MINI-REVIEW

Fruits of their labour: biotransformation reactions of yeasts during brewery fermentation

Natalia Svedlund1 · Simon Evering1 · Brian Gibson1 · Kristoffer Krogerus2

Received: 5 March 2022 / Revised: 26 June 2022 / Accepted: 2 July 2022 / Published online: 19 July 2022
© The Author(s) 2022

Abstract
There is a growing appreciation for the role that yeast play in biotransformation of flavour compounds during beverage fermentations. This is particularly the case for brewing due to the continued popularity of aromatic beers produced via the dry-hopping process. Here, we review the current literature pertaining to biotransformation reactions mediated by fermentative yeasts. These reactions are diverse and include the liberation of thiols from cysteine or glutathione-bound adducts, as well as the release of glycosidically bound terpene alcohols. These changes serve generally to increase the fruit and floral aromas in beverages. This is particularly the case for the thiol compounds released via yeast β-lyase activity due to their low flavour thresholds. The role of yeast β-glucosidases in increasing terpene alcohols is less clear, at least with respect to fermentation of brewer’s wort. Yeast acetyl transferase and acetate esterase also have an impact on the quality and perceptibility of flavour compounds. Isomerization and reduction reactions, e.g. the conversion of geraniol (rose) to β-citronellol (citrus), also have potential to alter significantly flavour profiles. A greater understanding of biotransformation reactions is expected to not only facilitate greater control of beverage flavour profiles, but also to allow for more efficient exploitation of raw materials and thereby greater process sustainability.

Key points
● Yeast can alter and boost grape- and hop-derived flavour compounds in wine and beer
● β-lyase activity can release fruit-flavoured thiols with low flavour thresholds
● Floral and citrus-flavoured terpene alcohols can be released or interconverted

Keywords Polyfunctional thiol · Terpene · β-lyase · β-glucosidase · Yeast · Fermentation · Beverages · Biotransformation

Introduction
The use of hops in the brewing process has increased dramatically in the past decades as a result of greater consumer demand for heavily hopped India Pale Ale (IPA)-style beers (Lafontaine and Shellhammer 2019a). In the USA, average hop use in breweries increased almost 50% from 2011 to 2019 (Lafontaine and Shellhammer 2019a; Cantwell and Swersey 2019). At the same time, global hop production has also grown. While hops were traditionally used mainly to impart bitterness and noble hop aroma (i.e. floral, herbaceous and woody) to beer, hops are today often used to impart a more intense hop aroma and taste, and newly developed hop varieties may contribute a wide range of fruity flavours to beer. In addition to increasing hop usage and diversity of hop varieties, brewers also exploit the ability of yeast to produce a wide range of flavour compounds in their attempts to meet consumer demand for more flavourful and diverse beers (Holt et al. 2019). A number of such flavour compounds are formed by enzymatic action from precursors originating from hops and malt. The term ‘biotransformation’ is often used to describe these types of flavour-releasing reactions during brewery fermentations. Exploiting these reactions is not unique to beer, as they occur and are exploited during wine and other beverage fermentations as well. In fact, the research on biotransformation reactions occurring during winemaking pre-dates that in brewing by

* Kristoffer Krogerus
kristoffer.krogerus@vtt.fi

1 Chair of Brewing and Beverage Technology, Institute of Food Technology and Food Chemistry, Technische Universität Berlin, Ackerstraße 76, 13355 Berlin, Germany
2 VTT Technical Research Centre of Finland, Tietotie 2, P.O. Box 1000, FI-02044 VTT Espoo, Finland

© Springer
many years, and the former has inspired and informed the latter. In this review, we focus on the recent work related to brewing yeast biotransformations but also refer to relevant work from the winemaking sphere.

Biotransformation reactions are of interest to brewers and other beverage producers for numerous reasons. Foremost, they allow for an increased yield of aroma-active compounds in the final product. As will be discussed in more detail below, many of these compounds are present in conjugated odourless forms in the raw materials, often at concentrations many-fold higher than that of the free form. Through biotransformation, increased concentrations of the free aroma-active forms can be obtained in the beverage, in addition to compounds not originally found in the raw materials. This allows the introduction of novel flavours to the beverage by converting precursors from the raw materials. As hops typically constitute the bulk of the raw material costs in brewing and they exert a considerable agricultural demand (Denby et al. 2018; Hauser and Shellhammer 2019; Lafontaine and Shellhammer 2019b), there is also potential to increase the sustainability and lower the environmental impact of the brewing process by exploiting biotransformation to produce similar flavour from less raw material.

In this mini-review, we cover the latest developments in using biotransformation as a tool to release additional flavour during beverage fermentations. The main focus is on beer fermentation and precursors derived from hops, but other beverage fermentations are covered as well. We discuss four different types of reactions, including β-lyase activity to release polyfunctional thiols, β-glucosidase activity to release terpene alcohols, reduction of terpene alcohols and esterification of polyfunctional thiols and terpene alcohols (Fig. 1). Furthermore, we also present strategies that can be used to enhance biotransformation capabilities of yeast strains and process changes that increase the yield of aroma-active compounds.

Polyfunctional thiols

Polyfunctional, sulphur-containing, thiols are an important group of compounds that influence the aroma of a broad range of beverages, both fermented and non-fermented (Roland et al. 2011; Holt et al. 2019; Bonnaffoux et al. 2021). These molecules typically have strong aroma and are detectable at very low concentrations (ng per litre levels). The aromas of such thiols can range from unpleasant (e.g. onion- and skunk-like) to pleasant (e.g. black currant and tropical fruits) (Table 1). In fermented beverages, such as wine, beer and saké, two pleasant-smelling volatile thiols are of particular importance: 3-mercaptohexan-1-ol (3MH; also referred to as 3-sulfanylhexan-1-ol, 3SH) and 4-mercaptop-4-methylpentan-2-one (4MMP; also referred to as 4-methyl-4-sulfanylpentan-2-one, 4MSP) (Darriet et al. 1995; Tominaga et al. 1998a; Kishimoto et al. 2006; Kishimoto et al. 2008a; Iizuka-Furukawa et al. 2017). Wines made from Sauvignon blanc grapes, for example, have been shown to contain particularly high concentrations of these thiols (Roland et al. 2011), while recent studies have shown they are vital to fruity hop aroma in modern highly hopped beer (Kankolongo Cibaka et al. 2017; Dennenlöhr et al. 2020; Biendl et al. 2021). In addition to the two previously mentioned thiols, 3-sulfanyl-4-methylpentan-1-ol (3S4MP) and 3MP (3-mercaptopentanol; also referred to as 3-sulfanylpentan-1-ol, 3SP) have also been shown to contribute to the fruity aroma of certain hop varieties, such as Nelson Sauvin (Sarrazin et al. 2007; Takoi et al. 2009a).

Thiol precursors in raw materials

While hops and grapes contain volatile thiols that can be directly transferred to the fermented beverage (Kishimoto et al. 2008a; Takoi et al. 2009a; Capone et al. 2011, 2012), the majority of volatile thiols that are present in beer, wine and saké are released by enzymatic action from glutathionylated or cysteinylated precursors found in the malt, hops, grapes and rice (Tominaga et al. 1998b; Peyrot des Gachons et al. 2000; Kishimoto et al. 2008b; Roland et al. 2010, 2016; Gros et al. 2012; Iizuka-Furukawa et al. 2017; Chenot et al. 2019). Early work using bacterial enzymes revealed that free

![Fig. 1 An overview of the biotransformation reactions occurring in yeast. Abbreviations: 3MH 3-mercaptohexanol, 3MHA 3-mercaptohexyl acetate, Cys cysteine, GSH glutathione, TPA terpene alcohol. Credit: Henrik Svedlund](image-url)
volatile thiols could be released from the conjugated precursors by action of a carbon–sulphur lyase enzyme (Tominaga et al. 1998b; Wakabayashi et al. 2004). In *Saccharomyces cerevisiae*, the main β-lyase enzyme involved in releasing volatile thiols from conjugated precursors is encoded by the *IRC7* gene (Howell et al. 2005; Thibon et al. 2008; Roncoroni et al. 2011). In addition to *IRC7*, the β-lyase enzyme encoded by the *STR3* gene has also been shown to release volatile thiols from cysteinylated 3MH and 4MMP (Holt et al. 2011). The β-lyase activity of yeasts will be discussed in more detail in the following section.

The amount of thiol precursor found in the raw materials varies greatly with variety. In grapes, where barely any free thiols are detectable, high 3MH and 4MMP precursor concentrations have been found in, e.g. Sauvignon blanc, Gewürztraminer, Semillon, Chardonnay and Riesling grapes (Concejero et al. 2014; Bonnaffoux et al. 2017). Precursor concentrations in these varieties have even been measured in the mg/L range. Hops, which also contain considerable amounts of free thiols, have also been shown to contain high concentrations of 3MH and 4MMP precursors (Roland et al. 2016). Concentrations of 6.5 mg/kg 3MH precursors have been reported for Cascade hops, and these concentrations are 1000-fold higher than the concentrations of free 3MH. In addition, a recent survey revealed the presence of cysteinylated and glutathionylated sulphanylalkyl aldehydes and acetates in hops and grapes for the first time (Chenot et al. 2022a). Despite a large pool of thiol precursors existing in the fermentation media, only a small fraction is typically converted into the free form during fermentation. In wine, the fractions of glutathionylated or cysteinylated precursors in the grape must that are converted into free thiols typically remain below 1% regardless of yeast strain or grape variety (Murat et al. 2001; Subileau et al. 2008b; Roland et al. 2011; Bonnaffoux et al. 2018). A similarly low conversion yield (0.1–0.5%) has been observed in beer wort (Nizet et al. 2013; Michel et al. 2019; Chenot et al. 2019, 2021). This low conversion could be linked to poor activity of β-lyase activity in acidic media and inhibition by polyphenols (Chenot et al. 2022b).

