Research article

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First evidence of the glutathione *S*-conjugate of 3-sulfanylheptanol in green malt: discrepancy with the ubiquitous 5- and 6-C analogues

Cécile Chenot,[1] Raphaël Robiette[2] and Sonia Collin[1]*

While cysteinylated (Cys-) and glutathionylated (G-) precursors of 3-sulfanylhexanol (3SHol) and 3-sulfanylpentanol (3SPol) appear to be ubiquitous in hop varieties, no data are available on precursors of their seven-carbon analogue 3-sulfanylheptanol (3SHptol), although the free form has been found in both hops and beer. Chemical synthesis of Cys- and G-3SHptol enabled determination of their chromatographic elution times (14.3-14.8 and 16.2-17.1 min, respectively) and ESI(+) mass spectra (main m/z fragments: 219 and 293, for Cys- and G- 3SHptol, respectively). Here, for the first time, we report the occurrence of G-3SHptol in a natural matrix. RP-HPLC-ESI(+) MRM analysis of selective extracts eluted from a cation exchange resin quantified G-3SHptol in green malt (0.10 mg/kg), while no trace was found after kilning. Neither the cysteinylated nor the glutathionylated conjugate was found in hop or grape extracts. G-3SHptol is formed in-situ from *trans*-2-heptenal and free glutathione, and its synthesis most likely involves aldol condensation between acetaldehyde and pentanal instead of lipid oxidation. © 2022 The Institute of Brewing & Distilling.

Keywords: polyfunctional thiols; cysteine-conjugates; glutathione-conjugates; alpha, beta-unsaturated aldehyde

Introduction

Polyfunctional thiols (PFTs), among the most powerful aromas, have been studied in several matrices *(1)*, mostly wine *(2–6)*, beer and corresponding raw materials *(7–10)*. The discovery of a substantial pool of non-odorous PFT S-conjugates in grapes *(11–16)*, malt *(17,18)*, and hops *(10,16,19–21)* has stimulated more research.

The number of identified S-conjugates is limited to the cysteinylated and glutathionylated forms (and in some cases the cysteinylglycinylated and glutamylcysteinylated conjugates) of sulfanylalkyl alcohols (3-sulfanylpentanol, 3SPol *(21)*; 3-sulfanylhexanol, 3SHol *(11,12)*; and 3-sulfanyl-4-methylpentanol, 3S4MPol *(19)*), corresponding aldehydes (3-sulfanylpentanal, 3SPal *(16)*; 3-sulfanylhexanal, 3SHal *(15)*), acetates (3-sulfanylpentyl acetate, 3SPA *(16)*; 3-sulfanylhexyl acetate, 3SHA *(16)*), and one sulfanylalkyl ketone (4-sulfanyl-4-methylpentan-2-one, 4S4M2Pone *(11,13)*). Of these, the 3SHol and 3SPol conjugates are ubiquitous to all hop varieties while others are specific to some varieties *(20,21)*.

In addition to seeking efficient ways to release this aromatic potential *(22–26)*, it is necessary to identify other PFTs S-conjugates to get a better view of the potential available to the grape, malt, or hop variety used. Of the free PFTs identified (41 in hops), 3-sulfanylheptanol (3SHptol, 7-C, Figure 1) is described as having an odour of 'lemon' and 'hoppy' with a threshold of 35 ng/L in hydroalcoholic solution. It has been recognised in hop varieties as key contributor to the lemon-like aroma of Cascade (up to 52.3 μg/kg), and has been found in lower concentrations in Nelson Sauvin and Nugget (1.2-2.0 and 14.1-21.2 μg/kg, respectively) *(7)*. This sulfanylalkyl alcohol is also found in botrytised wines from various Bordeaux regions (mean level of 51 ng/L) *(27)*, while its aldehyde counterpart has only been identified in Sauternes wines *(28)*. In botrytised wines, 3-sulfanylheptanol plays, together with 3SPol, a considerable role in the overall citrus aroma, even though both compounds exhibit low individual odour activity values. This is explained by the additive effect with 3SHol.

In this paper, direct liquid chromatography coupled with mass spectrometry (HPLC-MS) was used to investigate the occurrence of cysteinylated and glutathionylated 3-sulfanylheptanol (Cys-3SHptol and G-3SHptol, Figure 1) in raw materials - malt, hops, and grapes – involved in the brewing and wine-making processes. Both compounds were chemically synthesised and characterised by high-resolution mass spectrometry (HRMS) and nuclear magnetic resonance (NMR). The presence of both compounds was analysed by high-pressure liquid chromatography—positive electrospray ionisation—multiple reaction monitoring (HPLC-ESI(+)-MRM) in several samples of malt, hops, and grapes. Possible explanations for why 3SHptol S-conjugates are rare in natural matrices, compared to their 5- or 6-C counterparts, and potential synthetic pathways involving either lipid oxidation or aldol condensation are discussed.

* Correspondance to: Sonia Collin, Unité de Brasserie et des Industries Alimentaires, Louvain Institute of Biomolecular Science and Technology (LIBST), Faculté des Bioingénieurs, Université catholique de Louvain, Croix du Sud, 2 box L7.05.07, B-1348 Louvain-la-Neuve, Belgium. Email: sonia.collin@uclouvain.be

[1] Unité de Brasserie et des Industries Alimentaires, Louvain Institute of Biomolecular Science and Technology (LIBST), Faculté des Bioingénieurs, Université catholique de Louvain, Croix du Sud, 2 box L7.05.07, Louvain-la-Neuve B-1348, Belgium

[2] Institute of Condensed Matter and Nanosciences (IMCN), Université catholique de Louvain, Place Louis Pasteur 1, Box L4.01.02, Louvain-la-Neuve B-1348, Belgium

