

Scent of a mite: origin and chemical characterization of the lemon-like flavor of mite-ripened cheeses

Adrian Brückner¹ · Michael Heethoff¹

Received: 14 December 2015 / Accepted: 31 March 2016
© Springer International Publishing Switzerland 2016

Abstract Cheese infested with cheese mites is usually treated as unpalatable. Nevertheless, some traditional cheese manufactories in Germany and France intentionally use mites for fermentation of special varieties (i.e. Milbenkäse and Mimolette). While their production includes different mite species, both are characterized by a “lemon-like” flavor. However, the chemical nature and origin of this flavor-component is unknown. The cheese mites possess a pair of opisthosomal glands producing blends of hydrocarbons, terpenes and aromatics. Here, we describe the chemical profiles of the astigmatid mite species *Tyrolichus casei* (Milbenkäse) and *Acarus siro* (Mimolette). Although the chemical profiles differ in several aspects, both mite species produce neral (a volatile flavor component of lemon oil), which was absent from the headspace of both cheeses without mites. We conclude that the lemon-like flavor of mite cheese is not a consequence of fermentation of the cheese itself but a component from secretions of the cheese mites.

Keywords Cheesemaking · Würchwitzer Milbenkäse · Boule de Lille · Acari · Astigmata

Introduction

Numerous astigmatid mite species (Acari, Astigmata) are widespread vermin invading many kinds of stored food (Krantz and Walter 2009). Furthermore, they are one of the most important allergenic sources causing various syndromes e.g. rhinitis, atopic dermatitis and the oral mite anaphylaxis (=pancake syndrome; Sanchez-Borges and Fernandez-Caldas

✉ Michael Heethoff
heethoff@bio.tu-darmstadt.de

Adrian Brückner
brueckner@bio.tu-darmstadt.de

¹ Ecological Networks, Department of Biology, Darmstadt University of Technology, Schnittspahnstraße 3, 64287 Darmstadt, Germany

2015). While generally being characterized as pest species on cheese, they have also been used as beneficial organisms for fermentation in some traditional cheese manufactories in Germany (“Würchwitz Milbenkäse”) and France (“Mimolette”) for centuries (e.g. Hase 1929; Hughes 1959; Melnyk et al. 2010). In Würchwitz, cheese mites have become so famous that they are honored with a memorial (Alberti 2004), and a piece of Milbenkäse with its inhabitants has even travelled to the International Space Station (ISS) in 2004. Milbenkäse and Mimolette are popular among gourmands all over the world. However, in 2013, the US Food and Drug Administration (FDA) refused the import of 1.5 tonnes of Mimolette as being a “filthy, putrid, or decomposed substance” [Barry, 06-Aug-2013, <http://www.foodqualitynews.com/Industry-news/Mimolette-mite-be-blocked-if-levels-are-too-high-says-FDA>; Sanchez-Borges and Fernandez-Caldas (2015)], thereby provoking national and international protests. In an editorial, the Washington Times (June 26, 2013) stated “The nannies at the FDA should cut a slice of Mimolette, brush off the bugs, pour a glass of Kendall-Jackson pinot noir 2009, break out a package of crackers and leave the rest of us alone. And let the rest of the Mimolette, mites and all, out of jail.” Meanwhile, Mimolette has become available in the USA again, especially because no medical implications have been reported due to the consumption of mite ripened cheeses (Sanchez-Borges and Fernandez-Caldas 2015).

Mimolette is produced from pasteurized bovine milk with the addition of Annatto (grain of achiote, *Bixa orellana*) to gain an orange appearance. It ripens between 2 and 24 months. During the ripening, cheese mites feed on the crust and produce microscopic holes through which the cheese can breathe. Additionally, the excreta of the mites support the ripening process. The cheese mites are brushed from the crust before shipment, but a number of living specimens remains and may reproduce and increase in number when stored at home (own observation).

On the other hand, Milbenkäse is produced from dried low fat curd cheese containing salt, caraway and a number of herbs. The young cheese ripens in wooden boxes containing the cheese mites. Rye flour is added as food for the mites to prevent them from feeding on the cheese itself; the digestive juices support the fermentation. After ripening between 1 and 12 months, the cheese is consumed together with the living mites (about 2000 individuals/cm²).

Both cheeses are characterized by a fruity, lemon-like aroma (e.g. www.cheese.com; www.milbenkasese.de; Sanchez-Borges and Fernandez-Caldas 2015), which has been reported already in the late nineteenth century for the Milbenkäse [Küchenmeister and Zürn (1881), cited in Hase (1929)]. This typical lemon-like flavor is, however, absent when the cheeses are consumed without the mites. Mimolette has a salty, “cheddar-like” taste and Würchwitz Milbenkäse is characterized by a caraway flavoring. The chemical basis of this taste and the role of the cheese mites for its production remain unknown (Melnyk et al. 2010). In a recent study, Melnyk et al. (2010) demonstrated that Milbenkäse and Mimolette are infested by two different astigmatid mite species, *Tyrolichus casei* Oudemans and *Acarus siro* L., respectively (Fig. 1).

Astigmata belong to the glandulate Oribatida (Norton 1998; Rasputnig 2010) which are characterized by an evolutionary old (at least of Silurian origin; Schaefer et al. 2010) pair of opisthosomal exocrine glands. These glands produce blends composed mostly of hydrocarbons, mono-, sesqui- and diterpenes, aldehydes, ketones, fatty acids, fatty acid esters, aromatic compounds and alkaloids (Kuwahara 2004; Rasputnig et al. 2011). Although the gland chemistry of numerous astigmatid mites has been studied (Kuwahara 2004), secretions of *T. casei* remain to be uncovered. For *A. siro*, headspace analyses of mite-infested food (Curtis et al. 1981; Tuma et al. 1990) and some data from diethyl ether

