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2022 ASBC Meeting

BREWING SUMMIT 2022
Providence, Rhode Island | August 14–16

How to Get the Most of the Aromatic Potential of Hops by Enabling the Release of Free Polyfunctional Thiols from their Non-Aromatic Precursors?

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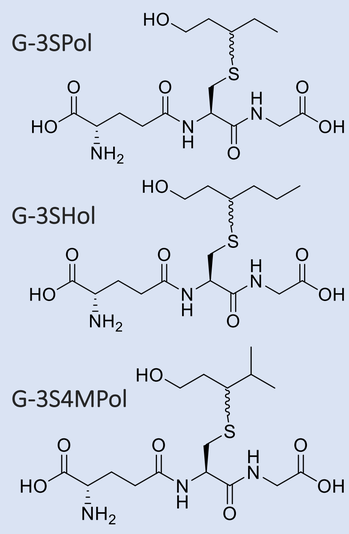
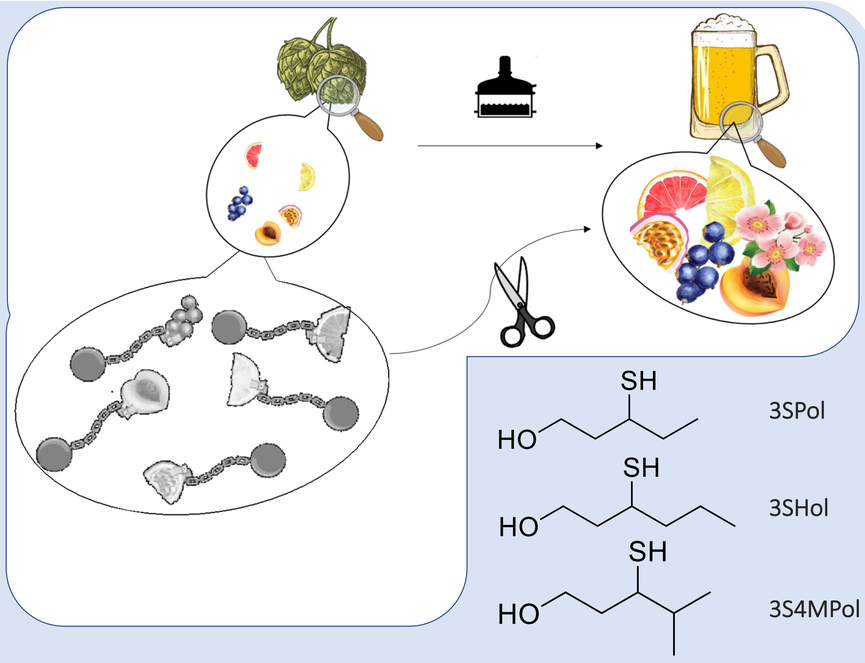
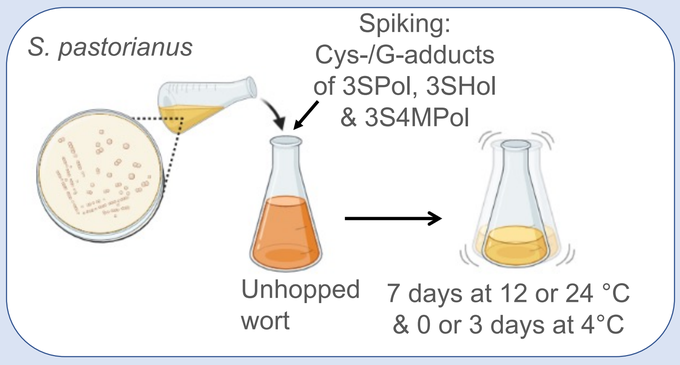
Introduction. The occurrence of a substantial pool of cysteinylated (Cys-) and glutathionylated (G-) forms of polyfunctional thiols (PFTs) has been evidenced for several dual-purpose hop varieties (Cys- and G- 3-sulfanyl-hexanol (3SHol) and 3-sulfanyl-pentanol (3SPol) ubiquitous to all studied varieties, Cys- and G- 3-sulfanyl-4-methylpentanol (3S4MPol) peculiar to some varieties). In the brewing field, the ability of yeast to hydrolyze cysteinylated adducts was first confirmed after bottle refermentation. Very recently, the ability of brewing yeast to release free PFTs from both Cys- and G-adducts, using synthetized adducts, was confirmed, in primary fermentation. It was evidenced that, regarding *S. cerevisiae* yeasts, a cold maturation period was required for thiol release. The present work aimed to investigate the effect of temperature, nitrogen levels, maturation time, and strain on the efficiency of sulfanylakyl alcohols release and sulfanylalkyl acetates formation from Cys- or G-adducts by *S. pastorianus* yeasts.



