Effects on varietal aromas during wine making: a review of the impact of varietal aromas on the flavor of wine.

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ABSTRACT

Although there are many chemical compounds present in wines, only a few of these compounds contribute to the sensory perception of wine flavor. This review focuses on the knowledge regarding varietal aroma compounds, which are among the compounds that are the greatest contributors to the overall aroma. These aroma compounds are found in grapes in the form of nonodorant precursors that, due to the metabolic activity of yeasts during fermentation, are transformed to aromas that are of great relevance in the sensory perception of wines. Due to the multiple interactions of varietal aromas with other types of aromas and other nonodorant components of the complex wine matrix, knowledge regarding the varietal aroma composition alone cannot adequately explain the contribution of these compounds to the overall wine flavor. These interactions and the associated effects on aroma volatility are currently being investigated. This review also provides an overview of recent developments in analytical techniques for varietal aroma identification, including methods used to identify the precursor compounds of varietal aromas, which are the greatest contributors to the overall aroma after the aforementioned yeast-mediated odor release.

KEYWORDS

Wine, Aroma, Wine matrix, Thiols, Terpenes, Esters, Higher alcohols

INTRODUCTION

Wine aroma is one of the characteristics that can best reflect wine quality. The hedonic effects of wine are greatly influenced by the volatile compounds in wine, which can be considered one of the most complex products among many other foods and beverages. Experts in the wine industry (winemakers, sommeliers, critics, etc.) can detect many different nuances in a wine, which has led to interest in characterization of the complexity wine aroma. Furthermore, the complexity of wine aroma can also vary depending on many variables of different origin, such as the type of wine, grape variety, terroir, microbial starter, fermentation process, aging, and bottling. This complexity makes the study of the aroma compounds in wine very interesting in terms of all the factors that can be improved or corrected to refine wine quality.

The detection of new compounds and their sensory relevance is a task of increasing difficulty because of the sub-parts-per-billion (ppb) levels of many aroma compounds in wines. Many reviews have examined the complexity of wine aroma from an analytical perspective. Currently, it is known that the overall flavor compounds of wine, detected using different techniques, are not directly correlated with the perceived sensations during wine consumption. Recently, the effects of the non-aroma compounds of the wine matrix have been shown to be important determinant factors for the perception and release of wine aroma. It has been determined that specific nonvolatile components of the wine matrix interact with specific volatiles, influencing the sensory characteristics of wines (Dufour and Bayonove 1999a, b; Dufour and Sauvaitre 2000; Jones et al. 2008; Muñoz-González et al. 2014; Rodriguez-Bencomo et al. 2011a; Saenz-Navajas et al. 2010).

Therefore, most certifications that teach wine description in their tasting protocols use common descriptors for several sensory parameters, such as mouthfeel, color and aroma, including the Master of Wine Institute (MW),Wine and Spirit Education Trust (WSET3 and dipWSET) (Robinson et al. 2016), the Master Sommelier (MS) certification (Zraly 2016) and associated materials (MacNeil 2015), and the Society of Wine Educators (CSW and CWE) certification (Nickles 2017). These protocols use several categories, such as primary, secondary and tertiary aromas, to denote aroma descriptors. The primary aromas are those associated with grapes and alcoholic fermentation (AF). These certification protocols divide this group of primary aromas into several families, such as floral, green fruit, citrus fruit, stone fruit, tropical fruit, red fruit, black fruit, dry fruit, herbaceous, herbal, spices and others. These families are divided into specific fruit descriptors. By this methodology, it is possible to objectively describe any wine. Based on these descriptors, some authors characterize grape varieties as possessing common aroma characteristics that are often used to describe representative wines from those specific varieties (Puckette 2015). There also exist commercialized standards that represent these descriptors (Renoir 2006) and several studies that describe the main molecules that represent these aromatic descriptors (thegoodscentscompany 2018). Table 1 shows a correlation between the main descriptions of the most well-known international grape varieties and the chemical molecules associated with those descriptors, identified by the corresponding CAS numbers. Some of these molecules have been identified by compositional analysis of wines (Francis and Newton 2005), and others have been used in the food industry to mimic descriptors (thegoodscentscompany 2018). All of this information is useful for Sensorial Analysis Panel training, determination of wine quality and origin, and market evaluation.

Several hundred aroma compounds have been identified in wine and classified into different chemical families. The most important families of volatile compounds in wine are higher alcohols and esters, but wine contains many other types of compounds, such as carbonyls, acids, terpenes, norisoprenoids, sulfur compounds, and methoxypyrazines (MPs) (Henryk and Szczurek 2010; Tetik et al. 2018). Each family of aroma compounds, and the complex nonodorant matrix in which these compounds are dissolved, varies greatly among different types of wines, with different predominant aromas in each case, conferring a specific typicity to each wine (Belda et al. 2017; Henryk and Szczurek 2010; Tetik et al. 2018). These differences are not truly perceptible in must or at the initial stages of the fermentation. In general, wine aromas can be classified into varietal, fermentative and aging aromas. Most wine aroma compounds, including those present as precursors, are produced or released during wine fermentation due to microbial activity. AF, mainly achieved by *Saccharomyces cerevisiae*, leads to the formation of several higher alcohols and esters (Álvarez-Pérez et al. 2012; Belda et al. 2017a). Generally, the volatile compounds derived from fermentation are the most important contributors to the overall aroma of the wine (Bartowsky 2005; Belda et al. 2016).

In this review, we will focus on the families of aromas originating from flavorless precursors present in grapes and musts that, due to microbial action, are transformed into aromas. Although these aromas are considered less important than fermentative aromas, they play a fundamental role in the characteristics of many wines. These compounds are called varietal aroma compounds because they originate in the vine. We will now briefly introduce the three families of very powerful odorants that contribute to the varietal characteristics of wines: terpenes, MPs and pleasant-odor thiols.

Terpene glycosides were the first glycosidic compounds identified in grapes (William et al. 1981, 1982a), with monoterpene glycosides being the most significant aroma precursors in many grape varieties (Noble et al. 1987; Park et al. 1991; Rodríguez-Bencomo et al. 2011a, b). Monoterpenes, as aroma glycosides, can be found as free volatile compounds; however, these compounds are present at much higher concentrations as nonvolatile precursors linked to sugar moieties than as free compounds in grapes and musts (Baumes et al. 2009). Hydrolysis of the glycoside precursor leads to the release of the free volatile aroma compound. This review summarizes the results obtained from the characterization of monoterpenes in grapes and wines, including the hydrolytic mechanisms and new analytical methods for identification of glycosidic aromas.

