Identification of a Powerful Aroma Compound in Munster and Camembert Cheeses: Ethyl 3-Mercaptopropionate

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With the view to investigate the presence of thiols in cheese, the use of different methods of preparation and extraction with an organomercuric compound (*p*-hydroxymercuribenzoate) enabled the isolation of a new compound. The analysis of cheese extracts by gas chromatography coupled with pulse flame photometry, mass spectrometry, and olfactometry detections led to the identification of ethyl 3-mercaptopropionate in Munster and Camembert cheeses. This compound, described at low concentrations as having pleasant, fruity, grapy, rhubarb, and empyreumatic characters, has previously been reported in wine and Concord grape but was never mentioned before in cheese. A possible route for the formation of this compound in relation with the catabolism of sulfur amino acids is proposed.

KEYWORDS: *p*-Hydroxymercuribenzoate; ethyl 3-mercaptopropionate; thiols; ripened cheeses

INTRODUCTION

Curd undergoes a biochemical dynamic change during the cheese-ripening process. These biochemical modifications are responsible for the production of the characteristic flavors of each variety. It was originally believed that cheese flavor resulted from one single compound or class of compounds. Nowadays, after extensive studies, it is well-established that hundreds of compounds, including aldehydes, alcohols, and ketones, are involved in the overall cheese flavor. Among them, sulfur compounds are of particular interest because of their very powerful odors and low perception thresholds. Although they are considered as quality flavors or off-flavors (*1*), depending on their concentration and on the type of food, they are of great importance for a lot of foods. The contribution of sulfur compounds to the typical flavors of various cheeses has often been reported by many researchers (*2, 3*). As reviewed by Molimard and Spinnler (*4*), odorants like methanethiol, dimethyldisulfide (DMDS), dimethyltrisulfide (DMTS), *S*-methyl thioacetate (MTA), and *S*-methyl thiopropanoate (MTP) play an important role in the flavor of several cheese varieties. These compounds are probably also involved in the aroma of smear soft and mold surface-ripened cheeses (*5*).

On the other hand, until now, little attention has been given to the possible occurrence of thiols. Such compounds possess a wide variety of odors ranging from cheese, catty, cooked, blackcurrant, rhubarb, meaty, brothy, flowery, onion, and rotten potatoes depending upon their chemical structures (*6*) and their concentrations. Thiols have been found to be associated with the flavor of different foods, including wine, beer, fruits, cooked meat, and roasted coffee (*7–12*). Because of the multiple sulfur descriptors, usually used to characterize smear soft cheeses like Munster, it is reasonable to assume that thiols may be involved in their characteristic flavors. To date, only one of them has been reported in cheese by Badings et al. (*13*) who found a catty flavor compound identified as 4-mercapto-4-methyl-pentan-2-one in Gouda cheese. More recently, Kleinhenz et al. (*14, 15*) carried out studies that highlighted the hypothetical presence of several thiols in Cheddar cheese. However, they have used a phosphine reagent, TCEP (Tris carboxyethyl phosphine) to prevent the oxidation of thiols and to enable their recovery from Cheddar cheese oil. Given that TCEP is a very powerful reductor, the hypothetical thiols that they found could have been formed by the reduction of the polysulfur molecules (e.g., DMDS and DMTS), which have been reported to be part of the cheese aroma. Moreover, these authors were not able to unequivocally characterize the identified thiols.

The difficulty of carrying out volatile thiols isolation in cheese is due to the complexity of its matrix and to the very low concentrations of these compounds, which make them undetectable by the classic means. The purpose of this study was therefore to develop a method for the detection and identification of thiols in cheese. To investigate this track, we developed a method modified from the one developed by Tominaga et al. (*10, 11*) on wine and based on the specific thiol extraction using *p*-hydroxymercuribenzoate (*p*HMB). This method was

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considerably modified to overcome problems associated with the fat content and heterogeneity of cheese. Furthermore, we have used gas chromatography (GC) coupled with several detectors [(mass spectrometry (MS), pulsed-flame photometric detector (PFPD), and human nose (O)] to identify the thiol compounds.

MATERIALS AND METHODS

Chemicals. Tris(hydroxymethyl)aminomethane, *p*HMB, hydrochloride L-cysteine monohydrated, 37% hydrochlorhydric acid, sodium acetate, 99% sodium sulfate, ethyl acetate, dichloromethane (99.9%), hexanethiol, thiazole (99%), DMDS, DMTS, ethyl 3-mercaptopropionate (99.9%), and 2,4-dithiapentane were supplied by Sigma-Aldrich Chemicals (Saint Quentin Fallavier, France). Ethyl 2-mercaptopropionate (98%) was purchased from Coger (Paris, France).

Cheese Samples. All of the samples were purchased from a local supermarket, and most of them were labeled "Protected Designation of Origin" French cheeses. These cheese varieties (and their fat contents in fresh matter) were as follows: Munster brands "Frech" (22%), "Ermitage" (27%), and "Les Petits Amis" (27%); Livarot brand "Auchan" (22%); and Camembert brand "Jean Vernier" (30%). Munster and Livarot cheeses are smear soft cheeses, whereas Camembert is a mold-ripened one.

Preliminary Sensory Analysis. To investigate the contribution of thiols in cheese, a triangle test was performed prior to the detection experiments. Munster "Ermitage" cheese was chosen for the analysis because of its characteristic sulfur aroma. Munster samples were prepared as follows: 100 g of cheese was crumbled in 200 mL of a 0.1 M Tris buffer solution at pH 8.5 containing 2.5-di-*tert*-butyl-4-methoxyphenol (0.02 mM) as the antioxidant and *p*HMB (2 mM). The mixture was subsequently adjusted to pH 7 and agitated for 30 min. The control sample was prepared in the same way without *p*HMB addition. The two solutions, around 300 mL each, were divided into small tinted glasses with covers. Twelve subjects from the laboratory staff familiar with cheese flavors participated in the triangle test. The test was balanced for the identity of the odd sample (both ABB and BAA were used) and its rank of tasting (ABB, BAB, and BBA) to avoid carryover effects.

