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The sensory contribution of thiols to hop and beer aroma – a review

Over the past two decades, research has established volatile polyfunctional thiols as important contributors to hop and beer aroma. Their low odour thresholds and diverse sensory properties make them relevant to hop-forward beers, but their concentrations alone do not adequately describe their sensory contribution. This review examines the development of thiol research in brewing, analytical approaches, free thiols and their precursors in hops and malt, yeast-dependent release, and stability during beer storage. Particular emphasis is placed on interactions with other aroma compounds and on the limitations of translating chemical measurements into sensory predictions. The available literature identifies opportunities to influence thiol formation through raw material selection and fermentation management, while also showing that maximising individual thiol concentrations does not necessarily maximise tropical-fruity aroma. Practical implications are discussed alongside unresolved questions concerning precursor conversion, sensory interactions and aroma stability.

Descriptors: Thiols, sensory, hops, beer

1 Thiols – Introduction

Sulfur-containing aroma compounds form a very large group of substances that shape the aroma and flavour of numerous foods. Accounting for 13% of the 9,514 aroma compounds identified in foods to date, they represent the second-largest compound group (Chen et al., 2019). Thiols are a subgroup of sulfur-containing aroma compounds, alongside thiazoles, thiophenes, polysulfides and thioesters (Lermusieau & Collin, 2003). Ethanethiol (CH_3CH_2SH), the first thiol to be described, was discovered by Zeise in 1834 from the reaction of barium hydrogen sulfide with calcium ethyl sulfate (Zeise, 1834).

Thiols are the sulfur analogues of alcohols: the hydroxyl group (–OH) is replaced by a sulfhydryl or thiol group (–SH). The historical term mercaptan refers to their ability to bind mercury(II), whereas sulfanyl is a nomenclature prefix used to designate an –SH substituent. The term thiol is derived from the Greek word theion, meaning sulfur, and their general structure is:

R–SH

Here, R denotes an organic residue, for example an alkyl or aromatic group. Compared with the O–H bond, the S–H bond is only weakly polar because sulfur has an electronegativity similar to that of hydrogen. As a result, thiols form only weak hydrogen bonds or none at all. Their boiling points are therefore lower than those of the corresponding alcohols (Federle et al., 2017). The pKa values of thiols are also lower than those of the corresponding alcohols, making thiols stronger acids. According to IUPAC nomenclature, aliphatic thiols are named by adding the suffix -thiol to the name of the corresponding alkane, for example ethanethiol. If a functional group of higher priority determines the suffix, the –SH group is designated by a prefix: mercapto- is the traditional term, whereas sulfanyl- is the systematic term. These naming conventions distinguish the functions present in polyfunctional thiols. For example, 4-methyl-4-sulfanylpentan-2-one contains a thiol group and a ketone group, whereas 3-sulfanylhexyl acetate contains a free thiol group and an acetate ester group. The latter is a thiol-containing ester, not a thioester.

Thiols occur in many foods. With the analytical advances of the past 20 years, research on thiols in different food matrices has also progressed substantially. A broad structural diversity of volatile thiols has now been detected in foods and beverages. The publication landscape shows that research has so far focused mainly on fruits, meat, wine, beer and coffee. Initial studies date back to the 1960s, but the number of publications has increased sharply since the late 1990s. The qualitative and quantitative analysis of thiols remains challenging because of complex matrices and the

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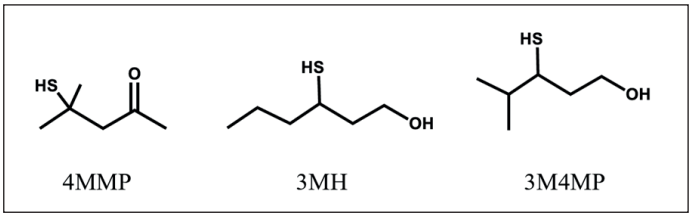

Fig. 1 **Molecular structures of the free thiols 4-sulfanyl-4-methylpentan-2-one (4-MSP/4-MMP), 3-sulfanylhexan-1-ol (3SH/3MH), and 3-sulfanyl-4-methylpentan-1-ol (3S4MP/3M4MP) (Schmidt et al., 2024)**

specific properties and instability of many thiols. This makes the analytical progress achieved over the past 25 years all the more important (Vermeulen et al., 2005) .

2 Analytical methods

Current approaches to thiol analysis are summarised in Table 1. The analysis of free thiols and their precursors is challenging because of low analyte concentrations, high reactivity and matrix-dependent losses. Free thiols occur in beer at ng/L concentrations and in hops at µg/kg concentrations; sample preparation must therefore control oxidation, evaporation and adsorption losses. For beer, on-fibre derivatisation combined with headspace solid-phase microextraction and GC-MS/MS provides an established analytical approach (Dennenlöhr et al., 2020). Isotope-labelled internal standards can improve quantification by compensating for losses and matrix effects, although their availability and the validation of the complete method remain important considerations. Free thiols in hops can also be quantified by LC-MS/MS following derivatisation with 4,4′-dithiodipyridine(Schmidt et al., 2024). Non-volatile precursor conjugates require a separate analytical strategy, commonly targeted LC-MS/MS or UHPLC-MS/MS.

3 Sensory properties of thiols

Thiols are among the aroma compounds with the lowest known sensory thresholds and are therefore often described as particularly potent aroma-active compounds or key aroma compounds. Their sensory properties are exceptionally diverse, ranging from diesel, onion and garlic notes to tropical-fruity impressions such as grapefruit, passion fruit and mango.

Among the thiols most extensively studied in hops and beer and considered most important from a sensory perspective are 4-MSP (4-sulfanyl-4-methylpentan-2-one), 3-SH (3-sulfanylhexan-1-ol), and 3S4MP (3-sulfanyl-4-methylpentan-1-ol) (see Fig. 1). Also 3-SHA (3-sulfanylhexyl acetate) plays an important role.

In food chemistry studies, the physiology of olfactory and gustatory perception is often considered only insufficiently. As a result, sensory findings may be interpreted incompletely or misleadingly. During olfactory perception, aroma compounds reach the olfactory mucosa, where they interact with approximately 400 olfactory receptors (GPCRs) located on the cilia of olfactory sensory neurons. A single aroma compound can activate several receptors, while one receptor can respond to different aroma compounds. Perception is therefore not determined by a single receptor but by a specific activation pattern, which is perceived as an odour only after processing in the brain. The brain evaluates whether an odour is familiar, perceived positively, evokes memories or emotions, and whether

Table 1 **overview of different analytical methods for thiol analysis (Dennenlöhr et al., 2020) (Tominaga et al., 1998) (Vermeulen et al., 2006) (Takazumi et al., 2017) (Ochiai et al., 2015) (Chenot et al., 2024)**