Bell pepper, vegetal and earthy are terms that are sometimes used to describe wine aromas from the “Bordeaux cultivars” (e.g., Cabernet Franc, Cabernet Sauvignon, Sauvignon Blanc, Merlot and Carmenère). MPs are the main source of these herbaceous aromas. These compounds are powerful odorants with very low (1-2 ng/L) sensory thresholds. Isobutyl-MP (IBMP) is the most abundant (5-30 ng/L) MP in wines, whereas isopropyl-MP (IPMP) and sec-butyl-MP (SBMP) are also present but typically at low levels. While MPs are considered appropriate for some wine varieties, adding complexity, these compounds are generally regarded as negative traits in terms of wine quality, especially in red wines. Therefore, viticultural and enological treatments to remove MPs (cultivars and clones, grape maturity, vine vigor, light, soil, water status, thermovinification, micro-oxygenation, use of activated charcoal, extended aging) have been used but with limited success because some practices to reduce MP-derived greenness may alter wine quality.

In this review, current strategies and new hypotheses for reducing MP levels (i.e., the use of yeast strains) are described (Alves et al. 2015; Lei et al. 2018).

Volatile thiols represent a large family of compounds that, positively or negatively, can influence wine aroma. This review is focused on varietal thiols that can be found as odorless nonvolatile precursors in grapes and are released by yeasts during fermentation. Varietal thiols have been identified in a wide range of grape varieties. Varietal thiols have strong effects on the sensorial properties of wines because of the low sensory perception thresholds of these compounds, despite their very low concentrations. *S. cerevisiae* strains are ineffective in release of varietal thiols from the corresponding nonvolatile precursors (usually less than 5%). Therefore, and considering that the production and extraction of thiol precursors are influenced by viticultural and enological practices, efforts to enhance the thiol content in wines is of great scientific and technical relevance (Belda et al. 2017a; Darriet et al. 1995; Ruiz et al. 2018; Swiegers et al. 2007; Tominaga et al. 1998a, b).

The analysis of the aroma compounds present in wines requires advanced analytical techniques depending on the aroma family and the concentration of the aroma compound. The considerable development of instrumental devices and analytical procedures has allowed improvement of the techniques that were first designed for identification of the major aroma compounds, in turn allowing the detection of other families of volatile compounds that are present at very low concentrations in wines but can be detected with high sensitivity. Due to the complex composition of the wine matrix, analysis of the minor but key aroma compounds might require different preanalytical steps (solvent extraction, microextraction, solid-phase microextraction (SPME), solid-phase dynamic extraction (SPDE), etc.) in combination with the use of sophisticated mass spectrometers. Furthermore, wine aroma detection can also be influenced by the presence of additional factors, such as the wine matrix, which could affect the volatility of aroma compounds, decreasing or increasing the release of these compounds from the aqueous phase to the headspace above the wine. In conclusion, given their importance, this review outlines the most recent advances in wine varietal aroma analysis.

In this review, we have also discussed the effect that the whole wine matrix could have on the sensory characteristics of wine.

TERPENES

Monoterpenoids (C_{10} compounds) are of great importance for wine aroma, as are sesquiterpenoids and C_{13}-norisoprenoids. All three groups of compounds belong to isoprenoids, which are the largest class of natural products with very high stereochemical and structural diversity. According to different estimations, there exist 25000 to 55000 isoprenoids (Christianson 2007, 2008; Gershenzon and Dudareva 2007; Humphrey and Beale 2006; Waterhouse et al. 2016a) that have been identified in all life forms. In addition to protecting many animals, plants and microorganisms against predators, pathogens and competitors, terpenes are also involved in providing signals regarding the presence of environmental dangers and food to conspecifics and mutualists (Gershenzon and Dudareva 2007).

The vast diversity of isoprenoid structures can be attributed to the very high variability of rearrangements and cyclizations of highly reactive carbocation intermediates and isoprenoid substrates, the ionization of which is triggered by terpenoid synthases. Terpenoid synthases also have the ability to catalyze the formation of one or several products, and because the family of terpenoid synthase genes in plant genomes contains 40-152 members (Chen et al. 2011), terpenoid synthases are the main contributors to the high diversity of terpenoid structures. (Christianson 2008; Gao et al. 2012; Kutchan et al. 2015). In many cases, the products generated by terpene synthases are further modified by reduction, oxidation, isomerization, acylation and glycosylation reactions (Kutchan et al. 2015).

The biosynthesis of mono- and sesquiterpenes is based on the formation of the isoprene C5 units dimethyl allyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP) and is described in several reviews (Humphrey and Beale 2006; Schwab and Wüst 2015; Wedler et al. 2015).

Approximately 800 different aroma compounds are present in wine (Rapp 1990), approximately 50 of which are monoterpenoids (Guth 1997a; Marais 1983; Rapp and Mandery 1986). In addition to influencing the aroma of several wines, monoterpenoids could also be used for identification of grape varieties. Rapp and Hastrich (1976) showed that grape varieties can be identified based on the typical varietal flavor compositions. The authors also discovered that the varietal flavor of Riesling grapes is independent of the location of the vines and that characterization of the terpene profile can be used for identification of grape variety (Rapp and Hastrich 1978; Rapp and Mandery 1986).

The highest monoterpenoid concentrations are detected specifically in Muscat varieties, such as Muscat of Alexandria, Muscat de Frontignan, Muscat Ottonel and Muscat Blanc, and these compounds are responsible for the typical aroma of these wines. In addition, monoterpenoids also contribute to the aroma of non-Muscat varieties such as Gewürztraminer, Müller-Thurgau, Riesling, Scheurebe, Sylvaner and Traminer. Monoterpenoids are also present in Cabernet Sauvignon, Carignan, Chardonnay, Merlot, Sauvignon Blanc and Shiraz, but the concentrations of monoterpenoids in these varieties are below the corresponding olfactory perception thresholds, and therefore, monoterpenoids have no significant influence on the overall aroma of these wines. (Marais 1983; Mateo and Jiménez 2000).

The most important monoterpenoids in wines are linalool, (*E*)-hotrienol, citronellol, geraniol, nerol, (−)-*cis*-rose oxide and α-terpineol. The chemical structures, odor impressions, concentration ranges and perception thresholds of these compounds are listed in Table 2. Citronellol, geraniol, linalool, nerol and α-terpineol are the most important odor-active monoterpenoids and contribute to the varietal aroma profiles of wines due to their floral, fruity and citrus aromas (Strauss et al. 1986).

In 1974, Cordonnier and Bayonove suggested that grapes contain not only free and volatile monoterpenoids but also nonvolatile glycosidically bound monoterpenoid precursors. Further studies showed that Muscat grapes consist of approximately 90% glycosidically bound monoterpenoids and only 10% free volatile monoterpenoids (Park et al. 1991). The chemical structures of the precursors have been intensively studied (Gunata et al. 1985a, b; Williams et al. 1995). The aglycones are mainly bound to disaccharides that connect β-D-glucopyranose with a second sugar molecule, such as α-L-arabinofuranose, α-L-rhamnopyranose or β-D-apiofuranose (Winterhalter and Skouroumounis 1997). The conversion of these compounds to free monoterpenoids can be carried out via acidic or enzymatic hydrolysis by enzymes (especially β-glucosidases) from grapes and/or microorganisms (non-*Saccharomyces* yeasts, *Saccharomyces* yeasts and lactic acid bacteria(LAB)) during the alcoholic and malolactic fermentation processes (Figure 1). The rates of acid hydrolysis in must have been observed as being too low for most of the released monoterpenoids (Ugliano et al. 2006; Williams et al. 1982).