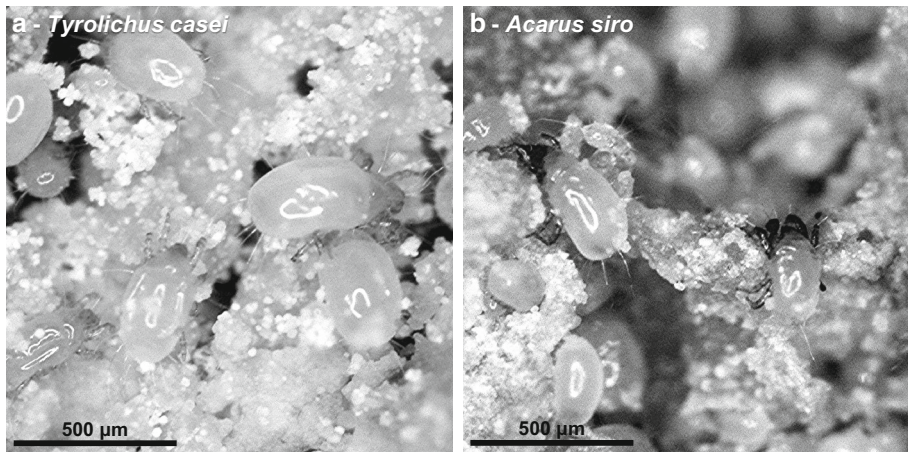


Fig. 1 Different cheese mite species on corresponding cheese. **a** *Tyrolichus casei* on Milbenkäse from Germany, **b** *Acarus siro* on Mimolette from France

extracts of the mites are available (Curtis et al. 1981). However, the results of both studies are controversial: Curtis et al. (1981) reported decane, undecane, tridecane (5) and perillen (a furanoid terpene) from the headspace, and 2-hydroxy-6-methyl-benzaldehyde (2,6-HMBD; 3) from diethyl ether raw extracts; but none of these compounds could explain the “minty smell”. Tuma et al. (1990) on the other hand, found tridecane (5), neral (4) and geranial in the headspace of mite-infested bin-stored wheat, but did not report 2,6-HMBD (3), perillen or other hydrocarbons. Citral [neral (4)/geranial], however, would explain the lemon-like smell/taste of the cheeses and is a common compound in gland secretions of Astigmata (Kuwahara 2004).

Here, we analyzed raw hexane-extracts of the gland secretions from *T. casei* reared on Milbenkäse and *A. siro* reared on Mimolette, as well as the headspace of Milbenkäse and Mimolette—after the removal of mites—with gas chromatography coupled with mass spectrometry (GC/MS) and show that both species have, among others, tridecane (5) and neral (4)—one of the main volatile compounds of lemon oil (Schieberle and Grosch 1998)—as main compounds. While tridecane (5) was also a minor compound in the headspace of both cheeses, neral (4) was completely absent. Hence, we conclude that the characteristic lemon-like taste of mite-cheese is in fact a component of the cheese mite exocrine glands rather than a characteristic of the cheese itself.

Materials and methods

Mite species and extraction of oil glands

German “Würchwitzer Milbenkäse” and French “Mimolette” were purchased from Schmelzer & Pöschel (Würchwitz, Germany) and Emilia (Dortmund, Germany), respectively. Milbenkäse is ripened by *Tyrolichus casei* and Mimolette by *Acarus siro* (Melnik et al. 2010). Oil gland compounds were extracted by submersion of clean, cheese-free and living adult individuals in hexane for 5 min—a common procedure for the analysis of oil gland secretions (e.g. Heethoff and Rasputnig 2011). Generally, pooled extracts of 5–20

individuals in 50 μ l hexane were prepared for qualitative analysis. For quantitative analysis several extracts of 5 individuals pooled in 20 μ l hexane with tetradecane (1 ng/ μ l) as internal standard were prepared.

Chemical analysis (GC/MS) of mite extracts

Oil gland secretion samples were analyzed with a QP2010 Ultra GC/MS (Shimadzu, Duisburg, Germany). The gas chromatograph (GC) was equipped with a ZB-5MS fused silica capillary column (30 m $\times$ 0.25 mm ID, df = 0.25 μ m) from Phenomenex (Aschaffenburg, Germany). 5 μ l sample aliquots were injected by using an AOC-20i autosampler-system from Shimadzu, Duisburg, Germany into a PTV-split/splitless-injector (Optic 4, ATAS GL, Eindhoven, Netherlands), which operated in splitless-mode. PTV-injection-temperature was programmed from initial 50 up to 250 $^{\circ}$ C (heating-rate of 5 $^{\circ}$ C/s) and then an isothermal hold until the end of the GC run (37 min), sampling-time was set to 3 min. Hydrogen was used as carrier-gas with a constant flow rate of 3.05 ml/min. The temperature of the GC oven was raised from initial 50 $^{\circ}$ C for 5 min, to 250 $^{\circ}$ C with a heating-rate of 8 $^{\circ}$ C/min, to 320 $^{\circ}$ C with a heating-rate of 25 $^{\circ}$ C/min and then an isothermal hold at 320 $^{\circ}$ C for 5 min. Electron ionization mass spectra were recorded at 70 eV with a scan rate of 2 scans/s from m/z 40 to 240. The ion source of the mass spectrometer and the transfer line were kept at 250 $^{\circ}$ C. Gas chromatographic retention indices (RI) for compound identification were calculated using an alkane standard mixture (C8-C33 dissolved in hexane) and the method described by Van den Dool and Kratz (1963).

Headspace sampling and analysis

Volatiles of Milbenkäse and Mimolette were sampled by putting activated charcoal (granulated 1.5–3.0 mm, Applichem, Darmstadt, Germany) along with a cheese sample in a clean, inert plastic box for 72 h. Subsequently samples for GC/MS analysis were prepared by submersion of charcoal pieces (about 100 mg per cheese) in 300 μ l dichloromethane for 15 min. Crude extracts were analyzed according to the procedure described above, only the acquisition method of the MS was slightly modified: Electron ionization mass spectra were recorded at 70 eV ion source voltage with a scan rate of 2 scans/s from m/z 40 to 450. Substances and substance classes in the headspace samples were tentatively assigned and identified by comparison of mass spectra with spectra from literature (e.g. Stein 2015) and mass spectrometric fragmentation patterns.

Reference compounds

Authentic standards of oribatid oil gland origin, which were known from previous studies, were used for comparison of chromatographic profiles. The oil gland secretion of *Oribotritia berlesei* was used as a natural source for 6,9-heptadecadiene (8) and 8-heptadecene (9) (Rasputnig et al. 2008). Furthermore, secretions of the model oribatid mite species *Archegozetes longisetosus* ran (laboratory strain from Roy A. Norton =ran; Heethoff et al. 2013), which were described by Sakata and Norton (2003) and more detailed by Heethoff and Rasputnig (2011), were used as references for the further characterization of the “astigmatid compounds” (Sakata and Norton 2001). Additional synthetic reference compounds such as 2-phenylacetaldehyde (1) was purchased from VWR International

(Darmstadt, Germany), several alkanes (**5**, **7**) and the alkane standard mixture were purchased from Lactan (Graz, Austria).

