

# Fate of polyfunctional thiols through beer ageing

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## INTRODUCTION

Beer flavour is known to deteriorate through ageing due to the release of compounds like trans-2-nonenal (1), dimethyltrisulfide (2), 4-vinylsyringol (3) , ...

Polyfunctional thiols have been recently identified as key-flavours of a fresh beer (4). Their evolution through ageing is therefore prime of concern for the brewers. Different kinds of lager (L1, L2, L3, L4), wheat (W) and top-fermented (T) beers have been studied for five months at 20°C. The polyfunctional thiols have been trapped by a specific extraction with pHMB (p-HydroxyMercuriBenzoic acid) (5). The extracts have been then analysed by GC-MS, GC-O and GC-PFPD.

### Roasted/burned flavours brought by mercaptoacetates

2-Mercaptoethyl acetate and 3-mercaptoethyl acetate are known as responsible of a roasted/burned flavour in wines (6). Vermeulen *et al.* (4) identified them in lager beers. Six commercial beers have been here analysed through a 5-months storage at 20°C. The GC-O analyses revealed that 2-mercaptoethyl acetate and 3-mercaptoethyl acetate are already present in the six fresh beers. However, their detection and quantification were possible by GC-PFPD only after ageing (Figure 1).

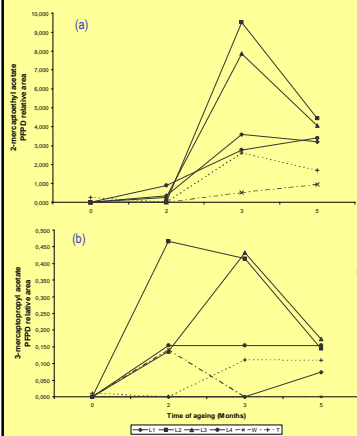


Figure 1. Evolution of (a) 2-mercaptoethyl acetate and (b) 3-mercaptoethyl acetate in six commercial beers during ageing at 20°C. L1, L2, L3 and L4 = lager beers, W = wheat beer, T = top-fermented beer.

In most beers, the concentration of 2-mercaptoethyl acetate revealed maximum after three months (up to 6.4 ppt IST equivalents) in L2. The concentration of 3-mercaptoethyl acetate was in all cases much lower (up to 313 ppt IST equivalents). Its concentration also increased during ageing except in the wheat beer. Given their threshold value (close to 40 ppt (6)) and probable synergy, they could impart strong defects in some aged beers.

As shown in Figure 2, mercaptoacetates are most probably synthesized from their corresponding mercaptoalcohols. However, neither 2-mercaptoethanol nor 3-mercaptoethanol have been detected by GC-olfactometry or GC-PFPD in fresh samples. Only two 5 months aged beers allowed us to detect them at the sniffing port (L3 and L4).

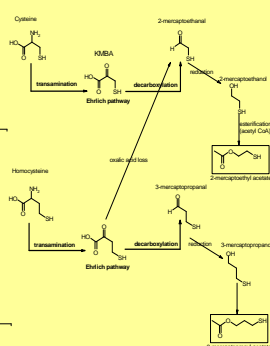


Figure 2. Hypothetical formation pathway of 2-mercaptoethanol, 3-mercaptoethanol and their corresponding acetates (4).

### Onion-like flavours brought by branched mercapto alcohols

GC-olfactometry analyses of fresh samples revealed the occurrence of three onion-like flavours ( $R_i = 964, 1000$  and  $1050$  on CP-Sil 5 CB). The former was identified as 2-mercapto-3-methylbutanol. It has been previously identified by Olsen *et al.* (7) as responsible of an onion-like defect in some beers. Vermeulen *et al.* (4) identified this thiol in only one lager beer. In our case, 2-mercapto-3-methylbutanol has been perceived at the GC-sniffing port in all fresh and aged samples and even detected by GC-PFPD in L2, L3 and L4 (Figure 3). Its concentration revealed strongly modified through storage (variation up to 341 ppt IST equivalents). Olsen *et al.* (7) defined its threshold value under 1 ppb in water.