Approach	Principle	Benefits	to consider
Selective extraction / p-HMB (para-hydroxymercuribenzoate)	Free thiols bind selectively through Hg–S (mercury–sulfur) affinity and are enriched/released.	Very high selectivity and substantial removal of matrix components.	Labor-intensive; risk of analyte losses; mercury-containing reagent.
Derivatization	Labeling of the –SH (sulfhydryl group) group: PFBBr (pentafluorobenzyl bromide) improves suitability for GC (gas chromatography) and extraction, while DTDP (4,4′-dithiodipyridine) stabilizes thiols and improves LC-ESI-MS (liquid chromatography–electrospray ionization–mass spectrometry) detection.	Higher sensitivity, stability and selectivity; PFBBr-OFD-HS-SPME (pentafluorobenzyl bromide on-fiber derivatization–headspace solid-phase microextraction) has been validated for beer.	Additional reaction step; yield and side reactions should be controlled.
SIDA (stable isotope dilution analysis)	Early addition of isotope-labeled analogues; quantification using the analyte-to-standard signal ratio.	Compensates for sample preparation losses and matrix effects, providing particularly robust quantification.	Labeled standards are expensive, may not be commercially available, or may require synthesis.
GC×GC-TOFMS (comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry)	Two largely orthogonal GC (gas chromatography) separations combined with rapid TOF (time-of-flight) detection.	Very high peak capacity and matrix resolution; well suited to broad screening and complex hop profiles.	High demands in terms of instrumentation, maintenance, method development and data processing.
LC-MS/MS (liquid chromatography–tandem mass spectrometry) precursor analysis	Targeted analysis of non-volatile cysteine, glutathione, CysGly (cysteinylglycine) and γGluCys (γ-glutamylcysteine) conjugates.	Direct, sensitive characterization of the bound thiol potential in hops, malt and wort.	Quantification of precursor structures, control of standards and matrix is challenging.

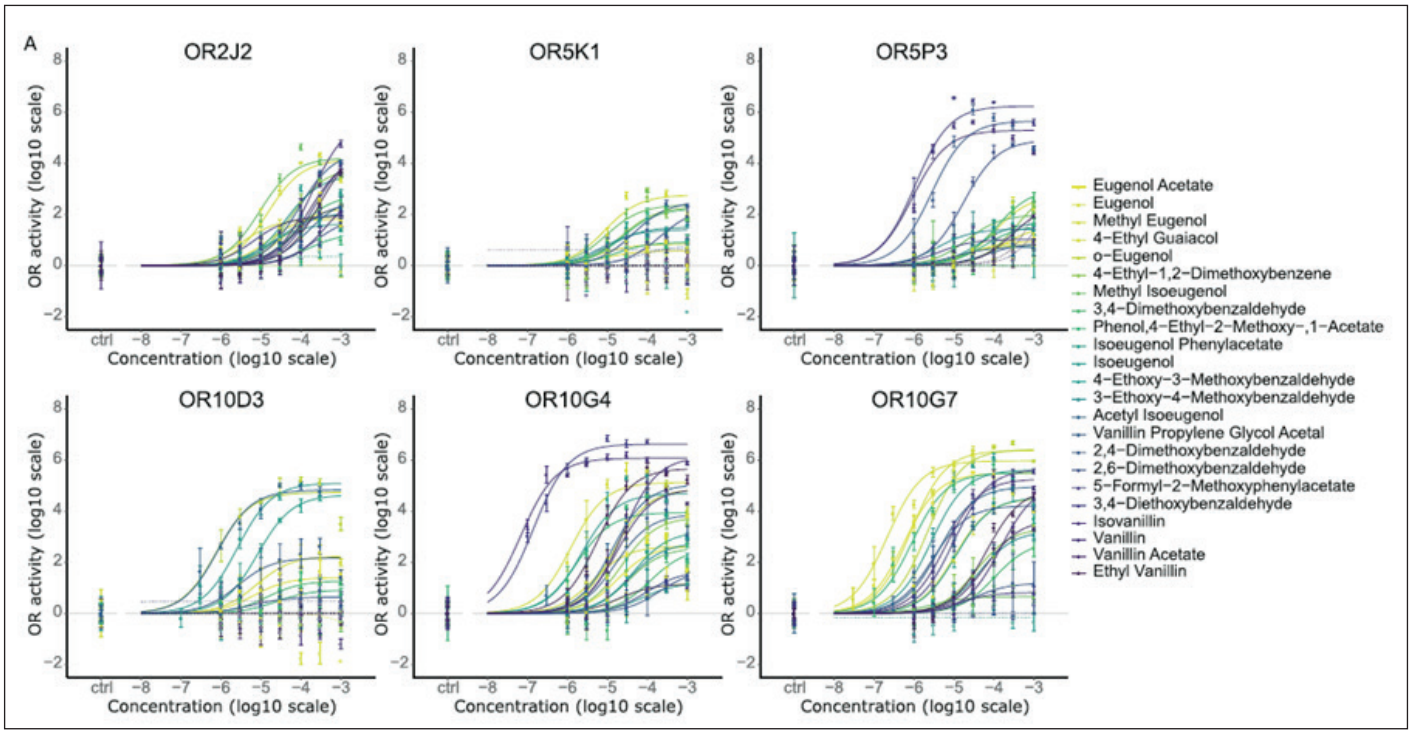

Fig. 2 **Example chose to show odor receptor activity measurement. Structurally related aroma compounds generate different activation patterns across six human olfactory receptors (OR): OR2J2, OR5K1, OR5P3, OR10D3, OR10G4 and OR10G7. Eugenol-like compounds activate OR10G7, OR2J2 and OR5K1, whereas vanillin-like compounds interact more strongly with OR10G4 and OR5P3 (Ihara et al., 2025)**

it is associated with food. Compared with other senses, the human sense of smell has a particularly high discriminatory capacity and can theoretically distinguish up to one trillion odours, although the number of aroma compounds actually known is much smaller (Bushdid et al., 2014). Odour perception also depends on experience, culture, attention and hedonic evaluation (Richardson, 2024).

This relative importance of receptor activation patterns puts into perspective the assumption, common until a few years ago, that aroma quality is determined solely by molecular structure. Instead, what matters is the activation pattern of olfactory receptors: molecules are perceived as similar when they activate comparable combinations of receptors (Ihara et al., 2025) .

Interestingly, the thiol 3-sulfanyl-2-methylpentan-1-ol is different in this regard. Unlike most other odorants, it selectively activated only the receptor OR2M3 among 391 human olfactory receptors tested against 190 key food odorants, with a submicromolar half-maximal effective concentration (Noe et al., 2017). Another receptor, OR2T11, also responds to thiols and interestingly is activated by copper ions (Li et al., 2016) .