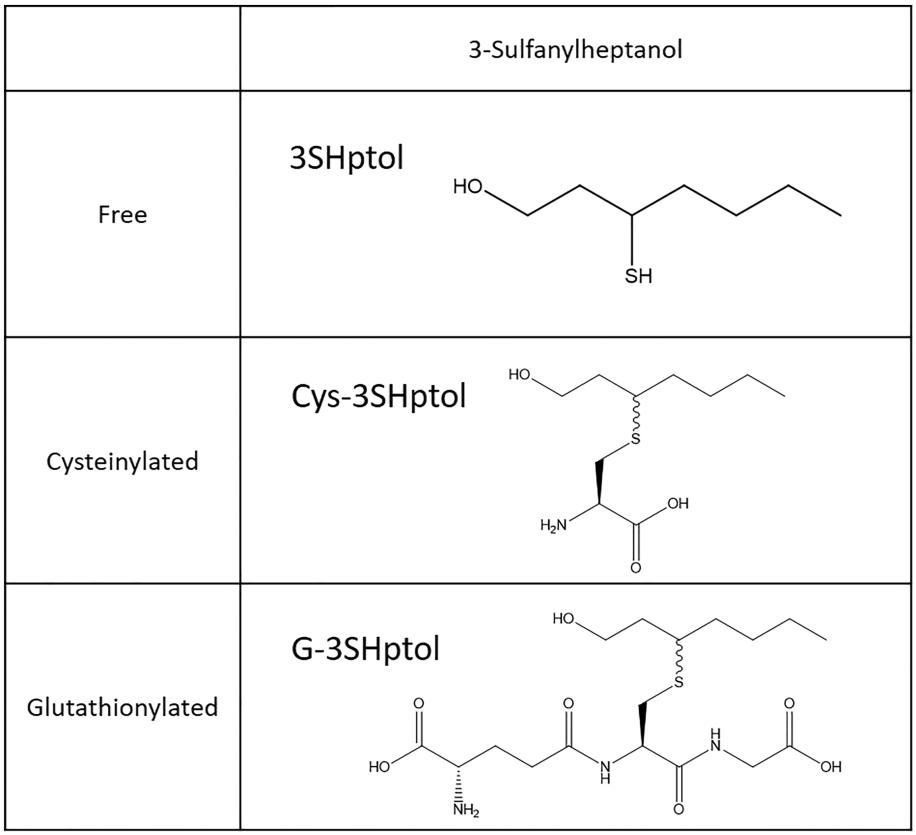


Figure 1. Chemical structures of free, cysteinylated, and glutathionylated 3SHptol.

Materials and methods

Chemicals

Absolute ethanol, acetonitrile, Amberlite IR-120 resin, 28% ammonia, dichloromethane, diethylether, ethyl acetate, formic acid, 37% hydrochloric acid and methanol were purchased from VWR (Leuven, Belgium). Anhydrous acetonitrile, cesium carbonate, deuterium oxide, >98% reduced L-glutathione, (E)-2-heptenal, S-benzyl-L-cysteine and sodium borohydride were purchased from Sigma-Aldrich (Bornem, Belgium). Anhydrous sodium sulphate and sodium hydroxide were purchased from Acros Organics (Geel, Belgium). N-Boc-L-cysteine, monosodium phosphate, trifluoroacetic acid, and disodium phosphate were purchased from Merck (Darmstadt, Germany). Milli-Q water was from Millipore (Bedford, MA, USA.). The 10g C18 Sep-Pak cartridges were purchased from Waters Millipore (Milford, MA, USA).

Synthesis of reference conjugates

Cys-3SHol, G-3SHol, Cys-3S4MPol, G-3S4MPol, Cys-3SPol and G-3SPol were synthesised according to the methods of Gros et al., *(10)*, Kankolongo et al., *(19)* and Chenot et al. *(21)*.

Malt samples

Three Pilsen malts (I, II, III) were purchased from Boortmalt (Belgium). Malt I was a 6-row winter variety (Etincel, harvest 2019) while the other two (II, III) were 2-row spring malts (harvest 2018 and 2020, respectively). Malt I was also provided as a green malt (sampled after 4 days of germination).

Hop samples

Vacuum packed T90 hop pellets (7−9% moisture) of different varieties, harvest years, and countries were arbitrarily selected and stored at -20°C. Saaz (Czech Republic, harvest 2020; α acids: 4.3%; oil content: 0.7%) and Mosaic (U.S.A., harvest 2016; α acids: 11.0%; oil content: 1.3%) were provided by Brouwland (Belgium); Hallertau Blanc (Germany, harvest 2015; α acids: 11.3%; oil content: 1.2%), Mandarina Bavaria (Germany, harvest 2019; α acids: 8.1%; oil content: 2.1%), and Polaris (Germany, harvest 2019; α acids: 19.1%; oil content: 3.6%) were provided by Hopsteiner (Germany); Nelson Sauvin (New Zealand, harvest 2018; α acids: 12.4%; oil content: 1.1%) was provided by Brouwerij Anders!; Amarillo (U.S.A., harvest 2015; α acids: 9.2%; oil content: 1.7%), Cascade (U.S.A., harvest 2020, α acids: 6.4%; oil content: 3.2%), Citra (U.S.A., harvest 2019; α acids: 12.3 %; oil content: 2.3%), and Sorachi Ace (U.S.A., harvest 2015; α acids: 13.5%; oil content: 2.3%) were provided by Yakima Chief (Belgium).

Grape samples

Different varieties of grapes were sampled at the end of September 2020. Chardonnay was collected at the Domaine de la Ferme du Chapitre (Baulers, Belgium) while Solaris and Johanniter were collected at the Domaine du Chenoy (Émines, Belgium). The grapes were crushed with a manual press and the must was stored at -20°C.

