proven reliability. We recently developed a new headspace cell for the determination of partition coefficients (Chaintreau et al., 1995). Its repeatability was established with model mixtures, and the system exhibited good accuracy when applied to quantitation in the vapor phase. This technique mimics well the aroma release conditions into a yogurt pot since the flavor is allowed to equilibrate between the matrix and the headspace at a controlled temperature, under atmospheric pressure. The air surrounding the yogurt is then collected without disturbing the partition of flavorings between both phases.

Table 1. GC/MS Identification of Yogurt Flavorings and Repeatability of GC Area

compounds	retention index			literature		GC area ^a	
	Tekmar ^b	vacuum transfer ^c	SDE ^d	yogurt	milk	area	rel. st. dev.
acetaldehyde	709			(e-g)	(e)	331354	5.8
dimethyl sulfide	757			(e-g)	(e)	1862	3.8
methylcyclohexane	756			—	—	—	—
propanal	800			—	(e)	—	—
2-propanone	802			(e-g)	(e)	123990	1
furan	802			—	—	—	—
methyl acetate	834			(e-g)	(e,i)	—	—
2-methylfuran	871			—	(e)	—	—
butanal	877			—	(e)	—	—
ethyl acetate	894			(e,g)	(e,i)	—	—
2-butanone	908			(e-g)	(e)	12264	1
methanol	916			—	(g)	8628	27.5
3-methylbutanal	919			(e)	(e)	—	—
dichloromethane	936			(h)	(e,h)	—	—
benzene	939			—	(e)	—	—
2-propanol	950			(f,g)	(e)	—	—
ethanol	956			(e-g)	(e,i)	10505	10.1
2-pentanone	988			(e,g)	(e,i)	2228	1.9
2,3-butanedione	993			(e-g)	(e,i)	15628	3.2
acetonitrile	1012			(h)	(h)	—	—
chloroform	1028			(h)	(e,h)	—	—
toluene	1044			(f,g)	(e,i)	—	—
2-butanol	1049			—	(e)	2092	11.1
S-methyl thioacetate	1057			—	—	—	—
1-propyl alcohol	1064			(g)	(g)	—	—
2,3-pentanedione	1076			(e-g)	—	1912	6.5
dimethyl disulfide	1081			(e-g)	(e)	—	—
butyl acetate	1086			—	(e)	—	—
hexanal	1094			(e,g)	(e,i)	—	—
2-hexanone	1094			(e)	(e)	—	—
2-methylthiophene	1102			—	(e)	—	—
2-methyl-1-propanyl alcohol	1121			—	(e)	—	—
ethylbenzene	1133			—	(e,i)	—	—
1,3-dimethylbenzene (1,4?)	1142			—	(i)	—	—
3-penten-2-one	1148			(e)	(i)	—	—
1,3-dimethylbenzene (1,3?)	1150			—	(i)	—	—
1-methylpyrrole	1155		1137	—	(e)	—	—
3-heptanone	1171			—	—	—	—
1-butanol	1179			(g)	(e)	—	—
1-ethyl-4-methylbenzene	1181			—	—	—	—
1-penten-3-ol	1192			—	—	—	—
limonene	1195			(f,g)	(g)	—	—
1,4-dimethylbenzene (1,2?)	1200			(f)	(i)	—	—
2-heptanone	1208		1182	(e-g)	(e,i)	2237	4.2
propylbenzene	1226			—	(i)	—	—
3-methyl-2-butenal	1230			—	—	—	—
2-pentylfuran	1252			(e)	—	—	—
pyrazine	1253			—	(e)	—	—
ethenylbenzene	1285			—	(e)	—	—
1-pentanol	1292			(e,g)	(e)	—	—
3-octanone	1300			—	—	—	—
2-methyl tetrahydrofuran-3-one	1304			—	—	—	—
trimethylbenzene or ethylmethylbenzene	1308			—	(i)	—	—
methylpyrazine	1312			—	(e)	—	—
octanal	1317			(g)	(e)	—	—
3-hydroxy-2-butanone	1333	1286		(e)	(e)	1950	18.1
1-methyl ethenylbenzene	1358			—	—	—	—
3-methyl-2-butenol	1362			—	—	—	—
2-nonanone			1389	(e,f)	(e,g)	—	—
2-butanol	1397			—	(e)	—	—
2-hydroxy-3-pentanone	1396			(e,f)	—	—	—
dimethyl trisulfide	1405			—	(e)	—	—
furfural	1495			(e)	(e,i)	—	—
acetic acid	1498	1442		(e)	(e,i)	4541	18.7
1H-pyrrole	1547			—	(e,i)	—	—
propionic acid		1536		(e)	(e)	—	—
benzaldehyde	1556			(e,g)	(e,i)	2809	19.5
2-methyltetrahydrothiophen-3-one	1561			—	—	—	—
2-methylpropanoic acid	1567			—	—	—	—
2-undecanone			1598	(e)	(e,i)	—	—
butyric acid		1623		(e)	(e)	—	—
2-furanmethanol		1659		(e)	(e,i)	—	—
3-methylbutanoic acid		1667		(e)	(e)	—	—
(phenylacetaldehyde) ^j				—	—	2605	6.8
pentanoic acid		1736		(e)	(e)	—	—

