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Occurrence of the ribes odorant 3-sulfanyl-3-methylbutyl formate in aged beers

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ABSTRACT: 2-Sulfanyl-3-methylbutyl formate and acetate were synthesized without purification steps, quantified with a pulsed flame photometric equimolar detector, and characterized by comparison with commercially available 3-sulfanyl-3-methylbutyl formate and acetate (retention indexes, mass spectra, odour descriptors, and intensities). Both formates exhibited a typical ribes flavour, in contrast to both acetates, which were much more piquant. The sensorial threshold of 3-sulfanyl-3-methylbutyl formate was much lower (57 ng/l in beer, BE-GC-LoADS = 0.0006 ng) than those measured for the three other esters. Only 3-sulfanyl-3-methylbutyl formate was perceived at the sniffing port in beer extracts. Concentrations up to 1230 ng/l were measured in pilot beers after 1 month at 20°C, although the compounds are rarely detected in commercial beers with highly oxygen-protected bottling. Accelerated ageing in the presence of oxygen confirmed the key role of oxygen. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: beer ageing; polyfunctional thiols; ribes; formate

Introduction

For brewers, the term 'ribes' refers to a characteristic taint encountered in some aged beers.[1] It is similar to the odour of the stems and leaves of currant plants. It has been associated with certain hop cultivars and in some cases with deteriorated hops. On the other hand, according to Clapperton,[1] ribes notes arise solely because of excessive amounts of air in the headspace. More recently, the key role of oxygen was confirmed by Saison *et al.*[2] The ribes odour can develop within 4 weeks of bottling. In many beers, the note is no longer discernible after 6 months.

In blackcurrant (*Ribes nigrum*), 2-sulfanyl-2-methyl-4-methoxybutane has been recognized as the main contributor to this ribes perception.[3] Its odour threshold has been shown to be as low as 0.1 ng/l.[4] The same compound has been detected by Grosch[5] and Flath *et al.*[6] as an important contributor to the aroma of olive oil. 2-Sulfanyl-2-methyl-4-methoxy-butane and 4-sulfanyl-4-methylpentan-2-one, furthermore, have been identified as the most important contributors to the odour of Japanese green tea.[4] In beer, 2-sulfanyl-2-methyl-4-methoxybutane has never been detected.[7,8] It is even often used as an internal standard for beer thiol extraction.[9]

With the aroma extract dilution analysis technique applied to an aged beer extract, Schieberle revealed the possible relevance of 3-sulfanyl-3-methylbutyl formate to the beer ribes note.[10] Its threshold in water is assessed at 2–5 ng/l.[11] It has been detected as catty-like in coffee,[12] with a very high flavour dilution factor (FD = 2048), especially in roasted Arabica powders. Recently, Kumazawa and Masuda[13] also identified the corresponding acetate, 3-sulfanyl-3-methylbutyl acetate, in roasted Arabica coffee brews, where it may contribute to the quality of the highly roasted flavour. It has also been found in passion fruit and grapefruit.[14,15] In coffee, the levels of both acetate and formate esters appear to increase with the degree of roasting, but changes in the amount of 3-sulfanyl-3-methylbutyl formate have been observed even at lower temperature, probably because of the higher reactivity of formic acid. In headspace gas chromatography of cat urine, 3-sulfanyl-3-methylbutan-1-ol and its formate have recently been identified as candidate felinine derivatives.[16]

In a previous study dedicated to polyfunctional thiols in lager beers,[7] no sulfanyl formate was found. Only two sulfanyl acetates were found: 2-sulfanylethyl acetate and 3-sulfanylpropyl acetate, both characterized by empyreumatic descriptors. Takoi *et al.* further found 3-sulfanyl-4-methylpentyl acetate to be responsible for interesting exotic notes in Nelson Sauvin hop-derived beers.[17] Very recently, Gros *et al.* described the presence of various other sulfanyl acetates in hop, especially in the Tomahawk and Nelson Sauvin varieties: 1-sulfanyl-3-butyl acetate (plastic/sprout), 3-sulfanylbutyl acetate (cheese), 3-sulfanyl-2-methylpropyl acetate (grilled nut descriptor), 3-sulfanyl-2-ethylpropyl acetate (floral), 3-sulfanyl-2-methylbutyl acetate (cooked meat), 3-sulfanyl-3-methylbutyl acetate (pepper), 4-sulfanyl-4-methyl-2-pentyl acetate (grilled nut), 3-sulfanylpentyl acetate (peach), 1-sulfanyl-3-pentyl acetate (cheese), 3-sulfanylhexyl acetate (candy) and 3-sulfanyloctyl acetate (catty).[9] Hop proved not to contain any sulfanyl formates. Likewise, only sulfanyl acetates were found in the corresponding Tomahawk-derived beers.[9,18]