Indeed, it could be shown that the concentrations of glycosides decreased between 22% and 28% during fermentation, while the decrease in nonfermented samples was only approximately 5% over the same duration (Ugliano et al. 2006).

The contribution of yeasts, in particular *S. cerevisiae,* to monoterpenoid release during fermentation due to enzymatic activities has been controversially discussed for many years. Initial investigations have demonstrated that *S. cerevisiae* exhibits β-glucosidase activity (Darriet et al. 1988), but this activity is lower than that in non-*Saccharomyces* yeasts (Rosi et al. 1994). In addition, it has been shown that grape β-glucosidase enzymes exhibit optimal activity at pH 5 and are strongly inhibited by glucose and ethanol (Aryan et al. 1987; Günata et al. 1990). Therefore, grape β-glucosidase is regarded as having a low contribution to the release of monoterpenoids from aglycones.

In particular, numerous extracellular hydrolytic enzymes, such as β-glucosidase, α-arabinosidase, α-rhamnosidase, α-xylosidase and α-apiosidase, have been detected in both *S. cerevisiae* and non-*Saccharomyces* species (Charoenchai et al. 1997; Darriet et al. 1988; Ugliano et al. 2006). In addition, it has been proposed that the hydrolysis of monoterpenoids could also be conducted by exo-β-glucanase enzymes of yeasts (Gil et al. 2005). Baffi et al. (2011) studied an extracellular β-glucosidase (Sp-β-gl) of *Sporidiobolus pararoseus* and, additionally, proposed an application for the development of aroma in wines using a preparation of *Aureobasidium pullulans* β-glucosidase enzymes (Baffi et al. 2013).

Several researchers have studied the importance of β-glucosidases in the release of monoterpenes from the corresponding glycoside precursors and have shown that non-*Saccharomyces* yeasts can contribute to the aroma of wines. For example, Cordero Otero et al. (2003) studied the β-glucosidase activity of 20 non-*Saccharomyces* yeasts in Chardonnay must fermentation and discovered that *Debaryomyces pseudopolymorphus* exhibits high β-glucosidase activity at the pH of wine and exhibits high resistance against ethanol, glucose and sulfur dioxide. Mixed fermentation with *D. pseudopolymorphus* and *S. cerevisiae* resulted in significantly enhanced release of citronellol and geraniol (Cordero-Otero et al. 2003).

Fermentation with the β-glucosidase-producing *Metschnikowia pulcherrima* in Muscat d'Alexandrie led to an increase in α-terpineol and nerol levels. However, wines produced by mixed fermentation with simultaneous or sequential inoculation with *S. cerevisiae* showed considerably lower concentrations of α-terpineol, nerol and geraniol than a monoculture with *C. pulcherrima* (Rodríguez et al. 2010). α-Terpineol was also released at high concentrations during fermentation of Gewürztraminer grapes with a mixture of *Torulaspora delbrueckii* and *S. cerevisiae*, although a control fermentation with only *S. cerevisiae* showed high concentrations of geraniol and nerol. (Čuš and Jenko 2013). Further mixed fermentation studies with *Debaryomyces vanriji* and *S. cerevisiae* in Muscat of Frontignan strongly indicated enhancement in the release of geraniol due to hydrolysis of the corresponding precursors (García-Carpintero et al. 2011).

According to Gonzalez-Pombo et al. (2011), *Issatchenkia terricola* can release monoterpenoids via β-glucosidase activity, and Arevalo-Villena et al. (2007) showed increased monoterpenoid levels by using an enzyme extract of *Debaryomyces pseudopolymorphus* in Airen, Riesling and Muscat wines. Further details on the contributions of different enzymes from grapes and various microorganisms were provided by Ugliano (2009) and Jolly et al. (2014).

Hanseniaspora yeasts isolated from grape must showed high β-D-glucosidase activity. *Hanseniaspora uvarum* strains showed the capability to produce β-glucosidase enzymes without glucose and low-pH repression (López et al. 2002). *M. pulcherrima*, *Meyerozyma guillermondii* and *Wickerhamomyces anomalus* also showed high β-D-glucosidase activity (Belda et al. 2016; Mendes-Ferreira et al. 2011). Screening of 370 strains of 20 species of yeasts (Rosi et al. 1994) showed that all of the strains of the species *Debaryomyces castelli, Debaryomyces hansenii, Debaryomyces polymorphus, Kloeckera apiculata* and *Hanseniaspora anomala* exhibited β-D-glucosidase activity.

In addition to the β-glucosidase activity of non-*Saccharomyces* yeasts, LAB, especially *Oenococcus oeni*, also exhibit glycosidase activity (Boido et al. 2002; Grimaldi et al. 2005a, b; Lerm et al. 2010; Spano et al. 2005). Sensory studies showed that the enzymes glucosidase and arabinosidase from *O. oeni* can contribute to the typical aroma of Riesling wines via the release of monoterpenoids from grape-derived aroma precursors (Michlmayr et al. 2012).

In contrast, the application of pectinases that also exhibit β-glucosidase activity contributes to only low-level release of monoterpenoids due to the inability of the enzymes to completely cleave the disaccharides of the precursors, whereas the so-called aroma enzymes exhibit specific β-glucosidase activity that enhances the floral aromas (Fischer 2007).

Carrau et al. (2005) demonstrated that some *S. cerevisiae* yeasts can perform *de novo* synthesis of monoterpenoids under specific conditions and circumstances.

Another approach for increasing the terpene concentration in wine is the engineering of *S. cerevisiae* wine strains that express enzymes for the hydrolysis of glycosylated terpenes. Zietsman et al. (2011) developed one such *S. cerevisiae* strain.

The coexpression of an α-L-arabinofuranosidase from *Aspergillus awamori* and a β-D-glucosidase from *Saccharomycopsis fibuligera* in *S. cerevisiae* led to the production of certain terpenes at high concentrations and to increased floral and fruity aromas in wine (Zietsman et al. 2011). An additional strategy is the development of *S. cerevisiae* strains that can express monoterpene synthase enzymes, which catalyze the conversion of the universal precursor geranyl diphosphate to monoterpenes, encoded by genes from plants such as *V. vinifera* (Cordente et al. 2012).