Table 1 (Continued)

compounds	retention index			literature		GC area ^a	
	Tekmar ^b	vacuum transfer ^c	SDE ^d	yogurt	milk	area	rel. st. dev.
2-dodecanone			1809	—	(e, i)	—	—
hexanoic acid		1845	1845	(e)	(e)	—	—
benzothiazole			1947	(e, f)	(e, i)	4407	1.2
heptanoic acid			1954	—	(j)	—	—
2-pentadecanone			2021	—	(j)	—	—
octanoic acid		2059	2060	(e)	(e, i)	—	—
nonanoic acid		2164	2164	—	(j)	—	—
decanoic acid		2271	2275	(e)	(e, i)	—	—
γ -dodecalactone			2368	—	(e, i)	—	—
δ -dodecalactone			2420	—	(e, i)	—	—
benzoic acid		2420	2436	(e)	(e)	—	—

^a Procedure 1c with an FID detector. ^b Tekmar purge and trap injection, retention indices on FFAP phase (sampling procedure 1a or b). ^c Direct solvent extraction and vacuum transfer, retention indices on DB-Wax phase (sampling procedure 2). ^d Vacuum SDE, retention indices on DB-Wax phase (sampling procedure 3). ^e Maarse, 1994. ^f Laye et al., 1993. ^g Imhof et al., 1995. ^h Imhof et al., 1994a,b. ⁱ Shiratsuchi et al., 1994. ^j No peak when injected using Tekmar but found using procedure 2 (ATD400/GC/FID).