From a process point-of-view, the temperature during beer fermentation was recently shown to affect the release of various thiols from their conjugated precursors (Chenot et al. 2021). However, the effect of temperature varied

### Table 1: A summary of hop- and grape-derived flavour compounds that can be released or formed through biotransformation reactions

| Compound | Abbreviation | Aroma description | Flavour threshold | Precursors found in | Enzyme activity to release | Molecular structure |
|----------|--------------|-------------------|------------------|---------------------|---------------------------|--------------------|
| 3-mercaptopentane / 3-sulfanyl-1-ol | 3MH / 3SH | Grapefruit | 55-60 ng/L | Malt, hops, grapes | β-lyase activity, to release from cysteinylated and glutathionylated precursors |
| 4-mercapto-4-methylpentan-2-one / 4-methyl-4-sulfanylpentan-2-one | 4MMP / 4MSP | Black currant, 1.5 ng/L | Hops, grapes | β-lyase activity, to release from cysteinylated and glutathionylated precursors |
| 3-sulfanyl-4-methylpentan-1-ol | 3S4MP | Grapefruit, Rhubarb | 70 ng/L | Hops | β-lyase activity, to release from cysteinylated and glutathionylated precursors |
| 3-mercaptohexyl acetate / 3-sulfanylhexyl acetate | 3MHA / 3SHA | Passion fruit, guava | 4 ng/L | Malt, hops, grapes | Acetyl transferase activity, to convert 3MH into 3MHA |
| Linalool | Lavender | | 5 µg/L | Hops, grapes | β-glucosidase, also α-L-arabinofuranosidase and/or α-L-rhamnosidase |
| Geraniol | Rose-like | | 6 µg/L | Hops, grapes | β-glucosidase, also α-L-arabinofuranosidase and/or α-L-rhamnosidase |
| Citronellol | Lemon-like and/or lime-like | | 8 µg/L | Hops, grapes | NADPH dehydrogenase 2 (encoded by OYE2) reduces geraniol into β-citronellol |

(Kishimoto et al. 2006; Czerny et al. 2008; Takoi et al. 2009a, 2009b, 2010a; Lafontaine et al. 2021)
between thiols, with 3MH and 3MP (3-mercaptopentanol; also referred to as 3-sulfanylpentan-1-ol, 3SP) release highest at 18–24 °C and 3S4MP (3-sulfanyl-4-methylpentan-1-ol) release highest at 28 °C. A post-fermentation maturation at 4 °C for up to 5 days was shown to steadily increase thiol concentrations (Chenot et al. 2021). In addition to temperature, addition of exogenous enzymes to beer (e.g. cystathionine β-lyase and apotryptophanase) was recently shown to release thiols from cysteinylated precursors (Chenot et al. 2022b). However, conversion rates were on a similar level to those observed by brewing yeast. Further addition of a γ-glutamyl transpeptidase enzyme also allowed the release of thiols from glutathionylated precursors (Chenot et al. 2022b). Preliminary unpublished work also suggests that adding hops to the mash could increase the amount of volatile thiols released into the beer during fermentation (Burns 2021). This might be a result of protease activity in the mash, which could release or modify the conjugated precursors in the hops. Finally, using copper in the brewing or winemaking process, or when treating raw materials (e.g. hops or grapes), has been shown to decrease 4MMP content of the beverage (Swiegers and Pretorius 2007; Kishimoto et al. 2008b; Morimoto et al. 2010).

### Thiol release through β-lyase activity

The release of volatile thiols from conjugated precursors during yeast fermentation has been directly linked to *IRC7* expression (Thibon et al. 2008). The resulting β-lyase enzyme is involved in sulphur metabolism and amino acid biosynthesis, and expression of the gene is linked to the nitrogen catabolite repression process (Subileau et al. 2008a; Thibon et al. 2008). In the presence of favourable nitrogen sources, such as ammonium, *IRC7* is repressed. *IRC7* regulation has been linked to the transcriptional activator Gln3p and its inhibitor Ure2p. Deletion of the activator-encoding *GLN3* decreased thiol release, while deletion of the inhibitor-encoding *URE2* increased thiol release (Thibon et al. 2008). Strains carrying loss-of-function mutations in *URE2* have also been shown to release higher amounts of thiols (Dufour et al. 2013). Selection of *ure2* mutants has been successfully demonstrated through growth on agar plates containing the toxic ammonium analogue methylamine and proline as sole nitrogen sources (Salmon and Barre 1998). A number of breeding strains carrying heterozygous nonsense mutations in *URE2* have also been identified among the ‘United Kingdom’ sub-group of the ‘Ale beer’/‘Beer 1’ clade (Krog-er us et al. 2021). Expression of *IRC7* in *S. cerevisiae* is also affected by its location in the sub-telomeric region of the right arm of chromosome VI (Holt et al. 2019). This region is silenced by the *SIR2*-encoded histone deacetylase, and both *SIR2* deletion and mutations in the subunit-encoding *SIR3* and *SIR4* genes have shown to increase *IRC7* expression (Ehrentraut et al. 2010; Samel et al. 2017).

In addition to regulation, thiol release by Irc7p is also affected by polymorphisms in the coding sequence of *IRC7*. The most well reported of these is a 38-bp deletion (*IRC7S*), which results in the formation of a truncated 360 amino acid protein with considerably lower activity than the full-length 400 amino acid Irc7p (Roncoroni et al. 2011). This 38-bp deletion is surprisingly widespread among wine strains and found in heterozygous form among many ‘Mosaic beer’/‘Beer 2’ brewing strains, but it is not so common in ‘Ale beer’/‘Beer 1’ brewing or other domesticated strains (Cordente et al. 2019; Ruiz et al. 2021; Krogerus et al. 2021). The *S. cerevisiae* S288C reference genome also contains the 38-bp deletion in *IRC7*. Strains carrying either homozygous or heterozygous alleles of *IRC7S* release lower amounts of thiols during fermentation (Roncoroni et al. 2011; Belda et al. 2016; Cordente et al. 2019). Along with *IRC7S*, a number of other inactivating mutations in *IRC7* have been identified. These include the T185A mutation, which reduces enzyme activity and thiol release by around 50% (Cordente et al. 2019). When coupled with other mutations, like K43R, P146R, G253R, G321D and E323G, enzyme activity and thiol release are decreased further. The T185A mutation is common among both wine and brewing strains (Cordente et al. 2019; Krogerus et al. 2021).

As β-lyase activity occurs inside the cell, the glutathionylated or cysteinylated precursors need to be transported inside the cell for any thiol release to occur. Limited studies on the topic have identified the *OPT1*-encoded oligopeptide transporter as the main transporter for glutathionylated 3MH and 4MMP (Subileau et al. 2008b; Santiago and Gardner 2015; Cordente et al. 2015). Deletion of *OPT1* significantly reduces release of both 3MH and 4MMP from their glutathionylated precursors, and 3MH release in particular is affected. Furthermore, deletion of *ECM38*, encoding a vacuolar transpeptidase, also increases release of 3MH from Glu-3MH (Cordente et al. 2015). Transport of cysteinylated precursors is not as well established. Preliminary work by Subileau et al. (2008a) suggested the *GAP1*-encoded general amino acid permease was involved, but no change in thiol release from cysteinylated precursors was observed when nine genes encoding known cysteine-transporting permeases, including *GAP1*, were deleted (Santiago and Gardner 2015).

A number of strategies have successfully been used to improve thiol release in yeast strains. Overexpression of the native full-length *IRC7* and *STR3* in wine strains has been shown to increase the release of 3MH and 4MMP (Holt et al. 2011; Roncoroni et al. 2011). Overexpression of the *tnaA*-encoded tryptophanase from *Escherichia coli* in wine strains also released 10- to 95-fold higher concentrations of 3MH in wine (Holt et al. 2011; Kiene et al. 2021). As described
above, deletion of *URE2* also increased 3MH and 4MMP concentrations fourfold in synthetic juice media (Thibon et al. 2008).