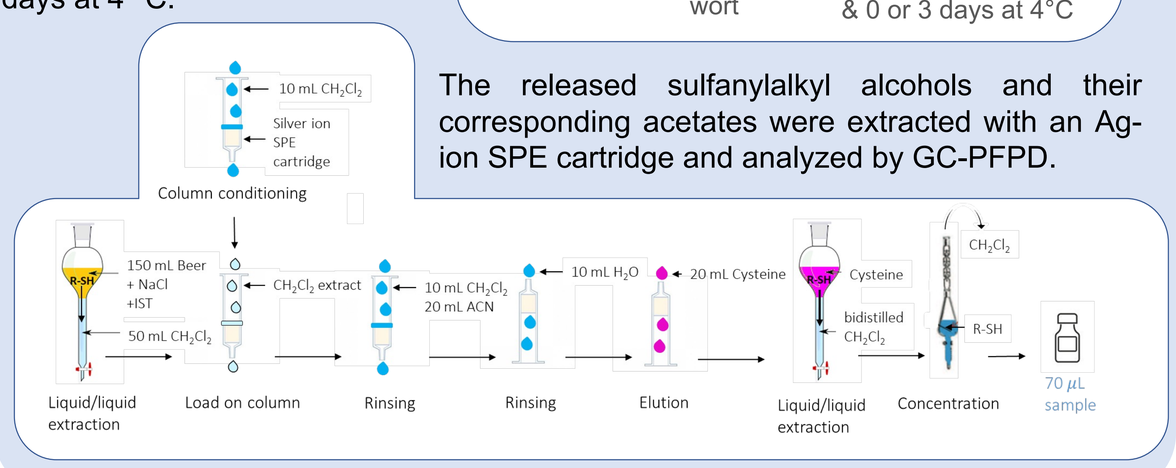


Materials & methods

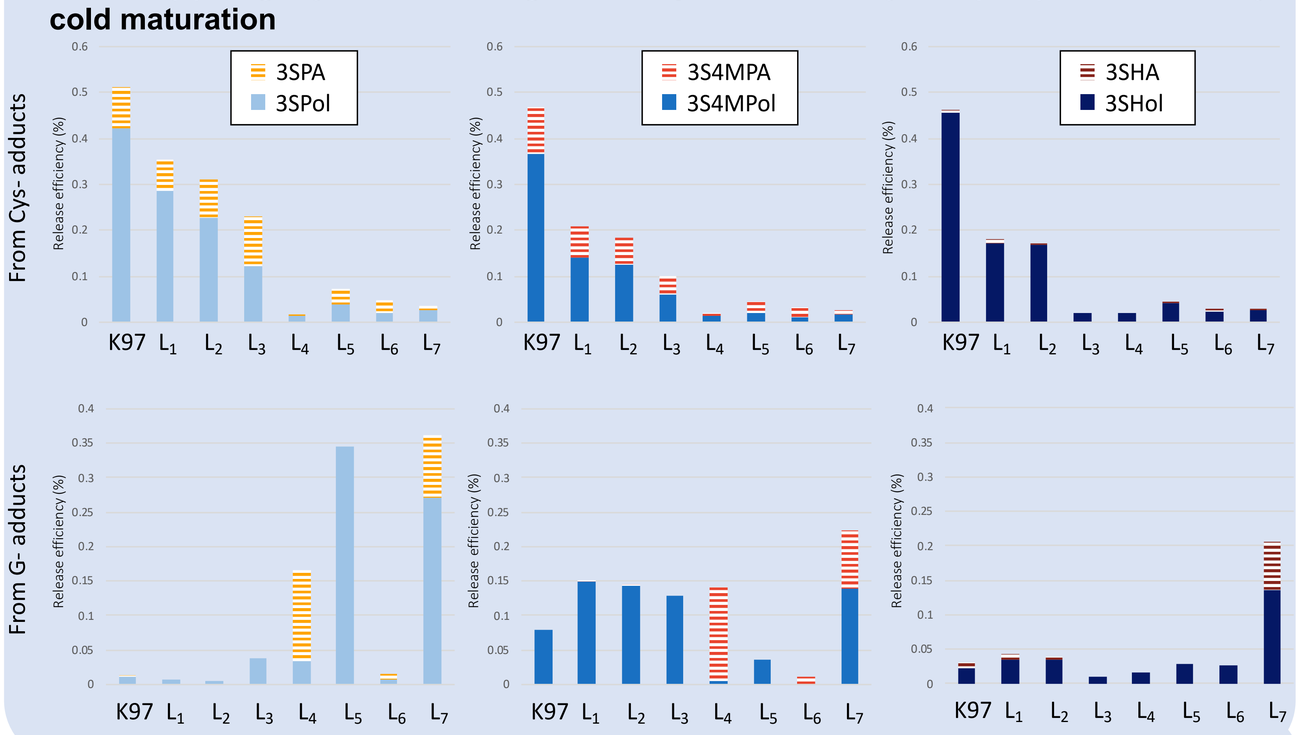
Unhopped worts (15°P) were spiked with Cys- (10 ppm) or G- (15 ppm) 3SPol, 3S4MPol and 3SHol, fermented for 4-7 days (at 12-24°C), with