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First evidence of cysteinylated and glutathionylated precursors of 3-mercaptohexan-1-ol in malts: Toward a better aromatic potential management?

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1. INTRODUCTION

Barley (*Hordeum vulgare*) is the major source for brewing malts, which constitute the most important raw material for beer production. Mainly composed of starch (more than 65% of dry weight), it also contains proteins, cell wall polysaccharides and in a smaller amount, fats and minerals. Previous hypotheses suggested that some aroma precursors might exist in malts. Indeed, Kishimoto and co-workers demonstrated that 3-mercaptohexanol (3MH) occurred in unhopped beers, suggesting that 3MH precursors might be present in malts.

3MH is a very strong smelling aroma which contributes to beer aroma with rhubarb, grapefruit and passion fruit odors. In wine 3MH was shown to be released during fermentation from cysteinylated and glutathionylated precursors initially present in the grape must. This gives opportunities to winemaker for managing the aroma in wine.

Thus, the aim of this work was

- to identify cysteinylated and glutathionylated precursors of both 3MH and 4-mercapto-4methylpentan-2-one (4MMP) in malts
- to quantify each molecule in various malt samples
- to study the evolution of glutathionylated and cysteinylated conjugates during sampling

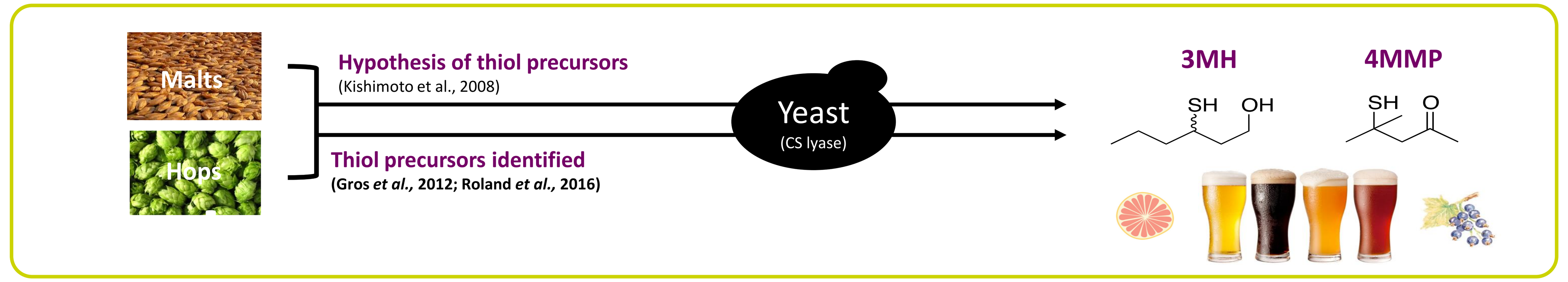

Fig 1: Hypothesis of biogenesis of 3MH and 4MMP during the alcoholic fermentation

2. RESULTS AND DISCUSSION

1. Identification of cysteinylated and glutathionylated precursors of 3MH in malts

Thiol precursors (Fig. 2) were synthesized according to published methods (Fedrizzi *et al.*, 2009, Roland *et al.*, 2010b) and fully characterized ^{1}H NMR.

In practice, a mixture of pure synthetic standards in water was analyzed by UPLC-MS/MS, and the retention times and MRM spectra were compared with those obtained from natural extract of malts.

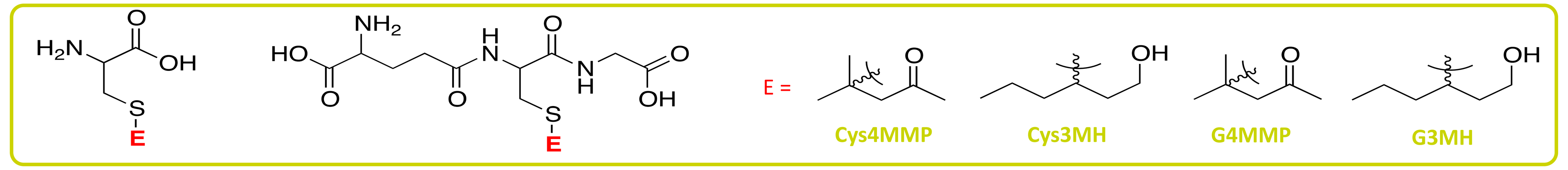

Fig 2: Cysteinylated (Cys) and glutathionylated (G) precursors of 3MH and 4MMP

Cys3MH and G3MH were formally identified for the first time in all malt samples, except in barley malt 12. Under our conditions, we did not detect any trace of 4MMP precursors (Fig.3).

They were considered as identified in the samples when meeting the following criteria specified in Commission decision 2002/657/EC [36]:

- The relative retention time of an analyte in a sample and a calibration solution has to be within the ± 5%.
- The presence of four identification points (parent ion and three daughter ions).
- The relative ion intensities have to comply with the ±30% permitted tolerances.