Synthesis of cysteinylated 3-sulfanylheptanol (Cys-3SHptol)

This procedure was adapted from the synthesis of Cys-3SPol, Cys-3SHol and Cys-3S4MPol described by Chenot et al. *(21)*, Gros et al. *(10)* and Kankolongo et al. *(19)*, respectively. Michael addition of N-Boc-L-cysteine (500 mg, 2.26 mmol, 0.9 equiv) on (E)-2-heptenal (0.328 mL, 2.51 mmol, 1 equiv) was performed overnight in anhydrous acetonitrile as solvent (7 mL) in the presence of cesium carbonate (350 mg, 1.13 mmol, 0.45 equiv). After evaporation of the solvent under reduced pressure, the aldehyde was dissolved in 5 mL of methanol and an aqueous solution of sodium borohydride (260 mg/4 mL, 6.87 mmol, 2.74 equiv) was added. The solution was stirred for 2 h. The pH was adjusted to pH 2 with hydrochloric acid (2 M), and 10 mL of water was added. The N-Boc protected product was extracted 3 times with 25 mL of ethyl acetate. The combined organic phases were washed with 25 mL of water, dried with sodium sulphate, and concentrated under reduced pressure. Deprotection of amine was achieved by reaction with trifluoroacetic acid (3.5 mL) in dichloromethane (10 mL) for 2 h. The solvent and excess trifluoroacetic acid were evaporated under reduced pressure. The product was dissolved in 5 mL of ethanol, and 5 mL of 2 M hydrochloric acid was added. After evaporation under reduced pressure, a pale-yellow solid was obtained. Yield of Cys-3SHptol, 38 %; ^{1}H NMR (300 MHz, deuterium oxide), δ 0.87 (t, 3H, $-CHCH_2CH_2CH_2\boldsymbol{CH_3}$), 1.21-1.46 (m, 4H, $-CHCH_2CH_2\boldsymbol{CH_2}CH_3$ and $-CHCH_2\boldsymbol{CH_2}CH_2CH_3$), 1.54−1.68 (m, 2H, $-CH\boldsymbol{CH_2}CH_2CH_2CH_3$), 1.68-1.95 (m, 2H, $-\boldsymbol{CH_2}$-CH_2OH), 2.89 (m, 1H, -C***H***S-), 3.14 (m, 2H, $-C\boldsymbol{H_2}S-$), 3.72 (m, 2H, $-CH_2\boldsymbol{CH_2}OH$), 4.28 (m, 1H, αCH in Cys); MS (ESI+) m/z [M + H]+ 236; HRMS (ESI+) calculated for $C_{10}H_{22}O_3NS$, 236.13149 Da; found, 236.13148 Da.

Synthesis of glutathionylated 3-sulfanylheptanol (G-3SHptol)

This procedure was adapted from the synthesis of G-3SPol, G-3SHol and G-3S4MPol described by Chenot et al. *(21)* and Kankolongo et al. *(19)*. (*E*)-2-Heptenal was added in three steps (0.33 equiv every 3 h) to a solution of glutathione (500 mg, 1.63 mmol, 1 equiv) in phosphate buffer (monosodium phosphate/disodium phosphate, 1 M, pH 8, 10 mL). After the first addition, the reaction mixture was stirred for 10 h at room temperature. The aldehyde derivative was reduced by adding dropwise an aqueous solution of sodium borohydride (177 mg/4 mL, 4.68 mmol, 2.87 equiv) to the reaction mixture. After 3 h of stirring at room temperature, the pH was adjusted to pH 2 with 6 M hydrochloric acid and the solvent was evaporated under reduced pressure. To purify the product, a 10g C18 Sep-Pak cartridge (Waters Millipore) was preconditioned with 200 mL of methanol and 300 mL of water. The product was dissolved in 5 mL of water and then loaded on the cartridge, washed with 100 mL of water, and eluted with 100 mL of acetonitrile/water/formic acid (89:10:1, v/v/v). The eluates were concentrated under reduced pressure. The resulting white solid residue was dissolved in 5 mL of aqueous 2 M hydrochloric acid and washed 3 times with 15 mL of diethylether. The final product was obtained after concentration of the aqueous phase under reduced pressure. Yield of G-3SHptol, 64 %; white powder; ^{1}H NMR (300 MHz, deuterium oxide), δ 0.90 (t, 3H, $-CHCH_2CH_2CH_2\boldsymbol{CH_3}$), 1.22-1.48 (m, 4H, $-CHCH_2CH_2\boldsymbol{CH_2}CH_3$ and $-CHCH_2\boldsymbol{CH_2}CH_2CH_3$), 1.52−1.68 (m, 2H, $-CH\boldsymbol{CH_2}CH_2CH_2CH_3$), 1.71-1.97 (m, 2H, $-\boldsymbol{CH_2}CH_2OH$), 2.19 (m, 2H, βC$\boldsymbol{H_2}$ in Glu), 2.56 (m, 2H, γC$\boldsymbol{H_2}$ in Glu), 2.80−2.98 (m, 2H, $-C\boldsymbol{H_2}S-$), 3.08 (m, 1H, $-C\boldsymbol{H}S-$), 3.73 and 3.84 (m, 2H, $-CH_2\boldsymbol{CH_2}OH$), 3.99 (s, 2H, αC$\boldsymbol{H_2}$ in Gly), 4.28 (m, 1H, αC***H*** in Glu), 4.57 (m, 1H, αC***H*** in Cys); MS (ESI+) m/z [M + H]+ 422; HRMS (ESI+) calculated for $C_{17}H_{32}O_7N_3S$, 422.19555 Da; found, 422.19545 Da.

HRMS of synthesised *S*-conjugates

To confirm the molecular formula of the synthesised products, they were analysed by HRMS as described by Chenot et al. *(21)*. The measured and calculated masses are given in Daltons.

