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Modulation of the Sulfanylalkyl Acetate/Alcohol Ratio and Free Thiol Release from Cysteinylated and/or Glutathionylated Sulfanylalkyl Alcohols in Beer under Different Fermentation Conditions

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ABSTRACT: The occurrence of a substantial pool of cysteinylated and glutathionylated forms of polyfunctional thiols has been evidenced for several dual-purpose hop varieties, and so is the ability of *Saccharomyces cerevisiae* yeast to release free thiols from these forms through fermentation. The present work aimed to investigate the effect of temperature, wort density, maturation time, and strain on the efficiency of free thiol release by *S. cerevisiae* yeasts. Model media at 12, 15, or 17°P were spiked with three cysteinylated (Cys-) or three glutathionylated (G-) sulfanylalkyl alcohols (Cys- or G-3-sulfanylpentan-1-ol, 3-sulfanyl-4-methylpentan-1-ol, and 3-sulfanylhexan-1-ol), fermented for 7 days at 18, 24, and 28 °C, and kept at 4 °C for varying number of days. The released sulfanylalkyl alcohols and their corresponding acetates were extracted with a Ag-ion SPE cartridge and analyzed by gas chromatography−pulsed-flame photometric detection. The wort density and yeast strain greatly affected the acetate/alcohol ratio. This ratio varied from 1 to 80% according to the yeast strain and was at its highest at 17°P and 24 °C. Maturation appeared as the crucial step for free thiol excretion from yeast cells (no thiol was recovered in the fermented worts without maturation). Among the five yeasts tested, the yeast strain SafAle K-97 released the highest level of sulfanylalkyl alcohols into the medium (up to 0.45% of the added cysteinylated adducts and 0.08% of the glutathionylated adducts), whereas S-33 or S-04 should be preferred when release of esters is sought out (release efficiencies up to 0.35% from cysteinylated adducts and 0.02% from glutathionylated adducts are observed if both the alcohol and its acetate are considered).

KEYWORDS: *polyfunctional thiols, cysteine conjugates, glutathione conjugates, S. cerevisiae, fermentation*

■ INTRODUCTION

Polyfunctional thiols (PFTs) are key contributors to flavors of numerous foods and beverages.[1−6] These aromatic compounds, made of a carbon chain carrying both a thiol function and an alcohol, aldehyde, ester, or a ketone moiety (usually three carbons apart from each other), are unusual due to their extremely low thresholds (ng L^{-1} level) and characteristic odors. PFTs with a shorter carbon chain exhibit rather unpleasant flavors (cheese, onion, grilled meat, etc.), while ones with a longer chain impart delightful fruity, citrusy, or flowery aromas.

In hops, 41 free PFTs have been evidenced, including many sulfanylalkyl alcohols and their corresponding esters.[7] Among them, 3-sulfanyl-hexanol (3SHol; grapefruit, rhubarb; threshold: 55 ng L_{beer}^{-1}), 3-sulfanyl-pentanol (3SPol; citrus, catty; threshold: 600 ng $L_{hydroalcoholic\ solution}^{-1}$), and 3-sulfanyl-4-methylpentanol (3S4MPol; passion fruit; threshold: 70 ng L_{beer}^{-1}) have been quantitated in beer at levels close to or above their sensory thresholds.

More recently, besides the free forms, cysteinylated (Cys-) and glutathionylated (G-) adducts of PFTs have been identified in various hop cultivars.[8−12] Both direct high-pressure liquid chromatography-multiple reaction monitoring (HPLC-MRM) analysis and indirect gas chromatography−pulsed-flame photometric detection (GC−PFPD) of free PFTs released upon incubation with apotryptophanase have revealed Cys-3SHol and Cys-3SPol in hops, Polaris having emerged as the richest in both precursors (up to 4.9 mg kg^{-1} Cys-3SHol and 0.2 mg kg^{-1} Cys-3SPol).[8−10] G-3SHol and G-3SPol also seem ubiquitous in hop varieties, at concentrations (determined by HPLC-MRM) much higher than their cysteinylated counterparts. Up to 118 mg kg^{-1}, G-3SHol has been found in Polaris and up to 18 mg kg^{-1} G-3SPol in Citra.[12] As for 3S4MPol, both the free form and its precursors appear more peculiar to certain varieties (the free form seems fairly specific to Hallertau Blanc and Nelson Sauvin, while G-3S4MPol is found at up to 0.3 and 3.6 mg kg^{-1} in Hallertau Blanc and Polaris, respectively).[12] Beyond a strong varietal effect, the harvest maturity may also greatly influence precursor levels within a same hop variety.[13]

Several *Saccharomyces cerevisiae* yeasts display the ability to release thiols from *S*-conjugates under both oenological and brewing conditions.[14−18] How *S. cerevisiae* breaks down

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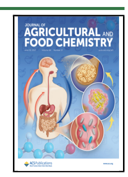