The onion-like 3-sulfanyl-3-methylbutan-1-ol and its isomer, 2-sulfanyl-3-methylbutan-1-ol, have been identified in fresh beer by gas chromatography–olfactometry (GC-O), gas chromatography–pulsed flame photometric detection (GC-PFPD) and gas chromatography–mass spectometry (GC-MS), suggesting a possible transformation to the corresponding formates through ageing. Gros *et al.*[19] have shown that these sulfanyl alcohols arise in beer

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through radical and electrophilic addition of yeast-excreted hydrogen sulfide to the hop allylic alcohol 3-methylbut-2-en-1-ol.

The aim of the present work was, first, to compare the chromatographic and sensorial properties of 3-sulfanyl-3-methylbutyl formate with those of 2-sulfanyl-3-methylbutyl formate and their corresponding acetates. Being commercially unavailable, both 2-sulfanyl derivatives were obtained by combinatorial syntheses. Second, specific *p*-hydroxymercuribenzoic acid (*p*-HMB) extracts were analysed in order to check for the occurrence of these compounds in various lager and special beers, before and after ageing. Accelerated ageing of model media was also investigated in order to assess how oxygen affects the ribes off-flavour in beer.

Experimental

Chemicals

p-Hydroxymercuribenzoic acid (*p*-HMB), thiazole, 3-sulfanyl-3-methylbutan-1-ol (compound **5** in Table 1), 3-sulfanyl-2-buten-1-ol, hydrogen sulfide, sulfuric acid, sodium hydroxide, hydrogen chloride, L-cysteine monohydrate hydrochloride, and Dowex resin 1 × 2, Cl^- form were purchased from Sigma–Aldrich (Bornem, Belgium). 3-Sulfanyl-3-methylbutyl formate (**1**) was purchased from Endeavour (Northamptonshire, UK). Dichloromethane was obtained from Romil (Cambridge, UK). 3-Sulfanyl-3-methylbutyl acetate (**2**) and 2-sulfanyl-2-methyl-4-methoxy-butane were obtained from Oxford Chemicals (Oxford, UK). Anhydrous sodium sulfate was obtained from Merck (Darmstadt, Germany), and tris(hydroxymethyl) aminomethane (Tris) from USB (Cleveland, OH, USA). Formic acid (99%) was from Acros (Geel, Belgium) and glacial acetic acid (99.7%) was from Fisher Scientific (Loughborough, UK). Sodium acetate and iron sulfide were obtained from UCB (Brussels, Belgium).

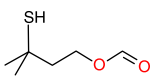
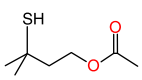
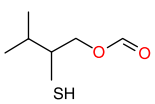
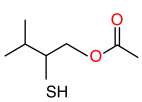
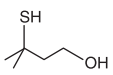
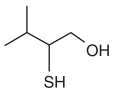
Synthesis of 2-Sulfanyl-3-methylbutanol, 2-Sulfanyl-3-methylbutyl Formate and 2-Sulfanyl-3-methylbutyl Acetate

2-Sulfanyl-3-methylbutanol (compound **6**) was synthesized according to Gros *et al.*[19] 3-Sulfanyl-2-buten-1-ol (2.5 g) was mixed with 5 ml hydrogen peroxide 30%; 95 ml acetate buffer (pH 4.2) was added. This solution was spiked with 100 mg FeS as catalyst and then was saturated with a continuous flow of hydrogen sulfide. The reaction was allowed to proceed for 3 h. Further esterification of the unpurified alcohol was performed, according to Furniss *et al.*[20] Formic acid (4.6 g, 0.1 mol) or 6 g (0.1 mol) acetic acid was mixed with 30 ml unpurified 2-sulfanyl-3-methylbutanol. Esterification was carried out for 24 or 6 h at room temperature. In the case of 2-sulfanyl-3-methylbutyl acetate (compound **4**), a few drops of concentrated sulfuric acid were cautiously added. Thiols were recovered by two successive extractions with dichloromethane and dried with anhydrous sodium sulfate. Quantification of 2-sulfanyl-3-methylbutyl formate (compound **3**) and of **4** was done with GC-PFPD (equimolar response).