^{1}H NMR spectra of synthesised *S*-conjugates

To confirm the chemical structure of the synthesised products, they were analysed by ^{1}H NMR as described by Chenot et al. *(21)*. All chemical shifts (δ) are reported in parts per million relative to the reference (tetramethylsilane (TMS)).

Extraction of cysteine and glutathione *S*-conjugates from malt, hop pellets and grape must

S-Benzyl-L-cysteine was used as the internal standard (IST) at 4-8 mg/kg according to the sample. For solid samples, the extraction method was identical to that reported by Chenot et al. *(29)* starting from either 150 mg milled malt or 100 mg milled hop pellets (solid/liquid extraction with acidified hydro-alcoholic solution; centrifugation; loading of the supernatant on cation exchange resin; elution with ammonia solutions of increasing concentrations). For the liquid must, 200 mL was acidified (1% formic acid) and directly loaded on the resin. The obtained extracts were dissolved in 2 mL of 0.1% formic acid aqueous solution for HPLC analysis. All extractions were performed in duplicate.

Reversed-Phase High-Performance Liquid Chromatography−positive Electrospray Ionization [RP-HPLC−ESI(+)-MRM] on Astec Cyclobond I 2000 RSP

Analyses were performed on a 250 × 4.6 mm, 5 μm, Astec Cyclobond I 2000 RSP chiral column (used here for its polarity and not chirality, as advised by Kankolongo et al. *(19)*). Water and acetonitrile with 0.1% formic acid were used as solvents A and B, respectively. The gradient elution was as follows: for solvent A, 95% for 5 min, from 95 to 80% in 5 min, 80% maintained for 15 min, from 80 to 10% in 1 min, 10% maintained for 8 min, and then back to the original conditions in 4 min for 17 min. The flow rate was set at 800 μL/min. A total of 10 μL of sample was injected into the column at 30°C. The equipment and software were as described by Chenot et al. *(21)*. For the MRM mode, m/z 236 → 229 was selected for Cys-3SHptol and m/z 422 → 293 for G-3SHptol.

Quantitation of cysteine and glutathione *S*-conjugates in malt, hop and grapes extracts

A calibration curve of G-3SHptol (the only compound detected) relative to IST was determined. Water with 0.1% formic acid, 5−10−15−20−25 mg/kg of synthesised conjugate, and 25 mg/kg IST were used to plot the linear curves (area ratio versus concentration ratio). The slope gave the conjugate-to-IST response coefficient ratio ($R^2 > 0.97$). The following equation was used for

the conjugate quantitation: concentration of the conjugate in the extract (mg/kg) = concentration of IST in the extract (mg/kg) × (conjugate peak area/IST peak area) × (response coefficient of IST/response coefficient of conjugate).

3SPol, 3SHol and 3S4MPol S-conjugates were also quantitated in green malt I, according to the procedure previously described by Chenot et al. *(29)* (data for grapes, hops and kilned malts obtained prior to this work *(16,18,21,29)*).

Results and discussion

Synthesis of S-3-(1-hydroxyheptyl)cysteine (Cys-3SHptol)

A pale-yellow solid was obtained with a 38% yield (weight ratio of final product to reagents in mole equivalents – mixture of the *R*- and *S*-diastereomers of 3SHptol conjugated to L-cysteine). As detailed in Figure 2A, the MS/MS-ESI(+) spectrum confirmed the