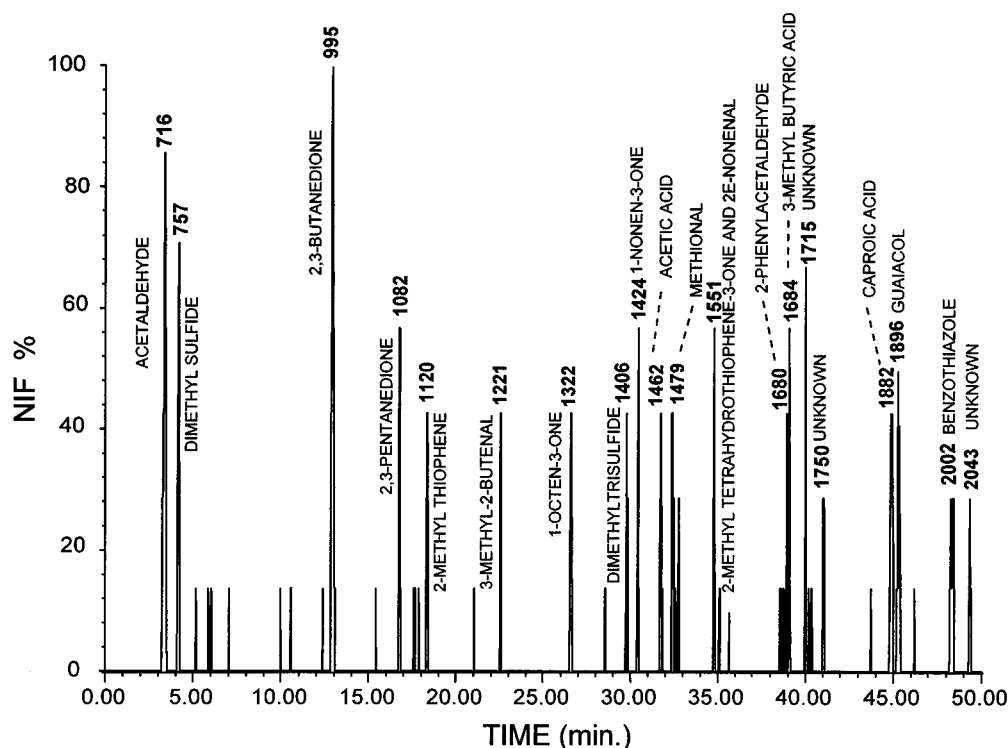


Figure 1. NIF profile of the yogurt aroma isolated according to procedure 1c (retention indices at the peak apex).

As the cell repeatability was tested with model mixtures in water (Chaintreau et al., 1995), it was evaluated again using yogurt flavorings themselves, according to procedure 1c, with FID detection. Starting from the same batch, five replications of the headspace trapping were performed. For 16 GC areas reported in Table 1 relative standard deviation was less than 10% for 10 peaks, and less than 20% for 15 peaks. Methanol area variations might be attributed to possible breakthrough due to its lower absorption in Tenax than other compounds (Maier and Fieber, 1988).

Further flavor generation by the strain during the analysis time probably did not occur because it would have given rise to a continuous increase or decrease of the peak abundances. As variations occurred randomly, other causes must be found (complex matrix, possible heterogeneity, etc.). However 10% or 20% was considered to be below variations recognized by the human nose with such a method (Pollien et al., in press).

Flavoring Identification. Yogurts were analyzed 2 weeks after their preparation since the flavor generation is known to mainly occur during the fermentation

step and it does not significantly change during the refrigerated storage at 4 °C (Imhof and Bosset, 1994). Flavorings were isolated from their matrix using three different mild sampling methods, described in Materials and Methods as procedures 1a, 2, and 3.

Table 1 summarizes results of the three isolations: most of the 86 compounds identified have already been mentioned in yogurt, milk, or skimmed milk powder, which are ingredients of the yogurt recipe. Among the newly identified compounds, some of them could contribute to the overall aroma: 1-penten-3-ol, 3-octanone, 2-methyl tetrahydrofuran-3-one, 3-methyl-2-butenol, 2-methyltetrahydrothiophene-3-one, and 2-methylpropanoic acid. Their contribution was determined by GC/sniffing.

Dichloromethane, chloroform, and acetonitrile were found: as no halogenated solvent nor acetonitrile was used in the sampling procedure 1 and since blank analyses using the Tekmar system did not exhibit any trace of these components, they seem to originate from milk itself (Maarse, 1994; Imhof and Bosset, 1994; Imhof et al., 1994a,b).

Table 2. Odor Descriptors of Yogurt Impact Flavorings (NIF > 12.5%)

ret. ind. ^a	ret. ind. (ref.)	descriptors	identification and descriptors proposed by the data-bank
716	710	fresh, green, pungent	acetaldehyde: fresh, fruity, pungent, ether
757	754	milk, lactone-like, sulfury, warm	dimethyl sulfide: sulfury, roasted
995	993	butter, diacetyl, vanilla	2,3-butanedione: buttery, fatty, oily, vanilla
1082	1082	butter, vanilla, mild	2,3-pentanedione: buttery, caramellic
1120	1109	gasoline, plastic, styrene	2-methylthiophene: gasoline, onion-like, paraffinic
1221	1222	metallic, aldehydic, herbaceous	3-methyl-2-butenal: aldehydic, caprylic, pungent
1322	1324	mushroom, earthy	1-octen-3-one: mushroom
1406	1403	sulfury, H ₂ S, fecal	dimethyl trisulfide: strong sulfury, cabbage, alliaceous
1424	1425	mushroom, earthy	no data
1462	1460	pungent, acidic, vinegar	acetic acid: sour, vinegar-like
1479	1479	soup, cooked vegetable, pungent, sulfury	methional: aldehydic, caprylic, cooked potato-like, green, sulfurous
1551	1557	green, leather, sulfury	(2E)-nonenal + 2-methyltetrahydrothiophen-3-one: fatty, green, pungent, leather
1680	1678	flowery	2-phenylacetaldehyde: floral, honey-like
1684	1688	sweaty, cheese, soy sauce, flowery	3-methylbutyric acid: acidic, cheese-like
1715	no data	flowery, warm, caramel	no data
1750	no data	metallic	no data
1882	1862–1885	rancid, flowery	caproic acid: acidic, caprylic, fatty
1896	1894	bacon, phenolic, smoked, spicy	guaiacol: aromatic, burnt, phenolic, spicy
2002	2000	burnt, rubbery	benzothiazole: roasted, rubbery
2043	no data	hydrocarbon, chemical, burnt rubber	no data

^a Retention index on DB-Wax phase, using headspace injection (procedure 1c).