Results

Identification of detected gland compounds

In total, nine chromatographically separable substances were recorded in the two astigmatic mite species investigated (Fig. 2). All substances belonged to three different chemical substance-classes: saturated and unsaturated n-hydrocarbons, aromatic compounds and monoterpenes (analytical data are summarized in Table 1).

Saturated and unsaturated n-hydrocarbons: peaks 5, 7, 8 and 9 (Fig. 2)

The EI-mass spectra of peaks **5** and **7** showed the typical fragmentation pattern of n-alkanes with characteristic series of C_nH_{2n+1} -ions and the molecular ions at m/z 184 (tridecane; **5**) and 212 (pentadecane; **7**), accordingly. The identities of the substances were affirmed by a comparison to an alkane standard mixture. Peak **9** showed a series of C_nH_{2n-1} -ions indicating monounsaturated hydrocarbons and the molecular ion at m/z 238 which is consistent with heptadecene. Peak **8** represented an alkadiene with diagnostic ions at m/z 95, 81 and 65 as well as the molecular ion at m/z 236, corresponding to heptadecadiene. Peak **9** was identified as 8-heptadecene (**9**) and peak **8** was identified as 6, 9-heptadecadiene (**8**) by comparison of retention times to authentic compounds from the secretion of *O. berlesei* (Rasputnig et al. 2008). The notations of absolute stereochemistry of double bonds in (Z)-8-heptadecene (**9**) and (Z,Z)-6,9-heptadecadiene (**8**) were presumed based on previous studies (Kuwahara 2004).

Aromatic compounds: peaks 1 and 3 (Fig. 2)

The EI-mass spectrum of peak **1** showed the base ion at $m/z = 91$, the molecular ion at $m/z = 120$ and diagnostic ions at $m/z = 77$, 65, 63, 53 and 51 indicating an alkyl benzene compound. Furthermore, $m/z = 92$ indicates a benzyl compound with a γ -hydrogen and low $m/z = 120$ compared to $m/z = 91$ from decarbonylation. Hence, peak **1** was identified as 2-phenylacetaldehyde (hyacinthin; **1**) by a comparison of retention time and mass spectrum of a synthetic standard and by comparison of literature mass spectra [NIST (Stein 2015)]. The EI-mass spectrum of peak **3** showed a typical fragmentation pattern of an aromatic compound with the molecular ion at $m/z = 136$. The identity of the substances was affirmed as 2-hydrox-6-methyl-benzaldehyde (2,6-HMBD; **3**) by comparison of mass spectrum and retention index to secretion of *A. longisetosus* (Heethoff and Rasputnig 2011; Sakata and Norton 2003).

Monoterpenes: peaks 2, 4 and 6 (Fig. 2)

The EI-mass spectrum of peak **2** showed a mass spectrum with the molecular and base ion at $m/z = 150$ and was confirmed as a monoterpene with three conjugated double bonds. Due to the atypical fragmentation pattern, a comparison to literature mass spectra (Hiraoka et al. 2001) confirmed the peak as (4E)-3,7-dimethylocta-2,4,6-trienal ((4E)-dehydrocitril, **2**),