VARIETAL THIOLS

Sulfur-containing compounds released by yeasts during fermentation are of great importance for the organoleptic quality of wine because of the abundance (approximately 10% of the volatile components detected in foods and beverages) and very low detection thresholds (Mestres et al. 2000) of these compounds. Volatile sulfur compounds are usually divided into two categories: highly volatile compounds, most of which are associated with aroma defects (carbon sulfide, ethanethiol, methanethiol, hydrogen sulfide), and low-volatility compounds, including the main desirable sulfur compounds that contribute to the enhancement of the sensorial quality of wines (Rauhut 2017; Tominaga et al. 1995). This group includes compounds with high molecular weights and low volatility, which are found at very low concentrations but at above the threshold value in wine. These compounds include “fruity volatile thiols”, mainly 4-methyl-4-sulfanylpentan-2-one (4MSP), 3-sulfanylhexan-1-ol (3SH) and its acetylated derivative 3-sulfanylhexyl acetate (3SHA) (Table 3). These compounds are among the most important sulfur compounds associated with the aroma of white wines (Darriet et al. 1995) and have been detected in many white wine varieties, such as Sauvignon Blanc, Macabeo, Gewürztraminer, Riesling, Verdejo, Merlot, and Cabernet Sauvignon, in which

3SH and 3SHA are more ubiquitous than 4MSP (Roland et al. 2011; Rauhut et al. 2017). These compounds also contribute a tropical characteristic to the wines, generally imparting box tree and blackcurrant bud aromas, in the case of 4MSP, and passion fruit, grapefruit, citrus zest, gooseberry and guava aromas, in the case of 3SH and 3SHA (Rauhut 2017; Roland et al. 2011). Other varietal thiols, which are also contributors of the characteristic flavor of these varieties, include 4-mercapto-4-methyl-pentan-2-ol, 3-mercaptopentan-1-ol, and 3-mercaptoheptan-1-ol (Tominaga et al. 1995; Sarrazin et al. 2007). Varietal thiols strongly influence wine quality despite their low concentrations (less than 400 ng/L in the case of 4MSP) because of their very low perception thresholds (Darriet et al. 1995; Roland et al. 2011).

Thiol aromas are not expressed in grape must but develop during the fermentative process (Dubourdieu et al. 2004). Thiol precursors are produced in vine plants as a detoxification mechanism via conjugation of unsaturated alkenals (forming 3SH precursors) and alkenones (forming 4MSP precursors) with glutathione (GSH). Then, the tripeptide GSH is hydrolyzed to the dipeptide Cys-Gly and to Cys. Therefore, GSH, Cys-Gly, and Cys must exist in grape as precursors of 3MH and 4MSP. The acetylated form of 3SH (3SHA) is formed by acetylation of 3SH after this compound is produced during fermentation (Waterhouse et al. 2016b) (Figure 2).

Yeasts can take up these thiol precursors from grape juice and then cleave the conjugated precursor, releasing the corresponding free thiols (Howell et al. 2004), using ammonium as a nitrogen source and pyruvate. Genes involved in the release of thiols from the corresponding precursors have been identified in *S. cerevisiae* (Santiago and Gardner 2015) and to a certain extent in related species such as *Torulaspora delbrueckii* (Belda et al. 2017a). Cysteinylated and glutathionylated precursors are taken up by general amino acid transporters, mainly *GAP1* and *OPT1*, respectively (Cordente et al. 2015; Subileau et al. 2008). In the cytoplasm, carbon-sulfur β-lyase enzymes cleave the cysteinylated precursors. *BNA3*, *CYS3, GLO1* and, mainly, *IRC7* have been identified as the genes encoding the enzymes responsible for 4MSP production from Cys-4MSP (Howel et al. 2004; Roncoroni et al. 2011). *STR3* has been described as the gene responsible for 3SH release but with low specificity (Holt et al. 2012). Glutathionylated thiol precursors, once in the cell, are transformed to cysteinylated precursors via a complex pathway that occurs in the vacuole and in which multiple genes are involved (Belda et al. 2017b). With regard to the acetylated thiol 3SHA, Swiegers et al. (2005) demonstrated that the gene encoding alcohol acetyltransferase, *ATF1*, is responsible for 3SHA formation from 3SH. Figure 3 shows the genes and metabolic pathways involved in thiol production in *S. cerevisiae*.

Nitrogen metabolism affects the regulation of thiol release pathways in yeasts (Harsch and Gardner 2013). Nitrogen catabolic repression (NCR) is one of the most important factors affecting thiol production in yeast (Dufour et al. 2013). Via this mechanism, preferred nitrogen sources (such as ammonia, normally supplemented as diammonium sulfate in winemaking to avoid stuck fermentation) inhibit the transcription of genes responsible for the use of poor nitrogen sources (Magasanik and Kaiser 2002). Amino-acid-conjugated thiol precursors represent a nonpreferred nitrogen source. Therefore, both genes involved in precursor transport and genes involved in precursor cleavage are controlled by NCR. Subileau et al. (2008a) and Thibon et al. (2008a) demonstrated the NCR effect on thiol production in synthetic grape must fermentation. Ure2p has been defined as the major regulator of NCR in yeast. This protein regulates GATA factors, namely, Gat1p and Gzf3p (active during NCR conditions) and Gln3p and Dal80p (active during nonrepressed conditions). Deed et al. 2011 showed that a dal80/gzf3 double-deletion mutant yeast upregulated NCR-related genes during wine fermentation.

The final thiol concentrations in wine depend on multiple factors. One of the most important factors is the concentration of thiol precursors in grapes. Thiol precursors are found in the skin and pulp at μg/L levels, and the concentration of these compounds depends on several factors (harvesting mode, SO_2 treatment, *Botrytis* infection (Waterhouse et al. 2016), ripeness (Cerreti et al. 2015), vine nitrogen conditions (Helwi et al. 2016), water deficit (Choné et al. 2000), grape variety, temperature (Roland et al. 2011), addition of grape skin tannins (Román et al. 2017), etc.).

After AF, oxygen affects the chemical stability of thiols; therefore, the storage and aging conditions are determinants of the thiol concentration in wines. Nevertheless, a lack of oxygen can reduce odor generation (Roland et al. 2011). Therefore, it is essential to develop appropriate storage and aging procedures to control the oxidation and to protect thiol aromas.

In addition to these technical factors, the yeast strain used to perform fermentation is one of the most important factors affecting thiol production (Cordente et al. 2012; Dubourdieu et al. 2004). *S. cerevisiae* is the main yeast involved in the fermentative process; therefore, multiple strategies have been used to improve thiol release via strain selection and genetic modification.

Belda et al. (2016) developed a medium based on yeast β-lyase activity using a thiol precursor-like substrate as the only nitrogen source to select strains with high potential for thiol production. The authors also demonstrated that most of the *S. cerevisiae* strains harbored a deletion in the *IRC7* gene, therefore encoding an enzyme with reduced activity (Roncoroni et al. 2011). Thus, selection of *S. cerevisiae* strains that harbor the complete allele of this gene can improve thiol production during fermentation.