Initial Sample Preparation. To date, thiols have only been detected in aqueous sample matrices like wine, beer, and olive oil (*16*). Because of the complexity of the cheese matrix, the method of Tominaga and Dubourdieu (*11*) was very difficult to carry out efficiently. Several preparation methods were therefore tested.

First, Tominaga's latest protocol applied to wine was adapted to cheese samples (*11*). It consisted of crumbling 100 g of cheese into 200 mL of a 0.1 M Tris buffer solution spiked with 2 mM *p*HMB and 0.02 mM *tert*-butyl-4-methoxyphenol as an antioxidant. Hexanethiol (20 nmol) was then added as internal standard before the mixture was adjusted to pH 7 and stirred using a magnetic stirrer for 30 min to improve the formation of thiols−*p*HMB complexes. At least two centrifugations (10 min at 6500*g*, 4 °C) in 30 mL Corex glass tubes, followed by filtrations, were necessary to clarify the sample and collect the aqueous phases. These layers, containing thiol−*p*HMB complexes, were immediately loaded on a strongly basic anion-exchanger column (Dowex 1WX2-100).

The second protocol was adapted from Kleinhenz et al. (*14*). Approximately 1200 g of cheese was cut in small pieces, placed in 500 mL Nalgene polypropylene bottles, and centrifuged for 40 min at 17500*g* and 38 °C. To improve the fat recovery, centrifugation was repeated twice. After each centrifugation, the fat was collected in a brown glass bottle to prevent oxidation. The total weight of the oil yielded was approximately 170 g (about 200 mL). Hexane (200 mL) was subsequently mixed with the oil, and hexanethiol was added as an internal standard. Finally, a *p*HMB solution containing 75 mL of ultrapure water, 0.1 M Tris, 2 mM *p*HMB, and 0.1 mM 2.5-di-*tert*-butyl-4-methoxyphenol was spiked with the oil−hexane mixture. Each solution was previously degassed by sonication and purged with nitrogen for 5 min. The combined solution of *p*HMB and hexane was also sonicated for 5 min before stirring for 6 h. After a 1 h settlement, the aqueous phase was recovered in a 100 mL brown glass bottle and

placed at 4 °C. The hexane phase was mixed again with a 1 mM *p*HMB solution, prepared as described above, and stirred for 1 h. The two *p*HMB solutions were subsequently mixed, and the pH was adjusted to 7. As for the first protocol, the *p*HMB phase was loaded on a column fitted with Dowex resin, and an extraction was performed in the same way.

In the third method, layers of approximately 0.5 cm were removed from the surface of each cheese sample. The layers were then cut into small cubes (measuring approximately 0.2 cm on each side) and put in a brown glass 1 L bottle until reaching a 100 g weight. Following the addition of 200 mL of dichloromethane containing 20 nmol of hexanethiol as an internal standard, the mixtures were left under agitation for 18−20 h. They were subsequently filtered through glass wool and then ready for extraction. The volatile thiols contained in the organic phases were extracted twice using 2 × 20 mL of a *p*HMB solution (2 mM *p*HMB and 0.1 M Tris) and purified by percolation through a strongly basic anion-exchanger column. After this step, the following procedure was identical for all three protocols described above.

Extraction of Thiols from the Columns. Whatever the procedure, once the *p*HMB extract was loaded, the resin was washed with 50 mL of sodium acetate buffer (pH 7). The volatile thiols were released by percolating a hydrochloride L-cysteine monohydrated solution. The three eluates, corresponding to each percolation, were successively extracted by 4 and 3 mL of dichloromethane, spiked with thiazole (external standard), and dried on anhydrous Na₂SO₄. They were finally concentrated under nitrogen to 20 μL, and 3 μL was injected for all analyses.

Analytical Methods. *GC-MS and GC-PFPD.* A Varian CP-3800 gas chromatograph equipped with an electronic impact mass spectrometer and a PFPD was used for this study. The separation of the analytes was performed using a 30 m × 0.25 mm i.d. DB-XLB capillary column (film thickness, 0.5 μm) for MS and a 30 m × 0.25 mm i.d. HP-5 MS capillary column (film thickness, 0.25 μm) for PFPD. The oven temperature was programmed as follows: 45 °C held for 2 min, heated to 160 °C at a rate of 3 °C/min, held for 1 min, and then heated to 250 °C at a rate of 10 °C/min with a final hold time of 5 min.

GC-O. The GC-O analysis was performed using a HP 6890 gas chromatograph (Hewlett-Packard) equipped with an effluent splitter, a sniffing port, and an intensity recorder (Sniffer 9000 system, Brechbühler, Basel, Switzerland). The apparatus was fitted with an HP-5 MS capillary column identical with the one used for MS. The oven temperature was set as previously described. The GC effluent was sent to the sniffing port and diluted with humidified air (30 mL/min). Upon emission of an aroma-active compound, each of the four judges was invited to use the intensity recorder to rate the relative intensity of the aroma.

Identification and Measurement of the Volatile Thiol. The volatile thiol and all of the other sulfur compounds were identified by comparing their spectra in SCAN mode (GC-MS) with those of the commercial references. The linear retention index (RI$_{MS}$) for MS was determined relative to a series of *n*-alkanes. As the PFPD is specific for sulfur compound detection, it did not detect *n*-alkanes. This issue was solved by using a homologous series of linear thiol compounds to estimate the PFPD indice (RI$_{PFPD}$) according to the following formula as proposed by Vermeulen (*17*):

$$RI_i = RI_n + \left[\frac{t_i - t_n}{t_{n+1} - t_n} \times (RI_{n+1} - RI_n) \right] \quad (1)$$

where RI$_i$ is the retention indice of the compound of interest *i*, RI$_n$ is the retention index of the sulfur reference preceding the compound of interest *i*, RI$_{n+1}$ is the retention index of the sulfur reference following the compound of interest *i*, t_i is the retention time of the compound of interest *i*, t_n is the retention time of the sulfur reference preceding the compound of interest *i*, and t_{n+1} is the retention time of the sulfur reference following the compound of interest *i*.