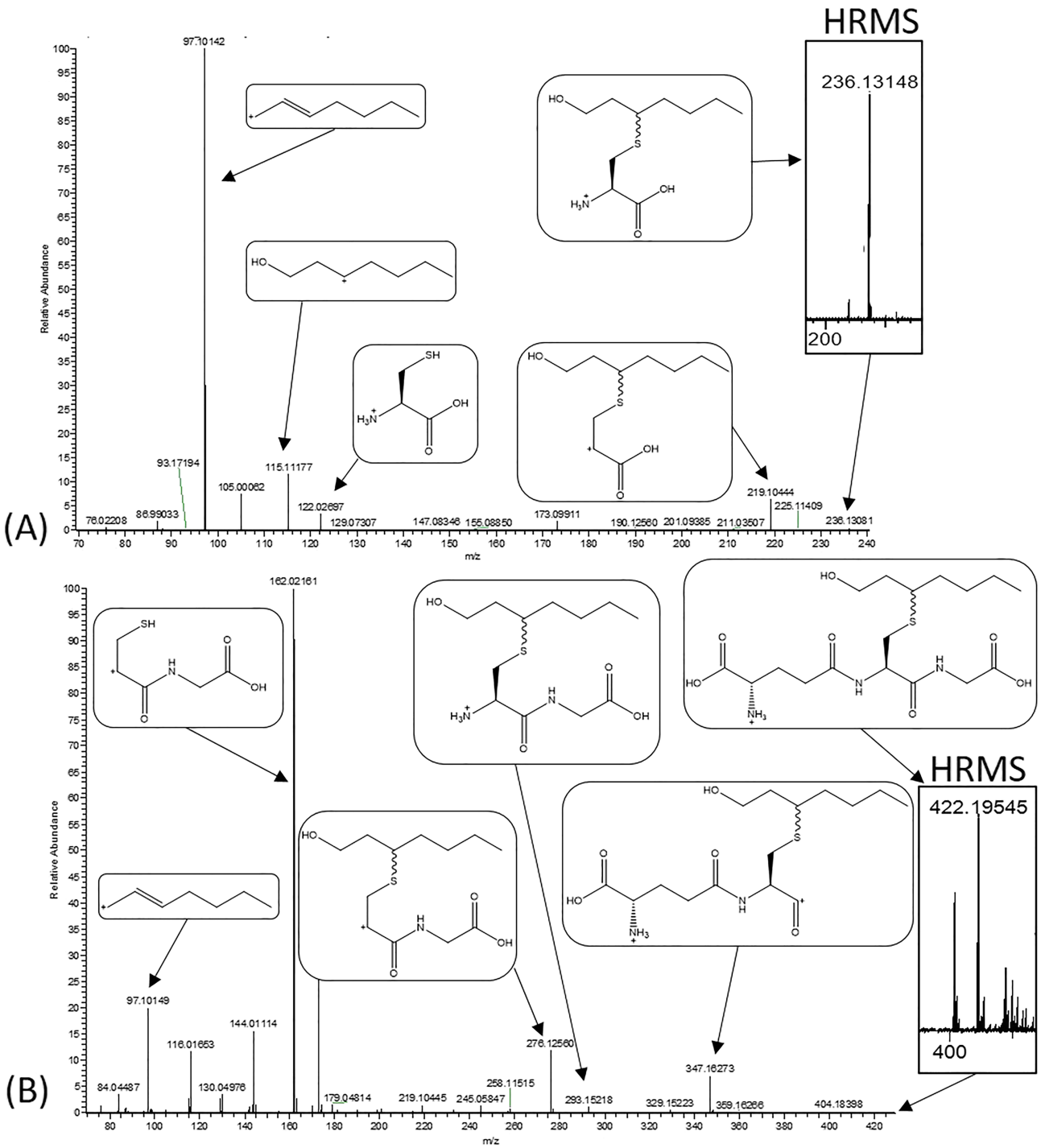
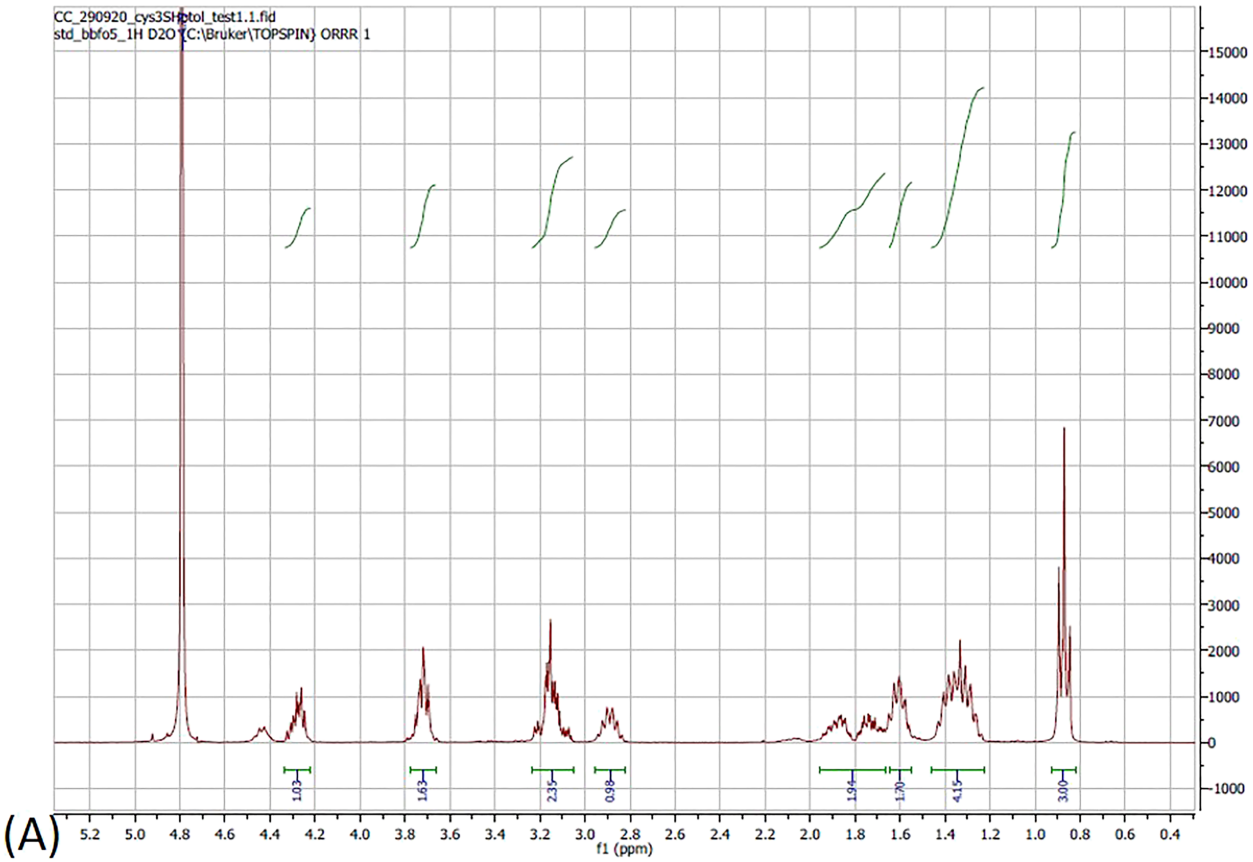


Figure 2. MS/MS-ESI(+) and HRMS mass spectra of (A) Cys-3SHptol and (B) G-3SHptol. [Colour figure can be viewed at wileyonlinelibrary.com]



