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Optimization of Headspace Solid-Phase Microextraction (SPME) for the Odor Analysis of Surface-Ripened Cheese

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Fifty volatile compounds of surface smear-ripened cheese were detected and identified using headspace solid-phase microextraction (HS-SPME) and vacuum distillation coupled to gas chromatography−mass spectrometry. Changes in the headspace of aroma compounds were monitored over the whole packaging period (47 days) using the HS-SPME method. Initially, the concentration of methanethiol increased before reaching a plateau. This evolution could be linked to the growth of *Brevibacterium linens*. During the shelf life of cheese, levels of acetic acid and 3-methylbutanoic acid remained constant, whereas butane-2,3-dione, 3-hydroxybutan-2-one, and hydroxypropan-2-one levels gradually declined and acetone and 3-methylbutanol levels dropped sharply to a plateau. Changes in odor could be related to changes of the rind, which behaved as a barrier, strongly influencing the distribution of volatile compounds in the headspace. Using a gas chromatography−olfactometry technique without separation, it was shown that the SPME extract was representative of the cheese odor.

KEYWORDS: Headspace; solid-phase microextraction; cheese; olfactometry; aroma compound

INTRODUCTION

The odor perception of cheese is one of the foremost criteria for its acceptance and preference by consumers. The typical odor of a food product results from a component balance (*1*) in a complex mixture of volatile compounds (acids, ketones, methyl ketones, alcohols, sulfur compounds, etc.) present in their correct proportions in the headspace around the cheese. However, during the shelf life, this balance changes and consequently the odor, too. To characterize the aroma of a cheese at a given time, the usual procedures consist of isolating volatile compounds by solvent extraction (*2*, *3*), vacuum distillation (*4*−*6*), or headspace analysis. The latter includes static (*7*) and dynamic headspace (*8*) techniques, as well as headspace solid-phase microextraction (HS-SPME), which involves concentration by adsorption on a fiber, a simple and sensitive technique (*9*). This technique has been applied to numerous determinations and identifications of aroma compounds in foods (*10*−*12*) and beverages. HS-SPME has been used to analyze highly volatile substances such as low molecular weight sulfur compounds in black and white truffles (*13*), beer (*14*), and wine (*15*). Moreover, when directly coupled (without chromatographic separation) with mass spectrometry, HS-SPME has been shown to be at least as powerful as electronic nose systems to characterize the odor (or the off-flavor) of cheeses (*16*) and milk samples (*17*).

In the present work, a smear-ripened cheese was studied, which has a weak but unpleasant odor. Because the ripening of this cheese takes place while it is already packaged, new packaging materials could limit this unpleasant odor by a selective absorption of volatile compounds (*18*). In the first step, this paper focuses on the development of a reliable HS-SPME method for monitoring the odor changes of cheese during shelf life. In a second step, the representativeness of the HS-SPME extract was evaluated by gas chromatography−olfactometry (GC-O).

MATERIALS AND METHODS

Cheese. Samples of three surface smear-ripened cheese (named A, B, and C) were either purchased from a supermarket (B and C) or obtained from the producer (A) and then stored at 4 °C, pending analysis. Cheeses B and C were used only in triangular tests (olfactometry) to evaluate the representativeness of the SPME extracts in cheese A. Cheeses A−C had shelf lives of ∼47 days.

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Chemicals. A mixture of medium-chain triglycerides (MCT), trioctanoic glyceride 55% (w/w) and tridecanoic glyceride 45% (w/w), was purchased from Stearinerie Dubois et Fils (Boulogne, France). 3-Methylbutanoic acid, dimethyl sulfide, and dimethyl trisulfide were purchased from Acros Organics (Noisy-Le-Grand, France). The other standard compounds were purchased from Sigma-Aldrich Chemical Co. (St Quentin Fallavier, France). The purity of compounds ranged from 98 to 99%.

Enumeration of *Brevibacterium linens* in the Smear of Cheese A over Packaging Time. Two agar media were employed: (i) mannitol salt agar (MSA, Difco) was added to fungizone (50 mg/L, Squibb) to inhibit yeast, and calcium carbonate was added to avoid acidification (*19*); (ii) brain heart infusion agar (BHI, Difco) was added to fungizone (50 mg/L) and nalidixic acid (40 mg/L) to inhibit the growth of Gram-negative bacteria.

At seven time points during the shelf life (47 days) of cheese A stored at 4 °C, two cheeses were analyzed simultaneously. At each time, 1 g of smear from the top face of both cheeses was scraped off with sterile materials and suspended in 10 g of a sterile 2% trisodium citrate solution. This suspension was homogenized with a blender (Waring blender type) for 2 min. Serial 10-fold dilutions of this suspension were plated on two different agar MSA and BHI media (use of spiral system apparatus, DS model, Interscience, St-Nom-la-Bretèche, France) for aerobic plate counts (27 °C, 72 h). The plates were then exposed to daylight at ambient temperature for 5 days, and orange-pigmented *B. linens* colonies, which were clearly differed from those of indigenous microflora present in cheese A, were counted separately. In addition, the identification of *B. linens* was confirmed by the color reaction of this microorganism with NaOH (5 M), which produces a characteristic carmine red color (*20*).

The other colonies underwent biochemical identification using the API Staps system (20500-Biomérieux, Marcy l'Etoile, France) and API 20 NE (20050- Biomérieux, Marcy l'Etoile, France).

Yeast populations were detected on yeast extract−glucose−chloramphenicol−agar and incubated at 30 °C for 2 days (*21*).

Vacuum Distillation. One hundred grams of cheese A was frozen at −20 °C, then ground while frozen, and placed in a 2 L round-bottom flask. Volatile components were distilled under vacuum (10^{-2} mbar, room temperature) for 2 h. The aqueous distillate was recovered from the traps (cooled with liquid nitrogen) situated between the sample and the vacuum pump and extracted three times for 20 min with 10 mL of dichloromethane. The extract was dried with sodium sulfate, which was then removed by filtration through glass wool, and then concentrated by distillation at 40 °C to 200 μl through a consecutive series of Kuderna-Danish columns.