Table 3. Compounds Identified Using Sampling Procedures or Identification Means Other Than Those of Table 1

compound	ret. index ^a	sampling ^b	mass spectrometric experiment	literature
1-octen-3-one	1322	proc. 3	GC/MS/MS, SIM in CI	none
1-nonen-3-one	1424	proc. 3	GC/MS/MS, SIM in CI	none
methional	1479	proc. 3	GC/MS, SIM in EI	unclear ^c
2-methyltetrahydrothiophen-3-one	1551	proc. 1b	GC/MS, full scan in EI	Schreier et al., 1976
(2E)-nonenal	1551	proc. 1c	ret. index on DB-Wax and SPB-1	Shiratsuchi et al., 1994
phenylacetaldehyde	1682	proc. 2 and 3	GC/MS-SIM in EI	Badings and Neeter, 1980
guaiacol	1896	proc. 3	GC/MS/MS, SIM in EI	none

^a Indices found by sniffing. ^b Procedure used for the sample preparation. ^c Bosset and Gauch, 1988.

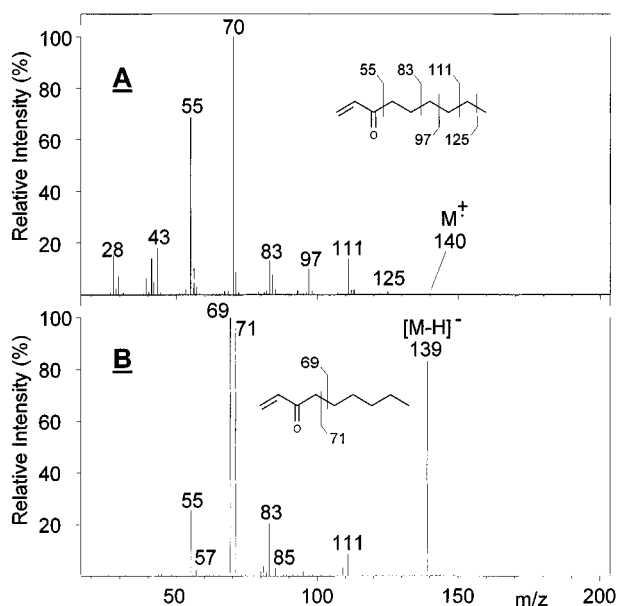


Figure 2. Mass spectra of 1-nonen-3-one. (A) EI ionization spectrum. (B) Negative CI spectrum.

Impact Flavorings. A SNIF profile of the yogurt flavor was established using the headspace sampling procedure 1c, from 8 sniffing runs as explained above. Each headspace sampling was performed with a new yogurt originating from the same fermentation batch.

Twenty main contributors to the overall aroma were detected (Figure 1 and Table 2). Among them, only 11 corresponded to volatiles that were identified using the three procedures mentioned in Table 1. On the basis of their GC and sensorial characteristics the identities

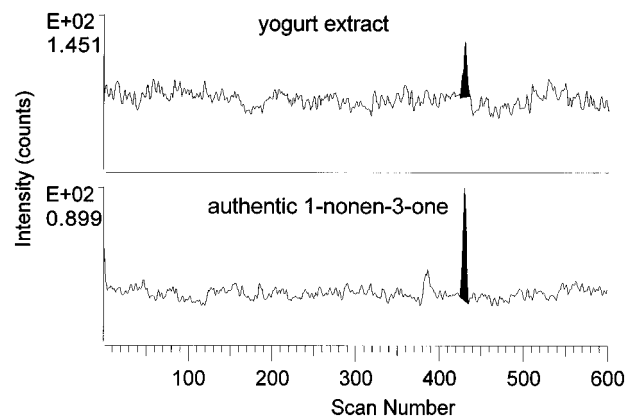


Figure 3. GC/MS/MS chromatograms of the daughter ion 69 obtained by SRM from the [M - H]⁻ ion at *m/z* 139: (lower trace) 50 pg of reference compound; (upper trace) 5 μL of yogurt extract.

of seven other compounds were proposed by comparing retention indices and odor descriptors to those recorded in our sensorial data-base. Their retention indices were checked by re-injecting authentic samples under the same conditions using a FID detector.