On the other hand, as stated above, it has been reported that NCR strongly affects thiol production, and this process is strongly dependent on the yeast strain. Genetic modification of yeast strains by alleviation of NCR can increase thiol concentrations in wines. Dufour et al. (2012) demonstrated that the use of natural *URE2* mutant strains produced by molecular breeding can enhance the production of volatile thiols, both 4MSP and 3SH, in wine. Subileu et al. (2008b) showed the effect of the preferred nitrogen source (diammonium phosphate) on 3SH thiol production in synthetic grape must fermentation. The NCR relief mutants showed an increase in 3SH production with increasing Cys-3SH consumption. In addition, the effect of NCR on precursor cleavage activity was also demonstrated (Thibon et al. 2008a). Thiol production is controlled by NCR via the regulation of *IRC7* by Ure2p and Gln3p.

Other strategies to enhance thiol production have been studied. An industrial yeast strain that was transformed with the cysteine β-lyase enzyme gene from *Escherichia coli*, namely, *tnaA*, showed a ten-fold increase in 4MSP production (Swiegers et al. 2000). Holt et al. (2012) carried out overexpression of the *STR3* gene in a commercial strain, increasing the production of 3SH by 25%. 3SHA thiol production was also enhanced by overexpression of *ATF1* in wine-associated yeast (Lilly et al. 2006).

Several studies have reported the limited capacity of most *S. cerevisiae* strains in thiol release, showing that less than 5-10% of the nonodorant precursors are transformed to free thiols under fermentation conditions (Murat et al. 2001; Swiergers and Pretorius 2007). Among *Saccharomyces* species, it was shown that a *S. bayanus/S. cerevisiae* hybrid strain could produce increased thiol content during wine fermentation (Murat 2001).

The use of nonconventional yeasts in winemaking has emerged as an important tool for improvement of the thiol profile. β-Lyase activity, as the main activity associated with thiol production, is a common characteristic among non-*Saccharomyces* species; however, most of these species exhibit moderate activity. Nevertheless, certain species, such as *T. delbrueckii*, *Kluyveromyces marxianus* and *M. pulcherrima*, show marked β-lyase activity and thiol production, but with high strain dependency (Belda et al. 2016; Zott et al. 2011). Additionally, thiol production has also been investigated in mixed fermentation with non-*Saccharomyces* yeasts and *S. cerevisiae*. Anfang et al. (2009) demonstrated an increase in 3SHA concentrations by fermentation using *Pichia kluyveri* with *S. cerevisiae* in Sauvignon Blanc wines. Mixed fermentation with *S. cerevisiae* and *Candida zemplinina* led to an increase in 3SH levels compared to the levels observed for single-species fermentation with *S. cerevisiae* (Englezos et al. 2018; Padilla et al. 2016). The ability of *T. delbrueckii* to enhance the thiol profile in winemaking has been well studied. Renault et al. (2016) demonstrated the effect of an industrial *T. delbrueckii* strain on 3SH production but not on 3SH and 4MSP production. In contrast, Belda et al. (2017a) showed a marked increase in 4MSP production in sequential fermentation with *T. delbrueckii* and *S. cerevisiae* compared to single-species fermentation with *S. cerevisiae*.

Thiol perception is associated with not only the thiol concentration in wine but also the chemical composition of the wine matrix (Frost et al. 2015). Therefore, decreased levels of the major aroma compounds, such as esters or higher alcohols, could diminish the masking effects of these compounds on the minor compounds, such as thiols. It was reported that *M. pulcherrima*, in combination with *S. cerevisiae*, can not only increase the 4MSP concentration but also reduce higher-alcohol production, increasing the fruitiness of wines (Ruiz et al. 2018).

Alternative pathways to volatile thiol formation during wine fermentation have been proposed. With regard to 3SH and 3SHA, the concentrations of the conjugated precursors of these compounds in must is not correlated with the final thiol concentrations (Pinu et al. 2012). Furthermore, high residual levels of the precursors have been found at the end of fermentation (Capone et al. 2011). These low conversion yields do not explain the observed final thiol concentrations in wine in most of the reported cases (Roland et al. 2010a; Winter et al. 2011). Similar results may be observed with 4MSP production in wine. The total conversion of the conjugated precursors of 4MSP does not explain the 4MSP concentration obtained in Verdejo must fermentation (Belda et al. 2017).

All the data indicate the existence of an alternative pathway for volatile thiol biogenesis during wine fermentation, in addition to the established pathway of amino-acid-conjugated precursor catalysis by the yeast beta-lyase Irc7p (Figure 4). According to the literature, certain unsaturated carbonyl compounds may act as alternative thiol precursors in musts. For example, due to its chemical similarity to 3-MH, *E*-2-hexenal has been suggested to be an alternative precursor of 3-MH, whereas mesityl oxide could be a precursor of 4MSP. Schneider et al. (2006) proposed that thiols could be produced by combination of H_2S, a byproduct of yeast metabolism during fermentation, and *E*-2-hexenal or mesityl oxide, which seem to be present in grape must at ppb concentrations. Similarly, Duhamel et al. (2015) described a reaction in which the corresponding sulfonic acid (1-hydroxyhexane-3-sulfonic acid or 2-methyl-4-oxopentane-2-sulfonic acid), is formed by the reaction of *E*-2-hexenal or mesityl oxide, respectively, with bisulfite (added during the winemaking process) is formed, and these sulfonic acids might be reduced to form the corresponding thiol, namely, 3SH and 4MSP, respectively.

THE WINE MATRIX IN VARIETAL AROMA PERCEPTION

A basic chemical-aromatic matrix is shared by a vast majority of wines, giving these wines the typical flavor of alcoholic beverages, commonly defined as vinous. This matrix, mainly composed of ethanol and other fermentation-derived compounds, establishes a buffer in which changes in the concentrations of single molecules have little to no effect on the general aroma profile of a wine. Ferreira et al. (2007) defined groups for classification of wine aroma compounds based on the roles of these compounds in the wine matrix. A large diversity of compounds are typically found at concentrations above their perception thresholds (higher alcohols, esters, fatty acids, etc.) but, as integrated components of the wine matrix buffer, the individual aroma descriptors cannot be perceived or differentiated on the basis of wine aroma. Despite not having direct individual contributions to the definition of the aroma of a particular wine, these compounds are critical for enhancing or depressing the perception of other aroma-impacting compounds. On the other hand, certain compounds or families of compounds (structurally similar compounds that contribute to the same aroma nuance) can significantly transmit the corresponding aroma descriptors to the wine. The presence and concentrations of these compounds/families define the specific aromatic signature of a wine, which is responsible for the primary aromatic nuance. A great example of this impact is the varietal aroma compounds (certain terpenes (i.e., linalool) and polyfunctional thiols (i.e., 4MSP, 3SH)), and the relationships of these aroma compounds with key compounds of the wine matrix are analyzed below.