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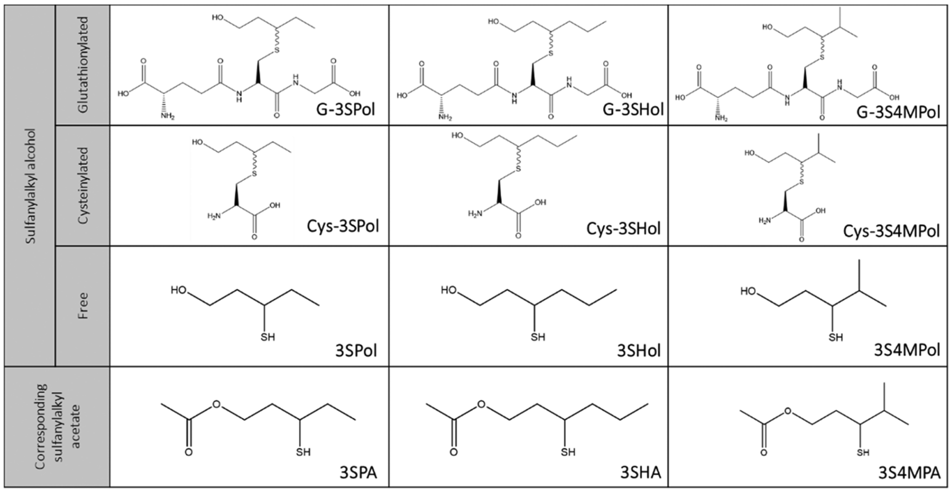

Figure 1. Chemical structures and abbreviations of the here-investigated bound and free sulfanylalkyl alcohols and derived acetates.

glutathionylated adducts to free PFTs has been investigated in grape must.[19−21] After transport into the yeast cell by the transporter *OPT1*, degradation to γ-glutamyl-cysteine, cysteine−glycine, and cysteine *S*-conjugates occurs. 3SHol release from its cysteinylated counterpart is mediated by *STR3*, while *IRC7* is likely responsible in the case of the sulfanylalkyl ketone 4-sulfanyl-4-methyl-pentan-2-one (4S4M2Pone; catty, black currant; threshold 55 ng L_{beer}^{-1}).[22,23] Interspecific hybrids between wine yeast strains show enhanced release of 4S4M2Pone.[14] Using a higher fermentation temperature (20 °C instead of 13 °C) appears as another way to increase the concentration of 3SHol and 4S4M2Pone in wine.[24]

In the brewing field, the ability of yeast to hydrolyze cysteinylated adducts was first confirmed after bottle refermentation. Up to 5.5 μg L^{-1} 3SHol was found after 3 weeks in a bottle-refermented beer spiked with 10 mg L^{-1} of synthesized Cys-3SHol (molar conversion 0.09%).[17] Other studies showed a transfer rate of 3SHol over 100% from hops to finished beer (12°P worts, hopped with Tomahawk, Nelson Sauvin, Cascade or Mosaic, 4−10 days of fermentation at 20−22 °C and 1−3 weeks of maturation at 0−2 °C). The temperature, pH, time, and concentration of enzyme and precursors were believed to be crucial parameters regarding the thiol release.[8,18] Very recently, the ability of brewing yeast to release free PFTs from both Cys- and G-adducts, using synthesized adducts, was confirmed, in primary fermentation.[12,25] When compared to another industrial ale strain, the dry yeast K-97 turned out to be more efficient. According to the dry yeast batch used, up to 0.16% of Cys-3SPol, -3SHol, and -3S4MPol added at 10 mg L^{-1} to a 12°P unhopped wort appeared cut after 10 days of fermentation at 22 °C and one night at 4 °C. These investigations also showed, for the first time, thiol release (up to 0.05%) from glutathionylated adducts.[25]

In these first experiments, the investigators did not consider the ability of yeast to produce esters from sulfanylalkyl alcohols. However, 3-sulfanylalkyl acetate (3SHA) is known to exhibit an even lower threshold (5 ng L_{beer}^{-1}) and more delicate odor descriptors (box tree and passion fruit) than 3SHol. Its production could thus greatly affect the resulting fermented product. For decades, fermentation parameters such as temperature and wort composition have been known to affect the esterification of fusel alcohols such as isoamyl acetate (e.g., overproduction in high-gravity worts or when the temperature is increased from 12 to 20 °C).[26,27] In beer and wine, the production of 3-sulfanylhexyl acetate (3SHA) from 3SHol can result from the alcohol acetyltransferase activity. The ability of various commercial wine yeasts to convert 3SHol to 3SHA has been studied, showing a broad variation between them.[28,29] The monitoring of free and bound 3SHol and 3SHA levels in malt, hops, and the resulting finished beer revealed that 3SHA could only be synthesized from 3SHol during brewing fermentation because no trace of free or bound 3SHA had been found in malt or hops used.[30] However, few years later, Kankolongo et al. evidenced 3SHA in several dual-purpose hop varieties (up to 27 μg L^{-1} in Citra).[31]

The aim of this work is to first assess the efficiency of 3-sulfanylalkyl alcohol release from *S*-conjugates and the esterification of these alcohols (structures and abbreviations are detailed in Figure 1) after different maturation times and at different wort densities and temperatures using one selected strain (K-97). After studying the fermentation parameters, a comparison with four other commercial dry top-fermentation yeasts: S-33, known to use maltotriose poorly, and US-05, S-04, and BE-256, all three with an apparent attenuation close to 80% (like K-97) will be performed at the chosen density and maturation time. Among these yeasts, S-33 and BE-256 have already been described in the literature as the best producers of fermentation fruity esters such as isoamyl acetate.[32]

■ MATERIALS AND METHODS

Chemicals. Acetonitrile, dichloromethane, 37% hydrochloric acid, and sodium chloride were purchased from VWR (Leuven, Belgium). 2-Acetylthiophene, Discovery Ag-ion SPE tube 6 mL, >98% L-cysteine hydrochloride monohydrate, and 4-methoxy-2-methylbutane-2-thiol were purchased from Sigma-Aldrich (Bornem, Belgium). Anhydrous sodium sulfate was purchased from Acros Organics (Geel, Belgium). Milli-Q water was used (Millipore, Bedford, MA, USA).