Breeding or hybridization has also been used to construct yeast strains with enhanced β-lyase activities. *ure2* mutations were introduced to various wine strains by first crossing them with a lab strain carrying a non-functional *URE2* allele and then backcrossing them with the wine strains (Dufour et al. 2013). *ure2* hybrids produced higher levels of both 3MH and 4MMP during wine fermentations. In a recent study, brewing yeasts with CRISPR/Cas9-aided mating type switching were bred in an attempt to construct strains with enhanced thiol release abilities (Krogerus et al. 2021). Using *IRC7* sequences as a marker, crosses between different brewing strains and between brewing and wild strains were performed. Selected hybrids produced higher amounts of 4MMP and 3MH-acetate. Interspecific hybridization between *S. cerevisiae* and *Saccharomyces uvarum* strains has also been used to enhance thiol release during both wine (Masneuf-Pomarède et al. 2002; da Silva et al. 2015) and beer fermentations (Krogerus et al. 2022). In the latter study, enhanced concentrations of 4MMP, 3MH and 3MH-acetate in beer were obtained through fermentation with brewing strains crossed with selected *S. uvarum* strains. Indeed, from limited studies, it appears as if *S. uvarum* strains tend to have higher thiol release abilities than *S. cerevisiae* strains (da Silva et al. 2015; Knight et al. 2018). A possible explanation could be that *IRC7* is located further away from the telomeres in *S. uvarum* and unlikely to be affected by subtelomeric silencing (Holt et al. 2019). A recent study in wine also revealed high thiol release by a strain of *Saccharomyces kudriavzevii*, highlighting the potential of other *Saccharomyces* species as well (Pérez et al. 2022).

Considerable β-lyase activity has been observed in non-*Saccharomyces* yeast as well. In wine fermentations, *Metschnikowia pulcherrima*, *Torulaspora delbrueckii*, *Lachancea thermotolerans* and *Candida zemplinina* have been shown to release high levels of 3MH or 4MMP in comparison to *S. cerevisiae* wine strains (Anfâng et al. 2009; Zott et al. 2011; Belda et al. 2016). To overcome the often poor fermentation performance of such non-*Saccharomyces* strains, co- or sequential fermentation with a *Saccharomyces* yeast can be employed. Studies focusing on the β-lyase activity of non-*Saccharomyces* yeasts during beer fermentations are however limited. In a recent study, the ability of two *T. delbrueckii* strains to release 3MH in beer was shown to be similar to the included *S. cerevisiae* and *Saccharomyces pastorianus* strains (Michel et al. 2019). β-lyase activity and 3MH release have also been demonstrated in *Lactobacillus plantarum* during grape juice fermentations, highlighting that the ability is not limited to yeast (Takase et al. 2018).

Yeast can be screened for β-lyase activity using growth media containing a cysteinylated substrate as the sole nitrogen source. Growth on S-methyl-L-cysteine (SMC) as the sole nitrogen source has been shown to positively correlate with β-lyase activity (Belda et al. 2016). However, when recently applied to brewing yeast, growth on SMC could not discriminate strains with good thiol release very well (Michel et al. 2019; Krogerus et al. 2021). High β-lyase activity has also been associated with the ability to grow on cysteine as the sole nitrogen source (Santiago and Gardner 2015). This was recently exploited to select brewing yeasts with enhanced β-lyase activity (Krogerus et al. 2021).

**Acetylation of 3-mercaptohexanol**

As described above, yeasts play a significant role in liberating free polyfunctional thiols from their cysteinylated or glutathionylated forms during fermentation. Biotransformation of polyfunctional thiols by yeast is not however limited to those reactions involving β-lyase. Fermented beverages, such as wine and beer, are also known to contain acetylated forms of polyfunctional thiols (Vermeulen et al. 2006). Of these, 3-mercaptohexyl acetate (3MHA), the acetylated form of 3MH, has received considerable attention due to its positive contribution to flavour. Its distinctive flavour, often described as passion fruit-like, is sensorially apparent at exquisitely low levels in beer (4 ng/L). It was, like 3MH, first detected in passion fruit juice (Engel and Tressl 1991) and later found in a range of wines, particularly in Sauvignon blanc wines where it is an important component of the style’s flavour profile (Tominaga et al. 1996). There is also a growing appreciation of this flavour note in dry-hopped beers (Dennenlöhr et al. 2020). Studies on 3MHA perception in red wine have also shown that its presence can accentuate the perception of other polyfunctional thiols such as 4MMP (Rigou et al. 2014).

3MHA has not been detected in substrates prior to fermentation and is not believed to occur commonly in a bound form (though glutathionylated 3MHA has been detected in grape, Chenot et al. 2022a). Available evidence suggests that this compound is created through esterification of 3MH by yeast during fermentation (Fig. 1). Swiegers et al. (2006) demonstrated that this esterification was primarily due to the activity of alcohol acetyltransferase, in particular that coded for by the gene *ATF1* (Swiegers and Pretorius 2007). Other enzymes may also have a role to play, as evidenced by the fact that *ATF1* deletion did not completely prevent the esterification reaction from occurring.

Given the clear link between *ATF1* activity and 3MHA, an obvious strategy to boost levels of the acetyl ester would be to engineer yeasts for greater *ATF1* activity, possibly in combination with a reduced esterase activity to reduce the risk of the acetylated form reverting to 3MH downstream. The work of Swiegers et al. (2006) and Kiene et al. (2021) suggests that such an approach would be effective.
Alternatively, one could screen yeasts for 3MHA production (Anfang et al. 2009), or screen yeasts that have a naturally high level of ATF1 expression, e.g. those which are known to produce high levels of acetate esters such as 3-methylbutyl acetate. Acetyltransferase activity may be increased via adaptive evolution. This has been seen, for example, when saké yeast have been exposed to toxic levels of 1-farnesylpyridinium, a disruptor of acetyltransferase activity. Cell lines that developed resistance to these compounds showed acetyltransferase activities several-fold higher than in the parental train and consequently a higher production of acetate esters (Hirooka et al. 2005). A similar approach could be taken to encourage 3MH acetylation. Likewise, reduced esterase activity in saké yeasts through adaptive evolution has been demonstrated. In this case, activity of the EST2 gene was disrupted via mutagenesis in an effort to maintain high levels of acetate ester (Fukuda et al. 1998), and a similar approach may be effective in preventing the loss of 3MHA.

Alternatively, one may take advantage of the esterification ability of non-Saccharomyces yeasts. Anfang et al. (2009), for example, demonstrated that a mixed culture of wine yeast and Pichia kluyveri was highly effective at promoting 3MHA levels. The synergistic effect observed may be due to liberation of 3MH by the wine yeast followed by esterification by the non-Saccharomyces partner (Anfang et al. 2009).

In addition to selecting or modifying yeast, one has the option of modifying the fermentation process to better support the formation and retention of 3MHA. Acetyltransferase activity in yeast is known, for example, to be influenced by oxygenation or the presence of unsaturated fatty acids in brewer’s wort, with both factors resulting in reduced ATF1 gene expression and enzyme activity (Fujii et al. 1997). One would therefore expect greater 3MHA formation where excess oxygenation is avoided, or where a particularly clear (trub-free) wort is utilized at the beginning of fermentation. Likewise, an increase in temperature or wort gravity would be expected to promote ATF1 expression and acetylation, as it does for acetate esters (Saerens et al. 2008).

3MHA levels may be determined to some extent by the type of conjugation of the precursor molecule. Winter et al. (2011) suggested that, despite the fact that 3MH is more readily released from cysteine-bound 3MH, higher levels of 3MHA were associated with higher level of the glutathione-bound precursor (Winter et al. 2011). While it is not yet clear why this would be the case, it is possible that the nitrogen fraction released through β-lyase activity in the cell may influence acetylation. Nitrogen concentration and composition are known to have an impact on acetyltransferase activity (Verstrepen et al. 2003). If it is indeed the case that the conjugation influences acetylation, this would suggest that those strategies designed to converted glutathionylated thiols to cysteinylated thiols (described above) may reduce the potential for 3MHA formation. Patel et al. (2010) noted that juice pasteurization resulted in wines with higher 3MHA levels. This occurred at the expense of 3MH, the levels of which were reduced by pasteurization (Patel et al. 2010). To what extent this might be influenced by the type of conjugation is not known. When considering 3MHA in fermented beverages, one must recognize that the molecule is highly volatile, and a gradual loss during fermentation or from the finished product is expected. As for other polyfunctional thiols, there is a risk of loss during active fermentation via CO₂-stripping, and hopping early in fermentation is not advised for this reason. 3MHA is also highly susceptible to loss by hydrolysis. This instability was demonstrated by Makhotkina and Kilmartin (2012), who observed a drop in 3MHA levels in Sauvignon blanc wines over time, particularly at higher temperatures. The 3MHA was apparently converted to 3MH and acetic acid during the process (Makhotkina and Kilmartin 2012).

As is the case for other biotransformation reactions, considerably more is known about changes occurring during wine fermentation relative to brewery fermentations. It remains to be seen if our knowledge on thiol acetylation is transferrable to the brewing system. Also, despite the existence of multiple acetylated thiols in fermented beverages, to date most attention has been focused on 3MHA due to its low flavour threshold and positive flavour attributes. If other acetate forms of polyfunctional thiols also contribute in a significant way to flavour profiles of fermented beverages has yet to be established.

