Combinatorial synthesis and sensorial properties of polyfunctional thiols

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Abstract

Sulphur compounds such as thiazoles, thiophenes or polysulphides have been investigated for many years by the food industry. Moreover, due to their commercially unavailability, their high reactivity and sub-ppb concentrations, the role of polyfunctional thiols is still misunderstood in most food products. They are very good nucleophiles, readily add to double bonds, are liable to form thiol radicals when exposed to light, and are thermally unstable. In the last few years, by using specific extraction and analytical techniques, polyfunctional thiols emerged, however, as key-constituents of wine, passion fruit and olive oil. The aim of this work was to characterise the chromatographic and sensorial properties of some new mercaptoketones, mercaptoalcohols and mercaptoaldehydes. Combinatorial chemistry proved to be a very useful way to synthesise them rapidly. Actually, this original method allowed us to intensify the process of flavour synthesis and analysis by handling "arrays" of homologous compounds rather than individual flavourings. Mass spectrometry and equimolar sulphur-selective detection enabled the target compounds to be identified and then quantified. Flavour profiles and Best Estimate – Gas Chromatography – LOwest Amount Detected by Sniffing (BE-GC-LOADS) values were further determined. As expected, new exceptionally odorous molecules (BE-GC-LOADS $\geq$ 0.001 ng) were revealed by this unusual approach.

Introduction

Since a very long time, sulphur compounds have been investigated in food products. However, very few is reported on polyfunctional thiols such as mercaptoketones, mercapto secondary alcohols and mercapto-aldehydes. This is mainly due to their commercially unavailability and their high reactivity. In addition, their minute concentrations in foods require concentration steps prior to analysis. This increases the risk of losing them through oxidation and photochemical and/or thermal degradation, in addition to the usual physical loss of the most volatile compounds. The use of a sulphur-selective flame photometric detector and careful extraction procedures have recently led to the discovery of new aliphatic polyfunctional thiols present as traces in food matrices. It turns out that these molecules, characterised by extremely low threshold values, contribute significantly to the sensory properties of food (Reiners and Grosch, 1998; Tominaga *et al.*, 1998; Werkhoff *et al.*, 1998).

The aim of the present work was to establish a strategy for quickly obtaining retention indexes and flavour descriptors for a greater number of polyfunctional thiols. As previously used by Khan et al. (1999) to obtain a series of thioester analogues, we applied combinatorial chemistry to get some mercaptoketones and their corresponding mercaptoalcohols (Vermeulen *et al.*, 2001). This original approach permitted us to intensify the process of flavour synthesis and analysis by handling "arrays" of homologous compounds rather than individual flavourings. By using a selective equimolar chemiluminescence detector (SCD), these mercaptans were quantified in a complex synthetic medium without commercially pure standards.

Experimental (Vermeulen *et al.*, 2001). Chemical reactions are given in tables 1 and 2.

Results and discussion

Five mercaptoketones, five mercapto secondary alcohols (Table 1) and thirteen mercapto-aldehydes (Table 2) were synthesised by combinatorial chemistry from the corresponding commercially available α,β-unsaturated ketones and α,β-unsaturated aldehydes.

The presence of a sulphur atom in the molecules was easily checked by gas chromatography coupled to SCD detection. Since the response is proportional only to the number of sulphur atoms, it was possible to quantify each compound in the complex synthetic medium without a specific calibration curve. All of the suspected structures were confirmed by mass spectrometry. In all cases, the molecular ion was easily recognised on the mass spectrum. Moreover, all the molecules revealed to be able to lose H_2S after electron impact bombardment. Sometimes, more than one peak was found for compounds with two or more chiral carbon atoms. These diastereoisomers were usually separated by about 10 retention index (R.I.) units on a CP-Sil5 CB column.