CATEGORY	DESCRIPTORS
floral	elderflower, camomile blossom, lily of the valley, jasmine, apple blossom, rose, geranium, carnation, lily, lilac, lavender, osmanthus
citrus	grapefruit, orange, lime, lemon, bergamot, lemongrass, ginger, tangerine, pomelo
sweet fruits	banana, watermelon, honeydew melon, peach, apricot, passion fruit, lychee, dried fruit, plum, pineapple, cherry, kiwi, mango, guava
green fruits	pear, apple, quince, gooseberry, white wine grapes
berries & currant	cassis, blueberry, raspberry, blackberry, strawberry, redcurrant, blackcurrant, wild strawberry, cranberry, mulberry
cream caramel	butter, chocolate, yogurt, honey, cream, caramel, toffee, coffee, tonka bean, vanilla, coconut
woody aromatic	tobacco, cognac, barrique, leather, woodruff, incense, myrrh, resin, cedar, pine, earthy
menthol	mint, lemon balm, sage, camphor, menthol, wine yeast, eucalyptus
herbal	marjoram, tarragon, dill, parsley, basil, fennel, coriander, rosemary, thyme, green tea, black tea, mate tea, oregano
spicy	lovage, pepper, chili, curry, juniper, aniseed, liquorice, fennel seeds, clove, cinnamon, gingerbread, coriander seeds, nutmeg
grassy-hay	fresh cut grass, hay, tomato leaves, green pepper, nettle, cucumber, bamboo leaves
vegetal	celery, leek, onion, artichoke, garlic, wild garlic, radish

Fig. 3 **Hop tasting scheme for hop varieties and beers. The descriptors highlighted in orange can be associated with thiol compounds. The different categories are assessed using intensity values from 0 to 10 and can be visualised as spider plots (McMillan, 2023)**

Nevertheless, specific structural features remain important for the sensory impact of aroma compounds. These include functional groups such as thiols, esters, aldehydes, ketones, terpenes and terpene alcohols, as well as molecular size, degree of branching, polarity, volatility and chirality.

As early as the late 1990s, Sonia Collin's group used

elaborate syntheses and GC-olfactometry to investigate how the chain length of S-methyl thioesters influences their aroma properties. The study showed at an early stage that even small structural differences within a homologous series of sulfur compounds can produce markedly different aroma qualities (Khan et al., 1999) (Berger et al., 1999).

Polster et al. investigated homologous series of different alkanethiols and alkanedithiols. The lowest odour thresholds were found for thiols with five to seven carbon atoms, whereas longer carbon chains led to an exponential increase in threshold values. Tertiary alkanethiols had markedly lower odour thresholds than primary or secondary thiols. A second sulfanyl or thiol group, or additional methyl substitution, did not further lower the odour threshold (Polster & Schieberle, 2015)

Aroma compounds in foods always interact with one another. As early as 2010, Hanke et al. showed that the perception thresholds of individual aroma compounds can change in the presence of other aroma compounds (Hanke et al., 2010). Beer can be assumed to contain 800 or more aroma compounds. These compounds may mutually mask, synergistically enhance or suppress one another, or generate new aroma impressions (Machado et al., 2021).

Takoi et al. showed in 2016 that 40 ng/L 4-MSP, together with terpene alcohols such as 70 µg/L linalool, 50 µg/L geraniol and 30 µg/L β-citronellol, can evoke tropical aroma impressions (Takoi, 2016). A comparable effect has been described for thiol–ester interactions in wine: 3-SH, 3-SHA and 4-MSP, at concentrations of 2 to 250 ng/L, produced impressions of passion fruit, guava, pineapple or tropical fruit in general when combined with fruity esters. Different concentrations resulted in differently pronounced aroma profiles (Iobbi et al., 2023).

In combination with esters, monoterpene alcohols and other aroma compounds, volatile thiols contribute substantially to the formation of diverse tropical aroma impressions. However, these impressions are also influenced by factors such as ethanol and CO_2 concentration, mouthfeel, bitterness and pH (Dietz et al., 2020). The resulting aroma quality therefore cannot be derived solely from the concentration of an individual thiol; rather, it depends on the composition, concentration ratios and matrix of the respective beverage. The possibilities of interaction of aroma compounds is illustrated in figure 4.

The reported aroma qualities of 4-MSP span fruity, green, animal-like and sulfurous impressions. Literature descriptors include catty, cat urine, box tree, blackcurrant, meaty, sulfurous, grapefruit, passion fruit, sweat, gooseberry, elderflower, chives and tomato plant. This diversity demonstrates the range of descriptions associated with the compound, but a compilation of descriptors alone does not establish how odour quality changes with concentration or between individuals. Such interpretation requires information on the assessment concentration, matrix and sensory procedure. In hops and beer, the contribution of 4-MSP must additionally be considered in the context of its interactions with other aroma compounds.

Suitable tasting schemes for evaluating hops and hop-forward beers were developed since 2011, when brewers' interest in the specific

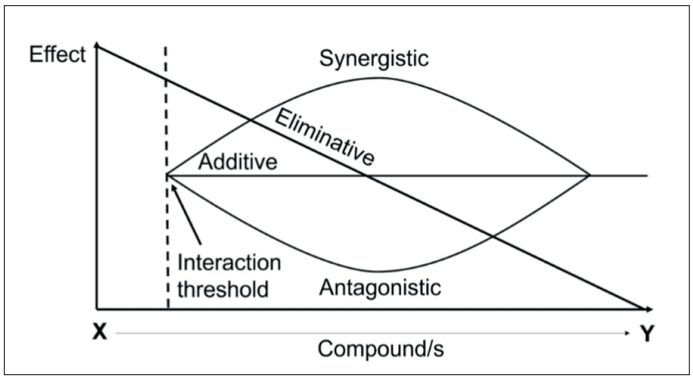

Fig. 4 **Graphical representation of sensory interaction effects caused by the combination of two or more compounds, leading to changes in sensory properties (Dietz et al., 2020)**

sensory properties of individual hop varieties increased markedly (C. Schönberger, 2011). Nevertheless, sensory analyses are still often given too little attention compared with the analytical effort invested. For robust conclusions about the sensory relevance of individual aroma compounds or aroma compound groups, sensory and analytical data should ideally be evaluated together (see FIg. 3).

4 Thiols in hops and beer – the discovery phase

A long-known and sensorially undesirable thiol in beer is 3-methylbut-2-en-1-thiol. It causes lightstruck flavour and is formed when light-induced radicals from unstable iso-α-acids react with thiol radicals (De Keukeleire, 2000). This off-flavour is often described as "skunky", skunk-like or pungent and has a perception threshold of only a few nanograms per litre. Interestingly, 3-MBT is also considered an important component of the characteristic aroma of coffee (Müller & Hofmann, 2005).

A review by Lermusieau et al. illustrates the structural diversity of volatile sulfur compounds in different hop varieties and describes how their concentrations strongly depend on growing region, cultivation conditions, sulfur treatment, drying and storage. The compounds described were mainly associated with negative aroma descriptors such as cheesy, cooked cabbage, vegetable, onion, garlic, burnt rubber, truffle and cardboard. Accordingly, the authors discussed how volatile sulfur compounds can be stripped during the brewing process (Lermusieau & Collin, 2003).

The synthesis and investigation of 13 mercapto primary alcohols by the same research group showed in 2003 that thiols with at least five carbon atoms were more likely to be associated with positive descriptors such as rhubarb or citrus. Branched structures, by contrast, were mainly described with onion, leek or pungent notes (Vermeulen & Collin, 2003).