### Terpenes

Numerous essential oils are found in hops and form a complex combination of different volatile substances. The characteristic aroma of dry-hopped beers is mainly derived from these hop oils transferred to the beer during the brewing process, either in the brewhouse during late hopping in the whirlpool or in the cellar via different dry-hopping techniques. In this section, we will focus on the terpene alcohols, which are the main contributors to the fruity, citrus and floral aromas in final beers (Inui et al. 2013). This complexity arises from the high number of possible combinations of compounds, and the synergistic and masking effects among volatile and non-volatile beer constituents (Rettberg et al. 2018). The flavour thresholds of typical monoterpane alcohols are low. Some examples of monoterpane alcohols are shown in Table 1. Geraniol is described as having a lime, flower, hyacinth and rose aroma, and can be detected at a concentration of 6 µg/L, while β-citronellol has a citrus, floral, lime aroma and has a flavour threshold of 8 µg/L (Meilgaard 1982; Takoi et al. 2010a). Hop oils other than monoterpane alcohols can be found in higher concentrations.
in hops, but these compounds (e.g. myrcene, α-humulene, β-caryophyllene, β-farnesene) are less important for the aroma of both fresh and dried hops (Steinhaus and Schieberle 2000). While predicting the aroma impact of hops on the finished beer remains difficult, recent work has revealed the contribution of many individual oil components and effect of various process conditions.

Hop oils are located mainly in glandular trichomes (lupulin glands), but also in leaves and flowers, as seen in Fig. 2. Myrcene is the most abundant hop oil > 80% v/w and is only available in the trichome, whereas linalool, α-humulene and β-caryophyllene are also available in leaves and flowers (Eri et al. 2000). Bitter acids, hop oils and prenyl flavonoids are all derived from pathways of terpene metabolism. Hop essential oils are synthesized from dimethylallylpyrophosphate (DMPP) and isopentenylpyrophosphate (IPP) to produce geranyl pyrophosphate (GPP), from which myrcene is synthesized through the action of terpene synthase (MTS) (Wang et al. 2008). Various species of hops convert many different terpenoids from the same substrate, and the subsequent addition of functional groups leads to different products (Tholl 2006; Nagegowda 2010).

The oil composition in hops is determined by genotype, with different hop varieties having different compositions (Oliveira et al. 1988; Saito et al. 1995). Not only does genetics determine the oil composition in hops but also the harvest time. Differences in the hop characteristics and chemistry of hops from different harvest maturities have been reported (Sharp et al. 2014; Matsui et al. 2016; Lafontaine et al. 2021). Studies have independently verified these results with Cascade and Willamette hops varieties in USA as well as with Saaz hop variety in the Czech Republic. Early harvesting resulted in lower essential oil composition from hops of the same variety, farm and year of harvest.

**Monoterpene alcohol glycosides**

Terpenes and monoterpene alcohols are found in hops in free form as well as bound to other molecules. Usually, they consist of an aglycone and a glycone, the latter being an activated sugar, and the former, a non-sugar moiety, such as linalool or geraniol. Monoterpene alcohol glycosides were first identified in grapes but are also found in other fruits such as apples. Plants use glycosides as a way to store...
In the last decade, research has also revealed how yeast can release and enhance characteristic hop aromas to the beer that were ‘hidden’ in the plant. These aromas can be liberated only through enzymatic activity, such as that of yeast during fermentation (Fig. 1). Yeast therefore has the ability to enhance the hop aroma characteristic of dry-hopped beer. Promotion of such biotransformation reactions in brewing may obviate the need for additional hops, or specialist hops, to achieve particular hop aroma profiles in beers (Goldstein et al. 1993; Kollmannsberger et al. 2006). The inter sugar bond present in glycosides can be cleaved by a 1,4-β-glucosidase (EC.3.2.1.21), first reported by Gunata et al. (1988) and Saray and Günata (2004). While hops contain glycosidically bound monoterpenoid alcohols that can be enzymatically cleaved, it is under debate whether their release has any perceivable impact on beer aroma.

Sharp et. al., after screening yeasts for their β-glucosidase activity, and performing fermentations with selected yeasts in wort dry hopped with three different hop cultivars, found no significant difference in the terpene alcohol concentrations. This was probably due to an inhibition in the expression of the enzyme by the presence of glucose, and/or the anaerobic conditions (Sharp et al. 2017a). Serra Colomer and colleagues had similar results when screening for β-glucosidase activity in Brettanomyces yeasts and found no correlation between the strains with high enzyme activity and the geraniol content in the beers after dry-hopping (Serra Colomer et al. 2020).

Van den Bremer used the term bio-generation to describe production of novel flavours in beer, by using the yeast Candida methanolovescens, the β-glucosidase enzyme of which produced salicyl aldehyde from salycylic acid, which has a characteristic almond aroma (Van Den Bremer et al. 2001). In 2003, Vanderhagen introduced the concept of beer bio-flavouring using different yeast strains, either to reduce aldehyde content or to release aromatic substances in hops (Vanderhagen et al. 2003). In S. cerevisiae, the enzyme exo-β-glucanase (encoded by the EXG1 gene) was found to cleave the intersugar bond in glycosides and to be released independently of the carbon source. Olivero et al. reported in 1985 that the overproduction of this enzyme resulted in an increase in volatile compounds (Olivero et al. 1985; Gil et al. 2005). Daenen et al. in 2008 looked for this enzyme activity in 59 yeasts, including 9 lager yeast strains, 27 ale yeasts strains, one yeast strain with deleted EXG1 gene/YLR300delta and 18 Brettanomyces spp. yeasts. Only two yeast strains exhibited β-glucanase activity, a brewing S. cerevisiae strain and a Brettanomyces custersii strain isolated from a lambic beer fermentation, the latter with 10 times more β-glucosidase activity than the first one, and even higher activity in co-culture with S. cerevisiae (Daenen et al. 2008). In 2017, Sharp et al. conducted extensive research on β-glucosidase activity of 80 different yeast strains including commercial S. cerevisiae strains for brewing, wine, saké and baking, as well as Brettanomyces anomalae, Brettanomyces bruxellensis, Candida versatilis, Kluyveromyces marxianus, Scheffersomyces stipitans, Saccharomyces pastorianus and Debaryomyces nepalensis. The β-glucosidase activity did not depend on the species, though highest activity was observed for two Brettanomyces strains. No clear benefits were seen when hopping regime was modified (Sharp et al. 2017b).
Colomer, inspired by the studies of Daenen et al. (2008) and Sharp et al. (2017a, b), continued yeast screening for β-glucosidase activity, this time focusing only on Brettanomyces spp. strains. The results showed that Brettanomyces anomalus and Brettanomyces bruxellensis exhibited the highest activity. And, because it has been reported that two open reading frames, which encode two β-glucosidase enzymes, are present in Brettanomyces spp., the study also focused on strains with and without these ORFs. In this study, there appeared to be no direct correlation between the β-glucosidase activity and hop aroma in the beers treated with Brettanomyces for primary or secondary fermentation (Serra Colomer et al. 2020).

Gunata et al. performed trials with an immobilized β-glucosidase enzyme isolated from Candida molischiana 35M5N and successfully released after 7 h the flavour compounds linalool and geraniol from wine and fruit juices (Gunata et al. 1988). In 2016, Vervoort et al. screened 428 Brettanomyces yeasts for β-glucosidase activity and isolated enzymes from these strains and compared them to β-glucosidases from Aspergillus niger and Prunus dulcis (almond). B. anomalus presented the highest activity (Vervoort et al. 2016).

Some commercial enzymes are already available in the market; these include Aromazyme, commercialized by Lallemand and released in 2020. This has been tested in some commercial breweries and has shown apparently promising results—releasing hop aroma volatiles. Other enzymes, such as Rapidase or Sumzyme, have been used to release monoterpenes such as α-L-arabinofuranosidase and/or α-L-rhamnosidase to release these bound compounds (Lafontaine et al. 2021).

### Terpene alcohol conversion

The importance of citrus, fruity and floral aromas in beer has transcended the craft beer scene to the non-alcoholic beer (NAB) trend; Rettberg et al. reported earlier this year that enhanced hoppy aromas are preferred in NAB (Rettberg et al. 2022). The positive synergy between geraniol, linalool and β-citronellol in beers was also reported by Takoi and his colleagues (Takoi et al. 2010a). The β-citronellol content in beer cannot be increased merely by adding hops, and it appears that yeast bioconversion is critical in determining levels of this compound. Already in 1986, it was reported that yeasts can transform enzymatically certain terpenoids into other terpenoids, including the reduction of geraniol to β-citronellol and the hydrolysis of geranyl acetate and isobutyrate to geraniol (Fig. 1) (Lam et al. 1986). Since then, conversions of various mono- and sesquiterpenes have been demonstrated (King and Dickinson 2000; Takoi et al. 2010b; Praet et al. 2012). During the fermentation of dry-hopped beer, Takoi et al. observed a slight decrease in linalool and α-terpineol, followed by an increase in β-citronellol and nerol, and then an increase of geraniol (Takoi et al. 2010b). Already in 2000, King et al. suggested a bioconversion of geraniol into linalool and linalool into α-terpineol (King and Dickinson 2000). These compounds can be further metabolized (e.g. via esterification/hydrolysis) (Lam et al. 1986; King and Dickinson 2000; Chatterjee and Bhattacharyya 2001). To enhance the citrus, fruity, floral aroma in beers through increasing geraniol and β-citronellol content in the final beer, Reyes et al. from Sierra Nevada Brewing Co. performed dry-hopping trials with Cascade hops and added the hops in different stages of fermentation. Higher concentrations of geraniol, linalool, nerol and some ethyl esters were found in these beers, and suggested that the hop dosing time with the presence or absence of yeast influences the flavour profile in beer (Moutsoglou et al. 2018; Reyes 2019).