Optimization of HS-SPME Procedures. Four types of fiber [poly(dimethylsiloxane) (PDMS) 100 μm, PDMS/divinylbenzene (PDMS/DVB) 65 μm, Carbowax/DVB (CW/DVB) 65 μm, and Carboxen/PDMS (CAR/PDMS) 75 μm] were tested (Supelco Co., Bellefonte, PA). A standard solution of pure volatile compounds (dimethyl sulfide, acetone, butanone, butane-2,3-diol, dimethyl disulfide, 3-methylbutanol, hydroxypropan-2-one, dimethyl trisulfide, and 3-methylbutanoic acid) was prepared by adding 200 μL of each compound to MCT (50 mL). After sonication in ice for 2 min, 100 μL of the standard solution was introduced into a gas sample cell (medium cell, 150 mL). Headspace equilibration was achieved after 1 h. Every hour, a fiber was inserted into the medium cell through a Mininert valve (Supelco) and then equilibrated with the headspace for 10 min at 25 °C prior to chromatographic analysis. All fibers were tested twice.

For all cheese experiments, the trapping procedure was carried out by inserting the SPME fiber (75 μm CAR/PDMS) into the analytical cell through a Mininert valve and then equilibrated with the headspace for 30 min at 25 °C.

Identification of the trapped volatile compounds was carried on $^1/_{32}$ of the whole cheese in bits following procedure 1 (**Table 1**). After accumulation of volatile compounds in the headspace for 40 min (in the small cell) the trapping procedure was started.

Distribution of Volatile Compounds between the Rind and Paste of Cheese A. Volatile compounds from the rind and paste of the cheese were compared after 30 days in packaging; 0.4 g of rind was scraped off, and 0.4 g of paste was removed from the central zone. Each sample was placed in a small cell (22 mL sample flask). After 1 h of equilibration, the trapping procedure was started, using both samples.

Table 1. Summary of HS-SPME Sample Preparation[a]

procedure	cell	sample	trapping time (CAR/PDMS fiber) (min)
1	small[b]	$^1/_{32}$ of cheese in pieces (11 g), rind and paste	30
2	large[c]	whole cheese (340 g)	30

[a] All experiment were carried out at 25 °C. [b] Small cell: 22 mL sample flask. [c] Large cell: 800 mL.

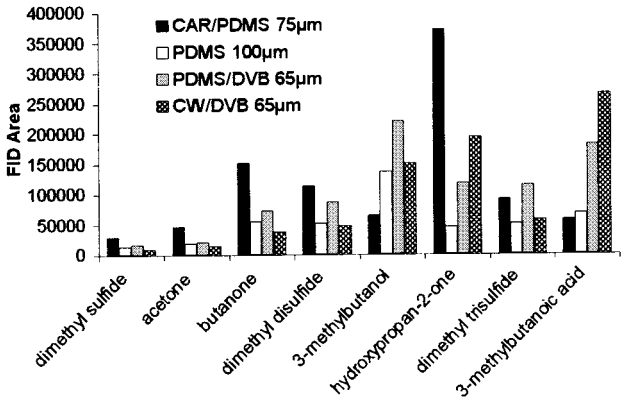

Figure 1. Extraction of the aroma compounds of cheese A (model solution, medium cell, 150 mL) by different SPME fibers.

Profile of Volatile Compounds in Cheese A during Its Shelf Life. Every hour, the trapping procedure was started and the profile of volatile compounds analyzed was followed according to procedure 2 (**Table 1**) to monitor the kinetics of appearance in the headspace during the equilibration time.

The profile of volatile compounds in the cheese was followed according to procedure 2 (**Table 1**) during 47 days. A solution (100 μL) of 4-heptanone (0.1 mL/L) in Milli-Q ultrapure water was added as an internal standard. After accumulation of volatile compounds in the headspace for 4 h, the trapping procedure was started. For longer accumulation times, the levels of some volatile compounds (sulfur compounds) decreased in the headspace (see **Figure 2** and corresponding discussion below). For each measurement during the shelf life (47 days), a new whole cheese of the same batch was tested.

During this evaluation, the pH of the cheese surface (top) and cheese paste (core) was determined five times per cheese using a surface electrode (Ingold, Steinbach, Germany).

GC-FID Analysis. Analysis of the different SPME extracts was performed using a Fisons (Les Ulis, France) gas chromatograph (GC 8000 Top) with a flame ionization detector. Volatile compounds were desorbed from the SPME fiber for 5 min into the split/splitless injector port, maintained at 260 °C. The injector port was in splitless mode, the split valve opening after 3 min. Separations were performed on a Supelcowax 10 fused-silica capillary column (0.32 mm × 30 m, 1 μm; Supelco). GC analysis of the SPME extracts was performed using a flame ionization detector, maintained at 260 °C. Hydrogen was used as the carrier gas (linear flow velocity = 40 cm/s). The column was kept at 35 °C for 3 min, and the temperature was increased at a rate of 3 °C/min to 52 °C, then by 7 °C/min to 240 °C, and maintained thus for 2 min. Signals were processed using data acquisition software (Borwin version 1.2, JMBS Developments, Le Fontanil, France) for quantification.

Analysis of Mass Spectra. Identification of the constituents was achieved using gas chromatography−mass spectrometry (GC-MS). The GC-MS system consisted of a Fisons GC-8000 chromatograph and an MD 800 mass spectrometer (Fisons Instruments). Separations were performed on an FFAP fused-silica capillary column (0.32 mm × 30 m; 1 μm; J&W Scientific Inc., Folsom, CA). The linear flow velocity of helium was 40 cm/s.

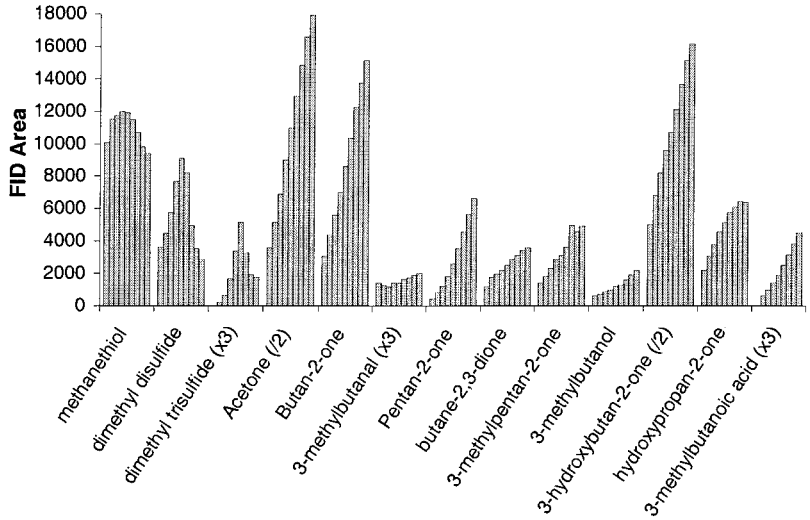

Figure 2. Variations in the amounts of each volatile compound in the headspace of a whole cheese placed into a large cell (procedure 2, **Table 1**) for 9 h.