A beer off-flavour already described by Clapperton in 1976 was characterised as "catty" and "ribes flavour" (Clapperton, 1976). Cosser first linked this sensory impression to 4-MSP in 1980 (Cosser, 1980). In their GC-O analysis of Saaz and Challenger pellets, Lermusieau et al. also assigned descriptors such as blackcurrant, grapefruit, strawberry, cat urine and citrus to unidentified compounds(Lermusieau & Collin, 2001). 4-MSP, as well as 3-SH,

Table 2 **Identified and quantified thiols in hops and beer. The reported values for the different thiols were obtained using different analytical methods. This data is intended only to provide an overview. (Chenot et al., 2026) (Uemoto et al., 2025) (Simon & Collin, 2024) (Molitor et al., 2023) (Chenot et al., 2022) (Molitor et al., 2022a) (Michel et al., 2019) (Chenot et al., 2019a) (Roland et al., 2017a) (Kankolongo Cibaka et al., 2016a) (Ochiai et al., 2015) (Gros et al., 2012) (Liu et al., 2024) (Svedlund et al., 2022)**

Thiol compound	Abbreviation	Free concentration in hops (µg/ kg)	Concentration in beer (ng/ L)	Sensory threshold in beer (ng /L)	Sensory descriptors in literature
4-Methyl-4-sulfanylpentan-2-one	4MSP / 4MMP	1–114	4–184	1.2–1.5	Blackcurrant, ribes, box tree, muscat-like, catty; fruity, passionfruit, cat urin, tropical, exotic fruits, grapefruit, mint, diesel, sulfury, body odor
3-Sulfanylhexan-1-ol	3SH / 3MH	2–125	60–1200	55	Grapefruit, rhubarb, exotic, citrus, guava, passionfruit, savory, onion
3-Sulfanylhexyl acetate	3SHA / 3MHA	0–27	10–36	4–5	Passion fruit, tropical, grapefruit, gooseberry, floral, herbal, body odor, onion
3-Sulfanyl-4-methylpentan-1-ol	3S4MP / 3M4MP	10–109	11–548	70	Grapefruit, rhubarb; associated with Sauvignon Blanc/ white-wine-like aroma
3-Sulfanyl-4-methylpentyl acetate	3S4MPA	no data	100–300	160	Grapefruit, melon; associated with white-wine-like/ Nelson Sauvin character

was later detected in a study by Vermeulen et al., in which twelve polyfunctional thiols were identified in fresh lager beers. Because these compounds were not detectable in wort, the authors attributed a central role in their formation to fermentation and hops, and discussed the 1,4-addition of hydrogen sulfide to α,β-unsaturated ketones or aldehydes from wort as a possible formation pathway (Vermeulen et al., 2006).

4-MSP attracted greater attention through the work of Steinhaus et al., who identified the compound in the US hop variety Cascade and described it sensorially as blackcurrant (Steinhaus et al., 2007). Building on this work, Kishimoto et al. analysed several US hop varieties for 4-MSP. Simcoe, Summit and Apollo in particular led to high concentrations of more than 100 ng/L in the beers brewed from them. This final concentration was only slightly influenced by fermentation; most of the 4-MSP was apparently transferred from the hops into beer as the free thiol. At the same time, other sources of free thiols came into focus. This marked the beginning of the discussion about possible precursor structures, especially cysteine conjugates in hops but also in malt, and about enzymatic pathways by which yeast releases free thiols (Kishimoto et al., 2008).

In 2009, Takoi et al. described two additional hop thiols: 3-sulfanyl-4-methylpentan-1-ol (3S4MP) and 3-sulfanyl-4-methylpentyl acetate (3S4MPA). Both were detected in the New Zealand hop variety Nelson Sauvin and in beers brewed from it (Takoi et al., 2009). 3S4MP is a structural isomer of 3-SH with the same molecular mass (Schmidt et al., 2024). In 2011, Gros et al. provided the first overview of the occurrence of thiols in different hop varieties and their detectability in beer. US and New Zealand hop varieties showed markedly higher concentrations of free thiols than European varieties. As a possible explanation, the authors discussed, among other factors, copper-containing plant protection products, since thiols can bind to copper ions. The results also indicated that additional thiols must either originate from malt or be released during fermentation (Gros et al., 2012).

Each hop variety has an optimal harvest window. Numerous studies over the past 20 years have investigated how harvest timing affects hop composition, particularly hop oils; increasingly, free thiols and thiol precursors have also been considered. For Cascade, it was shown that later-harvested hops contain higher concentrations of free thiols and lower concentrations of bound thiols. Accordingly, late-harvested Cascade can contribute more intensely fruity aromas to beer during dry hopping (Hauser et al., 2019). For Hallertau Blanc, Uemoto et al. showed that the 3S4MP content increased with later harvest, although this did not result in stronger fruity sensory expression (Uemoto et al., 2025). Overall, the increase in free thiols is variety-dependent and cannot be demonstrated for every variety. A study with five US hop varieties harvested early, at standard maturity and late also showed variety-specific developments in both thiol precursors and free thiols. Each variety must therefore be evaluated individually (Ch. , D. L. , Schönberger, 2020). Thiol concentrations may also remain stable during storage depending on the variety; this has been shown only recently for Citra and Strata (Chenot & Shellhammer, 2026).

5 The release of thiol precursors by yeast

The basis for thiol research in hops and beer was the research into aroma-active thiols in wine, which began somewhat earlier in the 1990s, when compounds such as 4MSP, 3SH and 3SHA were identified as key aroma compounds in Sauvignon Blanc (Darriet et al., 1995). Since then findings from wine research have certainly contributed to advances in research on hops and beer; however, their applicability to brewing science is limited because precursor structures, starting matrices, fermentation conditions and yeast

Table 3 Identified precursor structures of different thiols (Roland et al., 2016b) (Bonnaffoux et al., 2018) (Gros et al., 2012) (Chenot et al., 2024) (Kankolongo Cibaka et al., 2016b) (Chenot et al., 2019b) (Chenot et al., 2022)

Free thiol	Precursor structure	Occurrence
4MMP / 4MSP	Cys-4MMP (cysteine S-conjugate of 4-mercapto-4-methylpentan-2-one) / Cys-4MSP (cysteine S-conjugate of 4-methyl-4-sulfanylpentan-2-one)	Hops
4MMP / 4MSP	G-4MMP (glutathione S-conjugate of 4-mercapto-4-methylpentan-2-one) / G-4MSP (glutathione S-conjugate of 4-methyl-4-sulfanylpentan-2-one)	Hops
4MMP / 4MSP	CysGly-4MMP (cysteinylglycine S-conjugate of 4-mercapto-4-methylpentan-2-one) / CG-4MMP (cysteinylglycine S-conjugate of 4-mercapto-4-methylpentan-2-one)	Hops
4MMP / 4MSP	γGluCys-4MMP (γ-glutamylcysteine S-conjugate of 4-mercapto-4-methylpentan-2-one) / GC-4MMP (γ-glutamylcysteine S-conjugate of 4-mercapto-4-methylpentan-2-one)	Hops
3MH / 3SH	Cys-3MH (cysteine S-conjugate of 3-mercaptohexan-1-ol) / Cys-3Shol (cysteine S-conjugate of 3-sulfanylhexan-1-ol)	hops; barley; barley malt
3MH / 3SH	G-3MH (glutathione S-conjugate of 3-mercaptohexan-1-ol) / G-3Shol (glutathione S-conjugate of 3-sulfanylhexan-1-ol)	hops; barley; barley malt
3MH / 3SH	CysGly-3MH (cysteinylglycine S-conjugate of 3-mercaptohexan-1-ol) / CysGly-3Shol (cysteinylglycine S-conjugate of 3-sulfanylhexan-1-ol)	hops; barley; barley malt
3MH / 3SH	γGluCys-3MH (γ-glutamylcysteine S-conjugate of 3-mercaptohexan-1-ol) / γGluCys-3Shol (γ-glutamylcysteine S-conjugate of 3-sulfanylhexan-1-ol)	hops; specialty malts; rye; spelt; wheat
3M4MP / 3S4MP	Cys-3M4MP (cysteine S-conjugate of 3-mercapto-4-methylpentan-1-ol) / Cys-3S4MP (cysteine S-conjugate of 3-sulfanyl-4-methylpentan-1-ol)	Hops
3M4MP / 3S4MP	G-3M4MP (glutathione S-conjugate of 3-mercapto-4-methylpentan-1-ol) / G-3S4MP (glutathione S-conjugate of 3-sulfanyl-4-methylpentan-1-ol)	hops; malt
3Pol / 3SPol	Cys-3SPol (cysteine S-conjugate of 3-sulfanylpentan-1-ol) / Cys-3SP (cysteine S-conjugate of 3-sulfanylpentan-1-ol)	Hops
3Pol / 3SPol	G-3SPol (glutathione S-conjugate of 3-sulfanylpentan-1-ol) / G-3SP (glutathione S-conjugate of 3-sulfanylpentan-1-ol)	Hops