Quantification. Quantification of ethyl 3-mercaptopropionate (ET3MP) was performed using a standard addition procedure. Increasing quantities (30−150 ng/L) of ET3MP were added to a Munster or a Camembert

Table 1. Detection of Thiols and Some Oilier Sulfur Compounds in the Cheese Samples[a]

Compound	Structure	Cheese samples				
		Munster "Frech"	Munster "Ermitage"	Munster "Petis amis"	Livarot "Auchan"	Camembert "Le rustique"
DMDS		a, b, c	a, b, c	a, b, c	a, b, c	a, b, c
DMTS		a, b, c	a, b, c	a, b, c	a, b, c	a, b, c
2,4-Dithiapentane		a, b, c	a, b, c	a, b, c	a, b, c	a, b, c
2-Acetylthiazole		a, b, c	a, b, c	a, b, c	a, b, c	a, b, c
Ethyl 2-mercaptopropionate*		ND	a, b	ND	ND	ND
Ethyl 3-mercaptopropionate*		ND	a, b, c	ND	ND	a, b, c

[a] Key: a, detection by GC/O; b, detection by PFPD; c, detection by GC/MS; ND, not detected; and *only detected in the final L-cysteine extracts, whereas the other compounds were detected in the first steps of the extraction. All of the compounds except ethyl 2-mercaptopropionate were identified using their pure references.

cheese. For each concentration, the volatile was extracted from the cheese using the third sample preparation method as described above. Each cheese was analyzed three times to determine the standard deviations.

Recovery Rate. Because cheese extraction procedures involve numerous steps, the recovery rate during the isolation of ethyl 3-mercaptopropionate was calculated according to the method described by Tominaga et al. (11), using hexanethiol and thiazole as internal and external standards.

Determination of Gas−Liquid Partition Coefficient. ET3MP volatility in water and fresh cream was measured using the phase ratio variation (PRV) method described by Ettre et al. (18). Fresh cream was chosen because of its fat content (30%), which is very close to those of cheese samples. The PRV procedure, which enabled measurement of the gas−liquid partition coefficient (K_i), is based on the relationship between the K_i and the phase ratio (β) of the vial for concentrations at equilibrium between the two phases. The equation for the partition coefficient calculation is established as follows:

$$\frac{1}{A} = \frac{1}{f_i C_i^{\text{liq}}} \cdot \frac{1}{K_i} + \frac{1}{f_i C_i^{\text{liq}}} \beta \tag{2}$$

where A is the peak area obtained at equilibrium, f_i is a proportional factor that is substance-specific and dependent on the analytical conditions, and β is the V_g/V_1 ratio with V_g the headspace volume and V_1 the liquid volume of the sample (18). The previous relationship can be expressed as a linear equation: $1/A = a + b\beta$ (1) with $a = [1/(f_i C_i^{\text{liq}})](1/K_i)$ and $b = 1/(f_i C_i^{\text{liq}})$. Then, the b/a ratio corresponds to the partition coefficient expressed as a concentration ratio (K_i). For this experiment, increasing volumes of ET3MP in water or fresh cream (50, 200, 500, and 2000 μL) were inserted in 20 mL vials to obtain different β ratio phases. For each β ratio, vials were prepared in triplicate. After equilibration at 25 °C for 12−24 h, 2 mL of the headspace gases was analyzed as described by Athès et al. (19).

Determination of the Olfactive Perception Threshold. Ascending forced-choice procedures were used to measure the olfactive detection threshold of ET3MP (20). Stimulus intensity followed a geometric concentration series for ET3MP in cream (100 ppt, 300 ppt, 900 ppt, 2.7 ppb, and 8.1 ppb). The stimulus was increased in a series of triangle tests, in an ascending fashion, to find points where each individual panelist's responses changed from not correctly identifying the odd sample to correctly identifying the odd sample. The samples were provided to each panelist as a series of five blind-coded sets of three samples per set. The first set was the cream without adding ET3MP and the cream with the lowest concentration of ET3MP. The panelist had to make a choice of the odd sample before they would receive the second set and so on for five sets. Twenty-five untrained panelists participated in sensory analysis. The individual and group best estimated threshold (BET) during each sensory session was calculated as described by Meilgaard et al. (21). The individual BET was calculated as the geometric mean of the highest concentration missed and the next

concentration. For those panelists who gave correct answers at the lowest ET3MP concentration, their individual BETs were estimated as the geometric mean of the lowest ET3MP concentration tested in the study, and the hypothetical next lower ET3MP concentration that would have been given had the series been extended (20) (e.g., 33.33 ng of ET3MP/kg of cream). Similarly, for those panelists who failed to correctly identify the odd milk sample at the highest ET3MP concentration, their individual BETs were estimated as the geometric mean of the highest ET3MP concentration tested in the study and the hypothetical next higher ET3MP concentration that would have been given had the series been extended (e.g., 24.3 μg of ET3MP/kg of cream). For the panel, the group BET was the geometric mean of the individual BET (20, 21).