Production of the pilot lager beer

Experimental beers P1 and P2 were produced from pale malt (Malterie du Château, Beloeil, Belgium) in a 50-litre-scale pilot plant (Coenco, Oostkamp, Belgium). The 15° Plato wort was diluted to 11° Plato before

Table 1. Properties of 3- and 2-sulfanyl-3-methylbutyl formates and acetates and their corresponding alcohols

Compound	Structure	Retention index		BE-GC-LoADS (ng)	Odour threshold in beer (ng/l)	Odour at the sniffing port
		CP-Sil5 CB	FFAP			
(**1**) 3-Sulfanyl-3-methylbutyl formate		1002	1518	0.0006	57	Blackcurrant, catty
(**2**) 3-Sulfanyl-3-methylbutyl acetate		1089	1557	0.3	5300	Grapefruit, fuel
(**3**) 2-Sulfanyl-3-methylbutyl formate		1024	1548	0.05	—	Blackcurrant, fruity
(**4**) 2-Sulfanyl-3-methylbutyl acetate		1100	1567	0.4	—	Vinegar
(**5**) 3-Sulfanyl-3-methylbutan-1-ol		944	1656	0.2	—	Broth, onion, sweat
(**6**) 2-Sulfanyl-3-methylbutan-1-ol		964	1652	0.06	—	Onion, sulfur

boiling. A sample of 33 mg/l of supercritical CO_2 hop extract (Tomahawk–Yakimachief, Louvain-la-Neuve, Belgium) was added at the beginning of boiling (total time = 75 min, 12° Plato after boiling) and 1.5 g/l hop pellets (Saaz/P1 or Tomahawk/P2) were added 5 min before the end. Lager yeast (strain INBR 291; Université catholique de Louvain, Louvain-la-Neuve, Belgium) was pitched at 15×10^6 cells/ml into the cooled oxygenated wort. The fermentation temperature was maintained at 12–13°C for 7 days. The fermented wort was stored for 24 h at 7°C and for 1 week at 2°C. Yeast was separated from the beer by filtration before saturation with CO_2 for 1 week (1 bar, 2°C). The beer was bottled in 250-ml flasks.

Commercial beer samples

Three commercial Belgian lager beers (L1–3) and six Belgian top-fermentation beers (T1–6) were analysed before and after storage at 20°C (for 1, 2 and 3 months) in a dark room. Additionally, L3 was spiked with oxygen (flushing with pure oxygen for 3 min, at about 100 ml/min), before accelerated ageing for 1, 2, 3 and 4 weeks at 40°C or natural ageing for 1 and 2 months (20°C).

Extraction of beer thiols by p-hydroxymercuribenzoic acid

Thiols were selectively extracted according to Tominaga *et al.*[21] Five hundred millilitres of beer spiked with 0.67 μg/l 2-sulfanyl-2-methyl-4-methoxybutane (internal standard) were extracted with 200 ml dichloromethane. The mixture was allowed to clarify for 45 min and a 30 min centrifugation at 3500 rpm was necessary before collecting the organic layer. The organic layer was extracted with 2×20 ml *p*-HMB solution (360 mg *p*-HMB and 24.6 g Tris in 1 litre of Milli-Q water). The aqueous layers were loaded onto a strongly basic anion-exchange column (Dowex resin) washed sequentially beforehand with 50 ml sodium hydroxide (2 M), 150 ml Milli-Q water, 50 ml hydrogen chloride (2 M), 150 ml Milli-Q water. After loading, the resin was washed with 50 ml sodium acetate buffer (pH 6) and thiols were eluted with 60 ml cysteine solution (640 mg L-cysteine monohydrate hydrochloride in 60 ml Milli-Q water, pH 7.5). The eluate was extracted first with 4 ml and then with 3 ml dichloromethane. The extract was then dried with anhydrous sodium sulfate. The dried extract was first concentrated to 0.5 ml in a Kuderna–Danish (thiazole as external standard; spiking before concentration with 1 ml of the 0.20 mg/l stock solution) and to 70 μl in a Dufton apparatus. This final extract was stored at −80°C for further analyses.