strains, as well as thiol formation and release, differ considerably. The enzymatic release of thiols from cysteine conjugates by yeast was demonstrated in 2003 at the Nestlé Research Center (Huynh-Ba et al., 2003), glutathione and cysteine conjugates are formed in plant detoxification and stress metabolism when the cysteine SH group of glutathione binds to reactive electrophilic precursors (Noctor et al., 2024).

In 2006, a study from wine research also showed the potential of genetically modified yeasts to convert 3-SH into 3-SHA and thereby achieve aromatic benefits in the finished wine (Swiegers et al., 2006). The research group led by Roncoroni et al. identified the IRC7 gene as a central genetic factor in the formation of 4-MSP by Saccharomyces cerevisiae. Deletion and overexpression experiments showed that cysteine-bound 4-MSP either no longer yielded any 4-MSP or produced approximately 100 times more 4-MSP(Roncoroni et al., 2011). This work forms an important basis for the later development of so-called "thiolized yeasts", in the US i.e. genetically modified yeast strains with enhanced thiol release. In a subsequent publication, Gros et al. identified the precursor structure S-3-(1-hydroxyhexyl)cysteine in Cascade hops for the first time. Extensive brewing trials with different hop varieties also showed that 2-sulfanylethyl acetate, 1-sulfanylpentan-3-one and 1-sulfanylpentan-3-ol cannot originate from hops, whereas 3-SH, 3M4SP and 4-MSP where found to be particularly dominant from a sensory perspective (Gros et al., 2012). Additional hop varieties and hop products were also analysed for the corresponding cysteine adducts. Addition of β-lyase released high amounts of thiols from precursor structures, including 3-MBT. High amounts of 4-MSP could also be released from Nelson Sauvin (Gros et al., 2013).

These findings showed that hop varieties and hop products contain different amounts of thiol precursors, which can be released by yeast enzymes during the brewing process. The resulting free thiols contribute to intense, tropical-fruity and pleasant aroma profiles in the finished beer. Also in 2013, it was shown that thiols can also be released from cysteine conjugates in bottle-aged beers. Sensorially, this led to a clear increase in grapefruit, berry and blackcurrant notes, but also to an increase in onion and vegetable notes(Nizet et al., 2014).

An investigation of 148 strains, including Saccharomyces pastorianus, S. cerevisiae and Torulaspora delbrueckii, showed that the IRC7 gene is widespread in brewing yeasts and generally enables the release of 3-SH and 4-MSP. However, the extent of release also depends on pH, temperature, time, enzyme concentration and precursor content (Michel et al., 2019). A study from wine research further showed that the mere detection of a complete IRC7 gene is not sufficient to predict thiol release, because point mutations can strongly reduce enzyme activity. The formation of 4-MSP was more strongly affected than that of 3-SH. Furthermore increased thiol release can also be accompanied by enhanced formation of

undesirable sulfur compounds such as hydrogen sulfide and ethyl thioacetate (Cordente et al., 2019).

6 Thiol precursor in hops and malt

That 3-SH can also be formed from malt-derived precursor structures was shown by Roland et al. in 2016. Cysteinylated and glutathionylated precursors were detected in barley, rice, sorghum and wheat malt at concentrations of 40 to 700 µg/kg, but not in roasted malt (Chenot et al., 2024). Compared with different hop varieties, however, this represents only a small proportion (see Figure 5). During mashing, glutathione conjugates can already be degraded via CysGly-3MH to Cys-3MH(Chenot, Collin, et al., 2023). The work by Roland et al. shows that several precursor structures exist for 3-SH and 4-MSP in hops. In addition to glutathione and cysteine conjugates, intermediate dipeptide forms such as cysteinylglycine and γ-glutamylcysteine have also been described. The concentrations of 3-SH precursors are markedly higher than those of 4-MSP precursors and can reach up to 20 mg/kg in hops (Roland et al., 2016a). Precursor concentrations in raw materials do not by themselves indicate their relative contributions to wort or beer. Malt compard to hops is used about 100 times more per hL of beer, so a comparison of course requires the amount of each material used, precursor transfer and the conversion efficiency of the yeast. Consequently, a possible malt derived contribution to the total precursors pool should not be underestimated.

Based on the available data for different hop varieties, Roland et al. proposed using varieties with high levels of thiol precursors in the hot side of the brewing process in order to promote their degradation. Hop varieties with high concentrations of free thiols, by contrast, should preferably be used for dry hopping so that these compounds are not lost during processing but are instead extracted (Roland et al., 2017b). In the same year, Reglitz et al. provided further important data: in 53 hop varieties, they confirmed that American varieties contain significantly higher 4-MSP levels than European varieties and that drying and pelletisation have little influence on 4-MSP concentration(Reglitz & Steinhaus, 2017). The list of possible thiol precursor structures in hops was expanded after a glutathione conjugate of 3-sulfanylpentanal and a cysteine conjugate of 3-sulfanylhexanal were identified in different hop varieties(Chenot, Haest, et al., 2023).

7 Thiol release in practical brewing

On the basis of knowledge about which precursor compounds occur in which hop varieties, and the fact that Saccharomyces cerevisiae possesses the relevant enzymes for thiol release, brewing parameters that can promote this release were subsequently investigated. Maturation during the brewing process proved to be a decisive phase for the release of thiols from yeast cells; at the same time, conversion into acetate esters differed depending on the yeast strain. This suggests that the cleavage of cysteine and glutathione conjugates during primary fermentation takes place inside the yeast cell. Release from glutathione conjugates was substantially lower (max. 0.08%) than from cysteine conjugates (max. 0.45%). Because of the high initial concentrations, however, even this low conversion may be sufficient to form up to 100 ng/L 3-SH or 3-SHA. The yeast strains differ both in thiol release and in subsequent esterification; in addition, temperature and original gravity influence the amounts of thiols released (Chenot et al., 2021). Surprisingly, Saccharomyces pastorianus yeasts also showed the potential to release thiols from cysteine and glutathione conjugates. The yield from glutathione conjugates was 0.35%, and therefore markedly higher than the previously reported 0.08%. In addition, a lower nitrogen concentration in the wort and a lower fermentation temperature improved thiol release (Chenot et al., 2022).