Synthesis of Previously Investigated Reference Conjugates. Cys-3SHol,[8] G-3SHol,[11] Cys-3S4MPol,[11] G-3S4MPol,[11] Cys-3SPol,[12] and G-3SPol[12] were synthesized prior to this work according to the methods of Gros et al., Kankolongo et al., and Chenot et al.[8,11,12] Mixtures of *S*-conjugate diastereomers were obtained.

Yeasts. Five Fermentis active dry yeasts (*S. cerevisiae*) were used; SafAle K-97, SafAle S-33, SafAle US-05, SafAle S-04, and SafAle BE-256, hereinafter referred to as K-97, S-33, US-05, S-04, and BE-256.

Fermentation of Wort Spiked with Cys-3SPol/3SHol/3S4MPol or G-3SPol/3SHol/3S4MPol. Wort was produced from pale malt (Boortmalt) in a 50-L-scale pilot plant (Coenco, Oostkamp, Belgium). The 17°Plato unhopped wort was obtained after 90 min of boiling and freezing after clarification until the fermentation trials. The dry top-fermentation yeasts were pitched at 0.46 g/L into 150 mL wort at different °P densities (see Table 1, obtained by diluting the original 17°P wort). The worts were spiked beforehand with Cys-3SPol, Cys-3SHol, and Cys-3S4MPol (5 mg kg^{-1} each) or G-3SPol, G-3SHol, and G-3S4MPol (10 mg kg^{-1} each). The fermentations were conducted for 7 days at 18, 24, or 28 °C under shaking at 80 rpm (Labwit ZWY-240 incubator shaker). The fermented worts were kept at 4 °C for 0−5 days (see Table 1) before extraction of free thiols (see next section). Experiments were done in duplicate.

Table 1. Set of Fermentation Trials

trials	wort initial density (°P)	temperature of primary fermentation (°C)	days of maturation at 4 °C
A	15	24	3
B	15	18	3
C	15	28	3
D	15	24	0
E	15	24	5
F	12	24	3
G	17	24	3

PFT Extraction from Fermented Spiked Media with a Ag Cartridge. PFT extraction from fermented spiked media was adapted from Takazumi et al.[33] 4-Methoxy-2-methylbutane-2-thiol is added as an internal standard (IST, at 2 μg L^{-1}) into 100 mL of fermented wort which is then saturated with NaCl and stirred with 50 mL of dichloromethane for 15 min. The mixture was centrifuged at 4500 rpm for 15 min. The recovered organic phase was loaded on a Discovery Ag-ion SPE cartridge conditioned beforehand with 10 mL of dichloromethane. The cartridge was rinsed with 10 mL of dichloromethane, then with 20 mL of acetonitrile, and finally with 10 mL of ultrapure water (reversed cartridge in this last case). Free thiols were released from the Ag cartridge by percolating 20 mL of washed cysteine solution (4 × 20 mL dichloromethane for washing 215 mg of cysteine in 20 mL of water). The eluent was extracted twice with bidistilled dichloromethane (5 mL for 5 min and 10 mL for 10 min). The resulting organic phase was dried on anhydrous sodium sulfate and concentrated to 250 μL in a Danish−Kuderna distillation apparatus and to 70 μL on a Dufton column. 2-Acetylthiophene is added as the external standard (EST, 0.5 mL at 200 μg L^{-1} added before concentration).

Gas Chromatography−Pulsed-Flame Photometric Detection. One microliter of the free thiol extract was analyzed with a ThermoFinnigan Trace GC 2000 gas chromatograph equipped with a splitless injector maintained at 250 °C. Compounds were analyzed with a wall-coated open tubular apolar CP-Sil5-CB capillary column (50 m length, 0.32 mm i.d., and 1.2 μm film thickness). The carrier gas was helium and the pressure was set at 50 kPa. The oven temperature was programed to increase from 36 to 85 °C at 20 °C per min, then to 145 °C at 1 °C per min, and finally to 220 °C at 3 °C per min, and held for 30 min. The column was connected to the OI Analytical PFPD detector (model 5380, combustor internal diameter: 2 mm). The following parameters were selected for the PFPD detector: temperature, 220 °C; voltage, 590 V; gate width, 18 ms; gate delay, 6 ms; trigger level, 400 mV; pulse frequency, and 3.33 Hz. PFPD chromatograms were recorded throughout elution; ChemStation software was used to process the resulting data. Identifications were done as previously described by Gros et al.[7] The following equation was used for commercially available 3SHA and 3SHol (X) quantitation

$$\mu\text{g L}^{-1}\text{ of }X = \mu\text{g L}^{-1}\text{ of IST} \times \frac{X\text{ area}}{\text{IST area}} \times \frac{\text{IST molar response coefficient}}{X\text{ molar response coefficient}} \times \frac{X\text{ molar weight}}{\text{IST molar weight}} \times \frac{\text{IST recovery factor}}{X\text{ recovery factor}}$$

For commercially unavailable 3SPA, 3SPol, 3S4MPA, and 3S4MPol (X), the good equimolarity of the PFPD detector enabled us to set the IST-relative molar response coefficients at 1 and just the corrective molar weight ratio was applied. For all thiols, the IST-relative recovery factor was set at 1 (experimental values from 0.8 to 1.2, determined beforehand by standard addition).