Similar odour descriptors were found for many mercaptoketones and mercaptoalcohols, e.g.: catty-blackcurrant, sweat and cooked milk. The mercaptoaldehydes are often characterised by meaty, cheesy, onion-like descriptors. 3-Mercaptohexenal, 3-mercaptoheptenal, 3-mercaptooctenal, 5-methyl-4-mercaptohexan-2-one, and one diastereoisomer of 5-methyl 4-mercaptohexan-2-ol revealed to exhibit the most pleasant odours at the sniffing port. As initially suggested by Berger *et al.* (1999) for thioesters and Gijs *et al.* (2000) for sulphur compounds, we serially diluted our original extract to determine matrix-composition-independent sensorial threshold values. The so-called BE-GC-LOADS (Best Estimate – Gas Chromatography – LOwest Amount Detected by Sniffing) is defined as the lowest amount (in ng) of compound arriving at the detector which can be perceived by the panellist. By comparison to dimethyltrisulphide with a BE-GC-LOADS of 1.4 ng, these thiols often revealed 10 to 1000 times more potent (Tables).

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Table 1. GC-MS and sensorial properties of mercaptoketones and mercapto secondary alcohols synthesised.

Name	*MS fragments [relative percentages]*	*R.I. (CP-Sil5 CB)*	*R.I. (FFAP CB)*	*Odour at the sniffing port (CP-Sil5 CB) [BE-GC-LOADS in ng]*
4-Mercaptopentan-2-one	43 [100]; 85[29]; 41 [26]; **118**[a] [26]; 61 [19]	882	1422	Greenery, potato, blackcurrant [0.03]
4-Mercapto 4-methylpentan-2-one	43 [100]; **132**[a] [26]; 75 [21]; 55 [19]; 99 [12]	913	1382	Blackcurrant, broom, vinaigrette, citrus fruit [0.004]
4-Mercapto 3-methylpentan-2-one	43 [100]; 99 [69]; 55 [50]; 61 [19]; **132**[a] [6]	963[b] 965[b]	1469[b] 1477[b]	Sweat[b] [0.02] Cooked milk[b] [0.4]
5-Mercaptohexan-3-one	57 [100]; 61 [48]; 75 [44]; **132**[a] [30]; 99 [28]	983	1487	Box tree, fresh, empyreumatic [0.02]
5-Methyl 4-mercaptohexan-2-one	43 [100]; 112 [22]; 113 [19]; 55 [17]; **146**[a] [6]	1068	1585	Exotic fruit, sweet [0.03]
4-Mercaptopentan-2-ol	45 [100]; 86 [68]; 71 [63]; 61[53]; 69 [37] (**120**[a] [11] present)	914[b] 925[b]	1587[b] 1614[b]	Broom, blackcurrant, catty[b] [0.002] Raw onion[b] [0.02]
4-Mercapto 4-methylpentan-2-ol	57 [100]; 85 [97]; 45 [68]; 100 [58]; 41 [42] (**134**[a] [16] present)	951	1547	Broom, blackcurrant, solvent, fresh, sweet [0.009]
5-Mercaptohexan-3-ol	61[100]; 59 [96]; 71 [46]; 100 [43]; 116 [32] (**134**[a] [11] present)	1011[b] * 1022[b]	1658[b] 1682[b]	Sweat, meat broth, citrus fruit[b] [0.02] * Sweat, cooked milk[b] [0.06]
4-Mercapto 3-methylpentan-2-ol	56 [100]; 45 [96]; 55[71]; 61 [67]; 100 [58] (**134**[a] [13] present)	* 1022[b] 1036[b]	1674[b] 1677[b] 1687[b] 1739[b]	* Sweat, cooked milk[b] [0.06] Onion, leek, sweat, soup[b] [0.0001]
5-Methyl 4-mercaptohexan-2-ol	71 [100]; 45 [90]; 55 [76]; 61 [67]; 114 [45] (**148**[a] [14] present)	1096[b] 1107[b]	1747[b] 1762[b]	Rhubarb, lemon[b] [0.002] Spicy, peppery, meaty[b] [0.002]