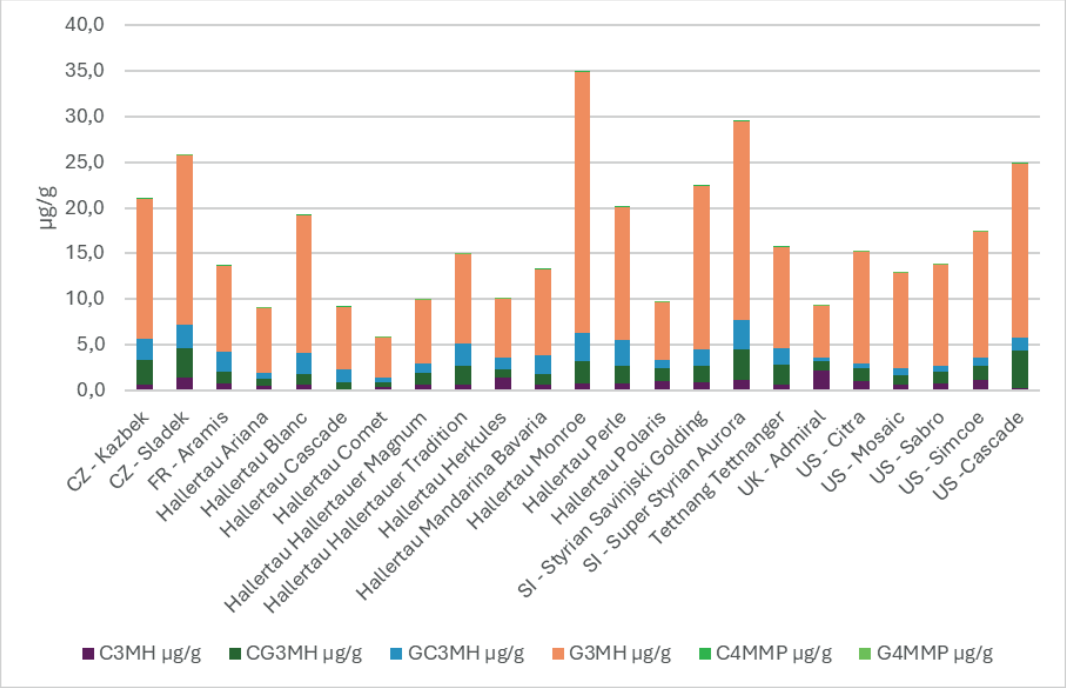

Fig. 5 **Concentrations of precursor structures in different hop varieties (Ch. , D. L. , Schönberger, 2020)**

The release of bound thiols can be substantially increased by suitably genetically modified yeasts. The team led by Molitor et al. worked with Saccharomyces cerevisiae brewing yeasts into which a gene encoding a highly active cysteine-S-conjugate β-lyase and an alcohol acyltransferase gene had been introduced in order to promote the formation of 3-SHA from 3-SH. As a result, the 3-SH concentration increased 73-fold compared with the parent yeasts, reaching more than 4000 ng/L; the 3-SHA concentration also increased eightfold. Sensorially, these concentrations led to intense tropical aroma notes such as mango, pineapple, passion fruit and guava, but were also partly described as sweaty.

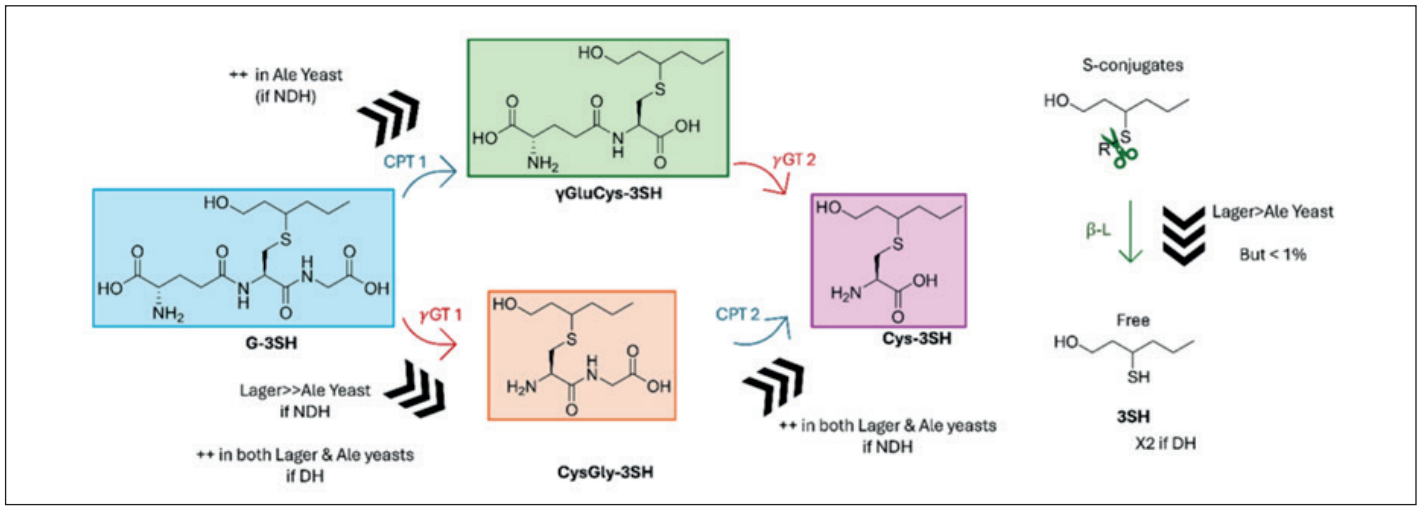

Fig. 6 **Highlighted observations regarding S-conjugate interconversions, based on S-conjugate content evolution through fermentation. NDH = non-dry-hopped; DH = dry-hopped (Chenot et al., 2026)**

It is particularly interesting that the aroma intensity and aroma quality of the beers were very similar despite strongly fluctuating 3-SH and 3-SHA levels. At the same time, the sensory difference between beers fermented with unmodified yeasts and those fermented with genetically modified yeasts was smaller than would have been expected from the large concentration differences. A possible explanation coud be sensory saturation. However, other aroma compounds such as 4-MSP, terpene alcohols or esters were not quantified in this study (Molitor et al., 2022b).

Krogerus et al. showed that increased thiol release can also be achieved by hybridising yeast strains: crosses with wild yeasts of S. uvarum and S. eubayanus led to increased formation of 4-MSP, 3-SH, 3-SHA and aroma-active esters. For 3-SH, however, concentrations reached only a maximum of 130 ng/L (Krogerus et al., 2023). In 2025, it was also shown that a low FAN content can promote thiol release because the yeast increasingly uses thiol precursors as alternative nitrogen and sulfur sources. Sensorially, however, beers with lower thiol levels and higher FAN values were described as more intensely fruity, citrus-like and tropical. This was attributed to higher terpene alcohol concentrations at low FAN levels, higher ester concentrations at higher FAN levels and aroma compound interactions. It is also noteworthy that, despite knockout of the IRC7 gene in one of the investigated yeasts, free thiols were still formed during fermentation (R. Samia et al., 2025).The addition of commercial enzymes such as apotryptophanase and cystathionine β-lyase to yeast can also efficiently release thiols, including 3-sulfanylhexan-1-ol, 3-sulfanylheptan-1-ol, 3-sulfanylpentan-1-ol, 3-sulfanyl-4-methylpentan-1-ol and 4-sulfanyl-4-methylpentan-2-one. This release can, however, be inhibited by polyphenols. Pretreatment of hop extracts with γ-glutamyltranspeptidase is therefore mentioned as a practical approach to degrade glutathione conjugates, since the optimal enzyme conditions in the brewing process itself, especially high pH and high temperature, are hardly achievable (Chenot, Willemart, et al., 2023).