Release Efficiency Determination. The efficiency of the release of free X_{OH} (sulfanylalkyl alcohol) from bound X_{OH} was calculated with the following equation

$$X_{\text{OH}}\text{ release efficiency (\%)} = \frac{\mu\text{g L}^{-1}\ X_{\text{OH}}}{\mu\text{g L}^{-1}\text{ added bound }X_{\text{OH}}} \times \frac{\text{bound }X_{\text{OH}}\text{ molar weight}}{\text{free }X_{\text{OH}}\text{ molar weight}} \times 100$$

For the corresponding esters, the efficiency of the release of free X_A (sulfanylalkyl acetate) from bound X_{OH} was calculated in alcohol equivalents

$$X_{\text{A}}\text{ release efficiency (\%)} = \frac{\mu\text{g L}^{-1}\ X_{\text{A}}}{\mu\text{g L}^{-1}\text{ added bound }X_{\text{OH}}} \times \frac{\text{bound }X_{\text{OH}}\text{ alcohol molar weight}}{\text{free }X_{\text{A}}\text{ acetate molar weight}} \times 100$$

The results are given as mean values of duplicates.

Acetate Ratio Determination. The ratio of acetate is calculated using the following equation

$$\text{ratio of acetate (\%)} = \frac{\mu\text{g L}^{-1}\ X_{\text{A}}}{(\mu\text{g L}^{-1}\ X_{\text{A}} + \mu\text{g L}^{-1}\ X_{\text{OH}})} \times 100$$

Table 2. Sulfanylalkyl Alcohols and Acetates Released and/or Biosynthesized (X_{OH} and X_A Release Efficiency in %) by K-97 Yeast after 7 Days of Fermentation and 3 Days of Maturation (4 °C) of a 15°P Wort Spiked with 5 mg L^{-1} Cys-3SPol, Cys-3S4MPol, and Cys-3SHol, or 10 mg L^{-1} G-3SPol, G-3S4MPol, and G-3SHol[a]

	spiking with					
	Cys-adducts			G-adducts		
trial→	B (18 °C)	A (24 °C)	C (28 °C)	B (18 °C)	A (24 °C)	C (28 °C)
3SPol	0.57^a	0.42^b	0.34^b	0.00^b	**0.01^a**	0.00^b
3S4MPol	0.26^b	0.36^a	0.43^a	0.02^c	**0.08^a**	0.04^b
3SHol	0.42^a	0.45^a	0.37^b	0.00^c	**0.02^a**	0.01^b
3SPA	0.00^b	**0.09^a**	0.00^b	0.00^b	**0.01^a**	0.00^b
3S4MPA	0.00^b	**0.10^a**	0.00^b	0.00	0.00	0.00
3SHA	0.00^b	**0.01^a**	0.00^b	0.00^b	**0.03^a**	0.00^b

[a]Standard deviations were considered in the Student−Newman−Keuls test. Values in the same row (in Cys-adduct and G-adduct columns independently) that do not share a common letter are significantly different (p > 0.05).