ENDOGENOUS/PRE-FERMENTATIVE COMPOUNDS

Among the major volatile compounds found in wines that are directly derived from grapes, some C6-alcohols such as 1-hexanol and cis-3-hexenol can be found at concentrations above the corresponding sensory thresholds (Waterhouse et al. 2016). Although these compounds can directly impart leafy, cut-grass aromas, they also contribute to the effects of other herbaceous compounds, such as MPs, in the perception of marked, usually undesired, green pepper aromas in wines (Escudero et al. 2007). However, the roles of these compounds in the final perception of wine aroma will depend on the concentrations of these compounds; depending on the grape variety and other climatic and viticultural factors, the 1-hexanol concentration can range from 1320 to 13800 µg/L (with a sensory threshold of 8000 µg/L), and the cis-3-hexenol concentration can range from 8 to 711 µg/L (with a sensory threshold of 400 µg/L) (Benkwitz et al. 2012; Ferreira et al. 2000; Guth 1997b). According to the classification of compounds described by Ferreira et al. (2007), this trend is typical of subtle or minor aroma compounds (when a combination of several groups of molecules that share a certain aromatic descriptor is necessary to disrupt the aroma buffer, affecting the overall aroma profile). However, among the prefermentative compounds that substantially interfere with the consumer's perception of the main fraction of compounds with varietal effects (terpenes and polyfunctional thiols), we should highlight the MP family. With a clearly recognizable earthy to vegetal odor, these compounds show an extremely low perception threshold of approximately 1 ng/L. The presence of these compounds at low concentrations can contribute to the complexity and typicity of some wines; however, at high concentrations, these compounds have dual undesirable effects: i) a direct effect, imparting undesirable green aromas, and ii) an indirect effect, as a depreciator of clear, fruity notes in both white and red wines.

FERMENTATIVE AROMAS

Higher Alcohols

Higher alcohols are considered to be a family of aroma compounds composed of volatile molecules with more than two carbon atoms; thus, these compounds have a higher molecular weights than ethanol; these compounds can also be called higher oils. Higher alcohols are generally considered to be the aromatic molecules with the strongest effects on the global wine aroma. The final concentrations of higher alcohols in wine depends mainly on yeast metabolism, in addition to other factors, such as wine type and chemical composition.

Many types of higher alcohols possess pleasant aromas, such as active amyl alcohol or isoamyl alcohol, with a marzipan aroma. Tyrosol and phenethyl alcohol can also be described as having honey and rose aromas, respectively (Lambrechts and Pretorius 2000). In addition, other higher alcohols may also contribute to the vinous character, masking, in some instances, the fruity aromas of wine. For example, propanol is described as having a stupefying odor, while butanol or isobutyl alcohol are described as having a higher-alcohol odor or alcoholic character (Lambrechts and Pretorious 2000). At total concentrations less than 300 mg/L, these compounds mostly contribute to increasing the general complexity of wine aroma (Rapp and Mandery 1986). In addition, concentrations of total higher alcohols more than 400 mg/L are thought to cause unpleasant sensory sensations that can dominate the wine aroma, inhibiting the perception of other volatile compounds present in wine (Rapp and Mandery 1986).

Thus, the most appropriate strategy during AF to favor the varietal aroma compounds of the grape, such as terpenes or thiols, is to maintain higher-alcohol production at concentrations less than 300 mg/L for the production of high-quality wines.

Initial assimilable nitrogen concentrations less than 150 mg/L usually cause stuck or sluggish fermentation. However, in modern enology, nutrient nitrogen correction is used to avoid obtain nondesired aromas derived from the increased concentrations of higher alcohols. Low concentrations of yeast-assimilable nitrogen (YAN) are associated with the production of higher alcohols at high concentrations. A study conducted by Schulthess and Ettlinger (1978) on the *Saccharomyces* genus showed that levels of nitrogen less than 500 mg/L increased the final concentrations of higher alcohols. An increase of approximately 50% in the final higher-alcohol concentrations occurred when the YAN concentration was 100 mg/L, compared to the controls with initial YAN concentrations more than 500 mg/L. These results indicate that if the main objective is to favor the impact of the grape varietal aroma compounds in wine, enologists should ensure an initial nitrogen concentration of more than 500 mg/L. This concentration can be easily controlled in winemaking by regulating initial nitrogen-related parameters such as YAN concentration, primary amino nitrogen content, ammonia content or amino acid profiles, which can be easily performed by using classical chemical techniques or advanced analyses such as enzymatic assays or fluorescence-based HPLC. The detected deficiencies can be easily corrected by nitrogen nutrient correction. Currently, there are numerous yeast nutrient products in the market that are used to increase initial nitrogen levels in grape juice prior to AF. Nevertheless, specific amino acids such as valine, leucine, isoleucine or threonine can increase the production of the corresponding higher alcohols (3-methylbutanol, 2-methylbutanol, isobutanol and propanol) (Schulthess and Ettlinger 1978). Therefore, oenologists should use nutrient products with low levels of these specific amino acids when aiming to reduce the impact of higher alcohols on the global aroma.

Several studies have demonstrated that yeast genetic factors directly influence the formation of higher alcohols. In addition, spontaneous fermentation usually leads to stronger production of higher alcohols than fermentation by selected starter cultures (Antonelli et al. 1999). Thus, selective processes for *Saccharomyces* species can aid the selection of strains that produce low quantities of higher alcohols. The final concentrations of most higher alcohols depend on the oxygenation conditions. Valero et al. (2002) reported decreased fermentation in the absence of oxygenation for *S. cerevisiae,* with the yields of 1-propanol, isobutanol, isoamyl alcohol, and phenyl ethyl alcohol and 1-butanol decreasing to approximately 50%, 90%, 66%, 70% and 20%, respectively (Valero et al. 2002). These data show oxygen control to be an interesting strategy to reduce the impact of higher alcohols on varietal aromas. The regulation of oxygen during fermentation is also very useful for preservation of grape varietal aroma compounds, such as thiols, the levels of which decrease under strongly oxidative conditions.