For vacuum distillation, the dichloromethane extract (1 μL) was injected into an on-column injector (20 °C). The oven temperature was then programmed to rise from 35 °C (for 3 min) to 240 °C at a rate of 5 °C/min.

For HS-SPME analysis, the column was kept at 35 °C for 3 min, and then the temperature was increased at rate of a 3 °C/min to 52 °C, then by 7 °C/min to 240 °C, and maintained for 2 min at 240 °C. The thermal desorption of volatile compounds on the SPME fiber was carried out in the injector port (250 °C) in a splitless mode. The split valve was opened 3 min after injection. The SPME fiber was held in the injector port for 5 min.

Electron-impact mass spectra conditions were as follows: capillary direct interface, 250 °C; ion source, 200 °C; ionization voltage, 70 eV; mass range, *m*/*z* 29−450; electron multiplier voltage, 450 V; scan rate, 3 scans/s.

Mass spectral matches were made by comparisons with NIST and INRAMASS mass spectra libraries. Kovats indices compiled using the INRAMASS library were employed to confirm identification.

GC-O Analysis of HS-SPME Extracts. For all sensory tests, the cheeses were analyzed for 30 days after the date of packaging (average time of consumption). Separation of the aroma compounds was performed on a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, Les Ulis, France) equipped with a split/splitless injector (250 °C), a flame ionization detector (FID, 250 °C), and a homemade sniffing port. The compounds were separated into a capillary column (Supelcowax 10, 0.32 mm × 30 m, 1 μm; Supelco). The carrier gas was helium, at 1.8 mL/min. The column was kept at 35 °C for 3 min, and the temperature increased at a rate of 3 °C/min to 52 °C, then by 7 °C/min to 240 °C, and maintained for 2 min at 240 °C. At the end of the column, the helium flow rate was split into two equal parts, one directed toward the FID and the other to the sniffing port. The connection between the capillary column and the sniffing port was made up of deactivated silica tubing (60 cm long, heated at 200 °C, 0.32 mm diameter). A nonhumidified airflow (50 mL/min) was added concentrically to the chromatography effluent at the bottom of the sniffing port (*22*).

Representativeness of the Odor of Cheese HS-SPME Extracts. The panel for representativeness experiments consisted of two men and two women, who were already experienced in sniffing procedures.

The representativeness of the odor of cheese extracts was carried out following procedure 1 (**Table 1**). The trapping procedure was immediately started after sampling, with no accumulation period of volatile compounds in the headspace of the small cell.

Three different cheeses (cheeses A−C) were used to obtain three types of HS-SPME extract (A−C). The equipment was identical to that used for GC-O), but the capillary column was replaced by deactivated silica tubing (diameter = 0.32 μm, length = 10 cm). The SPME fiber containing the adsorbed volatile compounds was introduced into the injector at 250 °C for 1 min (splitless mode). Because the compounds were not separated, the panelists sniffed all of the compounds simultaneously.

Each panelist evaluated the odor of the three cheese extracts by comparison with a reference (cheese A). The reference, smelled before the injection of each extract, was presented on a plate, at ambient temperature. The subjects scored the odors from 0 to 10, 10 being allocated if the odor of the extract was identical to that of the reference. The three extracts were presented in a random sequence, and the test was repeated three times by each panelist.

Screening of Representative Extracts of Cheese A. The panel for screening experiments consisted of two men and two women, who were already experienced in sniffing procedures and allocating descriptors. Attempts at describing odor intensity were unsuccessful. The panelists were free to chose their own descriptors.

The screening session consisted in the analysis of representative SPME cheese A extracts. The compounds were desorbed from the fiber into the injector of the gas chromatograph (splitless mode) and immediately transferred into the GC column. Subjects were asked to describe, using any terms they chose, the odor detected at the end of the column. The panelist was changed every 15 min. This experiment was repeated during several sessions. In total, four cheese A extracts were analyzed.

RESULTS AND DISCUSSION

Volatile Compounds of Cheese A, Isolated by Vacuum Distillation and HS-SPME. Because the extraction procedure may have a strong influence on the chromatographic profile of the volatile compounds of cheese (*5*), identifications of compounds in the headspace of cheese were made by comparing two methods: HS-SPME (with the CAR/PDMS fiber, known for its excellent properties of extraction of highly volatile compounds) (*23*, *24*) and the usual vacuum distillation procedure. Among the 50 chromatographic compounds seen by both techniques, ketones, methyl ketones, alcohols, aldehydes, esters, sulfides, and acids, the most important peaks were identified (**Table 2**) on the basis of electron-impact mass spectra and Kovats indices, when available. The data shown in **Table 2** agree with the findings in the literature (*16*, *25*), and 25 peaks were common to both types of extract. Both methods were complementary: the HS-SPME method was better for determining the most volatile compounds, especially methanethiol, carbon disulfide, dimethyl sulfide, acetone, ethyl acetate, butan-2-one, 3-methylbutanal, and ethanol, which are important to odor

Table 2. Volatile Compounds of Cheese A Identified in HS-SPME and Vacuum Distillation Extracts

