

HPLC-ESI(+)MS/MS quantitation of the newly evidenced glutathione S-conjugates in two dual-purpose hop varieties: Citra and Sorachi Ace

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Abstract

After the evidence in hop of the cysteinylated precursors of 3-sulfanyl-4-methylpentan-1-ol (3S4MPol) and 3-sulfanylhexas-1-ol (3SHol), S-glutathione precursors were recently investigated in Amarillo, Hallertau Blanc and Mosaic. The aim of the present work was to assess the linked-potential in two other dual-purpose hop cultivars, namely Citra and Sorachi Ace. The occurrence of S-3-(1-hydroxyhexyl)glutathione (G-3SHol) was confirmed in all cultivars, at levels well above those reported for the cysteinylated counterpart, while S-3-(4-methyl-1-hydroxypentyl)glutathione (G-3S4MPol) revealed more specific of the Hallertau Blanc variety.

Introduction

Dual-purpose hop cultivars are characterized by high contents of both bitter acids (>7% humulones) and essential oils. Among the essential oils, odorant polyfunctional thiols, present in much lower amounts (1-150 µg/kg) than terpenols (10–100 mg/kg), are viewed as key contributors to hop flavour in beer, especially when dry hopping or bottle refermentation are applied. Most of them have a 3-carbon distance between the SH group and the other chemical function (alcohol, ester, carbonyl, etc.). [1-5] 41 Volatile polyfunctional thiols have been found in hop, and each cultivar exhibits a unique thiol profile. [5-7] Among them are found 3S4MPol with its very nice grapefruit/rhubarb-like flavours (odour perception threshold = 70 ng/L in beer), and 3SHol, also with a grapefruit-like flavour (odour perception threshold = 55 ng/L in beer). [6,7] Hoppy flavours can be enhanced in beer by applying either late hopping (addition of hop at the end of wort boiling or in the whirlpool) or dry hopping (addition of hop during beer fermentation or maturation). As described by Gros *et al.*, the thiol content of the final beer reaches higher values than might be expected on the basis of hopping rate and hop free thiol contents, due to the presence of heavy precursors including cysteine adducts (levels 20-120 times higher than the free forms). [5] In plants, cysteine-S-conjugates usually arise through the glutathione detoxification pathway, where the tripeptide is added to an α,β -unsaturated carbonyl in the presence of glutathione-S-transferase. The resulting glutathione-S-conjugate is further converted to the corresponding S-cysteine conjugate after successive enzymatic cleavages of glycine and glutamate residues. [8,9] The occurrence in hop of glutathione S-conjugates was evidenced for the first time in 2016. [10] Very high concentrations of G-3SHol were quantitated in Amarillo, Hallertau Blanc and Mosaic cultivars (up to 32 mg/kg). The aim of the present work was to investigate G-3SHol and G-3S4MPol in two other dual-purpose hop varieties: Citra and Sorachi Ace.