[a] molecular ion [b] the data correspond to diastereoisomers * coelution

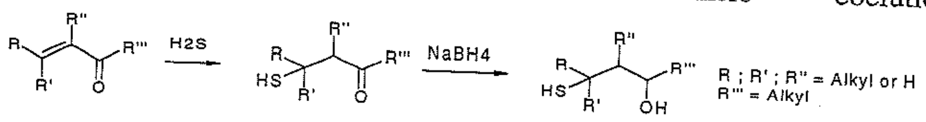
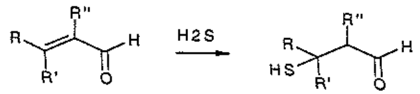


Table 2. GC-MS and sensorial properties of mercaptoaldehydes synthesised.

Name	*MS fragments [relative percentages]*	*R.I.* (*CP-Sil5 CB*)	*R.I.* (*FFAP CB*)	*Odour at the sniffing port (CP-Sil5 CB) [BE-GC-LOADS in ng]*
3-Mercaptopropanal	**90**[a] [100]; 72 [95]; 62 [77]; 47[69]; 57 [53]	756	1382	Rotten potatoes, broth [0.06]
3-Mercaptobutanal	41 [100]; 42 [90]; 86 [71]; 61 [54]; **104**[a] [46]	803	1372	Broth, cheese, pungent [4]
3-Mercapto 3-methylbutanal	41 [100]; 56 [75]; 57 [29]; **118**[a] [29]; 75 [25]	842	1353	Broth, cheese, pungent [0.06]
3-Mercapto 2-methylpropanal	41[100]; 42 [75]; 71 [75]; 47[60]; **104**[a] [38]	826	1400	Meat, broth, raw bread paste [3]
3-Mercapto 2-methylbutanal	56 [100]; 61 [73]; 41 [56]; 85 [50]; 100 [29] (**118**[a] [10] present)	892	1424[b] 1429[b]	Broth, onion, meat, cheese [0.002]
3-Mercaptopentanal	56 [100]; 41 [77]; 61 [38]; 100 [38]; **118**[a] [38]	910	1476	Broth, raw onion, flowery [1]
3-Mercapto 2-ethylpropanal	56 [100]; 85 [69]; 55 [55]; 41 [55]; 47 [55] (**118**[a] [22] present)	926	1481	Broth, rotten potatoes, plastic, groundnut [13]
3-Mercapto 2-methylpentanal	70 [100]; 41 [90]; 55 [86]; 43 [36]; 99 [33] (**132**[a] [17] present)	991[b] 995[b]	1508[b] 1524[b]	Broth, meat, onion[b] [0.003] Broth, pepper[b] [0.1]
3-Mercaptohexanal	55 [100]; 41 [61]; 70 [59]; 42 [49]; 61 [35] (**132**[a] [29] present)	1004	1560	Citrus fruit peel, fresh [0.003]
3-Mercaptoheptanal	41 [100]; 56 [77]; 55 [73]; 69 [67]; 61 [46] (**146**[a] [17] present)	1108	1659	Flowery, citrus fruit peel [0.03]
3-Mercapto 2-butylpropanal	55 [100]; 57 [95]; 41 [88]; 56 [75]; 69 [40] (**146**[a] [10] present)	1133	1667	Plastic, rhubarb, pungent [0.4]
3-Mercaptooctanal	55 [100]; 41 [88]; 56 [72]; 70 [55]; 131 [33] (**160**[a] [14] present)	1214	1764	Citrus fruit peel, grapefruit, verdure, fresh [0.001]
3-Mercaptononanal	55 [100]; 41 [94]; 70[75]; 43 [56]; 69 [50] (**174**[a] [13] present)	1320	1873	Stale odour [-]

[a] molecular ion [b] the data correspond to diastereoisomers



R ; R' ; R'' = Alkyl or H