volatile compd[a]	flavor note	no.[b]	Kovats index (DB-Wax)[c]	analytical method[d]
methanethiol[a]	cooked cabbage	1		A
carbon disulfide				A
dimethyl sulfide	cabbage	2		A
acetone[e]	ethereal, fruity	3	814	A
ethyl acetate[c]	solvent, pineapple, fruity	4	893	A
butanone[e]	acetone, ethereal	5	888	A
3-methylbutanal	green, malty	6	912	A
ethanol[c]	alcohol, mild	7	924	A
ethyl propanoate	pineapple, sweet, solvent	8	955	A, B
pentan-2-one[b]	fruity, acetone, sweet	9	969	A, B
butane-2,3-dione[c]	buttery	10	955	A, B
4-methylpentan-2-one	fruity, etheral		1000	B
3-methylpentan-2-one[e]	fruity, etheral	11		A, B
trichloromethane				A, B
methyl thioacetate	cooked cauliflower			A
dimethyl disulfide[a]	cauliflower, garlic	12	1077	A, B
isoamyl acetate	pear, banana, apple, solvent	13	1128	A, B
heptan-2-one	blue cheese, spicy, Roquefort cheese		1160	A, B
3-methylbutanol[d]	fruity, alcohol, whiskey	14	1168	A, B
3-methyl-3-buten-1-ol	herbaceous			A, B
2-methylpyrazine	popcorn			B
3-hydroxybutan-2-one[c]	buttery	15	1259	A, B
hydroxypropan-2-one[d]	fruity, ethereal	16	1268	A, B
dimethyl trisulfide[a]	alliaceous, meaty, over-ripened cheese	17	1365	A
2-butoxyethanol			1371	B
2,3,5-trimethylpyrazine	roasted nuts, cocoa, baked potato		1387	B
acetic acid[d]	vinegar, pungent	18	1410	A, B
2-propylpentanol				B
propionic acid	vinegar, pungent		1525	A, B
2-methylpropionic acid[c]	sweet, apple-like, rancid butter	19	1535	A, B
butanoic acid	rancid cheesy, putrid, sweaty	20	1588	A, B
3-methylbutanoic acid[d]	rotten fruit, mild, sweaty	21	1630	A, B
dodecanal			1682	A
pentanoic acid	sweaty, rancid, waxy		1700	A, B
2-phenylethyl acetate	floral, rose		1778	B
hexanoic acid	pungent, blue cheese, sour		1795	A, B
benzyl alcohol	sweet		1837	A, B
2-phenylethanol	rose floral		1865	A, B
2-ethylhexanoic acid	rancid fatty			A, B
heptanoic acid	rancid fatty		1904	B
phenol			1949	B
octanoic acid	goaty, waxy, soapy, musty, rancid, fruity		2008	A, B
nonanoic acid	cheese, waxy			A, B
decanoic acid	rancid fatty			A, B
diethyl phthalate				B
diisobutyl phthalate				B
acid				B
acid				B
acid				B
acid				B

[a] Superscripts indicate the following: a, present in rind only; b, predominantly in rind; c, present in paste only; d, predominantly in paste; e, equal distribution between rind and paste. [b] Numbering refers to **Figure 6**. [c] Retention indices on DB-Wax. [d] Analytical method: (A) SPME; (B) vacuum distillation.

perception. Using vacuum distillation, these peaks were not seen, either because of overlap with a solvent peak or because of losses during grinding or distillation; dimethyl disulfide was the only sulfur compound identified. Vacuum distillation favored the extraction of semivolatile compounds, mainly higher molecular weight acids. Therefore, we selected the HS-SPME method for further work on odor analysis.

Optimization of Headspace Extraction Conditions. Selection of the most appropriate SPME fiber depends on the compounds targeted and therefore on the food under study. During this work, a fleeting odor had to be defined, which required a fiber with high sensitivity for small volatile molecules, mainly sulfur compounds. Three other fibers were compared with the CAR/PDMS 75 μm fiber: PDMS 100 μm, PDMS/DVB 65 μm, and CW/DVB 65 μm, using a model solution of eight important odor compounds present in cheese A (**Table 2**) and representative of various chemical classes. The CAR/PDMS fiber (already used to compare SPME and vacuum distillation) produced the best results (**Figure 1**), with a very strong affinity to hydroxypropan-2-one. This fiber was selected for all subsequent work. **Figure 2** shows the evolution of the headspace of a whole cheese (A) over a period of 9 h at 25 °C in a large analytical cell (procedure 2, **Table 1**). Published procedures employ higher equilibration temperatures between cheese and headspace, up to 60 °C (*12*). However, in this case, the temperature was selected to maintain the physical integrity of the cheese, the paste of which melts at 30 °C.

The residence time of the fiber in the cell was optimized. After 30 min, the methanethiol peak was stabilized, while other compounds were still increasing. A residence time of 30 min was chosen as a good compromise between experimental duration and sensitivity.

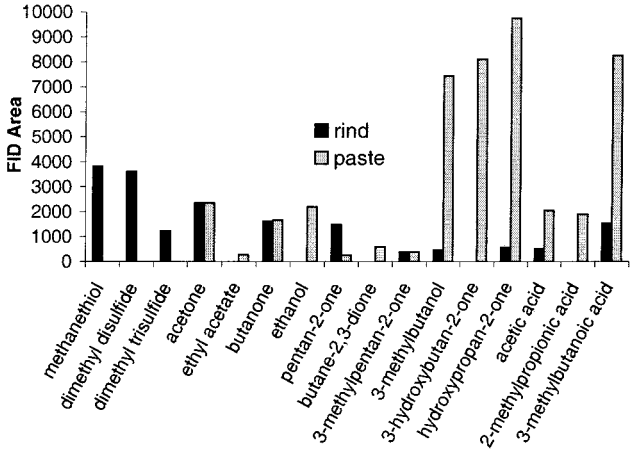

Figure 3. Distribution of each volatile compound between the rind and paste of cheese A.

Different desorption behaviors of volatile compounds from cheese A were obtained, depending on the volatility and the polarity of volatile compounds. Only 3-methylbutanal, 3-methylpentanone, butane-2,3-dione, and hydroxypropan-2-one reached equilibrium within 7−8 h. Acetone, butanone, pentan-2-one, 3-methylbutanol, 3-hydroxybutan-2-one, and 3-methylbutanoic acid were still increasing in the headspace after 9 h. Methanethiol, and especially dimethyl disulfide and dimethyl trisulfide, went through a maximum at $t = 5$ h. Such behavior has already been observed during HS-SPME (PDMS fiber) analysis of the most volatile compounds (*24*) and may be interpreted as a competition on the fiber between the different compounds in the headspace. When the concentrations of a larger number of volatile compounds increase in the headspace, the sorption equilibrium of the more volatile ones is shifted. A good compromise between all of these effects was to perform analysis of the headspace after 4 h of equilibration in the large cell (procedure 2, **Table 1**).

Distribution of Odor Compounds between Rind and Paste. Yeast and bacterial growth on the surface of soft smear cheese contributes to the development of a characteristic cheese flavor. It was therefore necessary to test the rind and paste separately (*26*) at $t = 30$ days ($t = 0$ being the date of packaging), a likely date of consumption. **Figure 3** shows the distribution of several volatile compounds between the rind and paste of cheese. Methanethiol and sulfur compounds (DMDS and DMTS) were observed only in the rind of cheese, in line with previous findings (*27*). Pentan-2-one was mainly present in the rind. Other compounds were observed only in the paste: ethyl acetate, ethanol, butane-2,3-dione, 3-hydroxybutan-2-one, and 2-methylpropanoic acid. 3-Methylbutanol, hydroxypropan-2-one, acetic acid, and 3-methylbutanoic acid were mainly found in the paste, with only low amounts in the rind. Acetone and 3-methylpentan-2-one were more or less evenly distributed in the rind and paste. The different distributions of these compounds may have been due to the activity of specific microorganisms (*26*) rather than a partitioning between phases.