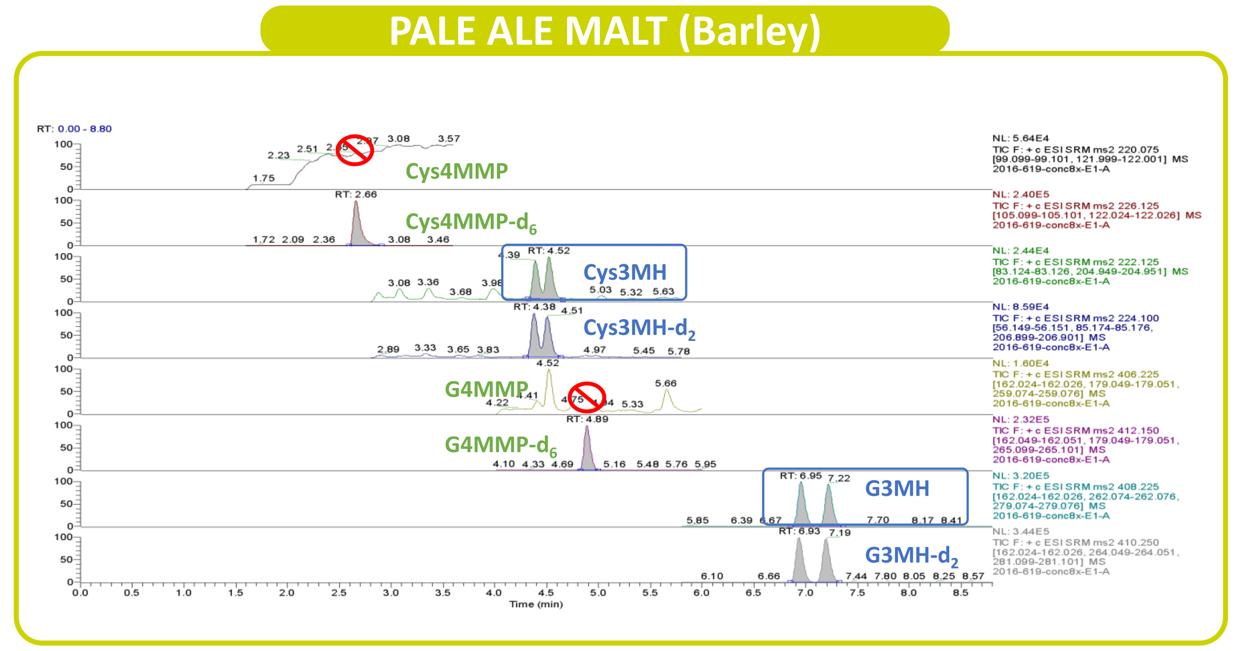

Fig 3: Chromatograms of cysteinylated and glutathionylated precursors of 3MH and 4MMP and in a Pale Ale malt

2. Quantification of precursors in malts

G3MH was the most abundant precursor whatever the malt considered. Barley malts exhibited the highest concentrations of G3MH and Cys3MH with levels ranging from 40 to 700 µg/kg and from 1 to 7 µg/kg, respectively (Fig.4).

Malt roasting levels influenced considerably the amount of 3MH precursors since no precursors were found in the most roasted (malt 12, 1150EBC).

Repartition of G3MH and Cys3MH differed in malt varieties. Indeed, G3MH/Cys3MH ratio was equivalent to 99/1 for all barley malts whereas it was close to 70/30, 80/20 and 90/10 for rice, wheat and sorghum malts, respectively.