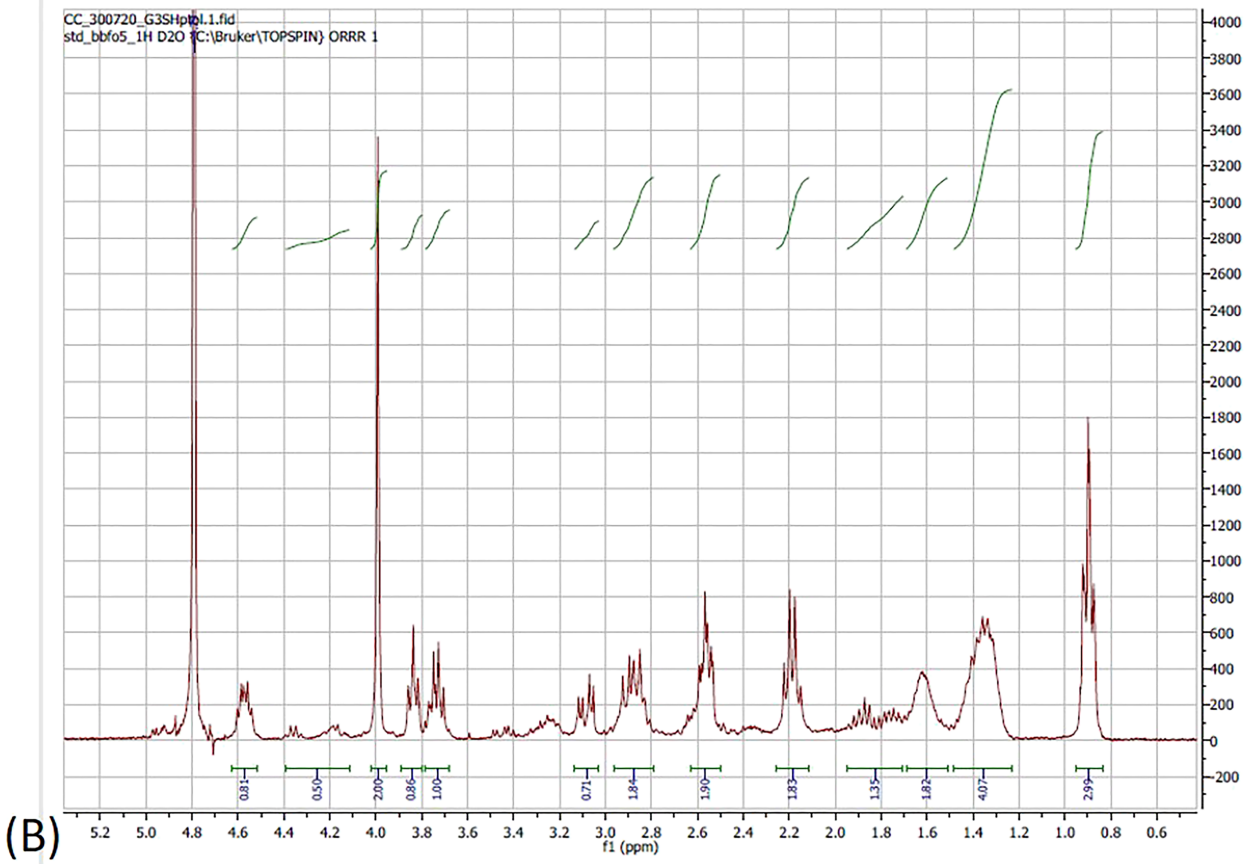


Figure 3. ^{1}H NMR spectra of (A) Cys-3SHptol and (B) G-3SHptol (300 MHz, D_2O). [Colour figure can be viewed at wileyonlinelibrary.com]

successful synthesis of Cys-3SHptol, revealing the presence of the pseudo-molecular ion (m/z 236), the ion corresponding to loss of NH_3 (m/z 219), the pseudo-molecular ion of cysteine (m/z 122), the carbocation of the volatile thiol (m/z 115), and its dehydrated form (m/z 97). The ^{1}H NMR spectrum shown in Figure 3A is also in agreement with the expected structure.

The m/z 219 fragment was used for RP-HPLC−ESI(+)−MRM quantitation. A double peak (a mixture of two diastereomers, slightly separated) was observed at 14.3−14.8 min after injection onto the Astec Cyclobond I 2000 RSP column (Figure 4).

Synthesis of S-3-(1-hydroxyheptyl)glutathione (G-3SHptol)

A white powder was obtained with a 64% yield (weight ratio of final product to reagents in mole equivalents − mixture of the *R*- and *S*-stereoisomers of 3SHptol conjugated to L-glutathione). The MS/MS-ESI(+) spectrum of the synthesised G-3SHptol is depicted in Figure 2B. From right to left are the pseudo-molecular ion (m/z 422), the ion corresponding to glycine loss (m/z 347), the ion corresponding to glutamyl loss (m/z 293), which can in turn lose NH_3 (m/z 276), the cysteinylglycine dipeptide ion (m/z 162), and the dehydrated carbocation of the volatile thiol (m/z 97). The ^{1}H NMR spectrum shown in Figure 3B is also in agreement with the expected structure.

The m/z 293 fragment was used for RP-HPLC−ESI(+)−MRM quantitation. Two distinct peaks (a mixture of two diastereomers) were observed at 16.2−17.1 min after injection onto the Astec Cyclobond I 2000 RSP column (Figure 4).