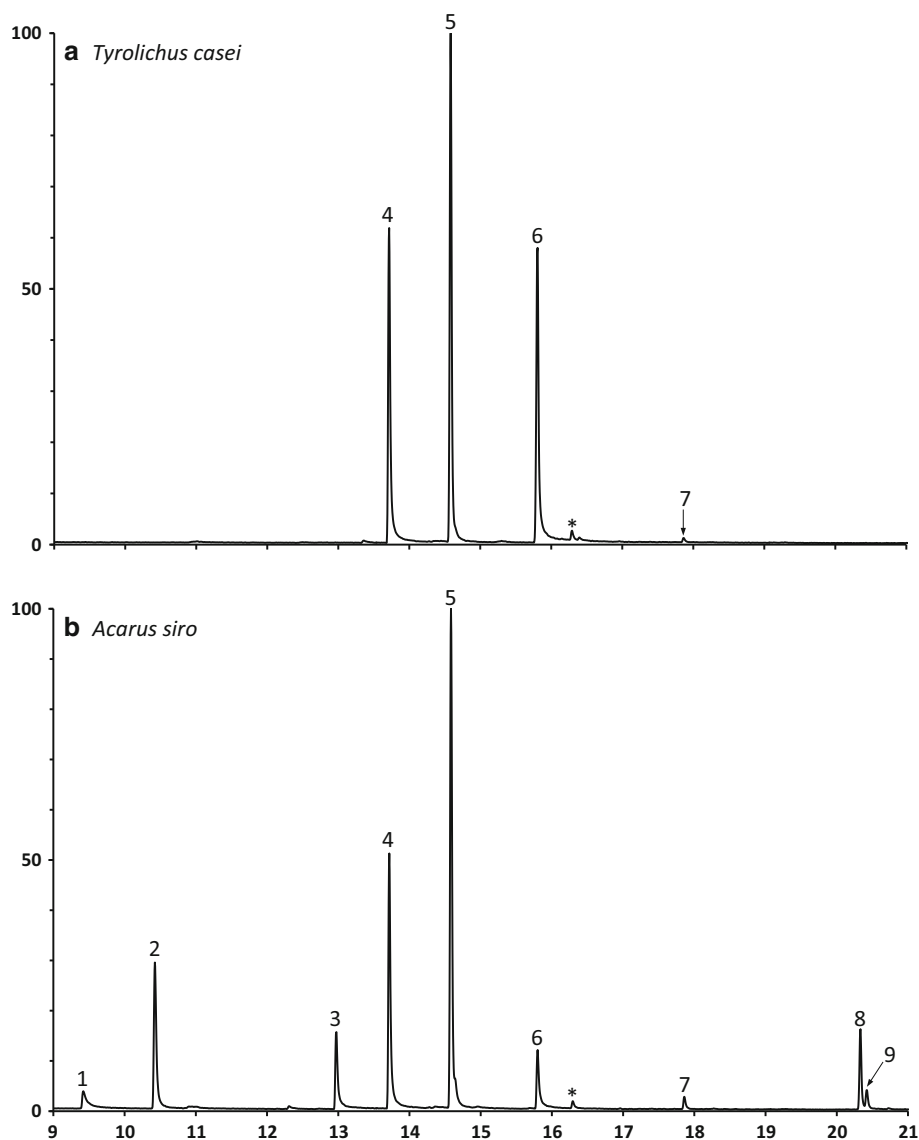


Fig. 2 Chromatographic profiles from extracts of different cheese mite species. Profile **a** from *Tyrollichus casei* from German “Milbenkäse” and profile **b** from *Acarus siro* from French “Mimolette”. Compound identity: *peak 1*—2-Phenylacetaldehyde (Hyacinthin), *peak 2*—(E)-3,7-Dimethyl-2,4,6-octatrienal ((4E)-Dehydrocitril), *peak 3*—2-Hydroxy-6-methylbenzaldehyde (2,6-HMBD), *peak 4*—(Z)-3,7-Dimethylocta-2,6-dienal (Neral), *peak 5*—Tridecane, *peak 6*—2-(4-Methyl-3-pentenylidene)-butanedial (β -Acaridial), *peak 7*—Pentadecane, *peak 8*—(Z,Z)-6,9-Heptadecadien, *peak 9*—(Z)-8-Heptadecene

known from other Astigmata (Kuwahara, 2004). The geometry of the second stereogenic center of **2** was not determined. Peak **4** showed typical monoterpene fragmentations and the molecular ion at $m/z = 152$, and was identified as neral by comparison of mass spectrum and retention index to secretion of *A. longisetosus* (Heethoff and Rasputnig 2011; Sakata

Table 1 Analytical data to compounds extracted from *Tyrolichus casei* and *Acarus siro*

Peak ID	RI ^a	Mass spectrometric fragmentation (<i>m/z</i>)	Identified as	Chemical structure
1 ^b	1022	120 (<i>M</i> ⁺ 17), 104 (2), 92 (33), 91 (100), 89 (6), 77 (3), 65 (28), 63 (10), 57 (2), 51 (9), 50 (5), 43 (6), 41 (9)	2-Phenylacetaldehyde (<i>Hyacinthin</i>)	
2	1098	150 (<i>M</i> ⁺ 100), 135 (87), 109 (7), 108 (12), 107 (40), 105 (23), 95 (44), 91 (67), 82 (30), 79 (58), 77 (30), 55 (24), 41 (79)	(<i>E</i>)-3,7-Dimethyl-2,4,6-octatrienal ((<i>4E</i>)- <i>Dehydrocitral</i>)	
3	1215	136 (<i>M</i> ⁺ 100), 135 (90), 118 (12), 107 (15), 90 (27), 89 (16), 79 (31), 77 (47), 63 (12), 53 (16), 51 (22)	2-Hydroxy-6-methylbenzaldehyde (<i>2,6-HMBD</i>)	
4	1255	152 (<i>M</i> ⁺ < 1), 134 (11), 119 (19), 109 (9), 94 (17), 91 (31), 84 (11), 79 (21), 77 (17), 69 (50), 67 (17), 59 (10), 53 (16), 43 (18), 41 (100)	(<i>Z</i>)-3,7-Dimethylocta-2,6-dienal (<i>Neral</i>)	
5	1300	184 (<i>M</i> ⁺ 1), 141 (< 1), 127 (2), 113 (2), 99 (5), 85 (28), 71 (53), 57 (100), 43 (96)	Tridecane (<i>C</i> -13)	
6 ^{b,c}	1371	166 (<i>M</i> ⁺ 1), 151 (6), 148 (11), 137 (7), 133 (18), 123 (13), 119 (20), 108 (12), 105 (26), 95 (25), 91 (37), 81 (22), 79 (39), 77 (30), 69 (28), 67 (55), 55 (34), 53 (35), 43 (38), 41 (100)	2-(4-Methyl-3-pentenylidene)-butanedial ("β-Acaridial")	
7	1500	212 (<i>M</i> ⁺ 1), 169 (< 1), 155 (1), 141 (1), 127 (2), 113 (4), 99 (7), 85 (29), 71 (55), 57 (100), 43 (93)	Pentadecane (<i>C</i> -15)	
8 ^d	1674	236 (<i>M</i> ⁺ 1), 165 (1), 152 (1), 138 (5), 124 (8), 110 (18), 95 (42), 81 (66), 67 (98), 55 (65), 41 (100)	(<i>Z,Z</i>)-6,9-Heptadecadien (<i>C</i> -17:2, Δ6,9) ^d	
9 ^d	1680	238 (<i>M</i> ⁺ 5), 205 (2), 125 (6), 111 (13), 97 (30), 83 (48), 69 (55), 55 (75), 41 (100)	(<i>Z</i>)-8-Heptadecene (<i>C</i> 17:1, Δ8) ^d	

^a Retention index (RI) on ZB-5MS column^b Only occurring in females of *A. siro*^c Tentatively assigned compound^d Notations of absolute stereochemistry of double bonds were presumed based on previous studies, see Kuwahara (2004)

and Norton 2003). A comparison of the mass spectrum of Peak 6 to literature mass spectra of other Astigmata (Leal et al. 1989; Shimizu et al. 2003) and an additional library search in the Wiley RegistryTM of Mass spectral data (9th edition) confirmed 6 to be most likely “β-acaridial”. The identity of the compound was tentatively assigned and geometry of the stereogenic center was not determined.

Description of chromatographic secretion profiles

The arithmetic mean of absolute amounts of gland secretions per individual (calculated via comparisons with the internal standard) were 8.5 (±4.8) ng in *T. casei* and 14.8 (±4.4) ng in *A. siro* which significantly differed from each other (two-sample *t* test₂₃ = 3.29; *p* = 0.003). Furthermore, chromatographic profiles of *T. casei* and *A. siro* were highly distinguishable (Fig. 2) and significantly differed from each other as well (one-way PERMANOVA; Jaccard-distance; pseudoF = 53.78; *p* < 0.001). All profiles of the two

species were clearly dominated by tridecane as main compound, constituting about 58 % of total secretion.

Tyrolichus casei: compounds 4, 5 and 6, (7)

All extracts of *T. casei* consistently showed four different compounds (Fig. 2a). The saturated n-alkane tridecane (**5**) was the main compound (60.7 ± 14.0 %), while the monoterpenes neral (22.0 ± 7.0 %; **4**) and “ β -acaridial” (17.0 ± 11.5 %; **6**) were minor compounds in more or less the same ratio. Pentadecane (**7**) was detected in traces (<0.3 %).