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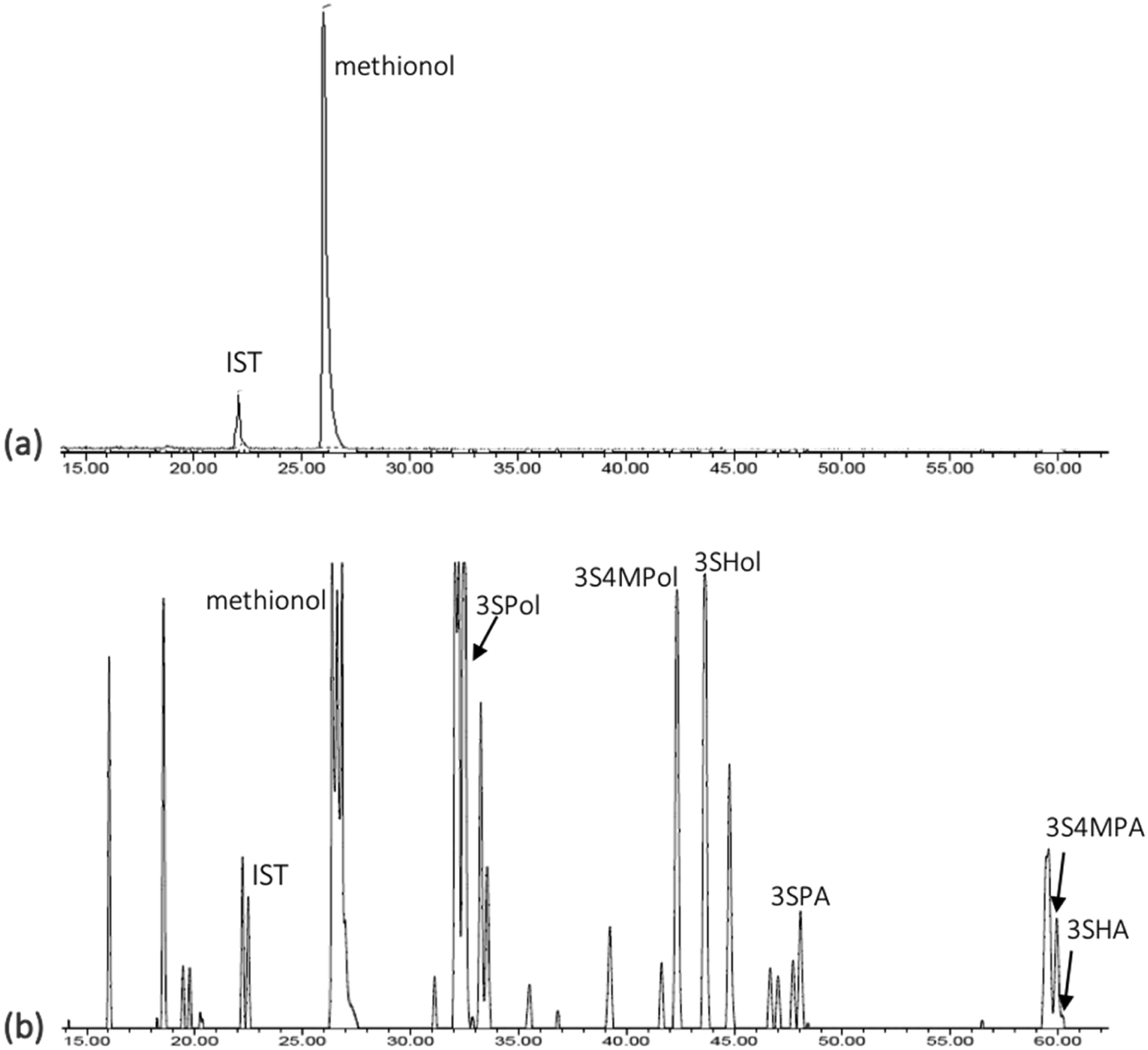

Figure 2. GC-PFPD chromatograms of a spiked unhopped 15°P wort fermented by K-97 yeast for 7 days at 24 °C and kept at 4 °C for (a) 0/trial D or (b) 3/trial A additional days.

■ RESULTS AND DISCUSSION

Yeast K-97 at Different Primary Fermentation Temperatures. In our first investigations conducted to evidence the ability of brewing yeasts to free PFTs from *S*-conjugates, primary fermentation was conducted for 10 days at 22 °C.[12,25] As limit attenuation was already reached in all assays after 5 days (no more weight loss), 7 day fermentations were here preferred to compare three temperatures: 18, 24, and 28 °C, before 3 days of maturation (4 °C). Spiking levels of the PFT precursors have been set arbitrarily to ease the chromatographic detection. As the obtained amount of PFTs do not correspond to the realm of what one might expect from a hopped beer, PFT releases will only be discussed as relative percentages (X_{OH} and X_A release efficiency).

Both for cysteinylated and glutathionylated adducts, only trial A (24 °C) allowed us to detect the acetates 3SPA, 3S4MPA, and 3SHA (Table 2). Although release from cysteinylated adducts appeared quite structure-dependent, fermentation at 24 °C (trial A) emerged as the best option for releasing a maximum of all three sulfanylalkyl alcohols from glutathionylated adducts (0.01% of 3SPol at 24 °C, not detected in trials B and C at 18 and 28 °C; 0.08% of 3S4MPol instead of 0.02−0.04%; and 0.02% of 3SHol instead of 0−0.1%).

As expected, PFT release from glutathionylated adducts was much (6−10 times) lower than from cysteinylated adducts. Glutathionylated adducts, however, are usually found in hop at levels 10−100 times higher than their cysteinylated counterparts. The impact of yeast activity on the conversion of this fraction can thus be determinant. Indeed, one can estimate based on these findings that if a 15°P wort late-hopped with 400 g.hL^{-1} Polaris (118 mg kg^{-1} G-3SHol)[12] was fermented with K-97 for 7 days at 24 °C and kept for 3 days at 4 °C, up to 94 ng L^{-1} 3SHol and 32 ng L^{-1} 3SHA would be found in the resulting beer (both above their respective perception thresholds).

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Yeast K-97 with Different Maturation Times at 4 °C. It is common for commercial-scale brewers to hold their beer at 0–4 °C for 1–4 weeks as a way to allow for carbonyl (e.g., diacteyl) reduction and other aroma refinements to occur.[34]

In our lab-scale trials, maturation appeared necessary in order to observe PFT release from cysteinylated and glutathionylated adducts (Figure 2a,b). When the spiked fermented product was analyzed directly after 7 days of fermentation, without any maturation (trial D), no PFTs were recovered: the chromatogram was practically flat (except for the methionol peak which, although methionol is not a PFT, is slightly extracted by the Ag cartridge). After 3 (trial A) or 5 (trial E) days of post-fermentation at 4 °C, similar amounts of sulfanylalkyl alcohols (Figure 3a) were recovered, but the last 2 days still brought significant amounts of esters (traces of 3SPA and 3S4MPA were found already after 3 days but 3SHA detection required 5 days).