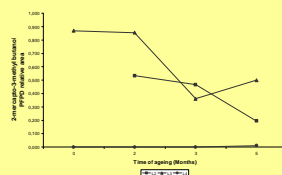


Figure 3. Evolution of the concentration of 2-mercapto-3-methylbutanol in three lager beers (L2, L3, L4) stored at 20°C

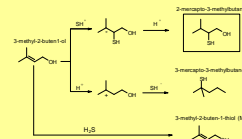


Figure 4. Hypothetical formation pathway of 2-mercapto-3-methylbutanol, 3-mercapto-3-methylbutanol and MBT (4)

Vermeulen *et al.* (4) proposed a hop constituent, 3-methyl-2-buten-1-ol, as the common precursor of 2-mercapto-3-methylbutanol, 3-mercapto-3-methylbutanol and 3-methyl-2-buten-1-thiol (MBT) (Figure 4). The two latter were detected by Vermeulen *et al.* in four different lager beers (4). 3-Mercapto-3-methylbutanol has been detected by GC-O in all our fresh and aged extracts, except for the wheat beer. 3-Methyl-2-buten-1-thiol (MBT), responsible of hop flavour at low concentration and the light-struck off-flavour at higher level, was also perceived by GC-O in all our extracts (beer stored without light).

Figure 5 (a) shows the evolution of the unknown U1 at  $R_{i,CP-Sil 5 CB} = 1000$  (PFPD). This onion-like compound is present as traces in most fresh samples but concentrations can reach up to 651 ppt IST equivalents after a few months at 20°C. Three other unknowns with an onion-like flavour, absent in the four fresh lager beers, were detected in all aged samples (GC-O detection at  $R_{i,CP-Sil 5 CB} = 725$  (U2), 780 (U3) and 1050 (U4)). Among them, only U2 has been quantified by GC-PFPD. Figure 5 (b) shows the evolution of this unknown in L1, L4 and T. The highest concentrations (100 ppt and 87 ppt IST equivalents) were measured after three (T) or five (L4) months.

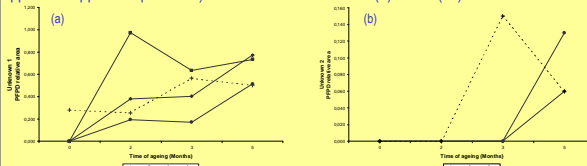


Figure 5. Evolution of the unknowns (a) U1 ( $R_{i,CP-Sil 5 CB} = 1000$ ) and (b) U2 ( $R_{i,CP-Sil 5 CB} = 725$ ) in commercial beers during ageing at 20°C. L1, L2 and L4 = lager beers, T = top-fermented beer

Table 1 shows the occurrence of the unknowns U3 and U4 at  $R_{i,CP-Sil 5 CB} = 780$  and 1050. No PFPD signal was detected. In lager beers, both unknowns appeared after two months. The wheat beer revealed to contain only U3 (after three months of ageing). The here-investigated top-fermented beer already contained U3 and U4 before ageing.

Table 1. GC-olfactometric occurrence of U3 and U4 in the pHMB extracts of fresh (F) and aged samples (2, 3 and 5 months) of four lager beers (L1, L2, L3, L4), one wheat beer (W) and one top-fermented beer (T). ■ = detected by GC-O.

Unknown	L1		L2		L3		L4		W		T	
	F	2	3	5	F	2	3	5	F	2	3	5
780 (U3)												
1050 (U4)												

## CONCLUSIONS

Polyfunctional thiols are compounds which confer to beer its typical aroma. 4-Mercapto-4-methyl-2-pentanone, 3-mercaptohexanol and traces of MBT most probably participate to the fresh hoppy flavour required in a lager. The present study shows that some thiols are generated through ageing. They could induce some roasted/burned (2-mercaptoethyl acetate and 3-mercaptoethyl acetate) and onion-like (U2 and U3) off-flavours after a few months of ageing.

(1) Noël *et al.*, J.A.F.C., 47, 4323-6, 1999 (2) Gijs *et al.*, J.A.F.C., 50, 5612-5616, 2002 (3) Callemien *et al.*, J.A.F.C., 54, 1409-1413, 2006 (4) Vermeulen *et al.*, J.A.F.C., 54, 5061-5068, 2006 (5) Tominaga *et al.*, J.A.F.C., 46, 1044-1048, 1998 (6) Lavigne *et al.*, Sciences des Aliments, 18(2), 175-191, 1998 (7) Olsen *et al.*, Carlsberg Res. Commun., 53, 1-9, 1988