RESULTS AND DISCUSSION

Preliminary Sensory Analysis. Before performing this study, the possible impact of thiols and their influence on the overall aroma of cheese was tested. A triangular sensory test with Munster "Ermitage" cheese was performed alone or mixed with *p*HMB, using the well-known property of thiols to react with mercury. This chemical bond leads to the loss of the thiol sensorial properties. Nine judges out of 12 were able to identify the odd sample as compared to the others. According to statistical tables, the number of correct answers is high enough to conclude that the difference between the two samples, with and without *p*HMB, is significant (probability < 0.01). The subjects were also asked to explain the differences that they perceived between the samples. The flavor descriptors used for samples with bound thiols (containing *p*HMB) were as follows: solvent, ammonia, cheese rind, alcohol, and white cheese, whereas those of the controls were as follows: fruity, sulfured, cheese, and Munster. The odor of samples supplemented with *p*HMB was generally described as weaker than the controls and was not representative of the Munster cheese aroma. The conclusion that some unidentified thiol compounds could play a decisive role in the characteristic aroma of Munster cheese was therefore possible. It was then reasonable to think that these results might be due to thiols such as hydrogen sulfide, methanethiol, and butanethiol, which were reported in cheese flavors (22). However, given that hydrogen sulfide and methanethiol are very reactive and that cheese flavor is a complex mix of a wide range of compounds, thiol occurrence was also considered. We extrapolate this result for other cheese varieties.

Evaluation of the Sample Preparations. The three sample preparation methods were evaluated to determine the best protocol to be used for the extraction of thiols from the cheese
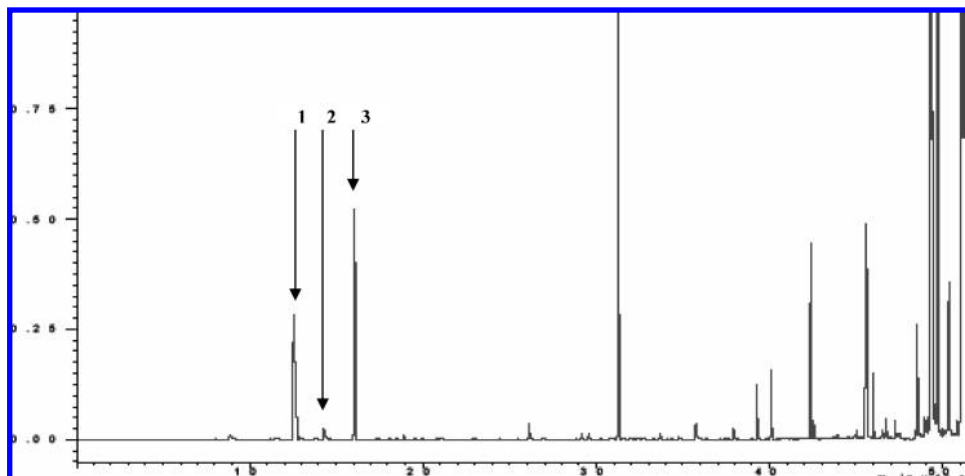
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Figure 1. GC-PFPD chromatogram of a final ʟ-cysteine extract from Munster "Ermitage". Peaks: 1, hexanethiol (IST); 2, ethyl 2-mercaptopropionate (tentatively identified); and 3, ethyl 3-mercaptopropionate.
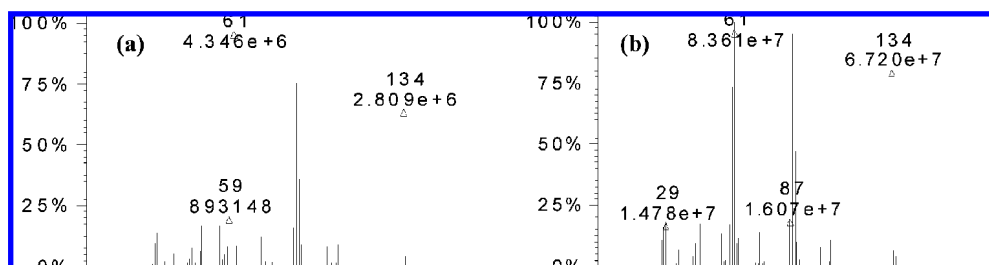

Figure 2. Mass spectra of ethyl 3-mercaptopropionate detected in a final ʟ-cysteine extract of Munster "Ermitage" (**a**) as compared to that of its commercial analogue (**b**).

matrices. Parameters like the length and the number of the steps were taken into consideration in the overall extraction efficiency. The first method, which consisted of crumbling cheese samples in a *p*HMB solution, was not suitable probably because the thiols were still retained by the proteins and the lipids of the cheese matrix and by the fact that the mercury reagent could interact with these compounds. The thiols were not therefore able to be complexed by the *p*HMB molecules. The second method including cheese fat extraction was a long process. These numerous steps could cause a potential loss of thiols as a result of oxidation. Furthermore, the thiol extraction from cheese oil was difficult, and there is also a risk to form byproducts between thiols and oil oxidation products. Even if the preceding protocols did not work in the present study, it must be emphasized that they could be suitable for other cheese varieties. Finally, the third protocol, which only considers the thiol content of the cheese surface layers, enabled us to eliminate the fat content drawback. This procedure gave the best results for the compound isolation and was therefore selected for further experiments. Given that flavor formation in smear-ripened and mold-ripened cheeses often begins at the surface where microbial activity is the highest before diffusion into the core, this result is not surprising. Moreover, the layers were wide enough to include a small part of the cheese core, which probably improved the extraction efficiency.

Method Specificity. As *p*HMB has the ability to combine specifically, in a reversible reaction, with compounds containing a thiol function (*23*), other sulfur compounds, including polysulfides, like DMDS and DMTS, which were previously reported to be present in various cheeses, must not be extracted. As expected, none of these compounds were found in the final ʟ-cysteine eluates. These volatiles were nevertheless found in

the first eluate (obtained after the percolation of the initial organic extract) and in the second one (rinsing buffer solution). All detected compounds are summarized in (**Table 1**).