We hypothesized that while both the release and esterification of PFTs probably occur inside the yeast cells during primary fermentation, their excretion seems to require a period at a low temperature.

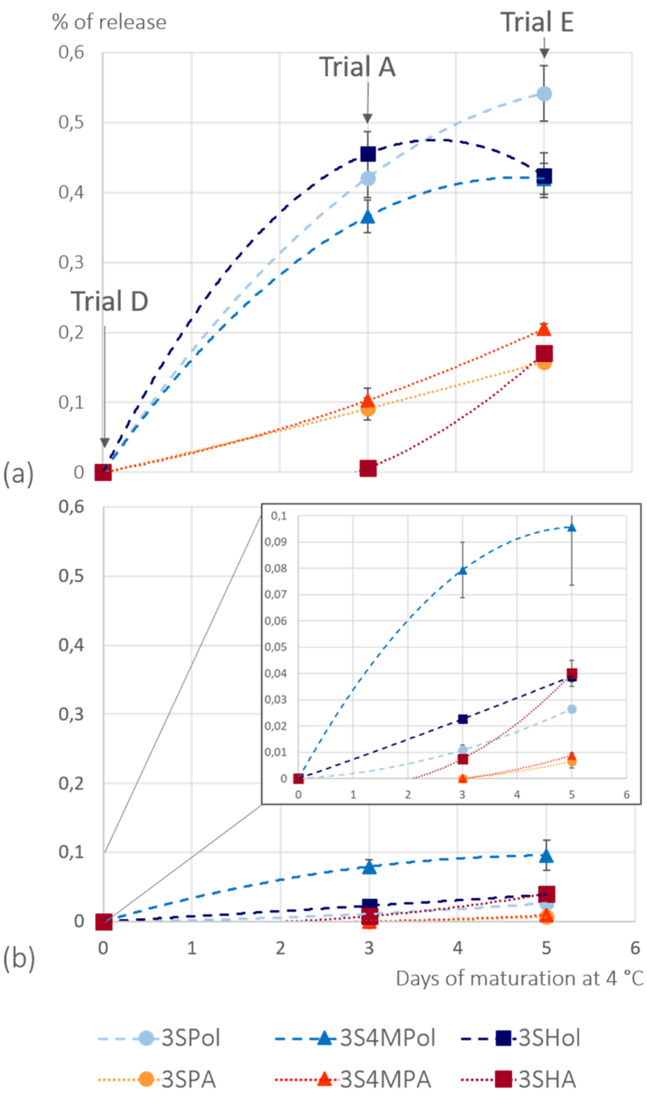

Figure 3. Sulfanylalkyl alcohols and acetates released and/or biosynthesized (%) by K-97 yeast from (a) Cys-adducts and (b) G-adducts as a function of maturation duration (trials D, A and E). Error bars illustrate the variation between duplicates.

When glutathionylated adducts were tested (Figure 3b), 3S4MPol appeared to be released preferentially (X_{OH} release efficiency of 0.08–0.09% after 3–5 days of maturation, as compared to the mere 0.04 and 0.03% reached by 3SHol and 3SPol, respectively). Another surprising result is the higher production of 3SHA (reaching, like 3SHol, a X_A release efficiency of 0.04% after 5 days) than that of 3S4MPA and 3SPA (barely reaching 0.01%).

Yeast K-97 in Worts of Different Densities. Wort density is known to greatly affect the yeast condition and metabolism during fermentation. Therefore, three wort densities were tested: 12, 15, and 17°P (trials F, A, and G).

Figure 4a shows that for cysteinylated precursors, the lower the initial density (i.e., the better the condition of the yeast), the