Looking into the transformation of geraniol into other monoterpenes, such as β-citronellol, Takoi noted that the yeast growth phase has an influence on the transformation of geraniol (Takoi et al. 2010a). Geraniol is converted mainly 2–4 days after initiation of fermentation, and it appears that the enzyme NADPH dehydrogenase 2 (encoded by OYE2) is responsible for the reduction of this monoterpenic alcohol to β-citronellol (King and Dickinson 2000; Yuan et al. 2011). Later, in 2013, it was reported that overexpression of OYE2 increased the reduction to 87% in comparison to 50% with a control strain (Steyer et al. 2013). Further confirmation came from Zhao and colleagues, who reported that the deletion of OYE2 or ATF1 genes led to an improved geraniol production by 1.7-fold or 1.6-fold in batch fermentation (Zhao et al. 2017). The activity of this enzyme has been observed in the cytoplasm, mitochondrion and nucleus of the yeast (Holt et al. 2018).

Serra Colomer et al. in 2020, while screening for β-glucosidase activity in Brettanomyces spp. species, found that the strains with lowest β-glucosidase activity showed the highest concentrations in β-citronellol (up to 31.5 µg/L). It was also suggested in that study that the reduction from geraniol to β-citronellol could be directly influenced by the oxidoreductase proteins BbHye2 and BbHye3 (Serra Colomer et al. 2020). Similar results to the ones from Reyes et al. in 2019, on hop timing and effect on biotransformation, were reported by Williams (2019), as a mid-fermentation dry hop addition increased the β-citronellol concentration in the final beers (Williams 2019).

Terpene contents in fermented beverages are not only influenced by reduction and isomerization reactions but also by changes in acetylation that are likewise mediated by fermentative yeasts (King and Dickinson 2003). As for the thiol ester 3MHA described above, the yeast creates acetylated...
forms of monoterpene alcohols via acetyltransferase activity, with the enzyme produced by the \textit{ATFI} playing a significant role (King and Dickinson 2000; Steyer et al. 2013; Zhao et al. 2017). Examples include geranyl- and citronellyl-acetates (King and Dickinson 2003). It is also believed that the ester forms, e.g. geranyl isobutyrate or geranyl acetate, may be hydrolysed back to the geraniol form via acetate esterase activity of the yeasts (Peacock et al. 1981). Levels of monoterpene alcohols in beer may therefore be the net result of these competing reactions. These changes may be significant as the form of the compound may influence the flavour threshold level or the flavour attribute. Geranyl acetate has been reported to have a lower flavour threshold than geraniol, and also a more lavender character (Pardo et al. 2015).

It can be noted here that monoterpene alcohols can exist in acetylated and non-acetylated forms in the raw material. Hops, for example, contain both geranyl acetate and geraniol, with hop varieties varying greatly in their relative contents of both (Forster et al. 2014). The work of Forster and co-workers (2014) showed how hop varieties, e.g. Cascade, Hallertau Blanc and Polaris, all containing high concentrations of geranyl acetate, typically produced beers with a low level of geranyl acetate but a high level of geraniol. This suggested that hydrolysis of the compounds occurred during fermentation, as suggested previously by Lam et al. (1986).

The potential impact of non-conventional yeast on terpene alcohol ester levels is not clear. At least one study, involving \textit{Williopsis saturnus}, showed the production of geranyl acetate from a hopped wort. This compound was not observed in the reference ale yeast (Liu and Quek 2016). Similarly, a higher concentration of geranyl acetate was observed in wine co-fermented with \textit{L. thermotolerans} (Korenika et al. 2020). It may be assumed that yeast choice has an influence on the relative concentration of terpene alcohols and esters; further studies are however required to prove this assumption.

Hop terpenoids undergo significant chemical and functional modification during the brewing process (oxidation, hydrolysis and isomerization). For example, the amount of hop terpenoids increases with increasing boiling time (Kishimoto et al. 2005), and a later addition is recommended in order to achieve a higher hop oil content in the beer, i.e. addition in the whirlpool or dry hop additions are more effective than traditional wort boil addition. Takoi et al. (2016) demonstrated the benefit of later dry hopping to boost the concentrations of geraniol. Later hopping appeared to avoid the consumption of geraniol, either derived directly the hop or via hydrolysis of an ester form, during active yeast growth (Takoi et al. 2016). It has been reported that oxygenated terpenoids, as they have a higher solubility, remain in higher concentration in the final beer (Sharp et al. 2017a). Apart from the above-mentioned biotransformation reactions, during fermentation, the terpene hydrocarbons from hops are lost due to their hydrophobic nature; either the yeast cell wall components adsorb them, or they migrate to the foam layer (Lam et al. 1986; King and Dickinson 2003; Praet et al. 2012).

**Alternative ways to increase terpene alcohols**

Monoterpene alcohol concentrations in beer can also be increased through de novo formation by yeast. Genetic engineering of brewer’s yeast for monoterpene alcohol production has been demonstrated, but these are not widely used yet because of legislation and lack of consumer acceptance. Carrau et al. (2005) demonstrated that low levels of geraniol and linalool are naturally synthesized de novo by strains of \textit{Hanseniaspora uvarum} and \textit{S. cerevisiae} through the isoprenoid pathway. By introducing a linalool synthase encoding gene from the plant \textit{Clarkia breweri}, increased linalool yields could be achieved (Herrero et al. 2008). Similarly, heterologous expression of a geraniol synthase from basil in \textit{S. cerevisiae}, together with mutagenesis of the native \textit{ERG20} gene, resulted in geraniol yields over 5 mg/L (Fischer et al. 2011). More recently, an industrial brewing yeast was engineered to produce both linalool and geraniol in similar ratios and amounts as those found in dry-hopped beers (Denby et al. 2018). Here, geraniol and linalool synthesis were accomplished by heterologous expression of a geraniol and linalool synthase from basil and mint, respectively. Monoterpene alcohol levels were controlled by modulating expression of the heterologous enzymes and two downstream enzymes in the isoprenoid pathway.

**Conclusions and future outlook**

The continued and growing interest in hop aromas in beers has focused attention on those factors in the process that can be modified to maximize aroma or steer hop aroma in particular directions. Fortunately, brewers have a number of tools at their disposal. Levels of hop oils and flavour compounds, as well as their composition in beer can be adjusted, not only by changing the amount, type or timing of hop addition, but also by promoting specific biotransformation reactions mediated by yeasts during the fermentation process. Biotransformation of hop compounds during brewing is a relatively new field of study; investigations in recent years have highlighted, not only the importance of these yeast-mediated reactions, but also our lack of fundamental knowledge regarding the genetic and biochemical processes responsible. Further investigations are expected to facilitate the effective exploitation of biotransformation reactions in brewing and other beverage fermentation processes.

In regard to β-lyase activity, challenges still remain in increasing the overall conversion rate of precursors to free thiols and better understanding the role of yeast enzymes.
The growing popularity of beers with hoppy aroma coincides with a growing interest in beers with low, or no, alcohol (Conway 2020). Methods used to produce these beers have the unfortunate side-effect of removing most of the volatile aroma compounds that are generated by yeast during fermentation. An increase in hop-derived aroma compounds from biotransformation reactions may serve to compensate for the loss of these fruity and floral aromas, thereby producing more palatable low-alcohol beers (Brendel et al. 2020; Lafontaine et al. 2020). Also, as highlighted in this review, Saccharomyces yeasts are not the only yeast species capable of increasing hop aroma compounds during fermentation. It is clear that much of the aromatic potential of hops could be tapped by exploiting the vast genetic diversity of available yeast species. In particular, it will be of value to test the biotransformation potential of those yeasts that are already being considered for low-alcohol beer brewing due to their maltose negativity (Gibson et al. 2017).

Furthermore, industrial trials considering the costs of these special process parameters should be performed. Continuous analysis of the impact of climate change on hop oil composition (low water and high-temperature stress), which will result in breeding new strains should be performed (Eriksen et al. 2021).