Thiols Identification. All of the organic extracts of cheeses containing volatile thiols were analyzed by GC-PFPD and GC-MS. Among the five cheese samples analyzed, thiols could only be identified in two of them. These cheeses were the Munster cheese brand "Ermitage" and the Camembert cheese brand "Jean Vernier". The PFPD chromatogram of the Munster cheese extract (**Figure 1**) shows a lot of peaks. Obviously, all of these peaks are not due to sulfur compounds but must come from other compounds that are probably present in large quantities in the extract. The compound corresponding to peak 1 was identified as hexanethiol, which was used as an internal standard. Peak 2 (RI_{MS} = 935 and RI_{PFPD} = 1012) has not matched with any compound of the GC-MS library (NIST Mass spectral database) perhaps because this detector is not sensitive enough and undoubtedly because the concentration of the compound was too low for detection. Nevertheless, it has been tentatively identified as ethyl 2-mercaptopropionate (ET2MP) by comparison to its PFPD retention time and that of the commercial reference. This volatile is well-known to be found in apple juice and strawberries (*24, 25*). After a comparison with the GC-MS identification and the injection in the same analytical conditions of a pure reference, peak 3 (RI_{MS} = 1024, RI_{PFPD} = 1101) was identified as ethyl-3-mercaptopropionate. The mass spectrum of this compound, presented in **Figure 2a**, tightly matched up (both R and F matches = 970) to that of the commercial reference (**Figure 2b**). The calculated linear retention index of ET3MP was also identical to that of the reference (on a DB-XLB column). In addition, ET3MP identification was confirmed in SIM mode by overlapping selected ions, *m/z* 61, 88, and 134,
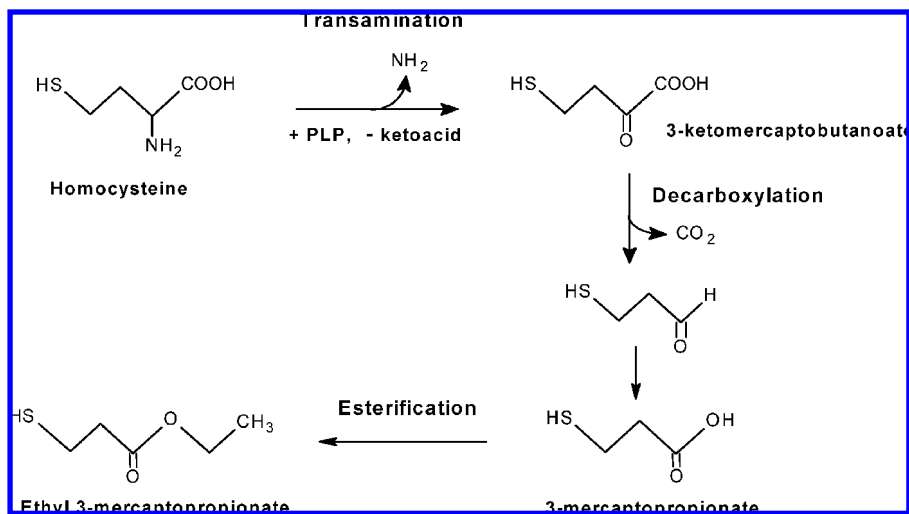
Figure 3. Hypothetical formation pathway of ethyl 3-mercaptopropionate.

at the linear retention index of the reference compound (data not shown). Moreover, an additional attribute for ET3MP identification was represented by the odor quality of this compound (assessed by GC-O), which was identical to that of the commercial reference at the same concentration. Ethyl 3-mercaptopropionate was previously reported in various foods like wine (*26*) or Concord grape (*27*). To our knowledge, however, this is the first time that it has been found in cheese.

The metabolic pathways for the formation of ET3MP are not yet elucidated. However, it can be supposed that they could be similar to those described for other thiol compounds in wine and beer. ET3MP synthesis must therefore be related to the catabolism of sulfur amino acids, principally methionine, which is present in higher concentrations than cysteine in caseins (*4*). As methionine can produce homocysteine by a methyl transfer, a possible route for the formation of ET3MP could be the Ehrlich degradation of this amino acid, which is well-known to occur in fermented beverages and cheese (*28, 29*). Homocysteine can undergo transamination and decarboxylation steps, leading to the corresponding aldehyde, 3-mercaptopropanal. The new-formed aldehyde can then be oxidized, depending on the redox of the cheese-ripening flora cells to the corresponding organic acid through aldehyde dehydrogenase activities, as reported for *Saccharomyces cerevisiae* (*30*) and *Oenococcus oeni* (*31*). So, we suggest that 3-mercaptopropanal can lead to 3-mercaptopropionate, which can finally undergo esterification to form ethyl-3-mercaptopropionate (**Figure 3**). This hypothetical pathway can be supported by the results of Kagli et al. (*32*) who have previously reported in *Kluyveromyces lactis* the formation of 3-methyl-mercapropionate esters like ethyl-3-methyl-mercaptopropionate from methionine. As underlined by several authors (*2, 5, 33*), some micro-organisms such as *Penicillium camemberti*, *Geotrichum candidum*, and *Brevibacterium linens* are able to produce methanethiol from methionine by an enzymatic pathway. Two pathways are likely to be involved depending on the strains. Coryneform bacteria, especially *B. linens*, are supposed to be the key producers of sulfur compounds in smear-ripened and mold-ripened cheeses such as Camembert (*5*) and other types of cheese (*34*). Moreover, the catabolic products of sulfur amino acids are known to be major contributors to the flavor of various cheeses. Low molecular weight sulfur compounds, deriving from methionine (e.g., hydrogen sulfide, methanethiol, DMDS, and DMTS) are thought to interact together and with other cheese compounds to produce a wide range of volatiles. Even though Kubickova
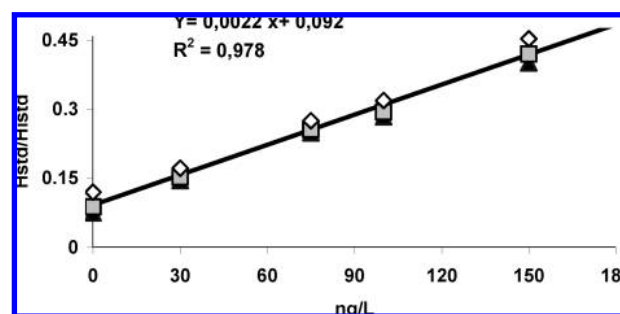