Identification proposals of the sensorial data base were confirmed by GC/MS and GC/MS/MS experiments (Table 3). Because of the low amount of each compound, full scan spectra could not be obtained by mass spectrometry in every case. Therefore, the compounds were selectively analyzed either by selected ion monitoring (SIM) of specific ions produced after electron impact or by collision-induced dissociation. Parent ions [M + NH₄]⁺ or [M - H]⁻ were generated after positive or negative chemical ionization, respectively. In this case,

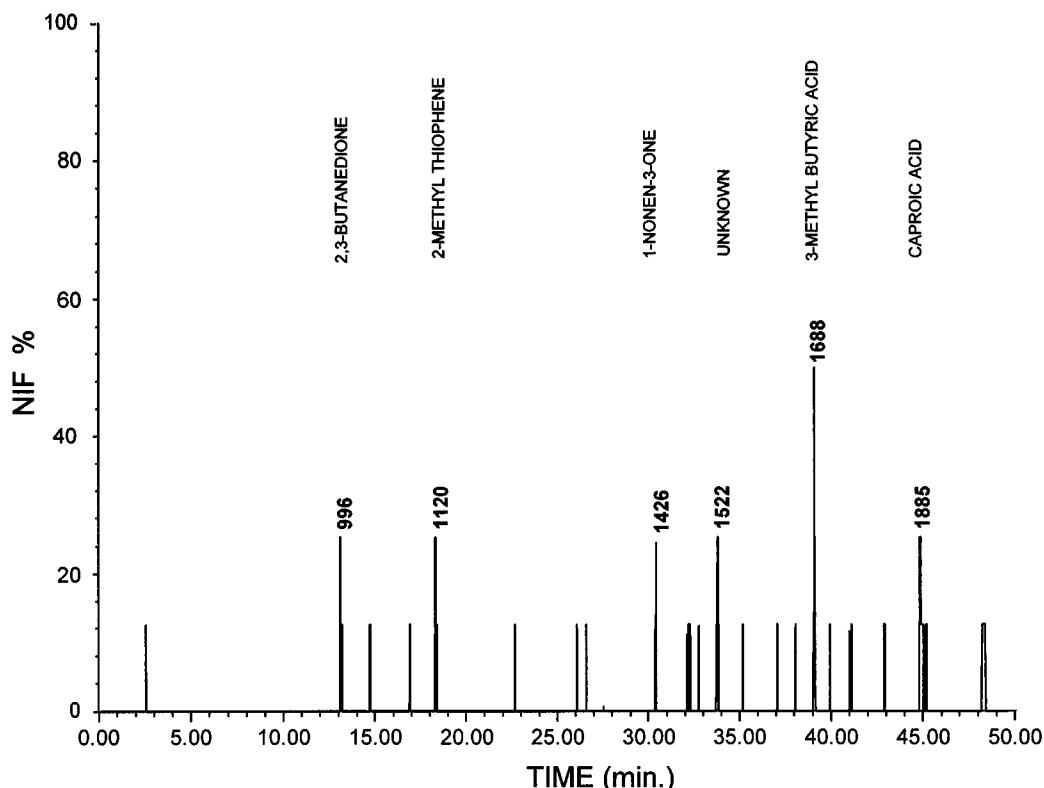


Figure 4. NIF profile of a blank experiment (headspace cell loaded with distilled water, procedure 1c).

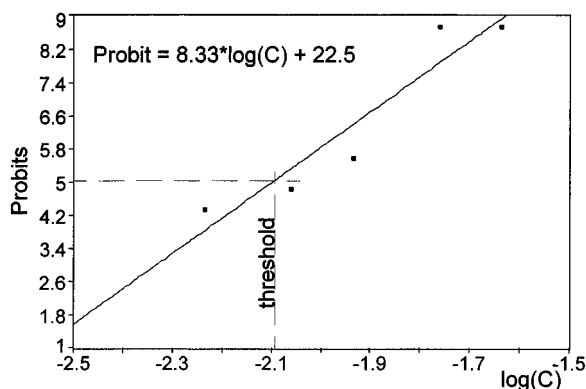


Figure 5. Determination of the 1-nonen-3-one threshold. C, concentration expressed in ng/kg.

the characteristic daughter ions obtained for each compound were recorded [selective reaction monitoring (SRM) experiment] if eluted from the GC column at the same retention time as the reference molecule.