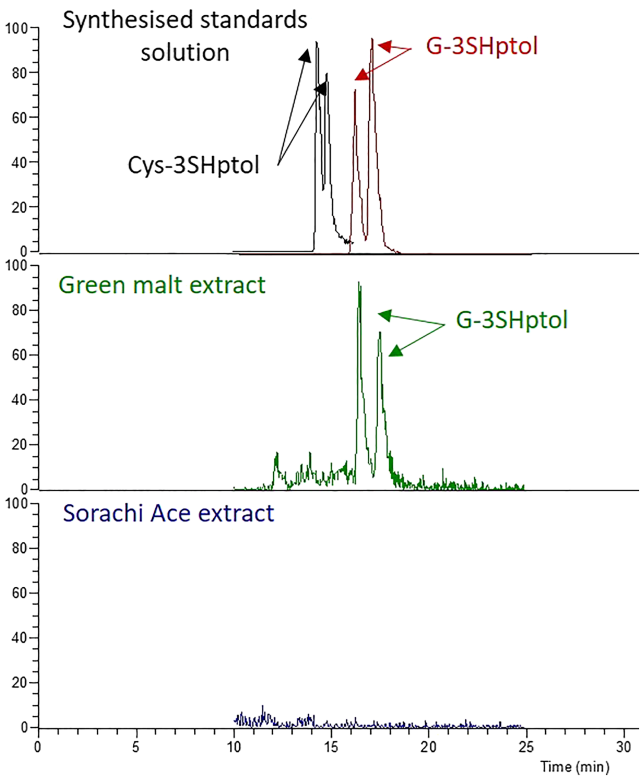


Figure 4. RP-HPLC-ESI(+)-MRM with the Cyclobond column (m/z 236 → 219) for Cys-3SHptol and (m/z 422 → 293) for G-3SHptol of synthesised standards solution and (m/z 422 → 293) for G-3SHptol of green Etincel malt and Sorachi Ace S-conjugates extracts. [Colour figure can be viewed at wileyonlinelibrary.com]

Investigation of cysteinylated and glutathionylated 3-sulfanylheptanol in malt

As shown in Figure 4 and Table 1(A), G-3SHptol was identified here for the first time in a natural matrix. Yet among all the malt extracts investigated, it was found only in the unkilned sample (green malt I, 0.10 mg/kg). Its counterpart Cys-3SHptol was not found in any of the malt samples.

Similarly, G-3SPol was found to be more concentrated in green malt (1.4 mg/kg detected after kilning). Yet this was not confirmed for G-3SHol (traces in malt I both before and after kilning, while kilned malt III contained up to 1.4 mg/kg) or G-3S4MPol (not detected in green malt I, although present at 35.2 mg/kg in kilned malt II) *(18)*.

According to Roland et al., G-3SHol is formed *in-situ* through malting from *trans*-2-hexenal and free glutathione, leading to an increase from barley to malt (except if G-3SHol is quickly transformed into Cys-3SHol) *(17)*. As for G-3SHptol, this mechanism would require the presence of *trans*-2-heptenal.

Investigation of cysteinylated and glutathionylated 3-sulfanylheptanol in hops

Neither Cys-3SHptol nor G-3SHptol was detected in any of the hop extracts investigated here (Table 1(B)). Sorachi Ace was chosen as an example to illustrate (Figure 4) an extract free of G-3SHptol, as compared to the green malt. Of the hops investigated, Cascade and Nelson Sauvin were the only varieties where free 3SHptol was found, albeit in a low amount. Previous studies have shown that samples that contain more free thiols are not always rich in the corresponding G- or Cys-precursors *(19,21)*.

Investigation of cysteinylated and glutathionylated 3-sulfanylheptanol in grapes

Neither Cys-3SHptol nor G-3SHptol was detected in any of the grape extracts investigated here (Table 1(C)). Botrytised grapes should now be investigated in order, possibly, to link the occurrence of free 3SHptol in these wines with bound forms in the grapes.

Potential pathways for the formation of 3SHptol conjugates

Glutathione S-conjugates arise from a detoxification pathway involving the addition of glutathione to an *alpha, bêta*-unsaturated aldehyde (a toxic compound for plants) through the action of glutathione S-transferase (GST) *(30,31)*. In an attempt to explain the scarcity of 3SHptol precursors (G-3SHptol was only found in one natural sample out of 17) compared to their 5- and 6-C analogues (both ubiquitous in hop, Table 1(B)), Figure 5 shows potential pathways for formation, either from lipid oxidation or by aldol condensation.

As depicted at the top of Figure 5 (compounds in shaded boxes), 6- and 9-C aldehydes are the main oxidation products of linoleic and linolenic acids (major fatty acids found in barley and hops) *(32–34)*. Lipoxygenase (LOX) produces 9- and 13-hydroperoxides, which are further degraded into hexanal, *trans*-2-hexenal, *trans*-2-nonenal or *trans*-2-*cis*-6-nonadienal (after *cis*-3 to *trans*-2 isomerisation).

In the case of *cis*-2-pentenal and pentanal (compounds in white boxes in Figure 5), it has been recently shown that their occurrence can be explained by the enzymatic oxidation (ADH, alcohol

Table 1. Concentration of Cys- and G- 3SHptol, 3SPol, 3SHol and 3S4MPol (in mg/kg) in (A) malts, (B) hops and (C) grapes

		3SHptol S-conjugates (mg/kg)		3SPol S-conjugates (mg/kg)		3SHol S-conjugates (mg/kg)		3S4MPol S-conjugates (mg/kg)	
Matrix		Cys-	G-	Cys-	G-	Cys-	G-	Cys-	G-
(A) Malt	Green malt I	nd	**0.10**	d	1.40	nd	d	d	nd
	Malt I	nd	nd	0.02	d	nd	d	0.01	nd
	Malt II	nd	nd	d	d	6	nd	d	35.20
	Malt III	nd	nd	nd	0.20	nd	1.40	nd	nd
(B) Hop	Amarillo	nd	nd	d	7.50	2.10*	101.00	2.10*	nd
	Cascade	nd	nd	na	na	na	na	na	na
	Citra	nd	nd	d	18.10	0.30*	91.00	0.30*	nd
	Hallertau Blanc	nd	nd	d	3.00	1.30*	77.10	1.30*	0.30
	Mandarina Bavaria	nd	nd	0.20	14.10	0.90	45.60	nd	nd
	Mosaic	nd	nd	na	na	0.08	20.73	nd	nd
	Nelson Sauvin	nd	nd	d	1.40	0.20*	20.10	0.20*	d
	Polaris	nd	nd	0.16	9.80	4.90*	118.20	4.90*	3.60
	Saaz	nd	nd	d	2.50	0.40*	95.70	0.40*	d
	Sorachi Ace	nd	nd	na	na	1.93	65.80	nd	nd
(C) Grape	Chardonnay	nd	nd	nd	nd	0.42	4.42	nd	nd
	Johanniter	nd	nd	nd	7.33	0.26	3.06	nd	nd
	Solaris	nd	nd	nd	0.01	0.05	1.18	nd	nd

na: not analysed; nd: not detected; d, detected at trace level (< 0.02 mg/kg in malts and hops, < 0.01 mg/kg in grapes). The shaded data were taken from already published results *(16,18,21,29)*. *value for Cys-3SHol+Cys-3S4MPol together.