In the past, most non-*Saccharomyces* yeasts were designated as strong producers of higher alcohols compared to pure cultures of *S. cerevisiae* (Lambrechts and Pretorius 2000). However, recent studies have reported that some specific non-*Saccharomyces* yeasts are weaker producers of higher alcohols than *S. cerevisiae* (Clemente-Jiménez et al. 2004; Gobbi et al. 2013; Parapouli et al. 2010). Other studies have also shown that some non-*Saccharomyces* yeasts produce lower aromatic alcohol concentrations than *S. cerevisiae* because these non-*Saccharomyces* species differ from *S. cerevisiae* in metabolic flux, influencing biomass generation, ethanol production, or byproduct synthesis (Benito 2018; Magyar and Tóth 2011; Milanovic et al. 2012). Nevertheless, new studies report substantial differences not only among different non-*Saccharomyces* species but also at the strain level (Escribano et al. 2018). The use of specific non-*Saccharomyces* species such as *P. kluyveri*, *Lachancea thermotolerans* and *M. pulcherrima* to obtain wines with decreased levels of higher alcohols has been previously described (Benito et al. 2014; Benito et al. 2015; Benito 2019). The latter study described 11%, 16% and 24% decreased production of higher alcohols compared to the *S. cerevisiae* control. *P. kluyveri* and *M. pulcherrima* exhibited approximately 20% and 40% decreased i-butanol production, respectively. *L. thermotolerans* produced 10% less 3-methyl-butanol than the *S. cerevisiae* control, while *M. pulcherrima* produced approximately 30% less 3-methyl-butanol than the *S. cerevisiae* control. The most significant differences were observed for hexanol, wherein *P. kluyveri* and *M. pulcherrima* exhibited an approximately 50% and 30% decrease in hexanol production, respectively. Consequently, the sensory analysis showed increased levels of Riesling typicity perception, as varietal aromas were not masked by the higher alcohols produced. *T. delbrueckii* has also recently been reported to produce lower levels of higher alcohols than *S. cerevisiae* (Belda et al. 2017), with values of approximately 18% to 39%. This difference in higher-alcohol production is considered to be viable strategy that can aid the production of wines containing less than the threshold level of 300 mg/L higher alcohols, avoiding the possible masking of grape varietal aromas. A new study also reported that *T. delbrueckii* and *L. thermotolerans* were weaker producers of total higher alcohols than the *S. cerevisiae* control, with values of 86 and 49 mg/L, respectively (Escribano et al. 2018). Nevertheless, other species, such as *D. hansenii*, *Candida zeylanoides* or *Saccharomyces bailli,* are reported to be more efficient in terms of that specific objective, producing higher alcohols at low levels of 250 mg/L, 200 mg/L and 134 mg/L, respectively (Escribano et al. 2018). The same study reported strain-level differences of up to 37 to 50% in higher-alcohol production in species such as *M. pulcherrima*, *T. delbrueckii* and *L. thermotolerans* (Escribano et al. 2018), which indicates the importance of considering this parameter during selection.

Esters

Ester molecules are compounds formed by condensation of a hydroxyl group of a phenol or alcohol and a carboxyl group from an organic acid. Esters are considered to be among the most important components of volatile aromas in wine, second only to higher alcohols; these compounds also directly influence the aromatic profiles and sensory perception of wines (Fujii et al. 1994). Esters are produced naturally by yeasts during AF. Several esters give pleasurable aromas, such as fruity or floral aromas, and improve the quality of wines made from neutral grape varieties with low varietal aroma characteristics. However, other esters are considered to be very undesirable when they dominate the aroma of wine. The total ester concentration in wine is quite significant and is usually higher than the perception threshold, substantially influencing the final sensory perception (Lambrechts and Pretorius 2000). More than 150 different esters can be detected in wine. However, most of these esters are present at trace concentrations and do not significantly influence the overall aroma of wine.

Acetate esters are composed of two main groups: an alcohol group from ethanol or from a higher alcohol derived from yeast amino acid metabolism and an acid group (acetate) (Saerens et al. 2008). These pleasant-odor molecules include isoamyl acetate and ethyl hexanoate, which are described as having a banana aroma. 2-Phenylethylacetate is commonly associated with a rose aroma. Ethyl octanoate and ethyl 2-methyl-butanoate are associated with pineapple and strawberry aromas, respectively, while ethyl butanoate and ethyl decanoate are associated with fruity and floral aromas (Lambrechts and Pretorius, 2000). Nevertheless, when present at high concentrations, especially at concentrations greater than 12 mg/L, some acetate esters, such as ethyl acetate, can negatively influence the wine, imparting a varnish and/or nail polish aroma. In addition, the main ester in wine (ethyl acetate) can also have a suppressive effect on the other esters and volatile molecules in the wine, inhibiting the perception of favorable fruity ethyl esters. A similar suppressive effect is observed on the grape varietal aromas.

Although several esters are recognized as having pleasant aromas, to preserve the varietal characteristics of the grape, production of esters at low concentrations is important, to avoid masking the grape varietal aromas. The use of some non-*Saccharomyces* yeast in combined fermentation is an efficient way to produce wines with lower ester concentrations than the *S. cerevisiae* controls. With regard to this biotechnological application, *M. pulcherrima* appears to be the most efficient, reducing the final total ester yield by approximately 33% (Benito et al. 2015). In that study, the wines that were fermented by using *M. pulcherrima*-based biotechnology showed high sensory scores in terms of varietal typicity for the Riesling grape variety. Most of the reduction was due to decreased total acetate formation, which was decreased by approximately 25%, while the production of ethyl esters was reduced by approximately 8%. When *P. kluyveri* was used, there was no significant difference in total ester production, but total acetate production increased by approximately 5%, while the total ethyl ester levels decreased by the same amount. When *L. thermotolerans* was used, the effect was the opposite, that is, the ethyl ester levels increased, but the acetate levels decreased. In that study, the wines that were fermented by non-*Saccharomyces* yeasts showed high sensory scores in terms of varietal typicity for the Riesling grape variety.

Volatile Fatty Acids

Most volatile fatty acids present in wine are saturated straight-chain fatty acids that vary in chain length from 2 to 18 carbon atoms. These fatty acids are divided into short- (C2-C4), medium- (C6-C10) and long-chain (C12-C18) fatty acids. Other small groups of branched-chain fatty acids include 3-methyl butanoic acid, 2-methyl butanoic acid and 2-methyl propanoic acid.

The main fatty acid in wine is acetic acid (Eglinton and Henschke 1991), usually present at concentrations varying from 150 to 900 mg/L (Lambrechts and Pretorious 2000). Acetic acid represents more than 90% of the total volatile acids in wine. Acetic acid might have negative effects at concentrations greater than 0.8 g/L, leading to a predominant vinegar aroma. However, acetic acid contributes to a warm sensation on the palate at concentrations less than the perception threshold. The final volatile acid concentration in wine depends on several environmental and physiological factors, such as the pH, dissolved oxygen tension, temperature and yeast nutrient concentration (Lambrechts and Pretorius 2000; Paltauf et al. 1992). Low acetic acid production, that is, below the negative threshold, is a basic criterion for the selection of yeast strains as proper commercial starters (Benito et al. 2016). The use of yeast strains with low acetic acid production appears to be a fundamental strategy for enhancing the varietal characteristics of wine.

The other volatile fatty acids in wine are formed in a manner similar to the specific acetic acid production by yeast species. This process is not only highly species dependent but also strain dependent (Benito et al. 2016; Benito et al. 2018; Erasmus et al. 2004; Ravaglia and Delfini 1993). The selection of species/strains with low fatty acid production would be the main strategy for the selection of appropriate strains to enhance the varietal characteristics without masking by fatty acids.

Some fatty acids, such as propionic acid, butyric acid, isobutyric acid, valeric acid, 2-methylbutyric acid, hexanoic acid, octanoic acid, nonanoic acid and decanoic acid, possess unpleasant aromas, which have been described as rancid, pungent, fatty or cheese-like (Lambrechts and Pretorious 2000). However, the fruity character of wine can be preserved if the total fatty acid ester concentrations are maintained at less than 50-100 mg/L.