Acarus siro: compounds 1–9

Extracts of *A. siro* showed nine different compounds (Fig. 2b) of which two, namely hyacinthin (**1**) and β -(*E*)-acaridial (**6**), occurred only in females (data not shown). The saturated n-alkane tridecane (**5**) was the main compound (55.3 ± 15.2 %), while the monoterpene neral (19.1 ± 7.4 %; **4**) was a medium compound. Minor substances were the saturated and unsaturated n-hydrocarbons pentadecane (1.4 ± 0.8 %; **7**), (*Z,Z*)-6,9-heptadecadiene (7.2 ± 8.0 %; **8**) and (*Z*)-8-heptadecane (1.7 ± 1.8 %; **9**), the monoterpenes dehydrocitral (7.9 ± 3.8 %; **2**) and “ β -acaridial” (1.4 ± 1.7 %; **6**) as well as the aromatics hyacinthin (1.1 ± 1.3 %; **1**) and 2,6-HMBD (4.9 ± 2.7 %; **3**).

Evaluation of headspace samples

There were no indications of neral (**4**) in the headspace of Würchwitzer Milbenkäse or Mimolette (Fig. 3), but several other compounds were found: Peak ranges indicated as **I** and **V** were identified as fractions of different unsaturated and saturated ketones with eight and nine carbon atoms, respectively. They indicated typical diagnostic ions at $m/z = 43$ and $m/z = 58$ (in saturated) and further fragments at $m/z = 55$ (in unsaturated) as well as the molecular ions at $m/z = 128$ and $m/z = 126$ (for ketones with eight carbon atoms) and at $m/z = 142$ and $m/z = 140$ (for ketones with nine carbon atoms). The MS fragmentation patterns of mass spectra of the ketone fractions and a comparison with spectra from the NIST library (Stein 2015) indicated different methylketones and not further determined unsaturated (methyl)-ketones. Peak **IX** showed typical diagnostic ions at $m/z = 43$ and $m/z = 58$ and the molecular ion at $m/z = 170$ and was identified as 2-undecanone based on the MS fragmentation and data from Raspotnig et al. (2005). Alkanes (peak **III** and **VIII**) were identified due to their typical fragmentation pattern of C_nH_{2n+1} -ions-series and the molecular ions at m/z 170 (dodecane; **III**) and 184 (tridecane; **VIII**). Peaks **II**, **VI** and **VII** were identified as a monoterpene and two monoterpene-ketones. Peak **II** showed a molecular ion at $m/z = 136$, a base ion at $m/z = 68$ and typical fragment ions at $m/z = 121$, 107, 93, 79 and 67. Furthermore, fragmentation patterns of peak **II** were compared to literature data (Adams 1995; Brückner et al. 2015) and assigned to limonene. Since limonene in the headspace originates from the added caraway (in the “Milbenkäse”) it is most likely R-limonene, because S-limonene is not present in the essential oil of caraway (Bouwmeester et al. 1998). Peak ranges indicated as **VI** were identified as dihydrocarvones based on their fragmentation pattern with a molecular ion at $m/z = 152$, a base ion at $m/z = 95$ (or $m/z = 67$), a typical ion series at $m/z = 137$, 123, 109, 95, and 82 and comparison with mass spectra from the NIST library (Stein 2015). Peak **VII** was assigned to be (S)-(+)-carvone by using a molecular ion $m/z = 150$, a base