different FAN levels (270-135) and kept 0-3 days at 4 °C.



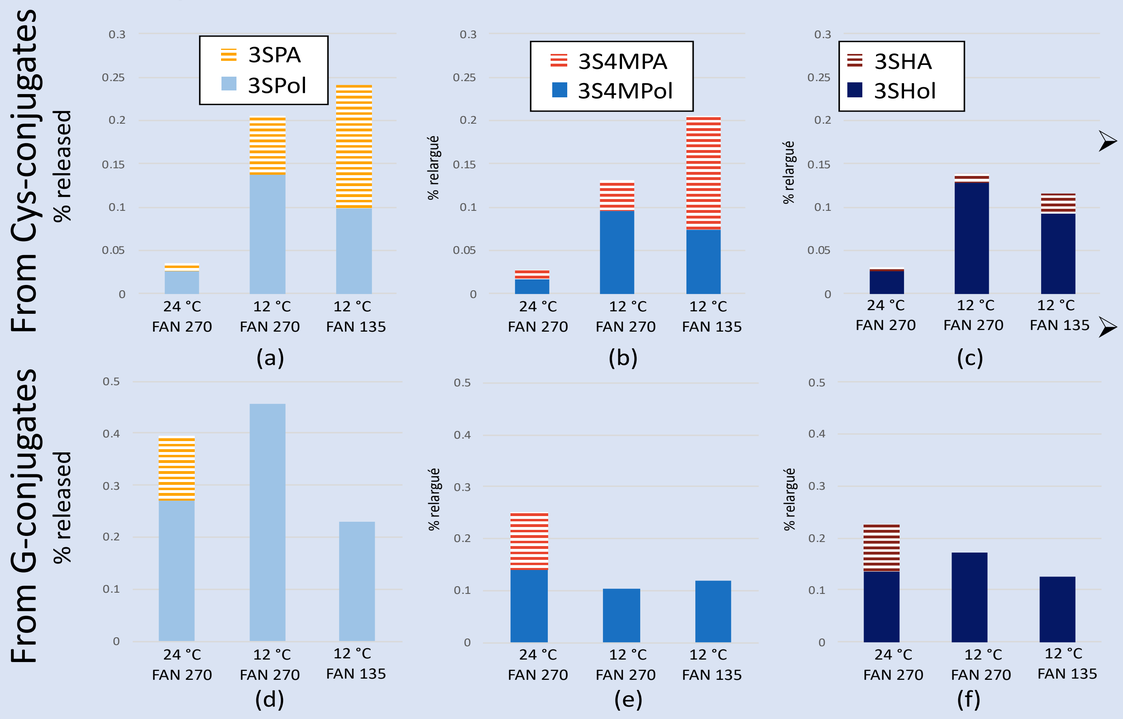
The released sulfanylalkyl alcohols and their corresponding acetates were extracted with an Ag-ion SPE cartridge and analyzed by GC-PFPD.