As an example, Figure 2A presents the electron impact ionization mass spectrum of 1-nonen-3-one and Figure 2B its mass spectrum after collision-induced dissociation of the $[M - H]^-$ ion. 1-Nonen-3-one was looked for in the yogurt extract after monitoring the daughter ion at m/z 69 from its parent ion at m/z 139. The GC/MS/MS traces obtained for a yogurt extract and for a standard of the reference compound are presented in Figure 3.

Of the seven flavorings of Table 3, six of them [1-octen-3-one, 1-nonen-3-one, methional, 2-methyltetrahydrothiophen-3-one, (2*E*)-nonenal, and guaiacol] were detected for the first time in the yogurt flavor. They were probably missed in the previous works due to their low concentrations, although their low odor thresholds strongly influence the aroma profile. Three odor contributors remain unidentified (indices 1715, 1750, and 2043 of the aromagram).

A blank experiment was run by loading the headspace cell with distilled water instead of yogurt. Some peaks corresponding to those found in yogurt were detected, but with a lower intensity (Figure 4). As 3-methylbutyric acid and hexanoic acid were also found without using a Tenax trapping (sampling procedures 2 and 3), they do not correspond to artefacts. In addition, all peaks of the blank, except 1-nonen-3-one, have already been mentioned as occurring in milk and/or yogurt (Table 1). Consequently, odor contributors of Figure 1 originate from the yogurt flavor itself.

Special attention was paid to 1-nonen-3-one, as no spectral evidence was ever given for this compound in any flavor.

1-Nonen-3-one: a New Impact Flavoring (Patent Pending; Chaintreau et al., 1996). To our knowledge, 1-nonen-3-one has not been positively identified in any food flavor (Maarse et al., 1994), and this compound is not commercially available. The only unambiguous occurrence was mentioned in the odorants produced by the thermal oxidation of polyethylene (Bravo et al., 1992). Even the corresponding alcohol, 1-nonen-3-ol, seems to be a non-common aroma component: only three natural occurrences are reported: chervil, beef fat, and banana (Maarse et al., 1994).

A reference sample was then prepared to elucidate its sensorial and analytical properties. In addition to its mass spectra (Figure 2), its retention indices on different phases were determined (Table 4). According to quantity of the reference compound used in Figure 3, the 1-nonen-3-one concentration in yogurt seems to be in the 2–5 ppt range. As 1-nonen-3-one was also detected in the blank sniffing experiment, one could wonder whether the Tenax degradation could generate this compound like in the Bravo's experiment of polyethylene degradation. As the positive spectral identification was performed on the SDE extract (procedure 3), this hypothesis can be excluded.