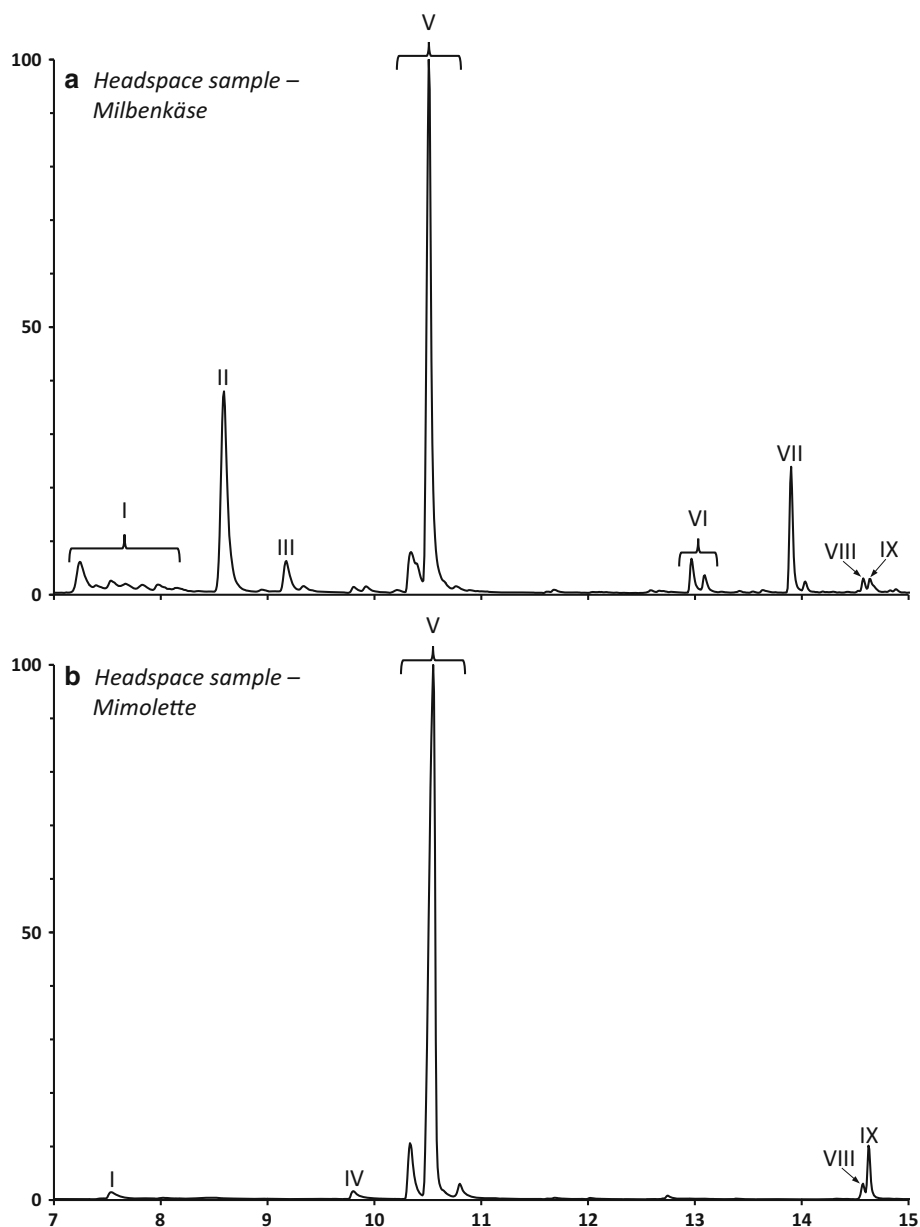


Fig. 3 Chromatographic profiles of headspace samples of cheese volatiles (**a** Milbenkäse; **b** Mimolette). In the order of retention times: *peak ranges I*—fraction of saturated and unsaturated C8-ketones, *II*—limonene, *III*—dodecane, *IV*—unknown ($m/z = 168$), *V*—fraction of saturated and unsaturated C9-ketones, *VI*—fraction of isomeric dihydrocarvones, *VII*—(S)-(+)-carvone, *VIII*—tridecane, *IX*—2-undecanone

ion at $m/z = 82$ and typical fragment ions at $m/z = 54, 67, 93$ and 108 . As well as by comparison with literature (Adams 1995) and because it is the main compound of caraway (Bouwmeester et al. 1995) which is added to “Milbenkäse”. Peak IV is an unidentified trace compound with a molecular ion at $m/z = 168$.

Discussion

Oil gland secretions from six other species from the genus *Tyrophagus* (*Tyrophagus casei* is a synonym of *Tyrolichus casei*) have already been chemically characterized (reviewed in Kuwahara 2004). Four species produced neral (4) in their oil gland secretions and two (*T. neiswanderi* and *T. longior*) did not. Hence, neral (4) seems widespread but not characteristic in this genus. The same pattern seems valid for the genus *Acarus*, although only one further species (*A. immobilis*) has been investigated (Kuwahara 2004). Here, neral (4) was absent from the secretions. Nevertheless, neral (4) is commonly distributed in about one third of all Astigmata so far investigated (Tagami and Kuwahara 2005), while its geometrical isomer geranial, which also has a lemon-like odor, seems to be more or less restricted to the family of the Histiotomatidae (and two further species) in Astigmata (Koller et al. 2012). (4E)-Dehydrocitral is a known compound from astigmatid mites, at least in *Histiogaster* sp. (Hiraoka et al. 2001), and as a part of the essential oil from some plants (Kumamoto et al. 1975; Asfaw et al. 1999). Hyacinthin (1) is a compound not yet known from astigmatid mites, it has been found in insects, as gland substance / attractor (Meagher and Mitchell 1999; Prokopy et al. 2001; Wood et al. 2002; Otienoburu et al. 2012). In addition, hyacinthin (1) is a volatile from different fruits, wine and roasted peanuts (Didzbalis et al. 2004; Wu et al. 2005; Escudero et al. 2007), and also known from cheese (Qian and Reineccius 2003; Whetstone et al. 2005). However, hyacinthin (1) was assigned to be of oil gland origin from *A. siro*, because headspace samples of corresponding cheese showed no hyacinthin (1) (Fig. 3). As stated above, hyacinthin (1) and “ β -acaridial” (6) were only present in female mite specimens of *A. siro*. A chemical sexual dimorphism is known for β -(E)-acaridial from the species *Caloglyphus polyphyllae* (Astigmata, Acaridae), where it functions as sex and aggregation pheromone (Shimizu et al. 2001). Hence, 1 and 6 may function as sex pheromones for *A. siro*.

Oil gland secretions of both mite species contained high amounts (about 20 %) of neral (4). However, neral (4) was absent from the headspace of both cheeses after the mites were removed and only odor compounds from the cheeses or their spices were detected. Furthermore, the typical lemon-like taste gets lost after the cheese mites are removed from the cheeses (own observation in some gustation tests). The different ketones (I, V, IX) and miscellaneous compounds, which were detected in the headspace of both cheeses, are typical substances in cheese bouquets (e.g. de Frutos et al. 1991; Kubíčková and Grosch 1997; Moio et al. 2000; Berard et al. 2007; Povoio et al. 2011). Especially the ketones derive from butterfat fatty acids in the cheese (Collins et al. 2003). Components such as methyl-ketones may also exhibit a fresh or fruity aroma as pure synthetic standards, but blended composition is responsible for a “normal” cheese flavor (e.g. Zehentbauer and Reineccius 2002; Nogueira et al. 2005) without a lemon-like bouquet. Limonene (a lemon-like monoterpene) as well as the dihydrocarvone fraction and (S)-(+)-carvone derived from the essential oil of caraway (Bouwmeester et al. 1995) were also detected in the headspace of Milbenkäse (which contains caraway) and possibly further expand the fruity bouquet – but they were absent from the headspace of Mimolette. Hence, it can be concluded that the lemon-like flavor of Mimolette and Milbenkäse are more characteristic for the cheese mites than for the cheese itself.

Acknowledgments We want to thank Sebastian Schmelzle for sharing his Milbenkäse with us. The first author was supported by the German National Academic Foundation (Studienstiftung des deutschen Volkes).

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical statement There are no legal restrictions on working with mites.