Evolution of Flora on the Surface of Cheese A during the Shelf Life of Cheese. In the flora of the surface of cheese A, two identified populations were predominant: *B. linens* and *Staphyloccocus xylosus* (99.9% of identification with API Staph system). Yeasts were also present at a low level (10^2-10^3 CFU/g of smear during the shelf life of cheese A), so they could not contribute significantly to the production of volatile compounds [no influence below 10^6 cell/g of cheese (*28*)]. To relate the evolution of volatile compounds with the evolution of the flora on the surface of cheese A during the shelf life, we monitored the growth of both microorganisms (**Figure 4**). The population of *B. linens* rose over the first 3 weeks and then reached a plateau, whereas the growth of *S. xylosus* was more progressive.

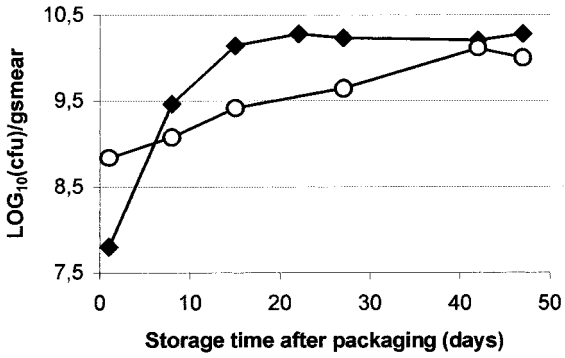

Figure 4. Cell populations of *B. linens* (◆) and *S. xylosus* (○) grown in a smear (CFU/g of smear) of the top surface of packaged cheese A during its shelf life.

Monitoring Changes in Volatile Compounds during the Shelf Life of Cheese A. The levels of volatile compounds in the headspace around a whole cheese (A) were monitored (**Figure 5**). Concentrations of methanethiol, dimethyl disulfide, and dimethyl trisulfide (**Figure 5a**) came close to equilibrium after ~20 days in the packaging, with dimethyl disulfide reaching higher levels than the two others. Dimethyl trisulfide was found at trace levels. Sulfur compounds exhibited lag times (3 days for dimethyl disulfide and 9 days for dimethyl trisulfide) consistent with their probable route of formation, by oxidation of methanethiol (*29*, *30*). Acetic acid and 3-methylbutanoic acid levels remained constant throughout the shelf life of cheese (**Figure 5b**). 3-Hydroxybutan-2-one (dairy note), butane-2,3-dione (dairy note), hydroxypropan-2-one (fruity note, **Figure 5c**), acetone, and 3-methylbutanol (**Figure 5d**) strongly decreased during this period, with 3-methylbutanol even disappearing from the headspace. Butanone and pentan-2-one produced results that varied from batch to batch.

In general, levels of compounds of the paste decrease and those in the rind increased. In the latter, the kinetics of methanethiol (**Figure 5a**) and probably of the other sulfur compounds, followed those of *B. linens* (**Figure 4**), supporting enzymatic production by this microorganism. Indeed, *B. linens* is known to produce methanethiol (*31*, *32*), which is not the case of *S. xylosus* (*33*). The fall in levels of paste compounds around the whole cheese (A) was not a result of a metabolic process, as can be seen from the behavior of 3-methylbutanol. Indeed, this compound was still present at high levels in the paste (**Figure 3**) at $t = 30$ days, but it disappeared from the headspace around the whole cheese (A) after this time (**Figure 5d**). This suggests that the increase in rind thickness played the role of a barrier.

The pH gradient between rind and paste was also likely to contribute to the heterogeneity of cheese odor. During the period of storage, the pH of the surface remained between 7.7 and 7.9, whereas in the paste it rose from 5.4 to 5.9. This may also explain why acid levels remained constant in the headspace: given the pH of the rind, only a weak proportion of acids could be present in their protonated form, which contributes to odor activity (*34*).

Representativeness of HS-SPME Extracts of Cheese A by Olfactometry Study. An olfactometry evaluation was made at $t = 30$ days, a likely date of consumption, when changes in the evolution of all volatile compounds had slowed.