Results (1). Lager yeast screening and comparison vs ale yeast (K97) at 24°C + 3 days cold maturation



Results (2). Optimisation of fermentation parameters on L7 (7 days fermentation + 3 days cold maturation)



- Fermentation at 12 °C = increased release of thiols from Cys-conjugates but no esterification from G-conjugates
- Nitrogen reduction = increased esterification of alcohols released from Cys-conjugates, no effect on G-conjugates

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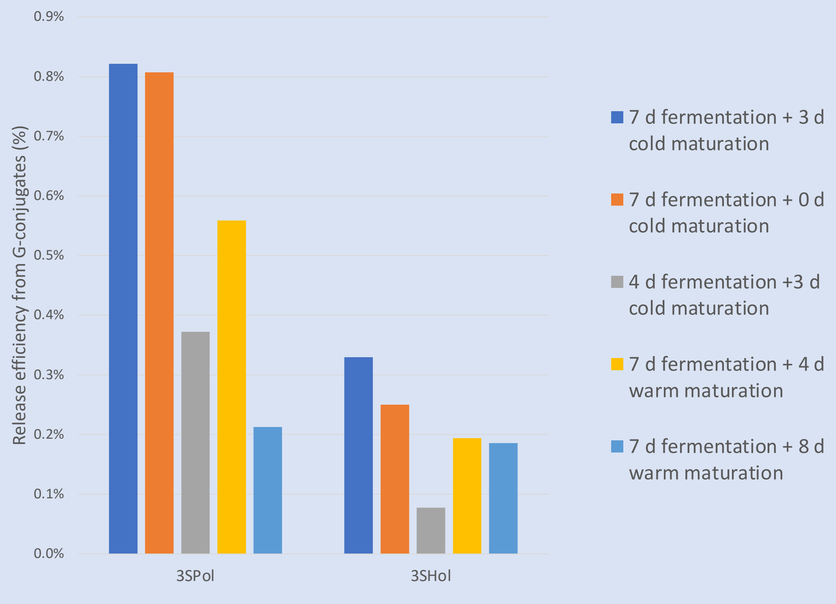
Results (3). Effect of fermentation (at 12°C) and maturation (at 4°C) time



- Free thiols observed in all samples : a fermentation at 12°C with lager yeast = sufficient to observe thiols release
- Higher release with 7 days fermentation, leading to the suggestion that a long contact time is required for thiols release
- Cold maturation is better for thiols preservation than warm maturation
- Best conditions: long fermentation and cold maturation = WHAT BREWERS KNEW FOR DECADES

- K-97 = highest rates of release from Cys-conjugates
- Increased release from G-conjugates for a majority of *S. pastorianus* (especially L7)
- Release selectivity according to the nature of the thiol varies according to the yeast strain

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Conclusion

- Free thiols are key odorant molecules in hopped beer, mostly originating from non odorant precursors found in huge amount in hops but still poorly recovered so far
- During fermentation, yeast has the potential to release odorant free thiols from their precursors
- Lager yeast show an interesting potential for the release from G-conjugates compared to ale yeast
- Cold temperatures seem to be required for thiol release, whether it is during maturation (ale yeast) or fermentation (lager yeast)
- Nitrogen concentration has an impact on alcohol esterification
- Mechanism is still poorly understood and requires further investigations

More information in Traité de brasserie, Volume 1, Chapitre 4 (Le houblon) & Volume 2, Chapitre 10 (Théorie de la fermentation)

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