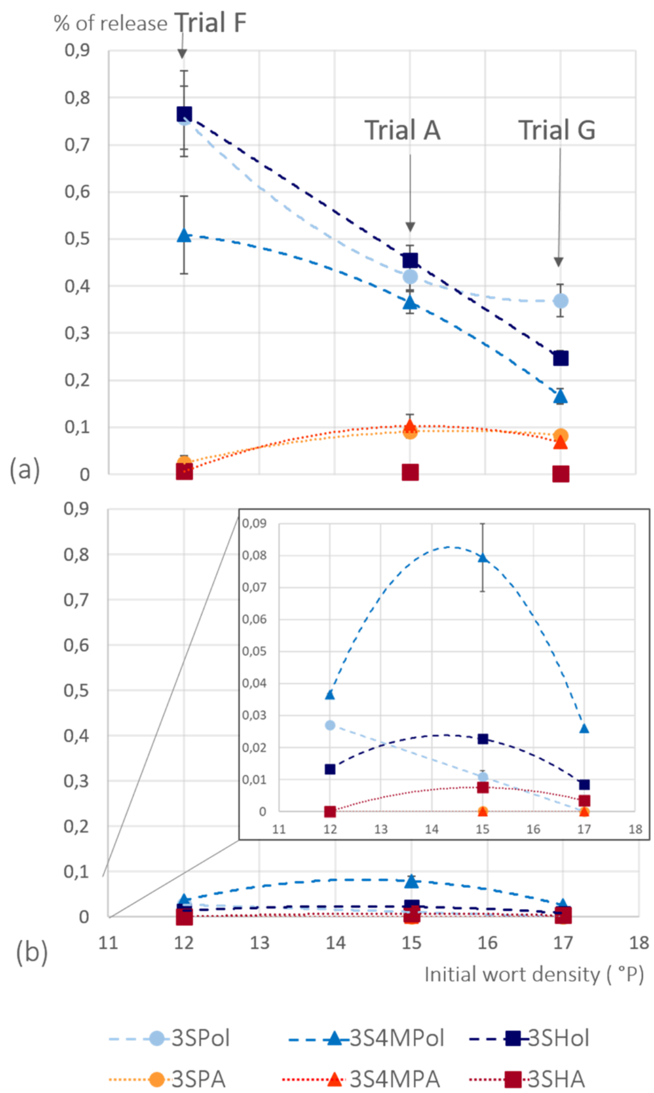

Figure 4. Sulfanylalkyl alcohols and acetates released and/or biosynthesized (%) by K-97 yeast from (a) Cys-adducts and (b) G-adducts as a function of initial wort density (trials F, A, and G). Error bars illustrate the variation between duplicates.

higher the sulfanylalkyl alcohol release percentage (X_{OH} release efficiency of 0.5–0.8% at 12°P vs 0.2–0.4% at 17°P). On the other hand, the higher the initial density (known to stress the yeast), the higher the ratio of acetate (Table 3). In the case of the sulfanylalkyl alcohols, no strong effect of the chemical structure

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Table 3. Ratio of Acetate (Released Acetate/Released Alcohol + Acetate, %) from Cys- and G-adducts in Different Trials with K-97 Yeast

	spiked with					
	Cys-adducts			G-adducts		
trial→	F (12°P)	A (15°P)	G (17°P)	F (12°P)	A (15°P)	G (17°P)
3SPA	3	18	**18**	0	1	0
3S4MPA	1	22	**29**	0	0	0
3SHA	1	1	**1**	0	25	**29**