Gas Chromatography–Olfactometry

Experiments were performed on a Chrompack CP9001 gas chromatograph (Chrompack, Middelburg, The Netherlands) equipped with a split/splitless injector maintained at 250°C (splitless mode; split vent opened 0.5 min post-injection; split flow = 20 ml/min). Compounds were analysed with a wall-coated open tubular (WCOT) CP-Sil5-CB ($50 \text{ m} \times 0.32$ mm i.d., 1.2 μm film thickness) apolar column (Varian, Middelburg, The Netherlands) and an FFAP ($25 \text{ m} \times 0.32$ mm i.d., 0.3 μm film thickness) polar capillary column (Varian). The carrier gas was nitrogen and the pressure was set at 50 kPa (for CP-Sil5-CB) or 30 kPa (for FFAP). The oven temperature was programmed to rise from 36°C to 85°C at 20°C/min, then to 145°C at 1°C/min, and finally to 250°C (CP-Sil5-CB) or 220°C (FFAP) at 3°C/min. To assess the olfactory potential of the extract, the column was connected to a GC-O port (Chrompack) maintained at 250°C (CP-Sil5-CB) or 220°C (FFAP). The effluent was diluted with a large volume of air (20 ml/min) pre-humidified with an aqueous copper(II) sulfate solution. All extracts were analysed immediately after extraction by three trained panellists. Aroma extract dilution analysis[22] was performed by two sniffers on *p*-HMB extracts resolved on the FFAP column. The extracts were diluted stepwise (two-fold) with dichloromethane. Flavour dilution (FD) is defined as the highest dilution at which the compound could be still detected ($\text{FD} = 2^n$ with $n + 1 =$ number of dilutions applied to the extract until no odour was perceived). The precision of this aroma extract dilution analysis is $n \pm 1$ (factor of 2 between FD values). In our experiment, for the same compound, there was never a difference between the two sniffers higher than one dilution (not significant). In these cases, the higher FD has been kept.

Gas Chromatography–Mass Spectrometry

Mass spectra (*m/z* 40–380) were recorded at 70 eV on a ThermoFinigan Trace MS mass spectrometer (Rodano, Italy) connected to a ThermoFinigan Trace GC 2000 gas chromatograph (Rodano, Italy) equipped with a split/splitless injector maintained at 250°C (splitless mode; split vent opened 0.5 min post-injection; split flow = 20 ml/min) and an apolar CP-Sil5-CB capillary column or a polar FFAP column (the same as described for GC-O). The carrier gas was helium and the pressure was set at 100 kPa (for the CP-Sil5-CB column) and 50 kPa (for the FFAP column). The oven temperature program was the same as described for GC-O. Spectral recording was automatic throughout elution. Xcalibur software was used. The injection volume was 1 μl.

Gas Chromatography–High Resolution Mass Spectrometry

The apolar column described above for GC-MS was connected to a gas chromatography–high-resolution mass spectrometry (GC-HRMS) set-up from Waters (HRMS, GCT Premier, ToF; Waters, Cheshire, UK). The same chromatographic conditions were applied. Perfluorotributylamine was injected on line as a reference for accurate mass determination. Electron ionization (EI) mass spectra were recorded at 70 eV (trap current = 200 IA, and emission current = 400 IA).

Gas Chromatography–Pulsed Flame Photometric Detection

Analyses were carried out on a ThermoFinnignan Trace GC 2000 gas chromatograph equipped with a split/splitless injector maintained at 250°C (splitless mode; split vent turned on 0.5 min after injection; split flow = 20 ml/min) and connected to the O.I. Analytical PFPD, model 5380 (College Station, Texas, USA). The oven temperature program and the column were the same as described for GC-O. Two microlitres of sample were injected. The carrier gas was helium at a pressure of 90 kPa (CP-Sil5-CB). At the detector, the following parameters were selected: 250°C as the temperature, 600 V as the voltage, 18 ms as the gate width, 6 ms as the gate delay, 580 mV as the trigger level, and 3.7 Hz as the pulse frequency. For quantification, calibration curves relative to the internal standard were used.

Determination of Best-estimated–GC–Lower Amount Detected by Sniffing

One microlitre containing all four esters at known concentration was injected into the GC-O system. As described by Berger *et al.*,[23] the parameter 'best-estimated–GC–lower amount detected by sniffing' (BE-GC-LoADS) was defined as the geometric mean between the lowest mass of compound perceived at the outlet of the GC–odour port and the highest undetected amount injected onto the column. Experiments were performed with the initial solution of a compound and its sequential dilutions as follows: 1/2, 1/5, 1/10, 1/20, 1/50, 1/100, 1/200, 1/500, 1/1000, and so on. Sensorial analysis was performed by two judges working independently, and a verbal description of the odour was obtained at the same time.