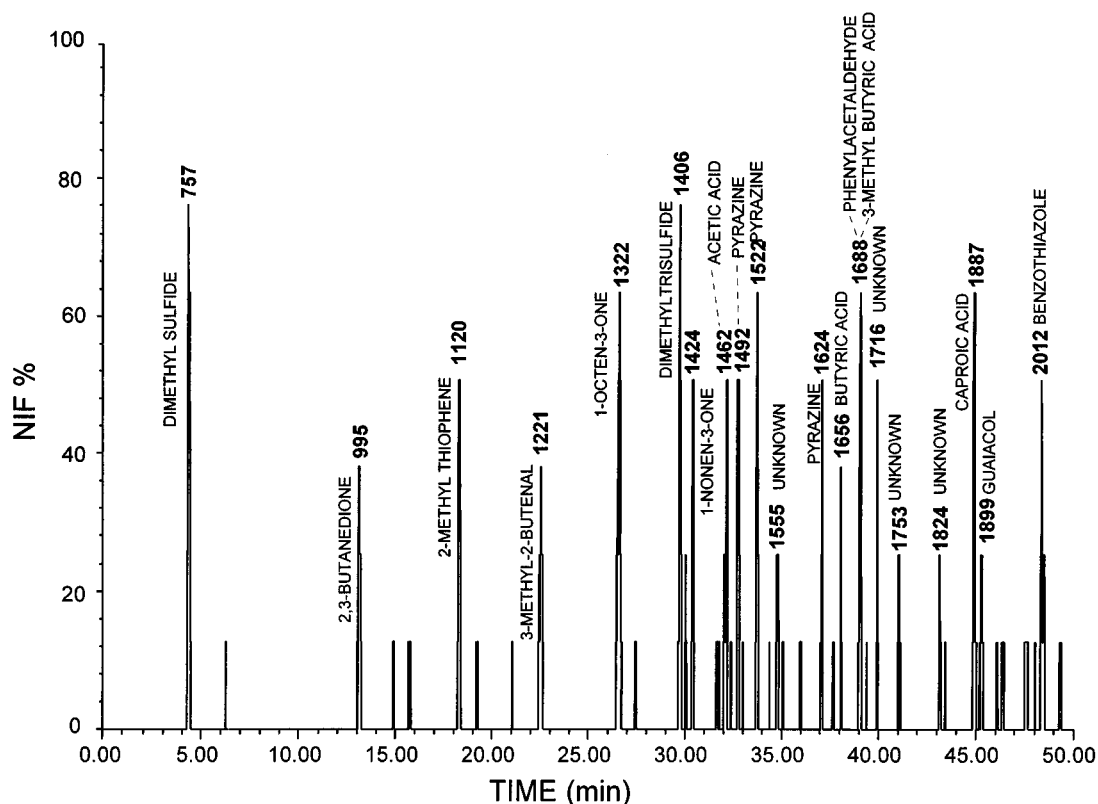


Figure 6. NIF profile of milk aroma (isolated according to procedure 1c).

Table 4. Retention Indices and Odor Description of 1-Nonen-3-one Occurring in Some Food Flavors

sample	DB-Wax	SE-54 DB-5	FFAP	OV-1701	SPB1	descriptor
1-nonen-3-one (authentic)	1400 ^a 1425 ^b	1080 ^a	1398 ^a	1171 ^a	1070 ^a 1065 ^b	mushroom, earthy
yogurt ^c	1424 ^b	—	—	—	1065 ^b	mushroom, earthy
raspberry ^c	1405	—	—	—	1052	mushroom
trout ^c	—	1081	—	—	—	mushroom, earthy
process flavor ^c	—	—	—	—	—	mushroom
toasted wheat bread ^c	1415	1077	—	—	—	mushroom
buttermilk off-flavor ^c	—	1076	—	—	—	mushroom and phenolic

^a 1-Nonen-3-one injected as solution. ^b 1-Nonen-3-one absorbed on Tenax and injected with the ATD400 system. ^c See bibliographic references in the text.

Table 5. Most Potent Flavors and Off-Flavors

compound	odor threshold (pg/kg)	reference
2,3,6-trichloroanisole	0.3	Ewender et al., 1995
2,3,6-tribromoanisole	8	Ewender et al., 1995
1-nonen-3-one	8	this work
2(5 <i>H</i>)-furanone, 5-ethyl, 3-hydroxy, 4-methyl	10	Kobayashi, 1989
2-isobutyl-3-methylpyrazine	20	Ewender et al., 1995

Odor Threshold. The threshold was determined by sniffing a series of triangle tests of an aqueous solution, and panelists answers were treated according a reported method (Voirol and Daget, 1986). In a forced-choice answer procedure some correct answers are due to chance. Results were therefore corrected using the following formula:

$$\% \text{ perception} = \% \text{ correct answers} - \frac{(1 - \% \text{ correct answers})}{2}$$

Perception percentages were transformed to probit values using the table established by Bliss (1967). Probit values were plotted against the logarithm of the

concentrations. The value for 50% perception (or for a probit value of 5) defined as perception threshold was calculated to be 8 pg/kg (Figure 5).

This extremely low threshold classifies 1-nonen-3-one among the most potent compounds identified in flavors and off-flavors up to now (Table 5). Consequently, its concentration must be very low in any flavor, which explains why its identification was not spectrally proved up to now.