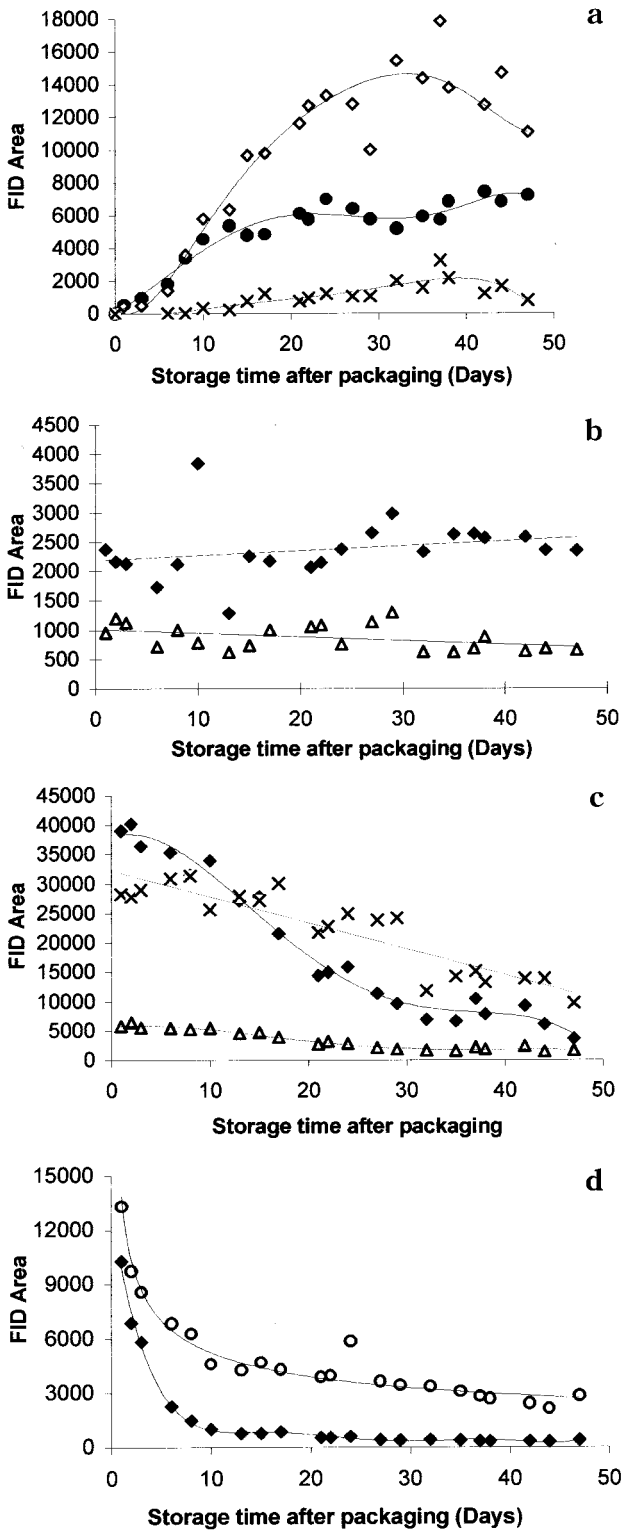

Figure 5. Changes to (a) methanethiol (●), dimethyl disulfide (◇), and dimethyl trisulfide (×) levels; (b) acetic acid (△) and 3-methylbutanoic acid (◆) levels; (c) 3-hydroxybutan-2-one (◆), butane-2,3-dione (△), and hydroxypropan-2-one (×) levels; and (d) acetone (○) and 3-methylbutanol (◆) levels in the headspace during the shelf life of packaged cheese A (each experimental value is for a different cheese A from the same batch).

First, we verified whether the HS-SPME extract following procedure 2 (**Table 1**, 4 h of equilibration in the large cell, 30 min of trapping by the fiber) was representative of the odor. The extract was assessed without chromatographic column separation, and panelists received the global odor of the HS-SPME extract of the whole cheese (A). All panelists felt that the cheese odor was completely masked by a garlic note (characteristic of sulfur compounds) and that the HS-SPME extract achieved under these conditions was not representative of the cheese odor. This indicated that the methodological conditions optimized as described above, and deemed to ensure significant and reliable quantities of the most volatile compounds in the headspace, produced a completely unbalanced odor profile, markedly distorted in terms of sulfur compounds (**Figure 6**). It was therefore necessary to optimize other methodological conditions, under which the importance of the sulfur compounds would be both more restricted and more realistic. This was possible by using a small analytical cell containing mixtures of rind and paste (procedure 1, **Table 1**), where equilibrium was rapidly achieved for all compounds. Four different mixtures of rind and paste were compared using an olfactometry approach (**Table 3**). A good balance was found at $t = 30$ days, with 20% rind and 80% paste ($^1/_{32}$ of cheese) and immediate exposure of the SPME fiber (30 min) at 25 °C. Panelists were able to recognize the global odor of cheese A, compared with the same type of surface smear-ripened cheeses (B and C) in a triangular test (**Table 4**). An average score of 8 on a scale of 1−10 (10 being total identity with reference cheese A) was allocated to this HS-SPME extract, which supported its representativeness. The extracts of cheeses B and C had been given average scores

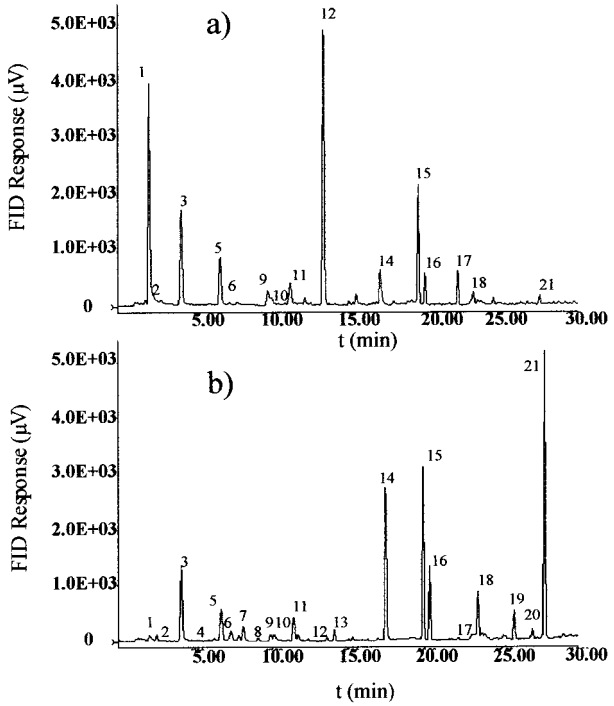

Figure 6. Comparison of both GC profiles of HS-SPME extract (CAR/PDMS), obtained with a whole cheese (a) (see procedure 2, **Table 1**) and with portions of rind and paste (b) (see procedure 1, **Table 1**).

Table 3. Composition of the Cheese A Sample Introduced in the Small Cell (Procedure 1, **Table 1**) and Global Odor Perception of Volatile Compounds Trapped on a Fiber ($n = 2$)

expt	% of rind (wt)	% of paste (wt)	recognition of the odor of cheese A
I	100	0	no
II	0	100	no
III	50	50	no
IV	20	80	yes

Table 4. Representativeness of the Odor of Cheese A Extracts Obtained by HS-SPME (Procedure 1, **Table 1**)

	panelist					
extracts obtained from	1	2	3	4	av	SD
cheese A	7[a]	8	8	9	8	0.8
cheese B	4	4	4	3	4	0.5
cheese C	2	2	2	0	2	1.3

[a] Average score obtained from three repetitions for each panelist with one reference of cheese A.

Table 5. Screening of Odor Compounds of Representative Cheese A Extracts Obtained by HS-SPME (Procedure 1, **Table 1**)

volatile compd	odor description[a]	odor threshold[b] (ppb)
methanethiol	unpleasant odor of cheese	0.02
3-methylbutanol	rind of cheese (A)	250–300
dimethyl trisulfide	unpleasant, garlic, over-ripened cheese	0.005–0.001
3-methylbutanoic acid	floorcloth, feet, sweaty	120–700

[a] Odor description as perceived by panelists during olfactometry. [b] Reference of odor detection thresholds in water: Leffingwell and Associates, www.leffingwell.com.

of 4 and 2, respectively. We therefore considered that the HS-SPME approach was validated for cheese A.