Experimental

Extraction of cysteine and glutathione S-conjugates

Thiols S-conjugates were extracted (according to Kankolongo *et al.* [10]) from the Citra and Sorachi Ace hop varieties. S-Benzylcysteine (Cys-IST) was used as an internal standard at 8 mg/kg of hop. Milled pellets (100 g) were stirred with 1000 mL of a 1% (v/v) formic acid aqueous solution for 2 h at 45 °C. After centrifugation for 30 min, the supernatants were collected and loaded on a column of IR-120 cation exchange resin (100 g preconditioned with 100 mL of aqueous 2M HCl followed by 1 L of water). The column was then washed with 500 mL of water and the thiol precursors were recovered by elution with aqueous ammonia solutions from 0 to 3.3 mol/L (increment of 0.3 mol/L). Glutathione adducts are eluted in the 1.2–2.4 mol/L fractions (also containing the cysteine S-conjugates). Those fractions were pooled and concentrated under reduced pressure. The obtained extract was dissolved in a formic acid aqueous solution for analysis by HPLC-ESI(+)-MS/MS with the Cyclobond I 2000 RSP chiral column. The elution solvents were water containing 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid (solvent B). An isocratic elution with 95% solvent A and 5% solvent B was applied, with a flow rate of 300 µL/min. 5 µL of sample was injected onto the column at room temperature. The mass spectra were acquired with a BrukerDaltonics Esquire 3000 ion trap mass spectrometer equipped with an electrospray ion source (Bruker) operated in positive mode (ESI+). The ESI inlet conditions were as follows: source voltage, 4.5 kV; capillary temperature, 360 °C; nebulizer, nitrogen, 12 Psi. Nitrogen was also used as drying gas, at a flow rate of 8 mL/min. For identification, collision-induced dissociation MS/MS spectra were recorded at a relative collision energy of 0.2 V. For quantitation, the MRM mode was applied (relative collision energy of only 0.05 V to maximize the [M + H⁺] ions). Calibration curves of G-T relative to Cys-IST were determined and the following equation was used: concentration of G-T (in µg/kg) = concentration of Cys-IST (in µg/kg) × (peak area of G-T/peak area of Cys-IST) × (mass response coefficient of Cys-IST/mass response coefficient of G-T). All analyses were carried out in duplicate.

Results and discussion

As depicted in Figure 1, HPLC-ESI(+)MRM analyses enabled us to evidence both diastereomers of G-3SHol in Citra and Sorachi Ace hop cultivars, at concentrations similar to those reported by Kankolongo *et al.* for the Amarillo, Hallertau Blanc and Mosaic hops. On the other hand, no bound 3S4MPol was found. [10]