Figure 4. Quantification of ET3MP in Munster "Ermitage" according to the standard addition procedure. The ratio of the area of the ET3MP peak to that of the internal standard (H_{std}/H_{istd}) is plotted vs the added amounts of the compound.

et al. (*35*) found that polysulfides are key odorants to the sulfurous garlic notes of the odor profile of Camembert cheese, it is reasonable to assume that the contribution of thiols may also be significant. The fact that ET3MP was only found in certain types of Munster or Camembert and not in all of the samples suggests that the microbial flora in the ripening chamber must be implicated in its synthesis. In the case of Munster "Ermitage" for instance, Feurer et al. (*36*) have reported that an *Arthrobacter* strain, which is not a part of the starter flora, was the dominant species at the surface of this cheese. Hence, differences between the cheese-making environments must be taken into consideration when assessing cheese samples from the same variety.

Quantification of ET3MP and Recovery Rate. The ratio of the areas of the peaks corresponding to ET3MP and the internal standard is expressed on the graph in relation to the concentrations mentioned. **Figure 4** shows as an example the standard curve for ET3MP in Munster "Ermitage" cheese. The average concentrations for ET3MP in the surfaces of Munster cheese brand "Ermitage" and Camembert cheese brand "Jean Vernier" were 3.88 ± 0.22 and 1.67 ± 0.19 μg/kg, respectively. Meanwhile, as cheese aroma formation often begins by the surface before diffusion into the core, the quantities of ET3MP in the whole cheeses must be higher than those of the surfaces. Leclercq-Perlat et al. (*37*) have previously reported that some volatile compounds from Camembert type cheeses, for example, DMDS, seem to diffuse from surface to core. They have shown that the concentration of this volatile during ripening was almost two times higher in the surface.
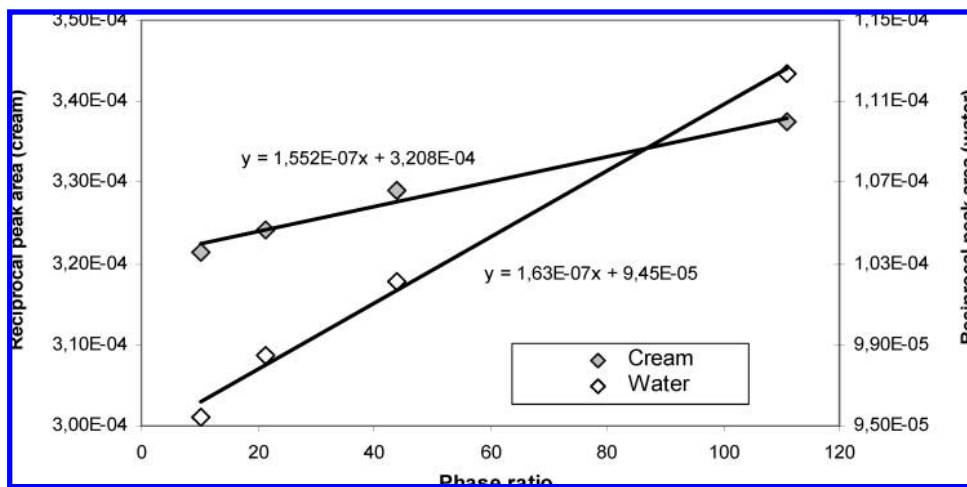
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Figure 5. ET3MP volatility calculation using the PRV method. Relationship between the reciprocal of the peak area (1/*A*) vs the phase ratio (*β*) in cream and water at 25 °C.

The recovery rate for the volatile thiol and the internal standard were over 70%, irrespective of the cheese variety.

Volatility Measurement. Using the linear equation previously described (*1*), we have plotted the reciprocal of the chromatographic areas for ET3MP vs the phase ratio β (V_g/V_l) to determine the partition coefficient in water and fresh cream (**Figure 5**). The volatility value was 1.75×10^{-3} in water and four times less in cream (4.87×10^{-4}), showing that ET3MP is more volatile in water. This result also shows that ET3MP is moderately retained in fresh cream, which is not surprising since it is slightly hydrophobic (log $P = 1.40$). The moderate retention of ET3MP in fresh cream is in agreement with the results of Gijs et al. (*38*) who found the same trend for some sulfur compounds in a lipidic medium.

Evaluation of the Sensorial Properties. The extracts of Munster cheese (brand "Ermitage") and Camembert cheese (brand "JeanVernier") were also analyzed using olfactometry to evaluate their sensorial properties. They showed many odorous zones, which received various descriptors from the judges. Odor corresponding to the peak 2 (**Figure 1**) was characterized as sulfur, animal, burnt, fruity, and pungent, which is very close to the descriptors for ethyl-2-mercaptopropionate. This odor may be indicative of the presence of ET2MP but not conclusive given that it was not possible to collect mass spectral data of this compound in our extracts. Odor descriptors for the zone including the peak 3 (**Figure 1**) were sulfur, fruity, grapy, rhubarb, and empyreumatic. These descriptors are in agreement with those commonly used to characterize ET3MP at low concentrations. Moreover, this volatile is known to be employed as a flavoring ingredient to provide pleasant Concord grape flavor and aroma notes to foodstuffs (U.S. Patent 4329372, CA Patent 1145198). Nevertheless, as all of the other thiols, ET3MP flavor characteristics depend on its concentration. Indeed, this volatile has a skunky or foxy animal-like aroma at higher concentration (*27*).