Screening of Cheese A HS-SPME Extracts. The volatile compounds from cheese A obtained from the representative HS-SPME extract (small analytical cell, 80% paste, 20% rind, immediate exposure of the fiber, procedure 1, **Table 1**) were separated on a GC capillary column, and panelists described the odor perception of each eluted peak (GC-O method, closely related to the Osme method, *35*). Among them, four volatile compounds, methanethiol, 3-methylbutanol, dimethyl trisulfide, and 3-methylbutanoic acid, were unequivocally detected (**Table 5**). Cheese A was globally described as having an unpleasant odor of "floorcloth", which was similar to the description of 3-methylbutanoic acid; it therefore seemed possible that this acid was responsible for the global odor. However, this acid could not contribute alone to the odor, as its concentration remained constant in the headspace (**Figure 5b**), whereas the unpleasant odor was perceptible in the laboratory at $t \geq 20$ days only. The contribution of methanethiol (which reached a plateau at $t = 20$ days, **Figure 5a**) and especially that of dimethyl trisulfide (because of its very low threshold) seemed likely. The odors of methanethiol and dimethyl trisulfide are often detected in cheese analysis. Methanethiol is responsible for the putrid aroma associated with Trappist-type cheese (*36*), the unpleasant odor of grana cheese (*37*), and the cabbage odor of Cheddar (*38*). Methanethiol, methional (not detected here), and dimethyl sulfide are key odorants in the sulfurous garlic note of Camembert (*39*, *40*). In Cheddar cheese, methanethiol has been shown to be essential to the odor, even if its odor differs from that of the cheese. An interaction between methanethiol and other compounds is often proposed to explain the complexity of cheese odor (*41*). Dimethyl disulfide, which has a relatively high threshold (0.16−12 ppb in air) and which was present at a low level in the representative extract, was not smelled by the panel. A similar situation was observed in the case of Cheddar cheese (*42*). 3-Methylbutanol was always detected by the panel, but because of its fleeting perception, a descriptor could not always be allocated. Nevertheless, this compound was always associated with the odor of cheese rind. This branched primary alcohol has a pleasant aroma of "fresh cheese" (*43*) ("cheese rind" in our study), which during the early stages of the evolution of cheese A after packaging may have had a positive impact on the odor, balancing 3-methylbutanoic acid and sulfur compounds. At $t \geq 10$ days (**Figure 5d**), the rapid decrease in 3-methylbutanol (together with other paste compounds, such as butane-2,3-dione, 3-hydroxybutan-2-one, and hydroxypropan-2-one; **Figure 5c**) in the headspace, compared with the marked increase in methanethiol, may have modified the global odor of cheese A, hence the unpleasant odor of "floorcloth" becoming predominant at $t = 20$ days.

The evolution of each compound in the headspace and of the global odor could be linked with changes to the rind during the shelf life of cheese A. During the first 2 days, the odor of cheese A was characterized by paste compounds, with "buttery" and "cream" attributes (3-methylbutanol, butane-2,3-dione, 3-hydroxybutan-2-one, and hydroxypropan-2-one). The rind then gradually built up and thickened; *B. linens* proliferated on the surface, releasing methanethiol (and thus other sulfur compounds) in the headspace. At the same time, the levels of "paste" compounds fell, with those of acids remaining constant. Thus, several days after packaging, 3-methylbutanoic acid, methanethiol, and dimethyl trisulfide were the compounds contributing to the main global odor of cheese A.

Conclusion. The HS-SPME technique performed using a CAR/PDMS fiber could be employed successfully to monitor changes to the headspace composition of cheese. When compared with vacuum distillation, HS-SPME provides a satisfactory assessment of the most volatile compounds that play a major role in odor perception. Moreover, by optimizing sampling methods, it was possible to obtain representative and reproducible HS-SPME extracts of the typical odor of cheese A. Olfactometry analysis of this representative HS-SPME extract made it possible to link the odor of cheese A with the combination of 3-methylbutanoic acid (the most active odor compound) with methanethiol and dimethyl trisulfide. Changes in odor could be related to changes of the rind, which behaved as a barrier, strongly influencing the distribution of volatile compounds in the headspace. HS-SPME was also of interest because it provided a good fingerprint of the compounds responsible for odor perception by consumers.

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LITERATURE CITED

(1) Mülder, H. Taste of flavor forming substances in cheese. *Neth. Milk Dairy J.* **1952**, *6*, 157.
(2) Wong, N. P.; Parks, O. W. Simple technique for extracting flavor compounds from fatty foods. *J. Dairy Sci.* **1967**, *51*, 1768−1769.
(3) Aishima, T.; Nakai, S. Pattern recognition of GC profiles for classification of cheese variety. *J. Food Sci.* **1987**, *52*, 939−942.
(4) Forss, D. A.; Holloway, G. L. Recovery of volatile compounds from butter oil practice. *J. Am. Oil Chem. Soc.* **1967**, *44*, 572−575.
(5) Dumont, J. P.; Adda, J. Isolement des constituants de l'arôme des fromages: comparaison de méthodes. *Lait* **1972**, *52*, 311−323.
(6) Dumont, J. P.; Roger, S.; Adda, J. Etude des composés volatils neutres présents dans les fromages à pâte molle et à croûte lavée. *Lait* **1974**, *54*, 31−43.
(7) Manning, D.; Moore, C. Headspace analysis of hard cheeses. *J. Dairy Res.* **1979**, *46*, 539−545.