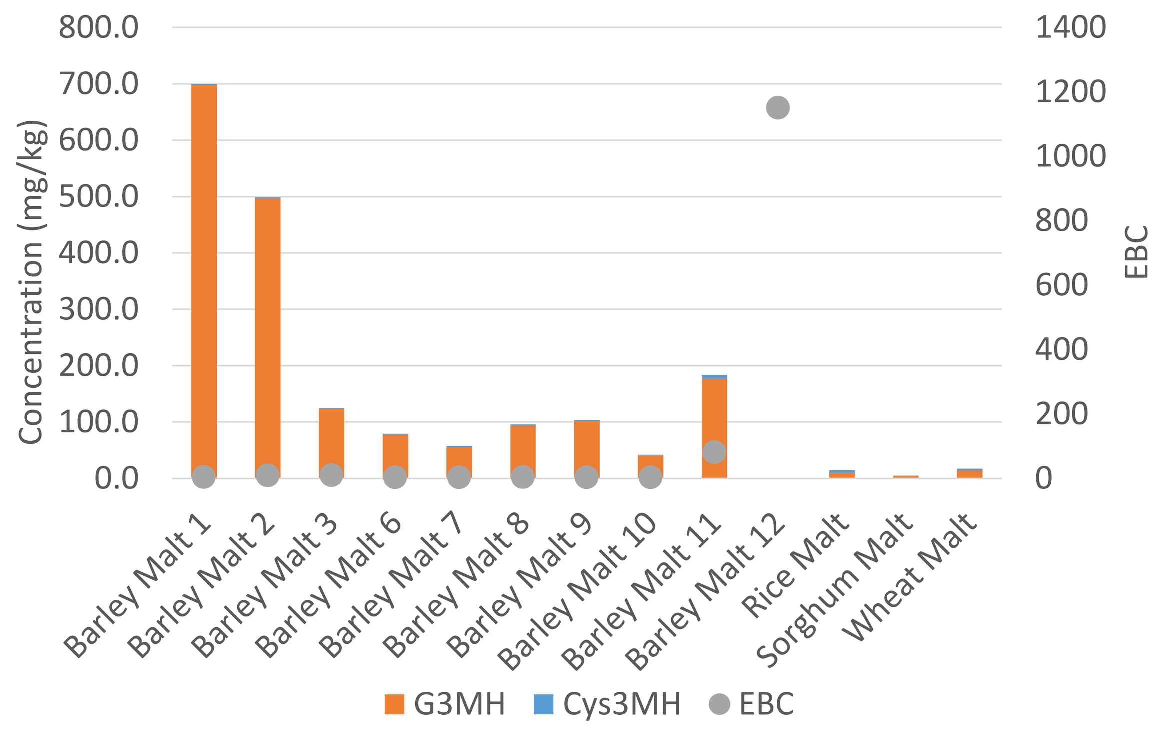

Fig 4: Quantification of thiol precursors in several malts

3. Study of conversion of G3MH to Cys3MH in water

Many peptidases are present in malts and some of them may modify the proportion of G3MH and Cys3MH during mashing. To study the evolution of thiol precursors, we used labelled G3MH as tracer. In practice, several crushed malts dissolved in water were spiked with G3MH-d_2 (400 µg/L) and we observed the kinetics for 1h at room temperature.

First we observed that a part of G3MH-d_2 disappeared, between 11% to 58% (Column 1 Table 1). And only a part of this lost can involve Cys3MH-d_2 formation for some samples, between 8 to 32% (Column 2 Table 1).

Results shows the filiation of G3MH-d_2 into Cys3MH-d_2 and suggests possible other thiol precursors formed from G3MH-d_2. This first evidence of precursor change at room temperature might be the result of endogeneous enzymatic activities. According to this hypothesis, mashing conditions could therefore have an effect on these precursors.

	Decrease of G3MH-d_2 (%)	Conversion rate of G3MH-d_2 into Cys3MH-d_2 (%)
Barley malt 11	18%	0%
Barley malt 12	34%	0%
Barley malt 13	19%	8%
Wheat malt	58%	16%
Barley malt 2	11%	32%

Table 1: Interconversion mechanisms of G3MH-d_2 into Cys3MH-d_2 in 5 different malts at room temperature for 1h

4. Possible contribution of thiol precursors from malts and hops to thiol release in beers

Thiol precursors of 3MH are present at very different levels in both malts and hops: ppb and ppm, respectively. In **Table 2**, the aromatic potential or "Eq3MH" of two malts and two hops was compared. By assuming an hypothetic conversion of 3%, we calculated the amount of 3MH that could be released during fermentation.

This example showed that both malts and hops may contribute to 3MH biogenesis. By adjusting the amount of raw material, the mashing, the hopping and the alcoholic fermentation conditions and by combining adequate malts and hops, brewers may be able to manage the release of thiols in beers, partly responsible for their fruity aromas.

Raw material	Eq3MH (mg/kg)	Amount	Hypothetic conversion yield (%)	3MH hypothetically released (ng/L)
Malt 1	227	25 kg/hL	3%	1704
Malt 10	14	25 kg/hL	3%	105
Hop (Cascade)	6534	400 g/hL	3%	784
Hop (Mandarina)	776	400 g/hL	3%	93

Table 2 : Comparisons of aromatic potential (Eq3MH) of 2 malts and 2 hops

3. CONCLUSIONS

In the present study, we identified and quantified for the first time the cysteinylated and glutathionylated precursors of 3MH in several barley, rice, sorghum and wheat malts. Malt can be considered as a source of new aroma precursors and the amount and the repartition of thiol precursors changed according to the malt variety, the roasting level and probably enzymatic activities.

Thiols can be controlled by brewers to create different beers. In this way thiol precursors and nitrogen source in wort are key parameters linked with fermentation, yeast metabolism and so thiol release. The study of the simultaneous role of enzymes on this two parameters may bring new usefull knowledge for brewers to modulate the corresponding fruity odors in beers.

4. ACKNOWLEDGEMENTS

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Kishimoto, T., *et al.*, Journal of the American Society of Brewing Chemists, 2008, 66(3), p. 192-196; **Gros *et al.*,** Journal of Agricultural and Food Chemistry, 2012, 60(32), p.7805-7816; **Roland *et al.*,** Flavour and Fragrance Journal, 2016, DOI 10.1002/ffj.3337; **Roland** *et al.*, Journal of Chromatography A, 2010a, 1217(10); p. 1626-1635; **Fedrizzi *et al.***, Journal of Agrilcultural and Food chemistry, 2009, 57(3), p.991-995; **Roland *et al.***, Journal of Agriculutral and Food Chemistry, 2010b, 58, p. 10684-10689