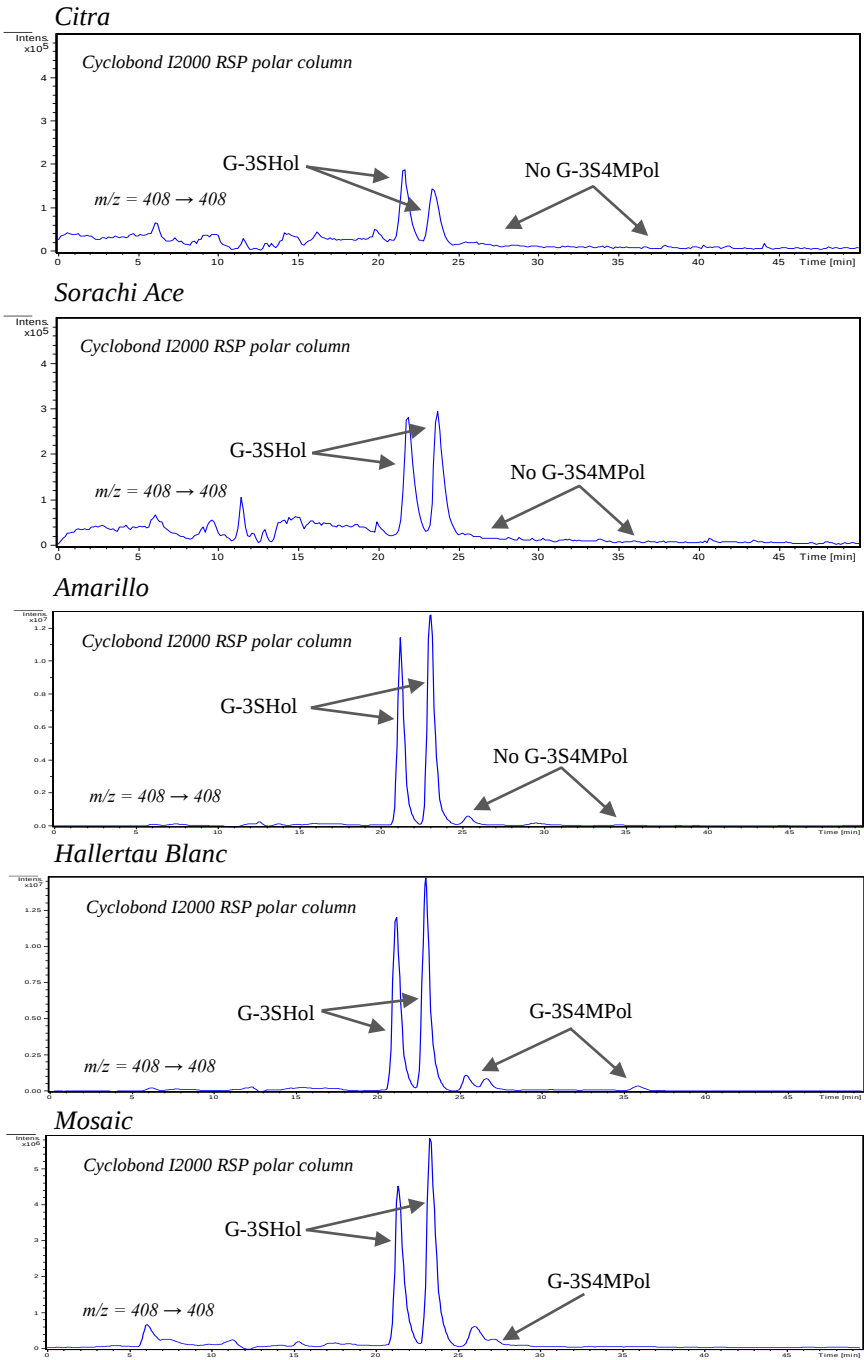


Figure 1: HPLC-ESI(+)MRM analysis of G-3SHol and G-3S4MPol diastereomers in Citra and Sorachi Ace hops on the Cyclobond I 2000 RSP column. Comparison with three previously investigated cultivars. [10]

Given in free thiol equivalents (Figure 2), the glutathionylated 3SHol emerged as the key fraction in all cultivars while for 3S4MPol, cysteinylated and free fractions remain important to be considered.

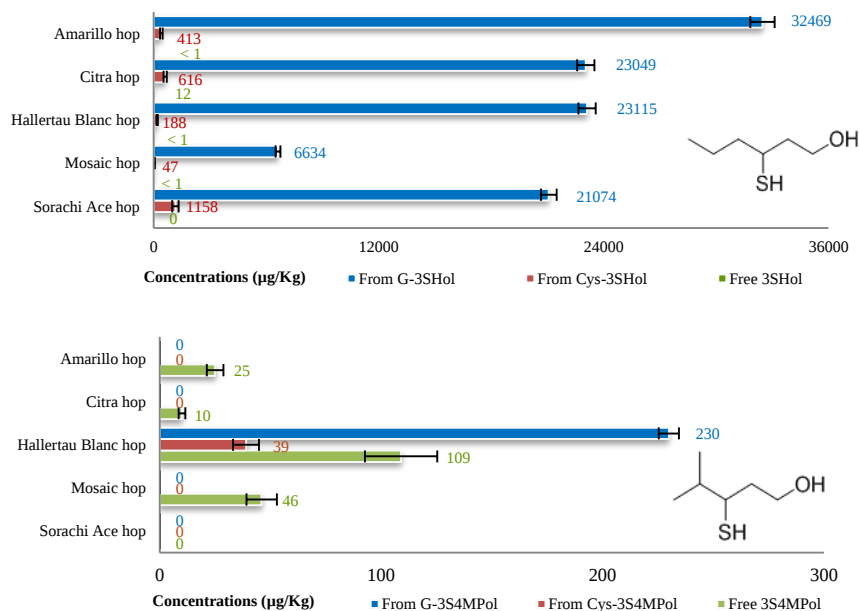


Figure 2: Concentration (µg/kg) of free and bound potentials (given in free thiol equivalents) of 3SHol and 3S4MPol in Citra and Sorachi Ace hop varieties. Comparison to Amarillo, Hallertau Blanc and Mosaic hops. [10]

In conclusion, 3SHol seems relatively ubiquitous in free, cysteinylated, and glutathionylated forms while the glutathione adduct of 3S4MPol was found only in the Hallertau Blanc variety. Further research is needed to understand how more thiols could be released from glutathione forms through the brewing process.

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