Determination of Odour Threshold Values

The ascending method of limits test described by the European Brewery Convention[24] and Meilgaard *et al.*[25] was used to measure odour thresholds of compounds **1** and **2** in beer. Each tested compound was added to a fresh Belgian lager beer at six increasing concentrations. A triangle test contained two samples without spiking compound and one spiked sample. The samples were provided to 16 panellists in six three-alternative forced choice tests. For each compound, the test was repeated twice. The best estimate threshold of a panellist is the geometric mean of the highest concentration missed and the next higher concentration. The group best estimate threshold was calculated as the geometric mean of the individual best estimate thresholds.

Identification of Beer Thiols

Identification of sulfanyl esters was confirmed by comparing their GC retention indices, mass spectra, and odours with those of the standards (commercially available or synthesized compounds) on two capillary columns (CP-Sil5-CB and FFAP) and by co-injection at the GC-PFPD (CP-Sil5-CB).

Results and Discussion

Chromatographic and Sensorial Data of 2- and 3-Sulfanyl-3-methylbutyl Formates and Acetates

2-Sulfanyl-3-methylbutyl formate (compound **3**) and acetate (**4**) were obtained by combinatorial synthesis without further purification, just to be compared with commercially available 3-sulfanyl-3-methylbutyl formate (**1**) and acetate (**2**). As depicted in Figure 1, GC-HRMS allowed us to confirm the structures of **3** and **4** (calculated *m/z* for **3**, 102.0503; found, 102.0504; difference, 1.0 ppm; calculated *m/z* for **4**, 102.0503; found, 102.0506, difference, 2.9 ppm; well within the variation range of the apparatus). The chromatographic resolution proved sufficient to provide the expected chromatographic, spectrometric, and sensorial data (Table 1). With the equi-molecular PFPD detector; moreover, accurate quantification was achieved in the crude extract.

Table 1 lists the retention indexes for all four sulfanyl esters on polar and apolar columns. On both columns, each acetate ester eluted after its formate counterpart. Similarly, each 2-sulfanyl-3-methylbutyl compound was retained slightly longer than its 3-sulfanyl counterpart by both the apolar and the polar column.

By EI mass spectroscopy, the molecular ion (*m/z* = 148 and 162) proved to be small for **1** and **2** and absent for **3** and **4** (Figure 1). Except for **4**, the major ion (*m/z* = 69) was obtained through loss of both the neutral acid and the SH radical. The radical cation of the allylic thiol (*m/z* = 102) and the allylic cation *m/z* = 41 were two other major ions for the four esters. The acetyl cation (*m/z* = 43) emerged, expectedly, as a major peak for both acetic esters.

At the sniffing port, the blackcurrant descriptor emerged for both formates (Table 1), while 2- and 3-sulfanyl-3-methylbutyl acetates were perceived as much more piquant (vinegar, grapefruit).

BE-GC-LoADS values given in nanograms enabled us to compare the olfactive power of the four esters. Solutions were diluted and sniffed until the odours were not detected by GC-O. All four compounds were characterized by low threshold values (BE-GC-LoOADS from 0.0006 to 0.4 ng, compared with 1.4 ng for dimethyltrisulfide[26]). Compound **1** emerged as 500 times more potent than the corresponding acetate and approximately 100 times more odorant than its 2-sulfanyl isomer. The odour threshold determination tests applied to both commercially available compounds confirmed the extremely low flavour threshold of **1** (57 ng/l) even in beer. On the other hand, 5300 ng/l was required in order to perceive compound **2**.