Yeasts have long been known to contribute greatly to beer quality, not only producing alcohol and CO₂, but also determining beer character through the production of various flavour-active metabolites and the removal of wort aldehydes, as well as stabilization of beers through the production of sulphite and removal of oxygen. To add to this, there is now a growing appreciation of the role that yeasts play in manipulating hop aroma. It is expected that as our understanding of this property improves, so will our ability to develop products with tailored hop aroma profiles in an efficient and sustainable way.
provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Anfang N, Brajkovich M, Goddard MR (2009) Co-fermentation with Pichia kluyveri increases varietal thiol concentrations in sauvignon blanc. Aust J Grape Wine Res 15:1–8. https://doi.org/10.1111/j.1755-0238.2008.00031.x

Belda I, Ruiz J, Navascués E, Marquina D, Santos A (2016) Improvement of aromatic thiol release through the selection of yeasts with increased β-lactase activity. Int J Food Microbiol 225:1–8. https://doi.org/10.1016/j.ijfoodmicro.2016.03.001

Biendl M, Schmidt C, Maye JP, Smith R (2021) New England IPA— the hop aroma champion of beers. MBAA Tech Q 58:38–42. https://doi.org/10.1094/TQ-58-1-0308-01

Bonnaffoux H, Roland A, Rémond E, Delpech S, Schneider R, Cavelier F (2017) First identification and quantification of S-3-(hexan-1-ol)-γ-glutamyl-cysteine in grape must as a potential thiol precursor, using UPLC-MS/MS analysis and stable isotope dilution assay. Food Chem 237:877–886. https://doi.org/10.1016/j.foodchem.2017.05.116

Bonnaffoux H, Delpech S, Rémond E, Schneider R, Roland A, Cavelier F (2018) Revisiting the evaluation strategy of varietal thiol biogenesis. Food Chem 286:126–133. https://doi.org/10.1016/j.foodchem.2018.06.061

Bonnaffoux H, Roland A, Schneider R, Cavelier F (2021) Spotlight on release mechanisms of volatile thiols in beverages. Food Chem 339:127628. https://doi.org/10.1016/j.foodchem.2020.127628

Bouwmeester HJ (2006) Engineering the essence of plants. Nat Biotechnol 24:1359–1361

Bowles D, Lim E (2010) Glycosyltransferases of small molecules: their roles in plant biology. In: eLS (ed). https://doi.org/10.1002/9780470015902.a0021537

Brendel S, Hofmann T, Granvogl M (2020) Dry-hopping to modify the aroma of alcohol-free beer on a molecular level-loss and transfer of odor-active compounds. J Agric Food Chem 68:8602–8612

Burns L (2021) Enhancing biotransformation with modern gene editing approaches. In: 2nd International workshop on brewing yeasts

Cantwell S, Dwyer C (2019) A new #1: 2019 BA hop usage survey. New Brew 36:87–93

Capone DL, Sefton MA, Jeffery DW (2011) Application of a modified method for 3-mercaptohexan-1-ol determination to investigate the relationship between free thiol and related conjugates in grape juice and wine. J Agric Food Chem 59:4649–4658. https://doi.org/10.1021/jf200116q

Capone DL, Sefton MA, Jeffery DW (2012) Analytical investigations of wine odorant 3-mercaptohexan-1-ol and its precursors. ACS Symp Ser: 15–35. https://doi.org/10.1021/bk-2012-1104.ch002

Capone DL, Ristic R, Pardon KH, Jeffery DW (2015) Simple quantitative determination of potent thiols at ultratrace levels in wine by derivatization and high-performance liquid chromatography—tandem mass spectrometry (HPLC-MS/MS) analysis. Anal Chem 87:1226–1231

Carrau FM, Medina K, Boito E, Farina L, Gaggero C, Dellacassa E, Versini G, Henschke PA (2005) De novo synthesis of monoterpenes by Saccharomyces cerevisiae wine yeasts. FEMS Microbiol Lett 243:107–115. https://doi.org/10.1111/j.1574-6968.2004.01105.x

Chatterjee T, Bhattacharyya DK (2001) Biotransformation of limonene by Pseudomonas putida. Appl Microbiol Biotechnol 55:541–546. https://doi.org/10.1007/s002530000538

Chenet C, Robiete R, Collin S (2019) First evidence of the cysteine and glutathione conjugates of 3-sulfanilpentan-1-ol in hop (Humulus lupulus L.). J Agric Food Chem 67:4002–4010. https://doi.org/10.1021/acs.jafc.9b00225

Chenet C, Thibault de Chanvalon E, Janssens P, Collin S (2021) Modulation of the sulfanylalkyl acetate/alcohol ratio and free thiol release from cysteinylated and/or glutathionylated sulfanylalkyl alcohols in beer under different fermentation conditions. J Agric Food Chem 69:6005–6012. https://doi.org/10.1021/acs.jafc.1c01610

Chenet C, Haest S, Robiete R, Collin S (2022a) Thiol S-conjugate profiles: a comparative investigation on dual hop and grape must with focus on sulfanylalkyl aldehydes and acetates adducts. J Am Soc Brew Chem 0:1–10.https://doi.org/10.1080/03610470.2021.195560

Chenet C, Willemart G, Gros J, Collin S, Chenet C, Willemart G, Gros J, Collin S (2022b) The science of beer ability of exogenous or wort endogenous enzymes to release free thiols from hop cysteinylated and glutathionylated S-conjugates ability of exogenous or wort endogenous enzymes to release free thiols from hop cysteinylated and glutathiol. J Am Soc Brew Chem 0:1–12.https://doi.org/10.1080/03610470.2021.217666

Concejero B, Peña-Gallego A, Fernandez-Zurbano P, Hernández-Orte P, Ferreira V (2014) Direct accurate analysis of cysteinylated and glutathionylated precursors of 4-mercapto-4-methyl-2-pentanone and 3-mercaptohexan-1-ol in must by ultrahigh performance liquid chromatography coupled to mass spectrometry. Anal Chim Acta 812:250–257. https://doi.org/10.1016/j.aca.2014.01.004

Conway J (2020) Market size of non-alcoholic beer worldwide from 2016 to 2024. In: Statista

Cordente AG, Capone DL, Curtin CD (2015) Unravelling glutathione conjugate catabolism in Saccharomyces cerevisiae: the role of glutathione/dipeptide transporters and vacuolar function in the release of volatile sulfur compounds 3-mercaptohexan-1-ol and 4-mercapto-4-methylpentan-2-one. Appl Microbiol Biotechnol 99:9709–9722. https://doi.org/10.1007/s00253-015-6833-5

Cordente AG, Borneman AR, Bartel C, Capone D, Solomon M, Roach M, Curtin CD (2019) Inactivating mutations in Irc7p are common in wine yeasts, attenuating carbon-sulfur β-lyase activity and volatile sulfur compound production. Appl Environ Microbiol 85. https://doi.org/10.1128/AEM.02684-18

Czerny M, Christlbauer M, Christlbauer M, Fischer A, Granvogl M, Hammer M, Hartl C, Hernandez NM, Schieberle P (2008) Reinvestigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. Eur Food Res Technol 228:265–273. https://doi.org/10.1007/s00217-008-0931-x

da Silva T, Albertin W, Dietmann C, Bely M, la Guerche S, Giraud C, Huet S, Sicard D, Masneuf-Pomarede I, de Vienne D, Marullo P (2015) Hybridization within Saccharomyces genus results in homoeostasis and phenotypic novelty in winemaking conditions. PLoS ONE. e0123834. https://doi.org/10.1371/journal.pone.0123834

Daenens L, Saisen D, Sterckx F, Delvaux FR, Verachtert H, Derdelinckx G (2008) Screening and evaluation of the glucoside hydrolase activity in Saccharomyces and Brettanomyces brewing yeasts. J Appl Microbiol 104:478–488. https://doi.org/10.1111/j.1365-2672.2007.03566.x

Darriet P, Tominaga T, Lavigne V, Boidron J-N, Dubourdieu D (1995) Identification of a powerful aromatic component of Vitis vinifera	
L. var. sauvignon wines: 4-mercapto-4-methylpentan-2-one. Flavour Fragr J 10:385–392. https://doi.org/10.1002/ffi.2730100610

Denby CM, Li RA, Vu VT, Costello Z, Lin W, Chan LIG, Williams J, Donaldson B, Bamforth CW, Petzold CJ, Scheller HV, Martin HG, Keasling JD (2018) Industrial brewing yeast engineered for the production of primary flavor determinants in hopped beer. Nat Commun 9:1–10. https://doi.org/10.1038/s41467-018-03293-x

Dennenlöh J, Thörn S, Retting N (2020) Analysis of hop-derived thiols in beer using on-fiber derivatization in combination with HS-SPME and GC-MS/MS. J Agric Food Chem 68:15036–15047. https://doi.org/10.1021/acs.jafc.0c06305

Dufour M, Zimmer A, Thibon C, Marullo P (2013) Enhancement of volatile thiol release of Saccharomyces cerevisiae strains using molecular breeding. Appl Microbiol Biotechnol 97:5893–5905. https://doi.org/10.1007/s00218-013-4739-7

Ehrentraut S, Weber JM, Dybowski JN, Hoffmann D, Ehrenhofer-Murko K, Tehrani P, Edler M, Hermsdorf D, Weiller R, Enge KH, Tressl R (1991) Identification of new sulfur-containing thiols in beer using on-fiber derivatization in combination with HS-SPME and GC-MS/MS. J Agric Food Chem 68:15036–15047. https://doi.org/10.1021/acs.jafc.0c06305

Gibson B, Geertman JMA, Hittinger CT, Krogerus K, Libkind D, Louis EJ, Magalhães F, Sampajo JP (2017) New yeasts-new brews: modern approaches to brewing yeast design and development. FEMS Yeast Res 17:1–13. https://doi.org/10.1093/femsye/fox038

Gil JV, Manzanera P, Genovesi S, Vallés S, González-Candelas L (2005) Over-production of the major exoglucanase of Saccharomyces cerevisiae leads to an increase in the aroma of wine. Int J Food Microbiol 103:57–68. https://doi.org/10.1016/j.ijfoodmicro.2004.11.026

Goldstein H, Rader S, Murakami AA (1993) Determination of 3-methyl-2-buten-1-thiol in beer. J Am Soc Brew Chem 51:70–74. https://doi.org/10.1094/asbcj-51-0070

Goldstein H, Ting PL, Navarro A, Ryder DS (1999) Water-soluble hop flavour precursors and their role in beer flavour. J Inst Brew 105:141