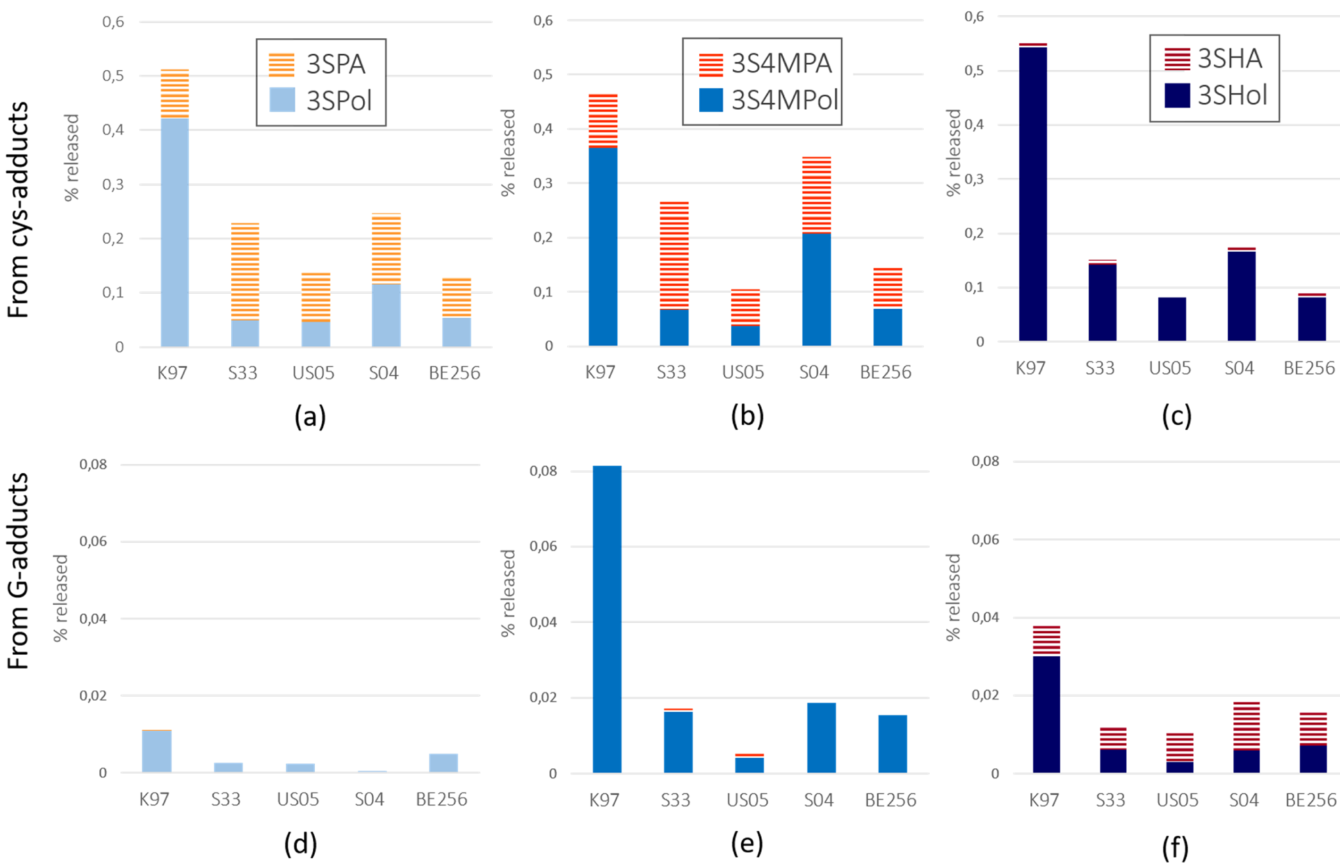

Figure 5. Sulfanylalkyl alcohols (solid fill) and acetates (striped pattern fill) released and/or biosynthesized (%) after 7 days of fermentation at 24 °C and 3 days of maturation at 4 °C (trial A) from Cys-adducts (a, b, c) or G-adducts (d, e, f) added to a 15°P wort pitched with yeast K-97, S-33, US-05, S-04, or BE-256.

Table 4. Ratio of Acetate (Released Acetate/Released Alcohol + Acetate, %) from Cys- and G-adducts for Different Yeast Strains (Trial A)

	spiked with									
	Cys-adducts					G-adducts				
yeast→	K-97	S-33	US-05	S-04	BE-256	K-97	S-33	US-05	S-04	BE-256
3SPA	18	79	66	53	58	1	0	0	0	0
3S4MPA	22	75	64	40	53	0	6	23	0	0
3SHA	1	6	4	7	8	20	50	71	68	54

on release was observed (3S4MPol was slightly less produced), whereas 3S4MPA and 3SPA were significantly more produced than 3SHA, especially at 15 and 17°P.

In worts spiked with glutathionylated PFTs (trial F in Figure 4b), X_A release efficiency was again the lowest at 12°P. In contrast, the production of the sulfanylalkyl alcohols 3S4MPol and 3SHol peaked at 15°P (trial A, X_{OH} release efficiency of 0.08 and 0.02%, respectively). Whatever the wort density, 3S4MPol was released preferentially to the other two sulfanylalkyl alcohols, and 3SHA was produced preferentially to the other two acetates (Table 3).

Comparison of K-97 with Four Other Dry Top-Fermentation Yeasts. On the basis of the results obtained with K-97 yeast, the release efficiencies of this strain and four other ale yeasts were compared under the following conditions: with an initial wort density of 15°P, 7 days of fermentation at 24 °C, and 3 days of maturation at 4 °C.

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As evidenced in Figure 5, K-97 shows the highest PFT-releasing efficiency, regardless of the thiol chemical structure or precursor type (total release efficiency is 1.5−5 times as high as for the other yeasts).

The yeast S-33, however, showed a greater ability to esterify the released thiols. When the wort was spiked with cysteinylated adducts of 3SPol, 3S4MPol, and 3SHol, the ratios of acetates 3SPA, 3S4MPA, and 3SHA reached, respectively, 75, 79, and 6% (Figure 5a−c, Table 4). As mentioned in the introduction, this enhanced production of pleasant esters could completely modify the final aromatic perception of the product. Taking into account a 10-fold lower sensory threshold for 3SHA than for 3SHol, and a confirmed synergic effect of 3S4MPA and 3S4MPol (perceived odor of 3S4MPA at a level much below its threshold when 3S4MPol is added at its threshold level)[35] the yeast S-04 emerged as the yeast with the most "analytical" perspective, combining efficient sulfanylalkyl alcohol release (with an X_{OH} release efficiency of 0.1−0.2%, the best after K-97) with efficient esterification (ratio of acetate is up to 53%) (Figure 5a−c).

As depicted in Figure 5d−f, a similar classification of thiol production emerged from the experiments conducted in the presence of glutathionylated adducts: K-97 > S-04 > S-33 > BE-256 > US-05. As already mentioned for K-97, 3SPol and 3S4MPol were more efficiently esterified when released from Cys-adducts, while 3SHol was better esterified when issued from glutathionylated adducts. For some adducts (cysteinylated or glutathionylated PFTs), esterification enzymes might also be involved before the action of β-lyase. As shown in trial E, differences between PFT structures disappeared after a longer maturation time.

Regardless of the strain used, these new results confirm the ability of ale-brewing yeasts to release PFTs from both cysteinylated and glutathionylated adducts. Our evidence further shows stronger-than-expected effects of wort density and maturation time. The ratio of acetate also varies greatly according to the fermentation conditions and yeast strain.

K-97 remains the best candidate for its ability to release sulfanylalkyl alcohols (X_{OH} release efficiency of up to 0.45 and 0.08% from Cys- and G-adducts, respectively), while S-33 and S-04 emerge as good challengers due to their better ester-producing efficiencies. To enhance the citrusy notes most probably conferred by sulfanylalkyl alcohols, fermenting a 12°P wort with K-97 can be recommended. A higher-gravity wort is more likely to produce more delicate and flowery notes characteristic of esters.

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Notes

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