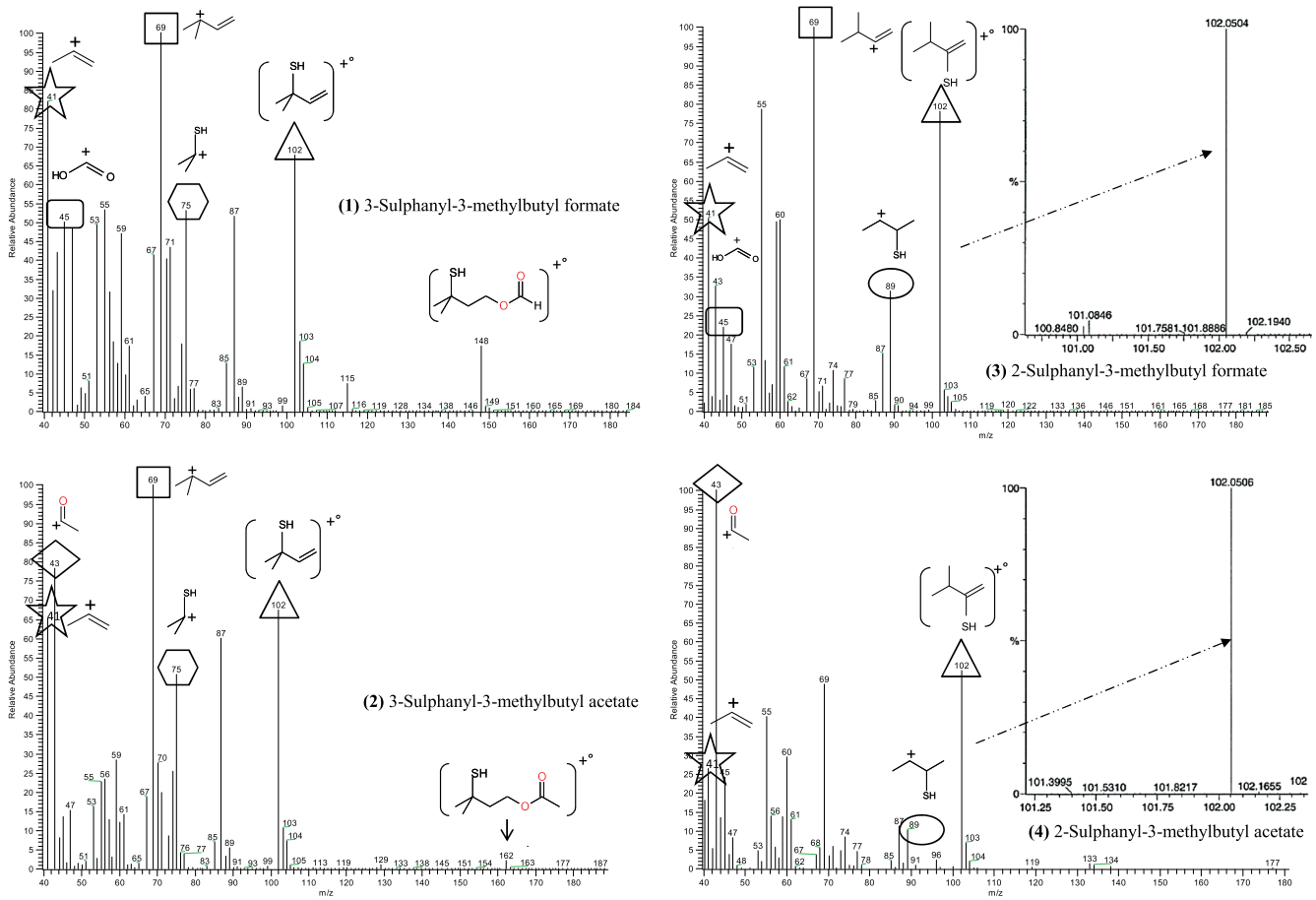


Figure 1. Mass spectra of compounds **1**, **2**, **3** and **4**. Confirmation of the structures of **3** and **4** by HRMS

Occurrence of 3-Sulfanyl-3-methylbutyl Formate and its Analogues in Commercial and Pilot Beers Before and After Ageing

Polyfunctional thiols were selectively extracted by *p*-HMB from five lager (L1–L3 and P1–P2) and six top-fermentation (T1–T6) beers and analysed by GC-PFPD (Table 2).

None of the investigated fresh commercial Belgian beers exhibited a PFPD peak at the retention time of 3-sulfanyl-3-methylbutyl formate (**1**) or acetate (**2**). Compounds **3** and **4** were also below the PFPD detection limit (7 ng/l) in all samples. Only in beer T1 was a ribes note detectable by GC-O at RI = 1002 (compound **1**, FD = 4).