Figure 6 shows the distribution of correct answers to triangle tests function of ascending flavor concentration. Significant results are observed for concentrations higher than 900 ppt. The group perception threshold for detection of ET3MP in fresh cream, as detected by panelists (geometric mean of the individual thresholds), was 723 ppt, giving evidence of its very powerful odor. Hence, its perception threshold in water must be considerably lower than the 0.2 ppm reported by Kolor (*27*). The presence of ET3MP in Munster "Ermitage" and Camembert "Jean Vernier" at concentrations higher than its perception
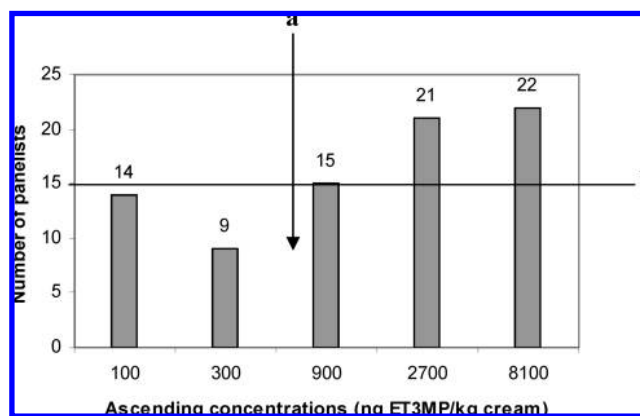

Figure 6. Number of panelists with a correct answer to triangle tests with ascending concentrations of ET3MP in fresh cream. Key: a, group BET was the geometric mean of the individual BET; b, critical number (minimum) of correct answers required for significance at the stated significance level (5%).

threshold in cream suggests that this thiol may have a significant contribution to the aroma of these cheeses.

In this study, we have demonstrated for the first time the unequivocal occurrence of a thiol in cheese, ethyl 3-mercaptopropionate. The additional experiments that were carried out to evaluate the sensorial and physicochemical properties of this volatile showed a very low perception threshold in fresh cream and a moderate affinity for fat, which can explain its successful extraction. To conclude, the promising results presented here are a first step toward a better understanding of thiols contribution to the cheese flavor. Nevertheless, identification of more hydrophobic thiols in cheese will require improvements of the extraction method. For example, the use of more apolar solvents (first step of extraction) or the use of "AFFi-Gel" (PhHgloaded Agarose gel) for purification and enrichment of volatile thiols could be tested. Further studies will also be necessary to investigate their pathway formation in cheese.

LITERATURE CITED

(1) Shankaranayana, M. L.; Raghavan, B.; Abraham, K. O.; Natarajan, C. P. Sulphur compounds in flavours. In *Food Flavours—Part A*; Morton, I. D., McLeod, A. J. Eds.; Elsevier Scientific Publishing: New York, 1982; pp 169–282.

(2) Sablé, S.; Cottenceau, G. Review: Current knowledge of soft cheeses flavor and related compounds. *J. Agric. Food Chem.* **1999**, *47*, 4825–4836.

(3) Curioni, P. M. G.; Bosset, J. O. Review: Key odorants in various cheese types as determined by gas chromatography-olfactometry. *Int. Dairy J.* **2002**, *12*, 959–984.

(4) Molimard, P.; Spinnler, H. E. Review: Compounds involved in the flavour of surface mould-ripened cheeses: Origins and properties. *J. Dairy Sci.* **1996**, *79*, 169–184.

(5) Berger, C.; Khan, J. A.; Molimard, P.; Martin, N.; Spinnler, H. E. Production of sulphur flavours by ten strains of *Geotrichum candidum*. *Appl. Environ. Microbiol.* **1999b**, *65*, 5510–5514.

(6) Vermeulen, C.; Pellaud, J.; Gijs, L.; Collin, S. Combinatorial synthesis and sensorial properties of thiols. *J. Agric. Food Chem.* **2003**, *49*, 5445–5449.

(7) Bouchilloux, P.; Darriet, P.; Dubourdieu, D. Mise au point d'une méthode de dosage de la 4-mercapto-4-méthylpentan-2-one dans les vins de Sauvignon. (Development of a method for mesuring 4-mercapto-4-methylpentan-2-one in Sauvignon wines). *J. Int. Sci. Vigne Vin*. **1996**, *30*, 23–29.

(8) Bouchilloux, P.; Darriet, P.; Henry, R.; Lavigne-Cruège, V.; Dubourdieu, D. Identification of volatile and powerful odorous thiols in Bordeaux red wine varieties. *J. Agric. Food Chem.* **1998**, *46*, 3095–3099.

(9) Darriet, P.; Tominaga, T.; Lavigne, V.; Boidron, J. N.; Dubourdieu, D. Identification of a powerful aromatic component of *Vitis vinifera* L.var. Sauvignon wines: 4-mercapto-4-methylpentan-2-one. *Flavour Fragrance J.* **1995**, *10*, 385–392.

(10) Tominaga, T.; Guimbertau, G.; Dubourdieu, D. Contribution of benzenemethanethiol to smoky aroma of certain *Vitis vinifera* L. wines. *J. Agric. Food Chem.* **2003**, *51*, 1373–1376.

(11) Tominaga, T.; Dubourdieu, D. A novel method for quantification of 2-methyl-3-furanthiol and 2-furanmethanethiol in wines made from *Vitis vinifera* grape varieties. *J. Agric. Food Chem.* **2006**, *54*, 29–33.

(12) Vermeulen, C.; Lejeune, I.; Tran, T. T. H.; Collin, S. Occurrence of thiols in fresh lager beers. *J. Agric. Food Chem.* **2006**, *54*, 5061–5068.

(13) Badings, H. T.; Stadhouders, J.; Van Duin, H. Phenolic flavor in cheese. *J. Dairy Sci.* **1968**, *51*, 31–35.

(14) Kleinhenz, J. K.; Kuo, C. J.; Harper, W. J. A method for the evaluation of thiols compounds in aged cheddar cheese. *Milk Sci. Int.* **2005**, *60*, 275–278.

(15) Kleinhenz, J. K.; Kuo, C. J.; Harper, W. J. Evaluation of thiols compounds in aged cheddar cheese: Identification. *Milk Sci. Int.* **2006**, *61*, 300–304.