Gros J, Peeters F, Collin S (2012) Occurrence of odorant polyfunctional thiols in beers hopped with different cultivars. First evidence of an S-cysteine conjugate in hop (Humulus lupulus L.). J Agric Food Chem 60:7805–7816. https://doi.org/10.1021/jf301478m

Gunata Z, Bitteur S, Brillout J-M, Bayonove C, Cordonnier R (1988) Sequential enzymic hydrolysis of potentially aromatic glycosides from grape. Carbohydr Res 184:139–149. https://doi.org/10.1016/0008-6215(88)80012-0

Hauser DG, Lafontaine SR, Shellhammer TH (2019) Extraction efficiency of dry-hopping. J Am Soc Brew Chem 77:188–198. https://doi.org/10.1080/03610470.2019.1617622

Hauser DG, Shellhammer TH (2019) An overview of sustainability challenges in beer production, and the carbon footprint of hops production. MBAA Technical Q 56:2–6. https://doi.org/10.1094/tq-56-4-0731-01

Herrero O, Ramón D, Orejas M (2008) Engineering the Saccharomyces cerevisiae isoprenoid pathway for de novo production of aromatic monoterpenes in wine. Metab Eng 10:78–86. https://doi.org/10.1016/j.meb.2007.11.001

Hirokka K, Yamamoto Y, Tsutsui N, Tanaka T (2005) Improved production of isoamyol acetate by a sake yeast mutant resistant to an isoprenoid analog and its dependence on alcohol acetyltransferase activity, but not on isoamyol alcohol production. J Biosci Bioeng 99:125–129. https://doi.org/10.1263/jbb.99.125

Holt S, Cordente AG, Williams SJ, Capone DL, Jitjaroen W, Menz IR, Curtin C, Anderson PA (2011) Engineering Saccharomyces cerevisiae to release 3-mercaptohexan-1-ol during fermentation through overexpression of an S. cerevisiae gene, STR3, for improvement of wine aroma. Appl Environ Microbiol 77:3626–3632. https://doi.org/10.1128/AEM.00309-10

Holt S, Mukherjee V, Lievens B, Verstrepen KJ, Thevelein JM (2018) Bioflavoring by non-conventional yeasts in sequential beer fermentations. Food Microbiol 72:55–66. https://doi.org/10.1016/j.fmicb.2017.11.008

Holt S, Miks MH, De Carvalho BT, Fouquié- Moreno MR, Thevelein JM (2019) The molecular biology of fruity and floral aromas in beer and other alcoholic beverages. FEMS Microbiol Rev 43:193–222. https://doi.org/10.1093/femsre/fuy041

Howell KS, Klein M, Swiegers JH, Hayasaka Y, Elsey GM, Fleet GH, Høj PB, Pretorius IS, de Barros Lopes MA (2005) Genetic determinants of volatile-thiol release by Saccharomyces cerevisiae during wine fermentation. Appl Environ Microbiol 71:5420–5426. https://doi.org/10.1127/AEM.71.9.5420-5426.2005

Hizuka-Furukawa S, Isogai A, Kusaka K, Fujii T, Wakai Y (2017) Identification of 4-mercapto-4-methylpentan-2-one as the characteristic aroma of sake made from low-gluten rice. J Biosci Bioeng 123:209–215. https://doi.org/10.1016/j.jbiosc.2016.09.002

Inui T, Tsuchiya F, Ishimaru M, Ōka K, Komura H (2013) Different beers with different hops. Relevant compounds for their aroma characteristics. J Agric Food Chem 61:4758–4764. https://doi.org/10.1021/jf3053737

Kankolongo Cibaka M-L, Ferreira CS, Decourrièrre L, Lorenzo-Alonso C-J, Bodart E, Collin S (2017) Dry hopping with the dual-purpose varieties Amarillo, Citra, Hallertau Blanc, Mosaic, and Sorachi Ace: minor contribution of hop terpenol glucosides to beer flavors. J Am Soc Brew Chem 75:122–129. https://doi.org/10.1007/ASBCJ-2017-2257-01

Kiene F, Pretorius IS, Rauhut D, von Wallbrunn C, van Wyk N (2021) Construction and analysis of a yeast for the simultaneous release and esterification of the varietal thiol 3-sulfanylhexan-1-ol. J Agric Food Chem 69:11919–11925. https://doi.org/10.1021/acs.jafc.1c03976

Kind C, Kaiser T (2020) Heat, hops, Hallertau: exploring implications of climate change for the German beer sector. In: Hoalst-Pullen N, Patterson MW (eds) The geography of beer: culture and economics. Springer International Publishing, Cham, pp 103–111

King A, Dickinson JR (2000) Biotransformation of monoterpene alcohols by Saccharomyces cerevisiae, Torulaspora delbrueckii and...
Klosteromyces lactic. Yeast 16:499–506. https://doi.org/10.1002/ (SICI)1097-0061(200004)16:6%3c499::AID-YEA548%3e3.0.
CO2-E

King AJ, Dickinson JR (2003) Biotransformation of hop aroma terpenoids by ale and lager yeasts. FEMS Yeast Res 3:53–62. https://
doi.org/10.1016/S1567-3565(02)00014-1

Kishimoto T, Wanikawa A, Kagami N, Kawatsuru K (2005) Analysis of hop-derived terpenoids in beer and evaluation of their behavior using the stir bar-sorptive extraction method with GC-MS. J Agric Food Chem 53:4701–4707. https://doi.org/10.1021/jf05
0728

Kishimoto T, Wanikawa A, Kono K, Shibata K (2006) Comparison of
the odor-active compounds in unhopped beer and beers hopped with different hop varieties. J Agric Food Chem 54:8855–8861. https://
doi.org/10.1021/jf061342c

Kishimoto T, Kobayashi M, Yako N, Iida A, Wanikawa A (2008) Comparison
of 4-mercapto-4-methylpentan-2-one contents in hop cultivars from different growing regions. J Agric Food Chem 56:1051–1057. https://
doi.org/10.1021/jf070217e

Kishimoto T, Morimoto M, Kobayashi M, Yako N, Wanikawa A (2008)
Behaviors of 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate
during brewing processes. J Am Soc Brew Chem 66:192–196. https://
doi.org/10.1094/ASBCJ-2008-070201

Knight SJ, Klaere S, Morrison-Whittle P, Goddard MR (2018) Fungal
diversity during fermentation correlates with thiol concentration in wine. Aust J Grape Wine Res 24:105–112. https://doi.org/10.
1111/jgwr.12304

Kollmannsberger H, Biendl M, Nitz S (2006) Occurrence of glycosidically
bound flavour compounds in hops, hop products and beer. Monatschrift For Brauwiss 59:83–89

Korenika A-MJ, Tomaz I, Preiner D, Lavrić M, Šimić B, Jeromel A (2020) Influence of L. thermotolerans and S. cerevisiae commercial yeast sequential inoculation on aroma composition of red wines (Cv Trnjak, Babic, Blatina and Frankovka). Fermentation 7:4. https://doi.org/10.3390/fermentation7010004

Kroggerus K, Fletcher E, Rettker N, Gibson B, Preiss R (2021) Efficient
breeding of industrial brewing yeast strains using CRISPR/Cas9-aided mating-type switching. Appl Microbiol Biotechnol 105:8359–8376. https://doi.org/10.1007/s00253-021-11626-y

Kroggerus K, Rettker N, Gibson B (2022) Increased volatile thiol release during beer fermentation using constructed interspecies yeast hybrids. bioRxiv. https://doi.org/10.1101/2022.06.12.495761

Lafontaine S, Senn K, Knobe L, Schubert C, Dennenlöhr J, Maximier J, Cantu A, Rettker N, Heymann H (2020) Evaluating the chemical components and flavor characteristics responsible for triggering the perception of ‘beer flavor’ in non-alcoholic beer. Foods 9:1914

Lafontaine S, Caffrey A, Dailey J, Varnum S, Hare A, Eichler B, Dennenlöhr J, Schubert C, Knobe L, Lerno L, Dagan L, Schönberger C, Rettker N, Heymann H, Ebeler SE (2021) Evaluation of variety, maturity, and farm on the concentrations of monoterpenes diglycosides and hop volatile/nonvolatile composition in five humulus lupulus cultivars. J Agric Food Chem 69:4356–4370. https://doi.org/10.1021/acs.jafc.0c07146

Lafontaine SR, Shellhammer TH (2019a) How hoppy beer production has redefined hop quality and a discussion of agricultural and processing strategies to promote it. MBAA Tech Q 56:1–12. https://doi.org/10.1094/mbaq-56-1-0221-01

Lafontaine SR, Shellhammer TH (2019b) Investigating the factors impacting aroma, flavor, and stability in dry-hopped beers. MBAA Tech Q 56:13–23. https://doi.org/10.1094/mbaq-56-1-0225-01

Lam KC, Deinzer ML, Foster RT (1986) Aging of hops and their con-
tribution to beer flavor. J Agric Food Chem 34:763–770. https://
doi.org/10.1021/jf00070a043

Liu S-Q, Quek AYH (2016) Evaluation of beer fermentation with a novel yeast Willopiosis saturnus. Food Technol Biotechnol 54:403–412. https://doi.org/10.17113/fib.54.04.16.4440