Table 2. Concentration of 3-sulfanyl-3-methylbutyl formate (ng/l) in fresh and aged Belgian beers

Type of beer	Fresh	1 month, 20°C	3 months, 20°C
Commercial lager beers			
L1	—	629 (64)*	(2)
L2 and L3	—	—	—
Commercial top-fermented beers			
T1	— (4)	—	—
T2, T3, T4, T5 and T6	—	—	—
Pilot beers			
P1	—	1230 (128)	253 (32)
P2	—	768 (64)	—

The flavour dilution factor (FD) is given in parentheses.
* Sample in which compound **2** was also detected (187 ng/l, FD = 1).

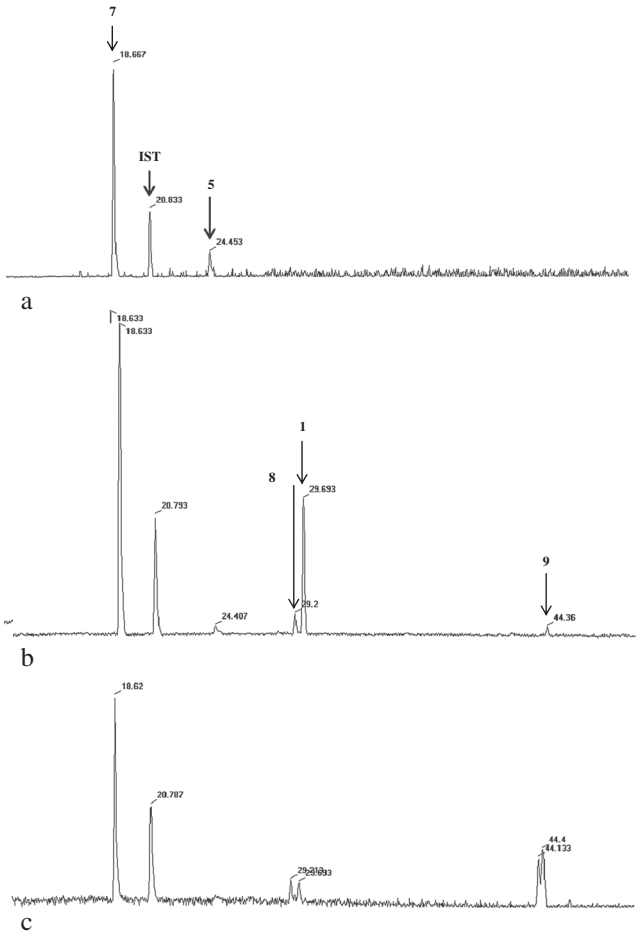


Figure 3. GC-PFPD chromatograms of *p*-HMB extracts of pilot beer P1: (a) fresh beer, (b) 1-month-aged beer, and (c) 3-month-aged beer