References

- Adams RP (1995) Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Corporation, Carol Stream
- Alberti G (2004) Tribute to the past-notes on the history of Acarology in Germany. *Phytophaga* 14:13–56
- Asfaw N, Storesund HJ, Skattebøl L, Aasen AJ (1999) (1, S,5, R)-(–)-2,4,4-Trimethylbicyclo[3.1.1]hept-2-en-6-one, from the essential oil of the Ethiopian plant, *Laggera tomentosa*. *Phytochemistry* 52:1491–1494. doi:[10.1016/S0031-9422\(99\)00373-8](https://doi.org/10.1016/S0031-9422(99)00373-8)
- Berard J, Bianchi F, Careri M, Chatel A, Mangia A, Musci M (2007) Characterization of the volatile fraction and of free fatty acids of “Fontina Valle d’Aosta”, a protected designation of origin Italian cheese. *Food Chem* 105(1):293–300. doi:[10.1016/j.foodchem.2006.11.041](https://doi.org/10.1016/j.foodchem.2006.11.041)
- Bouwmeester HJ, Davies JAR, Smid HG, Welten RSA (1995) Physiological limitations to carvone yield in caraway (*Carum carvi* L.). *Ind Crops Prod* 4:39–51
- Bouwmeester HJ, Gershenzon J, Konings MC, Croteau R (1998) Biosynthesis of the monoterpenes limonene and carvone in the fruit of caraway. I. Demonstration of enzyme activities and their changes with development. *Plant Physiol* 117(3):901–912
- Brückner A, Stabentheiner E, Leis H-J, Rasputnig G (2015) Chemical basis of unwettability in Liacaridae (Acari, Oribatida): specific variations of a cuticular acid/ester-based system. *Exp Appl Acarol* 66(3):313–335. doi:[10.1007/s10493-015-9914-3](https://doi.org/10.1007/s10493-015-9914-3)
- Collins YF, McSweeney PLH, Wilkinson MG (2003) Lipolysis and free fatty acid catabolism in cheese: a review of current knowledge. *Int Dairy J* 13(11):841–866. doi:[10.1016/S0958-6946\(03\)00109-2](https://doi.org/10.1016/S0958-6946(03)00109-2)
- Curtis RF, Hobson-Frohock A, Fenwick GR, Berreen JM (1981) Volatile compounds from the mite *Acarus siro* L. in food. *J Stored Prod Res* 17:197–203. doi:[10.1016/0022-474X\(81\)90006-0](https://doi.org/10.1016/0022-474X(81)90006-0)
- De Frutos M, Sanz J, Martinez-Castro I (1991) Characterization of artisanal cheeses by GC and GC/MS analysis of their medium volatility (SDE) fraction. *J Agric Food Chem* 39(3):524–530. doi:[10.1021/jf00003a019](https://doi.org/10.1021/jf00003a019)
- Didzbalis J, Ritter KA, Trail AC, Plog FJ (2004) Identification of fruity/fermented odorants in high-temperature-cured roasted peanuts. *J Agric Food Chem* 52:4828–4833. doi:[10.1021/jf0355250](https://doi.org/10.1021/jf0355250)
- Escudero A, Campo E, Farina L, Cacho J, Ferreira V (2007) Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *J Agric Food Chem* 55:4501–4510. doi:[10.1021/jf0636418](https://doi.org/10.1021/jf0636418)
- Hase A (1929) Zur pathologisch-parasitologischen und epidemiologisch-hygienischen Bedeutung der Milben, insbesondere der Tyroglyphinae (Käsemilben), sowie über den sogenannten „Milbenkäse“. *Parasitol Res* 1:765–821. doi:[10.1007/BF02284603](https://doi.org/10.1007/BF02284603)
- Heethoff M, Rasputnig G (2011) Is 7-hydroxyphthalide a natural compound of oil gland secretions?—Evidence from *Archegozetes longisetosus* (Acari, Oribatida). *Acarologia* 51:229–236. doi:[10.1051/acarologia/20112004](https://doi.org/10.1051/acarologia/20112004)
- Heethoff M, Bergmann P, Laumann M, Norton RA (2013) The 20th anniversary of a model mite: a review of current knowledge about *Archegozetes longisetosus* (Acari, Oribatida). *Acarologia* 53:353–368. doi:[10.1051/acarologia/20132108](https://doi.org/10.1051/acarologia/20132108)
- Hiraoka H, Mori N, Nishida R, Kuwahara Y (2001) 4e)-Dehydrocitrals [(2e,4e)- and (2z,4e)-3,7-dimethyl-2,4,6-octatrienals] from acarid mite *Histiogaster* sp a096 (Acari : Acaridae). *Biosci Biotechnol Biochem* 65:2749–2754. doi:[10.1271/bbb.65.2749](https://doi.org/10.1271/bbb.65.2749)
- Hughes TE (1959) Mites or the Acari. University of London, Athlone Press, London
- Koller LM, Wirth S, Rasputnig G (2012) Geranial-rich oil gland secretions: a common phenomenon in the Histiostomatidae (Acari, Astigmata)? *Int J Acarol* 38:420–426. doi:[10.1080/01647954.2012.662247](https://doi.org/10.1080/01647954.2012.662247)
- Krantz GW, Walter DE (2009) A manual of acarology. Texas Tech University Press, Lubbock
- Kubířková J, Grosch W (1997) Evaluation of potent odorants of Camembert cheese by dilution and concentration techniques. *Int Dairy J* 7(1):65–70. doi:[10.1016/S0958-6946\(96\)00044-1](https://doi.org/10.1016/S0958-6946(96)00044-1)
- Küchenmeister F, Zürn FA (1881) Die Parasiten des Menschen. A. Abel, Leipzig
- Kumamoto J, Scora RW, Payne WW (1975) Ambrosial, a dehydrocitrals isolated from the leaf oil of *Ambrosia confertiflora* DC. *J Agric Food Chem* 23:123–124