Makhotkina O, Klimtina PA (2012) Hydrolysis and formation of volat-	ile esters in New Zealand Sauvignon blanc wine. Food Chem 135:486–493. https://doi.org/10.1016/j.foodchem.2012.05.034

Masneuf-Pomarède I, Murat M-L, Naumov GI, Tominaga T, Doubardieu D (2002) Hybrids Saccharomyces cerevisiae X Saccharomyces bayanus var. awarum having a high liberating ability of some sulfur varietal aromas of Vitis vinifera Sauvignon blanc wines. OENO One 36:205. https://doi.org/10.2087/oenone-one.
2002.36.4.965

Matsui H, Inui T, Oka K, Fukui N (2016) The influence of pruning and
harvesting time on hop aroma, cone appearance, and yield. Food Chem 202:15–22. https://doi.org/10.1016/j.foodchem.2016.
01.058

Meilgaard MC (1982) Prediction of flavor differences between beers from their chemical composition. J Agric Food Chem 30:1009–1017. https://doi.org/10.1021/jf00114a002

Michel M, Haslebeck K, Ampenberger F, Meier-Dörnberg T, Stretz D, Hutzler M, Coelhan M, Jacob F, Liu Y (2019) Screening of brewing yeast β-lyase activity and release of hop volatile thiols from precursors during fermentation. Brew Sci 72:179–186. https://doi.org/10.23763/BrSc19-26michiel

Morimoto M, Kishimoto T, Kobayashi M, Yako N, Iida A, Wanikawa A, Kitagawa Y (2010) Effects of bordeaux mixture (copper sulfate) treatment on blackcurrant/muscat-like odors in hops and beer. J Am Soc Brew Chem 68:30–33. https://doi.org/10.1094/ASBCJ-2009-1118-01

Moutsoglou M, Cayler W (2018) Impact of dry hopping at different stages of fermentation on the physical and organoleptic quality of beer. In: Proceedings of the MBAA/ASBC Brewing Summit, San Diego

Murat ML, Masneuf I, Darrier P, Lavigne V, Tominaga T, Dombourdieu D (2001) Effect of Saccharomyces cerevisiae yeast strains on the liberation of volatile thiols in Sauvignon blanc wine. Am J Enol Vitic 52:136–139

Nagegowda DA (2010) Plant volatile terpenoid metabolism: biosyn-
thetic genes, transcriptional regulation and subcellular compart-
entation. FEBS Lett 584:2965–2973. https://doi.org/10.1016/j.
fleb.2010.05.045

Nagel J, Culley LK, Lu Y, Liu E, Matthews PD, Stevens JF, Page JE (2008) EST analysis of hop glandular trichomes identifies an O-methyltransferase that catalyzes the biosynthesis of xanthohumol. Plant Cell 20:186–200. https://doi.org/10.1105/tpc.107.055178

Nizet S, Gros J, Peeters F, Chaumont S, Robriette R, Collin S (2013) First evidence of the production of odorant polyfunctional thiol-
s by bottle refermentation. J Am Soc Brew Chem 71:15–22. https://doi.org/10.1094/ASBCJ-2013-0117-01

Oliveira MM, Salomk M, Pais S (1988) Glandular trichomes of Humu-
lus lupulus var. Brewer’s Gold: ontology and histochemical char-
acterization of the secretion. Nord J Bot 8:349–359. https://
doi.org/10.1111/j.1756-1051.1988.tb00510.x

Olivero I, Hernández LM, Larriba G (1985) Regulation of beta-exogluc-
ananase activity produced by Saccharomyces cerevisiae in batch and continuous culture. Arch Microbiol 143:143–146

Pardo E, Rico J, Gil JV, Orejas M (2015) De novo production of six key grape aroma monoterpenes by a geraniol synthase-engineered S. cerevisiae wine strain. Microb Cell Fact 14:1–8. https://doi.org/10.
1186/s12934-015-0306-5

Pate P, Herbst-Johnstone M, Lee SA, Gardner RC, Weaver R, Nicolau L, Klimtina PA (2010) Influence of juice pressing conditions on polyphenols, antioxidants, and varietal aroma of sauvignon blanc microferments. J Agric Food Chem 58:7280–7288. https://doi.org/10.1021/jf100200e
Takai K, Itoya Y, Takayanagi J, Matsumoto I, Nakayama Y (2009) Specific flavor compounds derived from Nelson Sauvin hop and synergy of these compounds. Brew Sci 62:108–118

Takai K, Itoya Y, Takayanagi J, Matsumoto I, Nakayama Y. Watarai J (2010) The control of geraniol metabolism to the citrus flavour of beer: synergy of geraniol and β-citronellol under coexistence with excess linalool. J Inst Brew 116:251–260. https://doi.org/10.1002/jf.2050-0416.2010.tb00428.x

Takai K, Itoya Y, Takayanagi J, Matsumoto I, Nakayama Y (2016) Control of hop aroma impression of beer with blend-hopping. J Agric Food Chem 64:11194–11203. https://doi.org/10.1021/acs.jafc.6b01457

Thibon C, Marullo P, Claissé O, Cullin C, Dubourdieu D, Tominaga T (2008) Nitrogen catabolic repression controls the release of volatile thiols in fresh and aged lager beers. Dev Food Sci 43:245–248. https://doi.org/10.1007/s11677-008-0058-0

Verstappen KJ, Derdelinckx G, Dufour J, Winderickx J, Thevelein JM, Pretorius IS, Delvaux FR (2003) Flavor-active esters: adding fruitiness to beer. J Biosci Bioeng 96:110–118

Vervoort Y, Herrera-Malaver B, Mertens S, Guadalupe Medina V, Duitama J, Michiels L, Derdelinckx G, Voordeckers K, Verstappen KJ (2016) Characterization of the recombinant Brettanozymes anomalus β-glucosidase and its potential for bioflavouring. J Appl Microbiol 121:721–733. https://doi.org/10.1111/jam.13200

Wakabayashi H, Wakabayashi M, Eisenreich W, Engel K-H (2004) Stereochemical course of the generation of 3-mercaptohexanal and 3-mercaptohexanol by β-lyase-catalyzed cleavage of cysteine conjugates. J Agric Food Chem 52:110–116. https://doi.org/10.1021/jf0305478

Wang G, Tian L, Aziz N, Broun P, Dai X, He Ji, King A, Zhao PX, Dixon RA (2008) Terpene biosynthesis in glandular trichomes of hop. Plant Physiol 148:1254–1266. https://doi.org/10.1104/pp.110.125187

Watanabe N, Watanabe S, Nakajima R, Shimokihara K, Inagaki J, Etoh H, Sakata K, Ina K, Asai T (1993) Formation of flower fragrance compounds from their precursors by enzymic action during flower opening. Biosci Biotechnol Biochem 57:1101–1106. https://doi.org/10.1271/bbb.57.1101

Wilhelm WP (2013) Charakterisierung qualitativer und quantitativer Unterschiede in wertgebenden Geruchsstoffen verschiedener Hopfenspezies: DSc thesis. Technische Universität München, Munich

Williams PJ, Strauss CR, Wilson B, Massy-Westropp RA (1982) Novel monoterpane disaccharide glycosides of Vitis vinifera grapes and wines. Phytochemistry 21:2013–2020. https://doi.org/10.1016/0031-1872(82)80334-3

Williams S (2019) The impact on hop-derived volatile compounds in beer by dry-hopping at different points during fermentation using different strains of hops and yeast. In: 2019 ASBC Meeting

Wills RBH, Scriven FM (1979) Metabolism of geraniol by apples in relation to the development of storage breakdown. Phytochemistry 18:785–786. https://doi.org/10.1016/0031-1872(79)80014-X

Winter G, Van Der Westhuizen T, Higgins VJ, Curtin C, Ugiano M (2011) Contribution of cysteine and glutathione conjugates to the formation of the volatile thiols 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) during fermentation by Saccharomyces cerevisiae. Aust J Grape Wine Res 17:285–290. https://doi.org/10.1111/j.1755-0238.2011.00127.x

Winterhalter P, Skouroumounis GK (1997) Glycoconjugated aroma compounds: occurrence, role and biotechnological transformation. Adv Biochem Eng Biotechnol 55:73–105. https://doi.org/10.1007/bfb010263

Yuan TT, Chen QQ, Zhao PJ, Zeng Y, Liu XZ, Lu S (2011) Identification of enzymes responsible for the reduction of geraniol to citronellol. Nat Products Bioprospect 1:108–111. https://doi.org/10.1007/s13659-011-0032-6

Zhao J, Li C, Zhang Y, Shen Y, Hou J, Bao X (2017) Dynamic control of ERG20 expression combined with minimized endogenous downstream metabolism contributes to the improvement of geraniol production in Saccharomyces cerevisiae. Microb Cell Fact 16:1–11. https://doi.org/10.1186/s13431-017-0641-9

Zott K, Thibon C, Bely M, Lonvaud-Funel A, Dubourdieu D, Mas-neuf-Pomarede I (2011) The grape must non-Saccharomyces microbial community: impact on volatile thiol release. Int J Food Microbiol 151:210–215. https://doi.org/10.1016/j.ijfoodmicro.2011.08.026

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.