(8) Yang, W. T.; Min, D. B. Dynamic headspace analysis of volatile compounds of cheddar and Swiss cheese during ripening. *J. Food Sci.* **1994**, *59*, 1309−1312.
(9) Zhang, Z.; Pawliszyn, J. Headspace solid-phase microextraction. *Anal. Chem.* **1993**, *65*, 1843−1852.
(10) Kataoka, H.; Lord, H. L.; Pawliszyn, J. Applications of solid-phase microextraction in food analysis. *J. Chromatogr. A* **2000**, *880*, 35−62.
(11) Song, J.; Gardner, B. D.; Holland, J. F.; Beaudry, R. M. Rapid analysis of volatile flavor compounds in apple fruit using SPME and GC/time-of-flight mass spectrometry. *J. Agric. Food Chem.* **1997**, *45*, 1801−1807.
(12) Chin, H. W.; Bernhard, R. A.; Rosenberg, M. Solid phase microextraction for cheese volatile compounds analysis. *J. Food Sci.* **1996**, *61*, 1118−1128.
(13) Pelusio, F.; Nilsson, T.; Montanarella, L.; Tilio, R.; Larsen, B.; Facchetti, S.; Madsen, J. O. Headspace solid-phase microextraction analysis of volatile organic sulfur compounds in black and white truffle aroma. *J. Agric. Food Chem.* **1995**, *43*, 2138−2143.
(14) Scarlata, C. J.; Ebeler, S. E. Headspace solid-phase microextraction for the analysis of dimethyl sulfide in beer. *J. Agric. Food Chem.* **1999**, *47*, 2505−2508.
(15) Mestres, M.; Busto, O.; Guash, J. Headspace solid-phase microextraction analysis of volatile sulphides and disulphides in wine aroma. *J. Chromatogr. A* **1998**, *808*, 211−218.
(16) Pérès, C.; Viallon, C.; Berdagué, J. L. Solid-phase microextraction-mass spectrometry: a new approach to the rapid characterization of cheeses. *Anal. Chem.* **2001**, *73*, 1030−1036.
(17) Marsili, R. T. SPME-MS-MVA as an electronic nose for the study of off-flavors in milk. *J. Agric. Food Chem.* **1999**, *47*, 648−654.
(18) Lecanu, L.; Ducruet, V.; Feigenbaum, A. Active packaging to stabilize the sensory quality of semi-soft cheese. In *Abstract Book of European Conference on Advanced Technology for Safe and High Quality Foods*; Eurocaft, Dec 5−7, 2001, Berlin; Elsevier: Amsterdam, The Netherlands, 2001; Abstract 4.07.
(19) Richard, J.; Zadi, H. Inventaire de la flore bactérienne dominante des camemberts fabriqués avec du lait cru. *Lait* **1983**, *63*, 25−42.
(20) Grecz, N.; Dack, G. M. Taxonomically significant color reactions of *Brevibacterium linens*. *J. Bacteriol.* **1961**, *82*, 241−246.
(21) FIL/IDF Laits et produits laitiers. Dénombrement des levures et des moisissures. Comptage des colonies à 25 °C; Standard 94B; 1991.
(22) Hanaoka, K.; Sieffermann, J. M.; Giampaoli, P. Effects of the sniffing port air makeup in gas chromatography−olfactometry. *J. Agric. Food Chem.* **2000**, *48*, 2368−2371.
(23) Shirey, R. E. Optimization of extraction conditions for low-molecular-weight analytes using solid-phase microextraction. *J. Chromatogr. Sci.* **2000**, *38*, 109−116.
(24) Roberts, D. D.; Pollien, P.; Milo, C. Solid-phase microextraction method development for headspace analysis of volatile flavor compounds. *J. Agric. Food Chem.* **2000**, *48*, 2430−2437.
(25) Sablé, S.; Cottenceau, G. Current knowledge of soft cheeses flavor and related compounds. *J. Agric. Food Chem.* **1999**, *47*, 4825−4836.
(26) Bosset, J. O.; Liardon, R. The aroma composition of Swiss Gruyère cheese. *Lebensm. Wiss. Technol.* **1985**, *18*, 178−185.
(27) Dumont, J. P.; Adda, J. Occurrence of sesquiterpenes in mountain cheese volatiles. *J. Agric. Food Chem.* **1978**, *26*, 364−367.
(28) Zadi, H. Etude de la flore bactérienne non lactique des camemberts fabriqués de façon traditionnelle. Ph.D., University of Orsay, France, 1981.
(29) Parliment, T. H.; Kolor, M. G.; Rizzo, D. J. Volatile components of limburger cheese. *J. Agric. Food Chem.* **1982**, *30*, 1006−1008.
(30) Chin, H. W.; Lindsay, R. C. Ascorbate and transition-metal mediation of methanethiol oxidation to dimethyl disulfide and dimethyl trisulfide. *Food Chem.* **1994**, *49*, 387−392.
(31) Sharpe, M. E.; Law, B. A.; Phillips, B. A.; Pitcher, D. G. Methanethiol production by coryneform bacteria: Strains from dairy and human skin sources and *Brevibacteriun linens*. *J. Gen. Microbiol.* **1977**, *101*, 345−349.
(32) Ferchichi, M.; Hemme D.; Nardi, M.; Pamboukdjan, N. Production of methanethiol from methionine by *Brevibacteriun linens* CNRZ 918. *J. Gen. Microbiol.* **1985**, *131*, 715−723.
(33) Hemme, D.; Richard, J. Utilization of L-methionine and production of methanethiol by bacteria isolated from raw milk Camembert cheese. *Lait* **1986**, *66*, 135−142.
(34) Brennand, C. P.; Ha, J. K.; Lindsay, R. C. Aroma properties and thresholds of some branched-chain and other minor volatile fatty acids occurring in milk fat and meats lipids. *J. Sens. Stud.* **1989**, *4*, 105−120.
(35) Miranda-Lopez, R.; Libbey, L. M.; Watson, B. T.; McDaniel, M. R. Odor analysis of Pinot Noir wines from grapes of different maturities by gas chromatography-olfactometry technique (Osme). *J. Food Sci.* **1992**, *57*, 985−1019.
(36) Grill, H.; Patton, S.; Cone, J. F. Aroma significance of sulfur compounds in surface-ripened cheese. *J. Dairy Sci.* **1966**, *49*, 409−413.
(37) Battistotti, B.; Bosi, F.; Scolari, G. L.; Bottazzi, V.; Chiusa, P.; Gonzaga, E. Development of unpleasant odour in Grana cheese, resulting from the formation of methyl-mercaptan. *Sci. Tec. Latt.-Casearia* **1985**, *36*, 77−97.
(38) Milo, C.; Reineccius, G. A. Identification and quantification of potent odorants in regular-fat and low-fat mild cheddar cheese. *J. Agric. Food Chem.* **1997**, *45*, 3590−3594.
(39) Kubickova, J.; Grosch, W. Evaluation of flavour compounds of Camembert cheese. *Int. Dairy J.* **1998**, *8*, 11−16.
(40) Kubickova, J.; Grosch, W. Quantification of potent odorants in Camembert cheese and calculation of their odour activity values. *Int. Dairy J.* **1998**, *8*, 17−23.
(41) Urbach, G. The flavor of milk and dairy products: II. Cheese: contribution of volatile compounds. *Int. J. Dairy Technol.* **1997**, *50*, 79−89.
(42) Arora, G.; Cormier, F.; Lee, B. Analysis of odor-active volatiles in cheddar cheese headspace by multidimensional GC/MS/sniffing. *J. Agric. Food Chem.* **1995**, *43*, 748−752.
(43) Moio, L.; Dekimpe, J.; Etievant, P. X.; Addeo, F. Volatile flavour compounds of water buffalo mozzarella cheese. *Ital. J. Food Sci.* **1993**, *1*, 57−68.

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