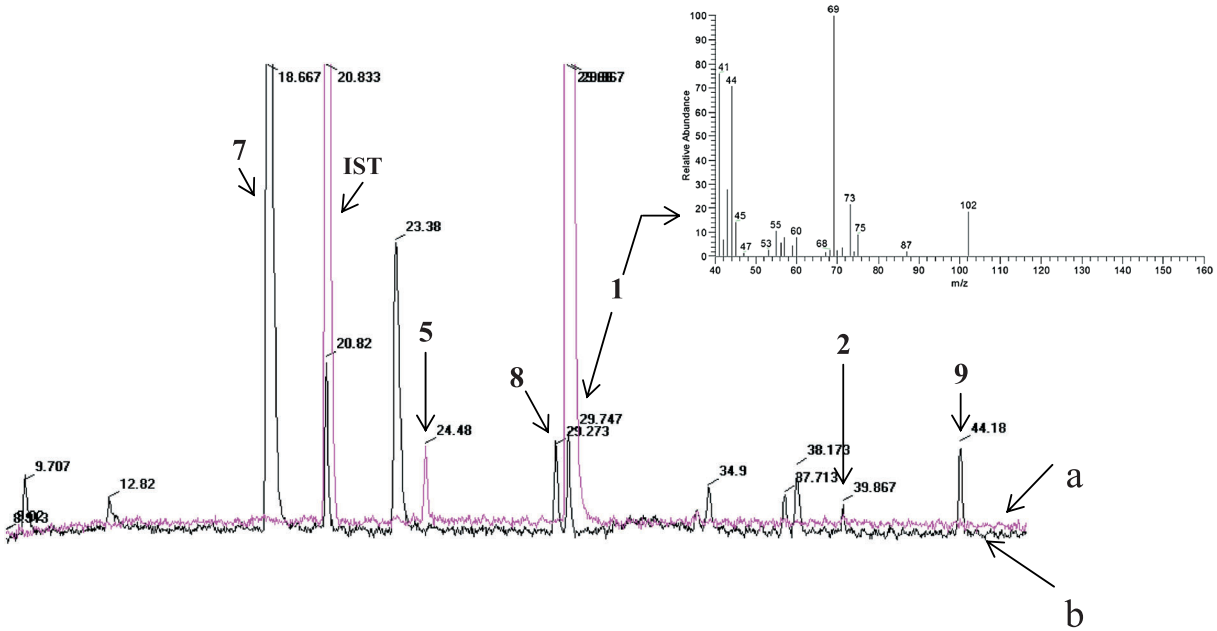


Figure 2. Superposition of the GC-PFPD chromatograms of a model mixture containing compounds **1**, **5**, and internal standard (line a) and of the *p*-HMB extract of 1-month-aged lager beer (L1) (line b). Experimental MS spectrum of **1**. Compound **7**, 2-sulfanylethyl acetate; **8**, 3-sulfanylpropyl acetate; and **9**, 3-sulfanylhexan-1-ol; IST, internal standard

Table 3. Pulsed flamed photometric detection concentrations (ng/l) and flavour dilution factor (given in parentheses) of 3-sulfanyl-3-methylbutyl formate in L3 through accelerated (40°C) and natural (20°C) ageing

Ageing temperature (°C)	Spiked with oxygen	Weeks of ageing					
		0	1	2	3	4	8
20	No	0	—	—	—	0	0
	Yes	0	—	—	—	100 (32)	UD (4)
40	No	0	0	0	0	0	—
	Yes	0	70 (32)	UD (2)	UD (0)	UD (0)	—

Assays were carried out in duplicate.
UD, undetected.

After a very short natural ageing period (4 weeks at 20°C), one commercial lager beer (L1) displayed the presence of **1** and **2** together (Figure 2 and Table 2), at 629 ng/l and 187 ng/l, respectively. With a concentration well above its odour threshold in beer (57 ng/l), **1** was expectedly strongly perceived at the sniffing port (FD = 64), while **2** was detected much more weakly (FD = 1). After 2–3 months, **1** and **2** were again undetectable by PFPD but the former was still perceived at the sniffing port (FD = 2). As in filtered lager beers no yeast survives in the bottle, the biosynthesis[27,28] of esters by yeast cannot explain the present results. These compounds were not observed in any of the aged commercial top-fermented beers, whatever the length of the ageing period (1, 2, 3 or 6 months).

The highest concentrations of **1** were found in the two pilot beers after 1 month of ageing, with values up to 1230 ng/l. Results for beer P1 are shown in Figure 3b, while values for both pilot beers are given in Table 2. After 3 months (Figure 3c), **1** was found to have decreased to 253 ng/l. According to Clapperton[1] and Saison,[2] the ribes defect can be linked to the presence of a higher level of oxygen in the bottle. Our two pilot beers were less oxygen-protected, as they were not bottled industrially.

To confirm the key role of oxidation, L3 was spiked with oxygen before natural ageing (for 1 and 2 months) at 20°C or accelerated ageing (for 1, 2, 3 and 4 weeks) at 40°C (Table 3). Compound **1** was quantifiable by GC-PFPD only in oxygenated samples aged naturally for 1 month and in oxygenated samples subjected to accelerated ageing for 1 week (100 ng/l and 70 ng/l, respectively), dropping drastically thereafter. In the control samples (not flushed with oxygen), **1** was absent at all times. The GC-O data confirmed that the ribes note peaked at 1 month in naturally aged samples and at 1 week in samples subjected to accelerated ageing.

Conclusion

3-Sulfanyl-3-methylbutyl formate and 2-sulfanyl-3-methylbutyl formate both exhibit a typical ribes flavour, unlike their corresponding acetates. Only 3-sulfanyl-3-methylbutyl formate is detected in beer, together with the grapefruit-like acetate (**2**). Their occurrence in industrially bottled beers seems rare, even after ageing. In the presence of oxygen, 3-sulfanyl-3-methylbutyl formate can be detected at concentrations above its threshold after 1 month at 20°C, but it drops quickly thereafter to a very low level by the end of month 3.

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