With the increasing success of non-alcoholic beers, the use of maltose-negative yeasts is also becoming more important. Accordingly, current research is investigating the extent to which these yeasts are able to release thiols. For Saccharomyces cerevisiae var. chevalieri (SafBrew LA-01), relatively high release rates from glutathione conjugates of 3-SH were observed (0.34%), whereas utilisation of cysteine precursors was low. At the same time, it was shown that the yeast strains investigated can release 3-SH from γ-GluCys-3MH(Simon & Collin, 2024). That other non-Saccharomyces yeasts are also capable of thiol release was demonstrated by Wang et al. (Wang et al., 2026) . This research is particularly important for all relevant maltose-negative yeast strains in the context of non-alcoholic beers with convincing flavour and intense aroma.

Further brewing trials with different yeast strains showed that higher fermentation temperatures can markedly increase the release of 3-SH and slightly increase the release of 4-MSP. At the same time, however, the concentrations of many other aroma compounds also increased. The sensory descriptions as fruity-tropical therefore did not correlate clearly with higher thiol concentrations; undesirable sulfur notes were also detected (R. Samia, 2024) . Another study showed that high γ-GT and carboxypeptidase activities do not necessarily predict high thiol release. β-Lyase activity is also only of limited predictive value if the precursor structures present are not known. Evidence suggests that γ-GluCys and glutathione conjugates may be degraded directly by β-lyase. Whether the thiols formed are measurable in beer, however, also depends on whether the transport systems out of the yeast cell are known and controllable (Calicis et al., 2025). A newer study also provided new practically relevant insights: in beers that were not dry-hopped, most 3-SH and 3S4MP was formed between days 0 and 3 of fermentation. During this phase, glycolytic activity and the number of yeast cells in suspension are highest, while the FAN content decreases strongly. Thereafter, thiol concentrations remain largely stable. Thiol release is therefore strongest during primary fermentation. Additional dry hopping, however, does not increase concentrations in proportion to the amount of hops used. The delayed increase between days 4 and 7 is presumably due not only to the direct extraction of free thiols (5–10%) but above all to enzymatic release from precursor structures. A further increase during maturation is attributed to active enzymes and release from autolysed yeast cells. Gene analyses also showed that neither the presence of a complete IRC7 allele nor the expression of other relevant genes can sufficiently explain the observed thiol release (R. S. Samia et al., 2026). The metabolic

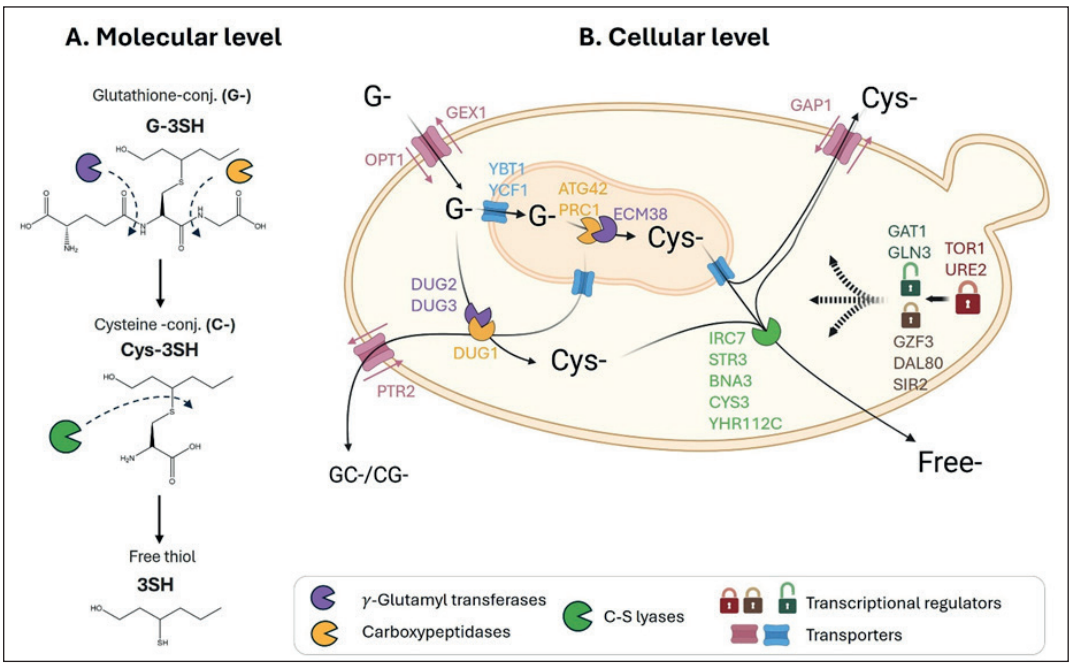

Fig. 7 **Representation of thiol precursor degradation pathways at the molecular and cellular levels using 3SH as an example: (A) pathways at the molecular level illustrating glutathione-3SH catabolism; (B) cellular-level scheme showing the intracellular localisation of thiol precursor degradation pathways and the genes involved in uptake, transport and enzymatic cleavage (R. S. Samia et al., 2026)**

framework is pictured in Figure 6 and shows the mechanisms between precursors, enzymatic transformation and thiol release for Ale and Lager yeasts and for dry-hoppend and non-dry-hopped beers. A more detailed insight is provided in Figure 7 illustrating how 3SH can be released from non-volatile, glutathione-bound precursors during fermentation. At the molecular level, the glutathionylated precursor G-3SH is degraded by removing glutamate and glycine, thereby forming intermediate dipeptide conjugates and ultimately the cysteinylated precursor Cys-3SH, β-lyases then cleave the bond between cysteine and the thiol moiety, releasing free 3SH. At the cellular level, figure 7 shows how specific transporters enable precursor uptake and intracellular trafficking, while peptidases and β-lyases catalyze the individual degradation steps in the cytosol and vacuole. The expression of the genes involved is additionally controlled by nitrogen catabolite repression, linking thiol release to the yeast's nitrogen status during fermentation.

