

# Odorant Polyfunctional Thiols Issued from Bottle Beer Refermentation

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## 43.1 INTRODUCTION

Bottle refermentation, which imparts beer effervescence and resistance against infection and oxidation, is also known to improve flavor profile and stability [1]. Through the process, some stale off-flavors exhaled by aldehydes (*trans*-2-nonenal, 3-methylthiopropionaldehyde, 3-methylbutanal, 5-hydroxymethylfurfural) can be easily reduced into alcohols, with lower flavor thresholds (*trans*-2-nonenol and nonanol, 3-methylthiopropanol, 3-methylbutanol, 5-hydroxymethylfurfuro) [2–3]. Unfortunately, yeast esterases can also strongly affect beers' fruity character by hydrolyzing isoamyl acetate, ethyl hexanoate and ethyl octanoate [4].

Thiols have a strong impact on the overall aroma of fermented beverages. Primary fermentation is known to generate odorant polyfunctional thiols [5]. By producing hydrogen sulfide, yeast is indeed able to transform hop allylic alcohols into onion-like sulfanylalcohols such as 2- and 3-sulfanyl-3-methylbutanol [6]. Yeast  $\beta$ -lyase activity is also suspected to transform hop cysteine adducts into beta-sulfanyl carbonyles,  $\beta$ -sulfanyl alcohols, and/or esters [7,8].

Thiols of refermented beer have been little studied. In this work, the polyfunctional thiol contents of commercial beers submitted or not submitted to bottle refermentation were compared. A specific pHMB thiol extraction [9] was applied, and the extracts analyzed by GC-MS, GC-PFPD, and GC-olfactometry (AEDA).

## 43.2 MATERIALS AND METHODS

Bottle refermentation was applied to half of the LB (Lager Beer) commercial samples (addition of 11 g/L saccharose and 600,000 cells/mL of

MUCL 34627 before storage for 1, 2 or 3 weeks at 27°C; LB1, LB2, and LB3). After each week, samples were directly extracted (LB1, LB2, LB3) and compared to the reference stored 3 weeks at 27°C without refermentation (LB0).

Beer (500 mL) and distilled CH<sub>2</sub>Cl<sub>2</sub> (200 mL) were stirred for 30 minutes. After decantation, the lower phase and the interfacial emulsion were centrifuged for 20 minutes at 10,000 rpm. The organic phase was then extracted by 2 × 20 mL of a pHMB solution (360 mg of pHMB, 24.6 g of Tris in 1 L of Millipore water). The combined aqueous phases were loaded into a strongly basic anion exchanger column (Dowex 1WX2-100 resin), having been previously washed with NaOH 2 M, HCl 2 M, and in between rinsed in ultrapure water. Next, 50 mL of sodium acetate buffer (0.1 M, pH 6) was poured onto the resin to remove impurities. Volatile thiols were then released by percolating a purified cysteine solution (640 mg of hydrochloride L-cysteine monohydrated in 60 mL of Millipore water). The eluate containing the volatile thiols was collected and extracted by 4 mL and then 3 mL of distilled CH<sub>2</sub>Cl<sub>2</sub>. The organic phases were pooled, dried on anhydrous Na<sub>2</sub>SO<sub>4</sub>, and finally concentrated to 70 μL with a Dufton system to be stored at -80°C before analysis. 4-Methoxy-4-methyl-2-butanethiol (added at the first extraction step) and thiazole (added before the final concentration step) were used, respectively, as internal and external standards (IST and EST).

Extracts were analyzed by GC-PFPD (selective sulfur compound detection) and GC-olfactometry. A sensorial aroma extract dilution analysis (AEDA) was applied to determine the odor intensity of each compound in beer [10].

### 43.3 DISCUSSION AND CONCLUSION

The sensorial comparison between LB0 and LB3 allowed us to evidence significant differences between both samples. The sulfur, onion, and cabbage descriptors were strongly associated to LB3.

GC-O (AEDA) and GC-PFPD enabled us to identify many thiols after refermentation (absent or less present in LB0). A large number of sulfanylalkylalcools (Table 43.1), sulfanylalkylacetates (Table 43.2), and sulfanylalkylcarbonyles (Table 43.3) were revealed, having been produced during the refermentation process, especially after 3 weeks. MBT (3-methyl-2-buten-1-thiol, RI = 811) even reached an FD olfactometric value of 32,768. 2-Sulfanylethylacetate also increased very significantly, up to

**Table 43.1** Sulfanylalkylalcohols Increasing During Bottle Refermentation

RI	Compound	LB0		LB3		Odor
		FD	µg/L in Beer	FD	µg/L in Beer	
981	1-Sulfanylpentan-3-ol (1S3Pol)	16	–	32	0.11	Green
1094	3-Sulfanylhexas-1-ol (3SHol)	4	–	64	3.96	Rhubarb
1291	4-Sulfanylnonan-2-ol (4S2NoI)	16	–	32	1.03	Lemon

**Table 43.2** Sulfanylalkylacetates Increasing During Bottle Refermentation

RI	Compound	LB0		LB3		Odor
		FD	µg/L in Beer	FD	µg/L in Beer	
890	2-Sulfanylethyl acetate (2SE-A)	–	0.58	8/*	9.53	Onion
1001	3-Sulfanylpropyl acetate (3SPr-A)	4	–	32	0.01	Burned
1130	3-Sulfanylpentyl acetate (3SP-A)	4	1.15	32	3.06	Flower

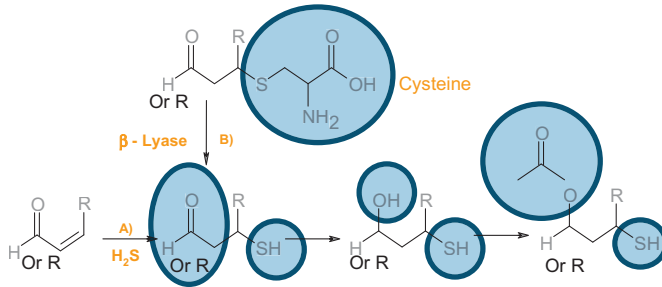
\*Slight co-elution.

**Table 43.3** Sulfanylalkylcarbonyles Increasing During Bottle Refermentation

RI	Compound	LB0		LB3		Odor
		FD	µg/L in Beer	FD	µg/L in Beer	
919	4-Sulfanyl-4-methylpentan-2-one (4S4M2Pone)	16	–	128	0.21	Catty
1011	3-Sulfanylhexasal (3SHal)	–	–	16	–	Floral

9.5 ppb after 3 weeks of refermentation. Most of these were only slightly detectable after 2 weeks, suggesting that yeast autolysis could make their synthesis easier.

Various formation pathways are suspected (Figure 43.1). During refermentation, by producing hydrogen sulfide, yeast is able to transform  $\alpha,\beta$ -unsaturated carbonyles into  $\beta$ -sulfanylalkylcarbonyles (path A in Figure 43.1). Hydrogen sulfide can also substitute alcohols (e.g.: MBT). For beta-sulfanyl structures, hop cysteine adducts are suspected to be hydrolyzed by refermentation yeast-derived lyases (path B in Figure 43.1). For 2SE-A



**Figure 43.1** Hypothetical formation pathways of thiols during refermentation: (A) H<sub>2</sub>S addition; (B) hydrolysis of cysteine adducts.

and 3SPr-A, the cysteine and homocysteine Ehrlich pathways should be also investigated.

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