(16) Reiners, J.; Grosch, W. Concentration of 4-methoxy-2-methyl-2-butanethiol in Spanish virgin oils. *Food Chem.* **1999**, *64*, 45–47.

(17) Vermeulen, C. Synthèse et caractérisation organoleptique de thiols polyfonctionnels, recherche de leur présence dans la bière. Ph.D. Thesis, Université Catholique de Louvain, 2005.

(18) Ettre, L. S.; Welter, C.; Kolb, B. Determination of gas-liquid partition coefficients by automatic equilibrium headspace-gas chromatography utilizing the phase ratio variation method. *Chromatographia* **1993**, *35*, 73–84.

(19) Athès, V.; Pena y Lillo, M.; Bernard, C.; Pérez-Correa, R.; Souchon, I. Comparison of experimental methods for measuring infinite dilution volatilities of aroma compounds in water/ethanol mixtures. *J. Agric. Food Chem.* **2004**, *52*, 2021–2027.

(20) Lawless, H. T.; Heymann, H. Measurement of sensory thresholds. In *Sensory Evaluation of Food: Principles and Practices*; Chapman and Hall, Eds.; Kluwer Academic/Plenum Publishers: New York, 1998; pp 173–207.

(21) Meilgaard, M.; Civille, G. V.; Carr, B. T. Determining thresholds. In *Sensory Evaluation Techniques*, 2nd ed.; CRC Press Inc.: Boca Raton, FL, 1991; pp 123–133.

(22) Mc Sweeney, P. L. H.; Sousa, M. J. Biochemical pathways for the production of flavour compounds in cheese during ripening: A review. *Lait*. **2000**, *80*, 293–324.

(23) Jocelyn, P. C. Spectrophotometric assay of thiols. In *Methods in Enzymology*; Jakoby, W. B., Griffith, O. W. Eds.; Academic Press: London, United Kingdom, 1987; Vol. 143, p 44.

(24) Maciel, M. L.; Hansen, T. J.; Aldinger, S. B.; Labows, J. N. Flavor chemistry of cashew apple juice. *J. Agric. Food Chem.* **1986**, *34*, 923–927.

(25) Teranishi, R.; Corse, J. W.; McFadden, W. H.; Black, D. R.; Morgan, A. I. Identification of volatile compound in strawberry oil. *J. Food Sci*. **1961**, *28*, 478–483.

(26) Tominaga, T.; Guimbertau, G.; Dubourdieu, D. Role of certain volatile thiols in the bouquet of aged champagne wines. *J. Agric. Food Chem.* **2003**, *51*, 1016–1020.

(27) Kolor, M. G. Identification of an important new flavor compound in Concord grape: Ethyl-3-mercaptopropionate. *J. Agric. Food Chem.* **1983**, *31*, 1125–1127.

(28) Bonnarme, P.; Arfi, K.; Dury, C.; Helinck, S.; Yvon, M.; Spinnler, H. E. Sulfur compound production by Geotrichum candidum from L-methionine: Importance of the transamination step. *FEMS Microbiol. Lett.* **2001**, *205*, 247–252.

(29) Bonnarme, P.; Amarita, F.; Chambellon, E.; Semon, E. C.; Helinck, S.; Spinnler, H. E.; Yvon, M. Methylthioacetaldehyde, a possible intermediate metabolite for the production of volatile sulphur compounds from L-methionine by *Lactococcus lactis*. *FEMS Microbiol. Lett.* **2004**, *236*, 85–90.

(30) Vuralhan, Z.; Morais, M. A.; Tai, S. L.; Piper, M. D.; Pronk, J. T. Identification and characterization of phenylpyruvate decarboxylase genes in *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* **2003**, *69*, 4534–4541.

(31) Pripis-Nicolau, L.; de Revel, G.; Bertrand, A.; Lonvaud-Funel, A. Methionine catabolism and production of volatile sulphur compounds by *Oenococcus oeni*. *J. Appl. Microbiol*. **2004**, *96*, 1176–1184.

(32) Kagli, D. M.; Bonnarme, P.; Neuveglise, C.; Cogan, T. M.; Casaregola, S. L-Methionine degradation pathway in *Kluyveromyces lactis*: Identification and functional analysis of the genes encoding L-methionine aminotransferase. *Appl. Environ. Microbiol.* **2006**, *72*, 3300–3335.

(33) Spinnler, H. E.; Berger, C.; Lapadatescu, X.; Bonnarme, P. Production of sulphur flavours by several yeasts of technological interest for cheese ripening. *Int. Dairy J.* **2001**, *11*, 245–252.

(34) Jollivet, N.; Bézenger, M. C.; Vayssier, Y.; Belin, J. M. Production of volatile compounds in liquid cultures by six strains of coryneform bacteria. *Appl. Microbiol. Biotechnol*. **1992**, *36*, 790–794.

(35) Kubickova, J.; Grosch, W. Evaluation of flavour compounds of Camembert cheese. *Int. Dairy J.* **1998**, *8*, 17–23.

(36) Feurer, C.; Vallaeys, T.; Corrieu, G.; Irlinger, F. Does smearing inoculum reflect the bacterial composition of the smear at the end of the ripening of a French soft, red-smear cheese. *J. Dairy Sci*. **2004**, *87*, 3189–3197.

(37) Leclerq-Perlat, M.-N.; Latrille, E.; Corrieu, G.; Spinnler, H.-E. Controlled production of Camembert-type cheeses. Part II. Changes in the concentration of the more volatile compounds. *J. Dairy Res*. **2004**, *71*, 355–366.

(38) Gijs, L.; Piraprez, G.; Perpète, P.; Spinnler, E.; Collin, S. Retention of sulfur flavours by food matrix and determination of sensorial data independent of the medium composition. *Food Chem.* **2000**, *69*, 319–330.