- Kuwahara Y (2004) Chemical ecology of astigmatid mites. In: Cardé RT, Millar JG (eds) Advances in insect chemical ecology. Cambridge University Press, Cambridge, pp 76–109
- Leal WS, Kuwahara Y, Suzuki T, Kurosa K (1989) Pheromone study of acarid mites. XXI. Beta-acaridial, the sex-pheromone of the acarid mite *Caloglyphus polyphyllae*. *Naturwissenschaften* 76:332–333. doi:[10.1007/Bf00368436](https://doi.org/10.1007/Bf00368436)
- Meagher RL, Mitchell ER (1999) Nontarget hymenoptera collected in pheromone- and synthetic floral volatile-baited traps. *Environ Entomol* 28:367–371
- Melnyk JP, Smith A, Scott-Dupree C, Marcone MF, Hill A (2010) Identification of cheese mite species inoculated on Mimolette and Milbenkäse cheese through cryogenic scanning electron microscopy. *J Dairy Sci* 93:3461–3468. doi:[10.3168/jds.2009-2937](https://doi.org/10.3168/jds.2009-2937)
- Moio L, Piombino P, Addeo F (2000) Odour-impact compounds of Gorgonzola cheese. *J Dairy Res* 67(2):273–285
- Nogueira MCL, Lubachevsky G, Rankin SA (2005) A study of the volatile composition of Minas cheese. *Food Sci Technol LEB* 38(5):555–563. doi:[10.1016/j.lwt.2004.07.019](https://doi.org/10.1016/j.lwt.2004.07.019)
- Norton RA (1998) Morphological evidence for the evolutionary origin of Astigmata (Acari: Acariformes). *Exp Appl Acarol* 22:559–594. doi:[10.1023/A:1006135509248](https://doi.org/10.1023/A:1006135509248)
- Otienoburu PE, Ebrahimi B, Phelan PL, Foster WA (2012) Analysis and optimization of a synthetic milkweed floral attractant for mosquitoes. *J Chem Ecol* 38:873–881. doi:[10.1007/s10886-012-0150-6](https://doi.org/10.1007/s10886-012-0150-6)
- Povolo L, Cabassi G, Profaizer M, Lanteri S (2011) Study on the use of evolved gas analysis FT-IR (EGA FT-IR) for the evaluation of cheese volatile fraction. *Open Food Sci J* 3:10–16. doi:[10.2174/1874256401105010010](https://doi.org/10.2174/1874256401105010010)
- Prokopy RJ, Phelan PL, Wright SE, Minalga AJ, Barger R, Leskey TC (2001) Compounds from host fruit odor attractive to adult plum curculios (Coleoptera : Curculionidae). *J Entomol Sci* 36:122–134
- Qian M, Reineccius G (2003) Potent aroma compounds in parmigiano reggiano cheese studied using a dynamic headspace (purge-trap) method. *Flavour Frag J* 18:252–259. doi:[10.1002/ffj.1194](https://doi.org/10.1002/ffj.1194)
- Rasputnig G (2010) Oil gland secretion in Oribatida (Acari). In: Sabelis MW, Bruin J (eds) Trends in acarology. Springer, Dordrecht, pp 235–239
- Rasputnig G, Fauler G, Leis M, Leis HJ (2005) Chemical profiles of scent gland secretions in the cyphophthalmid opilionid harvestmen, *Siro duricorius* and *S. exilis*. *J Chem Ecol* 31:1353–1368. doi:[10.1007/s10886-005-5291-4](https://doi.org/10.1007/s10886-005-5291-4)
- Rasputnig G, Kaiser R, Stabenheimer E, Leis HJ (2008) Chrysomelidial in the opisthotal glands of the oribatid mite, *Oribotritia berlesii*. *J Chem Ecol* 34:1081–1088. doi:[10.1007/s10886-008-9508-1](https://doi.org/10.1007/s10886-008-9508-1)
- Rasputnig G, Norton RA, Heethoff M (2011) Oribatid mites and skin alkaloids in poison frogs. *Biol Lett* 7:555–556. doi:[10.1098/rsbl.2010.1113](https://doi.org/10.1098/rsbl.2010.1113)
- Sakata T, Norton RA (2001) Opisthotal gland chemistry of early-derivative oribatid mites (Acari) and its relevance to systematic relationships of Astigmata. *Int J Acarol* 27:11
- Sakata T, Norton RA (2003) Opisthotal gland chemistry of a middle-derivative oribatid mite, *Archeogozetes longisetosus* (Acari : Trhypochthoniidae). *Int J Acarol* 29:345–350
- Sanchez-Borges M, Fernandez-Caldas E (2015) Hidden allergens and oral mite anaphylaxis: the pancake syndrome revisited. *Curr Opin Allergy Clin Immunol* 15:337–343. doi:[10.1097/ACI.0000000000000175](https://doi.org/10.1097/ACI.0000000000000175)
- Schaefer I, Norton RA, Scheu S, Maraun M (2010) Arthropod colonization of land-linking molecules and fossils in oribatid mites (Acari, Oribatida). *Mol Phylogenet Evol* 57:113–121. doi:[10.1016/j.ympev.2010.04.015](https://doi.org/10.1016/j.ympev.2010.04.015)
- Schieberle P, Grosch W (1998) Identification of potent flavor compounds formed in an aqueous lemon oil/citric acid emulsion. *J Agric Food Chem* 36:797–800
- Shimizu N, Mori N, Kuwahara Y (2001) Aggregation pheromone activity of the female sex pheromone, beta-acaridial, in *Caloglyphus polyphyllae* (Acari: Acaridae). *Biosci Biotechnol Biochem* 65:1724–1728
- Shimizu N, Mori N, Kuwahara Y (2003) Simple synthesis of mite pheromone beta-acaridial and its analogs in the secretion of *Caloglyphus polyphyllae* (Acari : Acaridae). *Biosci Biotechnol Biochem* 67:1732–1736
- Stein SE (2015) Mass Spectra by NIST Mass Spec Data Center. In: Linstrom PJ, Mallard WG (eds) NIST chemistry webbook, NIST standard reference database number 69, National Institute of Standards and Technology, Gaithersburg, MD, 20899. <http://webbook.Nist.Gov>
- Tagami K, Kuwahara Y (2005) Remarkable species of Histiostoma (Histiostomatidae, Astigmata) from japan with a pair of new identified ventral coxal organs and sexual dimorphism of chemical components. *Acarologia* 45:83–98
- Tuma D, Sinha RN, Muir WE, Abramson D (1990) Odor volatiles associated with mite-infested bin-stored wheat. *J Chem Ecol* 16:713–724

- Van den Dool H, Kratz PD (1963) A generalization of retention index system including linear temperature programmed gas–liquid partition chromatography. *J Chromatogr* 11:463–468. doi:[10.1016/S0021-9673\(01\)80947-X](https://doi.org/10.1016/S0021-9673(01)80947-X)
- Whetstone MEC, Cadwallader KR, Drake MA (2005) Characterization of aroma compounds responsible for the rosy/floral flavor in cheddar cheese. *J Agric Food Chem* 53:3126–3132. doi:[10.1021/jf048278o](https://doi.org/10.1021/jf048278o)
- Wood WF, Palmer TM, Stanton ML (2002) A comparison of volatiles in mandibular glands from three *Crematogaster* ant symbionts of the whistling thorn acacia. *Biochem Syst Ecol* 30:217–222. doi:[10.1016/S0305-1978\(01\)00099-0](https://doi.org/10.1016/S0305-1978(01)00099-0)
- Wu S, Zorn H, Krings U, Berger RG (2005) Characteristic volatiles from young and aged fruiting bodies of wild *Polyporus sulfureus* (bull.:Fr.) fr. *J Agric Food Chem* 53:4524–4528
- Zehentbauer G, Reineccius GA (2002) Determination of key aroma components of Cheddar cheese using dynamic headspace dilution assay. *Flavour Frag J* 17(4):300–305. doi:[10.1002/ffj.1102](https://doi.org/10.1002/ffj.1102)