Other Occurrences. In the literature, 1-nonen-3-one can be suspected: among the main odorants revealed by aroma extract dilution, Milo, Rychlik, and Heiler reported an unknown compound in boiled trout, in toasted wheat bread, and in buttermilk off-flavor, respectively, eluting exactly 100 retention index units after 1-octen-3-one (Milo and Grosch, 1993; Rychlik and Grosch, 1996; Heiler and Schieberle, 1996). In all cases, its odor description and its index (Table 4) fit well with our finding. More recently, Blank suspected its occurrence in a process flavor (no retention index), with lipid degradation products such as (2*E*)-nonenal (Blank et al., 1994). As trout, yogurt, and buttermilk are fat-containing foods as well, 1-nonen-3-one could also originate from unsaturated fatty acid oxidation. However, on the basis of its odor and retention indices, it was also

Table 6. Comparison of Odor Impact Contributions in Milk and Yogurt Using SNIF Values

ret. ind. ^a	compound	milk	yogurt
716	acetaldehyde	—	7006
757	dimethyl sulfide	5269	3969
995	2,3-butanedione	3069	8561
1082	2,3-pentanedione	0	3549
1120-1	2-methylthiophene	3487	2480
1220-1	3-methyl-2-butenal	2866	1804
1322	1-octene-3-one	5282	2357
1406	dimethyl trisulfide	3109	1804
1424	1-nonene-3-one	3114	1786
1462	acetic acid	3223	2051
1479	methional	0	2581
1492	pyrazine (isomer?)	2734	1007
1522	pyrazine (isomer?)	3421	0
1551	2-methyltetrahydrothiophene-3-one and (2E)nonenal	0	2410 (both peaks)
1555	unknown	1531	0
1624	pyrazine (isomer?)	1951	0
1680-8	2-phenylacetaldehyde	3199	1113 (both peaks)
1680-4	3-methylbutyric acid		2339
1715-6	unknown	1368	1344
1750-3	unknown	1017	2507
1824	unknown	1119	0
1882-7	hexanoic acid	4470	4048
1896-9	guaiacol	1315	3377
2002-12	benzothiazole	1779	3486
2043	unknown	0	1125

^a Retention index on DB-Wax phase, using headspace injection (procedure 1c).

detected in raspberry, a non-fat product (Roberts and Acree, 1996).

Origin of Yogurt Impact Flavorings. With the aim of identifying flavorings originating from the strain action, the aromagram of the milk mixture (skimmed milk powder and pasteurized full fat milk) was established (Figure 6). The milk amount was the same as used for the yogurt preparation. As frequencies of detection of a given peak (Pollien et al., in press) increased with concentration, yogurt and milk aromagrams were compared using SNIF values as the areas take into account possible minor variations of retention for each of the eight assessors. Many peaks were common to both aromas: impact flavorings already present in milk exhibited similar or lower SNIF values in yogurt (Table 6). On the other hand, SNIF values of several compounds [acetaldehyde, 2,3-butanedione, 2,3-pentanedione, methional, 2-methyltetrahydrothiophene-3-one, and (2E)-nonenal], were greatly increased by the fermentation step. Levels of dimethyl trisulfide, guaiacol, and benzothiazole could be also affected, and this should be verified by a quantitative analysis.

Pyrazines (isomers not determined) probably occurred in milk due to its thermal treatment, but they were not observed to contribute any more to the yogurt aroma.

Consequently, yogurt flavor appears to result from milk odor contributors and specific flavorings synthesized by the lactic acid bacteria as SNIF differences were equal or less than the least significant difference for this method (Pollien et al., in press).

CONCLUSION

Using the new SNIF approach for the data treatment of GC-olfactometric signals, the main contributors of the yogurt odor have been determined. Of the 91 volatiles identified in the flavor, 21 exhibit a key aroma impact. According to Imhof (1995), 2,3-butanedione,

2,3-pentanedione, and dimethyl sulfide are confirmed to be impact flavorings of yogurt. On the other hand, benzaldehyde does not seem to significantly participate to the overall aroma.

Five compounds [1-nonen-3-one, methional, 2-methyltetrahydrothiophen-3-one, (2E)-nonenal, and guaiacol] with intense odor were found for the first time in the yogurt flavor. This paper also reports the first spectral identification of 1-nonen-3-one in a flavor. This component appears to be one of the most potent flavorings which has been identified up to now. Its low odor threshold justifies its impact role in yogurt in spite of its low concentration.

This work also shows that the yogurt flavor results from the addition of aroma compounds originating from milk and of specific odor contributors generated by the metabolism of the lactic acid bacteria.

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