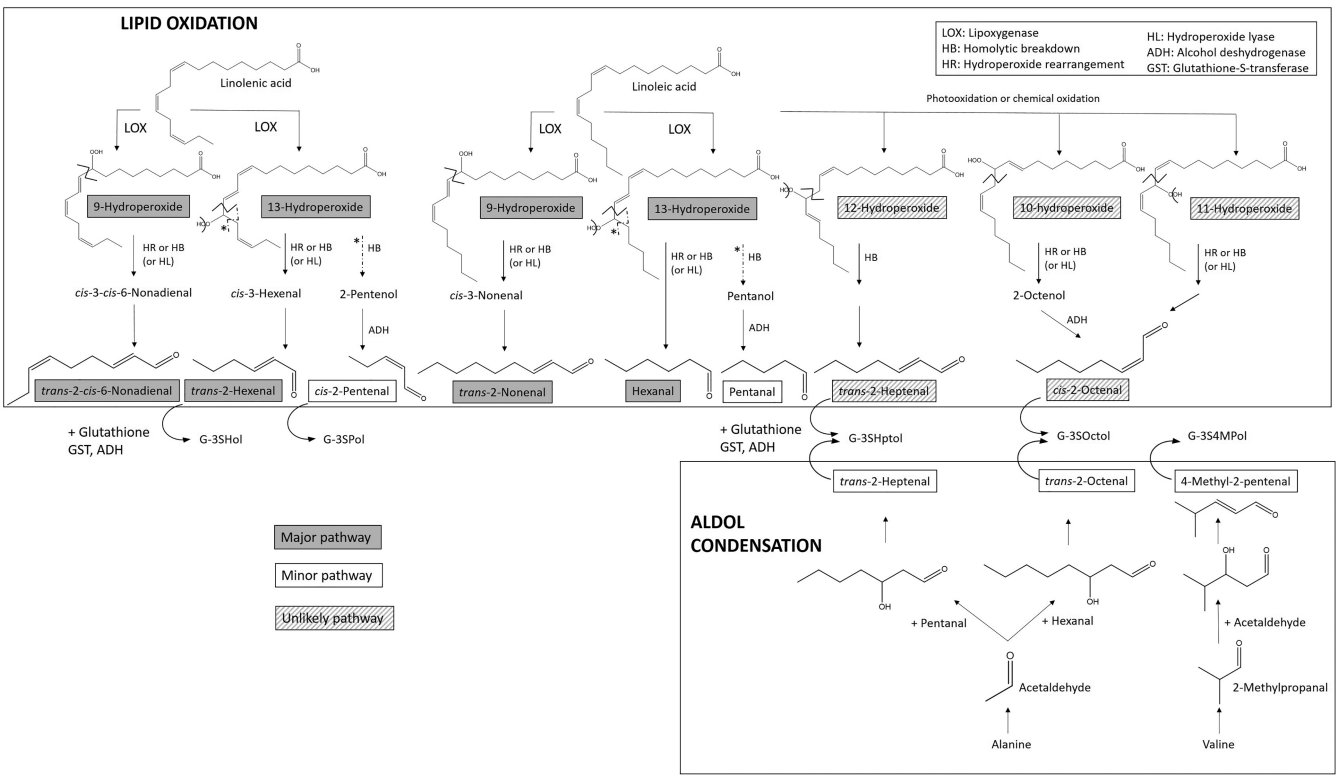


Figure 5. Potential pathways for the formation of *alpha, bêta*-unsaturated aldehydes required to produce S-conjugates in presence of glutathione.

dehydrogenase) of the corresponding alcohols derived from lipid oxidation. Therefore, as it is the case for G-3SHol, G-3SPol is ubiquitous, although at lower concentrations *(21)*.

To obtain *trans*-2-heptenal or *cis*-2-octenal (compounds in hatched boxes in Figure 5), unlikely lipid oxidation pathways would be required, involving 10-, 11- or 12-hydroperoxides (minor

products from photooxidation or chemical oxidation). Yet an alternative pathway could be by aldol condensation (bottom of Figure 5) of acetaldehyde (up to 34 mg/kg in malt *(35)*) with pentanal. Similarly, 4-methyl-2-pentenal (precursor of G-3S4MPol which was detected in pale malt II) could be formed by condensation of acetaldehyde and 2-methylpropanal (valine degradation product found at up to 1020 mg/kg in malt *(35)*).

Conclusions

For the first time, we report G-3SHptol in a natural matrix. The 0.10 mg/kg found in green malt is within the range of what has been found for G-3SPol or G-3SHol in kilned malt. However, no trace of G-3SHptol was found in Cascade and Nelson Sauvin hop pellets. Compared to *trans*-2-hexenal (a precursor of the ubiquitous G-3SHol after glutathione addition and reduction), *trans*-2-heptenal is not likely to be derived from an hydroperoxide but could be formed from the aldol condensation between pentanal and acetaldehyde.

Author contributions

Cécile Chenot: investigation, methodology, design, analysis and writing.
Raphaël Robiette: supervision and manuscript review.
Sonia Collin: conceptualisation, supervision and manuscript review.

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