8 Thiols and aroma stability

From a brewer's perspective, it is desirable for aroma and flavour to remain stable in the finished beer; in practice, however, beer remains a dynamic system. Understanding the reactions that take place after packaging is therefore essential for reducing sensory instability. Aged flavour is largely shaped by aldehydes, which are either formed de novo or arise from degradation or intermediate products, including α-carbonyls from Strecker degradation, 3-deoxyosones from pentosans, and trihydroxy fatty acids as precursors of aldehydes formed through lipid oxidation (Ditrych et al., 2025). Lehnhardt et al. showed that aldehyde concentrations decreased continuously during wort production; the initial concentrations in the malt used were the decisive factor. Efficient evaporation and reduced thermal stress contribute to the sensory stability of beer (Lehnhardt et al., 2026) . Late or split hop additions can scavenge free radicals and thereby reduce the formation of ageing aldehydes . Targeted strategies for removing metal ions, especially iron and copper, at the beginning of the brewing process could further reduce oxidative reactions(Wietstock et al., 2010). Barnette et al. also showed that low oxygen levels and low temperatures are particularly important for dry-hopped beers. Aged samples were described as less fruity, less citrus-like and less hoppy, while malty, dried-fruit and papery notes increased. These sensory changes did not correlate with the concentrations of hop-derived monoterpenoids; here too, Strecker aldehydes and compounds from lipid oxidation are suspected to play a role (Barnette & Shellhammer, 2019). This was also confirmed by Schubert et al who found that cold storage was the most effective measure for preserving hop-derived aroma compounds during beer ageing. Although the concentration of linalool remained relatively stable, hop-derived esters decreased over time, with pasteurisation and low storage temperatures slowing this decline(Schubert et al., 2024) .

During ageing, some compounds are formed rapidly and at higher concentrations, whereas others remain stable or decrease at different rates. Thiols readily react with metal ions. For copper ions, it has been shown that dipeptides such as Gly-His can protect thiols from copper-induced oxidation. In wort, various compounds may therefore act as potential protective partners (Vincent et al., 2025).

Rettberg et al. showed that higher storage temperatures cause greater losses. During storage for up to six months at 20 °C, 4-MSP was less stable than 3-SH. The extent to which oxygen influences degradation depends in turn on storage time and temperature. At room temperature, 4-MSP concentrations halved within the first four weeks. The concentrations of 3-SH decreased more slowly. The main cause of the loss is the formation of thiol disulfides. Current research is investigating potential additives such as glutathione as an antioxidant. Pasteurisation can stabilise the reaction dynamics in beer but is associated with higher aldehyde concentrations in fresh beer (Rettberg, 2024) .

9 Summary

Over the past twenty years, extensive knowledge about thiols in the brewing process has emerged. Today, it is well understood which hop varieties contain free thiols and what potential they have for bound thiols. Important, although still much less comprehensive,

insights are also available for malt. The main enzymatic release pathways are now known; this knowledge has led to the development of genetically modified "thiol yeasts". Brewers who are able to work with such yeasts do not need to make the brewing process unnecessarily complex, but they should consider that the resulting sensory diversity may be lower than when different hop varieties are used in a targeted way.

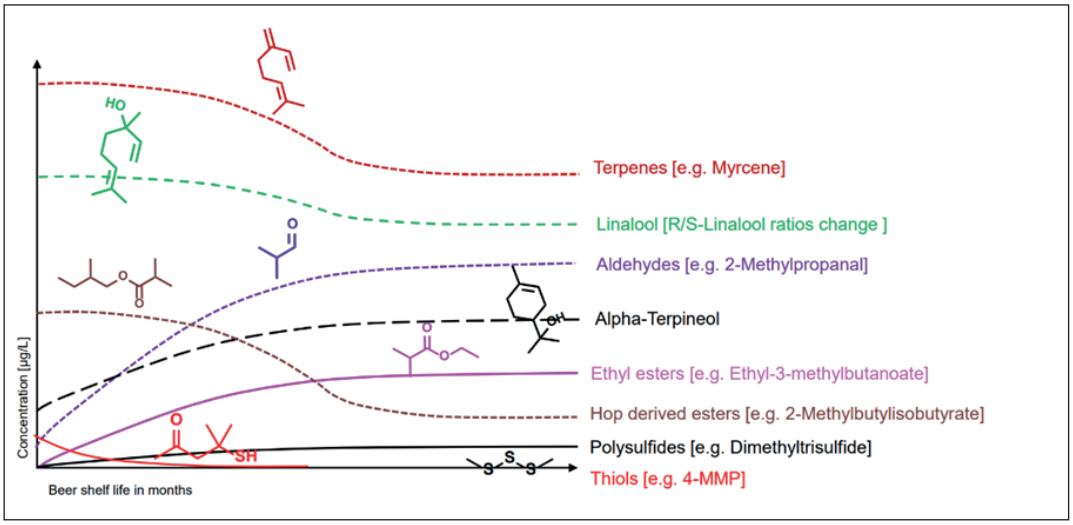

Fig. 8 **Schematic behaviour of different aroma compound groups over time in packaged beer (Rettberg, 2024)**

From a sensory perspective, the known free thiols can now be described well. Nevertheless, many questions remain open, and further discoveries in this research field can be expected. For the brewing industry, the decisive question is how this knowledge can be applied practically in beer production. If the aim is to achieve an intense tropical-fruity aroma profile in the finished beer, several control points are available. Hop varieties with high concentrations of free thiols are particularly suitable for dry hopping; yeast activity can further increase the levels of free thiols in the finished beer. Although it has not yet been conclusively clarified at the genetic level which gene expressions are decisive, there is now a good understanding of which brewing yeasts can generate higher thiol concentrations. Knowledge about the degradation of precursor structures enables targeted design of raw material selection, mashing, boiling and fermentation management. The possibilities for shaping primary and secondary fermentation to promote targeted biotransformation are now well described. Quantitatively, the greatest potential clearly lies in the precursor structures of 3-SH, whereas 4-MSP has a more diverse sensory impact.

However, the sensory evaluations in the cited studies also show that neither the individual concentrations of 4-MSP, 3-SH, 3-SHA or 3S4MP nor their sum correlate reliably with aroma intensity. More therefore does not necessarily mean more intense in this context. Thiols have distinctive individual aroma qualities that can change markedly depending on concentration. This applies particularly to 4-MSP.

The sensory contribution of thiols is therefore best assessed by combining compound-specific concentrations with sensory measurements and information on the beer matrix. Low ng/L concentrations can contribute to fruity and tropical impressions in combination with esters and terpene alcohols, but synergy can not be assumed for every mixture. Depending on composition and concentration ratios, interactions may also involve masking or suppression. The practical objective is to understand these relationships rather than to treat either thiol concentration or aroma interactions in isolation. Still available data so far supports the idea that even low concentrations in the nanogram-per-litre range are sufficient, in combination with esters and terpene alcohols, to evoke a fruity, intense and tropical aroma in olfactory perception.

To brew beers with a convincing aroma profile, good drinkability, clean flavour and high flavour stability, numerous process parameters must be precisely controlled and the raw materials carefully selected. Changing an established process solely to maximise the final thiol concentration is therefore not a sensible option. With the knowledge currently available, however, it should be readily possible to achieve a sufficient amount of free thiols in the finished beer in a targeted way.

The future of thiol research in beer will probably lie less in maximising individual thiol concentrations and more in understanding aroma interactions between thiols, esters, terpene alcohols and the beer matrix specifically in context with aroma stability.

Conflict of interest

The author declares no conflict of interest.

CrediT Authorship contribution

Christina Schönberger: conceptualisation, writing, visualisation

Use of AI

AI-assisted tools were used to refine language, including grammar and readability, of the text written by the author. The scientific content, data analysis, and conclusions are solely the responsibility of the author.

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