A promising biotechnology to reduce the final fatty acid content is the use of combined fermentation involving non-*Saccharomyces* species such as *D. hansenii, C. zeylanoides, M. pulcherrima, T. delbrueckii, L. thermotolerans* and *Z. bailii*. These species are reported to produce lower fatty acid levels than the *S. cerevisiae* controls (Escribano et al. 2018). Although the most popular industrial non-*Saccharomyces* yeasts *T. delbrueckii* and *L. thermotolerans* reduce the total fatty acid levels by approximately 50% to 60%, *D. hansenii* and *C. zeylanoides* can reduce the total fatty acid content by 10-fold, and these species appear to be the most appropriate option for this purpose (Escribano et al. 2018).

Aging aromas

Generally, the aging of wines, in bottles or oak barrels, leads to loss of grape varietal and fermentative aromas and to the formation of new aromas. This aromatic profile is a result of the aging process itself (oxidation), contact with lees, and presence of oak wood or atypical aromas associated with wine deterioration.

Varietal aromas and oxidation: Concentrations much higher than the olfactory perception threshold of 3SH, a thiol of varietal origin that it is expressed during AF, as seen above, were frequently detected in not only the Sauvignon Blanc or Verdejo varieties but also Merlot, Cabernet Franc, and Cabernet Sauvignon wines at the end of AF. These concentrations decreased during malolactic fermentation and aging. By the end of aging, the wines contained only a low percentage of the 3SH formed during AF. Oxygen dissolved in the wine during various handling operations led to a decrease in the 3SH content of red wine.

A synergistic effect of sulfur dioxide and anthocyanins in the stabilization of 3SH was observed. The combination of anthocyanins and sulfur dioxide reduced the oxidative decrease in 3SH levels. The findings also confirmed the important role of SO_2 in winemaking, mainly the protective effect of this compound against oxidation, which can decrease the 3SH concentration. High levels of free SO_2 can protect the 3SH thiol during handling operations, preserving the fruity aromas in red wines.

The role of wine phenolic compounds in the oxidation process was studied by Blanchard et al. (2004). The 3SH disappearance kinetics in red wine treated with oxygen exhibited a delay compared to the oxygen consumption kinetics; hence, the decrease in 3SH levels did not result from direct oxidation by oxygen. This effect is due to the previous oxidation of catechins, which accelerates oxidation of 3-MOH. In contrast, anthocyanins, another family of phenolic compounds, did limit the decrease in 3SH levels.

Sulfur compounds with thiol functional groups are also highly reactive compounds that are easily oxidized to disulfides in the presence of metals, particularly iron and copper, at trace concentrations (Jocelyn 1972). Moreover, the nucleophilic properties of these compounds result in numerous additional reactions, and in enology, reactions involving nonvolatile or volatile thiols in grape juice with oxidized phenolic compounds have been reported (Singleton et al. 1984, Cheynier et al. 1986). Recently, Murat et al. (2003) demonstrated the stabilization of a volatile thiol, 3SH, in the presence of anthocyanins in a model medium. It is said that redox levels during aging should be in dynamic equilibrium, as excess oxidation accelerates evolution, and the absence of oxygen leads to a reduced wine with off-odors due to the presence of sulfur compounds.

The vital role of sulfur dioxide in the protection of 3-mercaptohexanol in wines is evident, but in winemaking, this compound is restricted and limited, and some other alternatives are being studied. The biological antioxidant molecule GSH seems to protect wine from thiol oxidation (Dubourdieu and Lavigne-Cruege 2004) and decrease the evolution of volatile esters (isoamyl acetate) and terpenes (linalool) during aging (Papadopolou and Roussis 2008). GSH concentrations between 10 and 20 ppm in bottles allow sustained evolution and prevent the loss of volatile aromas (Roussis et al. 2007). GSH, as an alternative to sulfur dioxide, is currently being actively studied.

Aging under lees: Lees are formed from the yeast cells that have completed AF via a process called autolysis. Autolysis is relevant in enology because during this process, cells are lysed, releasing the intracellular content into the wine. The intracellular content contains nitrogen in the form of amino acids, peptides and proteins, including cell wall mannoproteins that protect against haze formation and increase the stabilization of wine color. During autolysis, lipids from the cells are also liberated, leading to increased fatty acid levels, which could impact the aroma and flavor via increased levels of volatile esters, aldehydes, and ketones in the wine. Specifically, during aging, the concentrations of ethyl esters of branched-chain fatty acids vary, the levels of fruity aroma compounds decrease, and the levels of long-chain alcohols and volatile fatty acids increase. Furthermore, because of their biosorbent qualities, lees can prevent some unpleasant odors, such as those of wine volatile phenols. Moreover, GSH is released from *S. cerevisiae* during yeast autolysis, contributing to maintenance of GSH levels in wines matured on yeast lees (Kritzinger 2003).

Aging with oak wood: The structure (grain, porosity and permeability) and chemical composition (polyphenols, tannins and volatile compounds) of wood determine some biochemical processes that occur during the aging of wine in wood barrels or other wood materials, adding a richness and complexity to the wine aroma and flavor and increasing the stability of the wine. There are five families of aroma compounds associated with the characteristic profile of oak-aged wine: furanic compounds, lactones, phenolic aldehydes, volatile phenols and phenyl ketones.

Unpleasant odors during aging: When high residual sugar levels remain during aging and the molecular form of sulfur dioxide is present at less than 0.5 mg/l, biological deterioration is possible. Tetrahydropyridines and 4-ethylphenol can be formed by *Brettanomyces*/*Dekkera* spp., conferring to the wine undesirable characteristics described as “medicinal” or “mousey”. LAB can degrade acids in the remaining wine and form unpleasant metabolites that can decrease the varietal and fruity wine aromas. Therefore, it is extremely important to maintain an appropriate level of sulfur dioxide, based on the pH of the wine (molecular form), and to carefully sanitize barrels or wood containers.

ANALYTICAL CHEMISTRY: UNLOCKING THE SECRETS OF WINE FLAVOR

The flavor of wine is the result of several volatiles that are present in wine and derived from the grape, including monoterpenes, norisoprenoids, some benzenoid compounds and polyfunctional sulfur compounds; the fermentation process, such as fatty acids, esters and higher alcohols; and wine aging. In grape, aroma compounds are mainly present in a nonvolatile state as these compounds are glycosylated and, in the case of polyfunctional sulfur compounds, cysteinylated or glutathionylated. The winemaking process allows the transfer of these molecules in both free and bound forms. Free aroma compounds are released from the corresponding bound compounds via the enzymatic activities of fermenting yeast and chemical acid-catalyzed reactions at the wine pH, leading to decreased or altered levels of the aroma compounds (Versini et al. 2008). Wood aging also plays a role in the complexity of wine aroma, because several compounds are released from wood, conferring spicy, toasted, caramel-like notes and the typical aged character (Cadahía et al. 2003; Cutzach et al. 1997; De Rosso et al. 2009).

For assessment of aroma compounds and their precursors in grape and wine, different analytical methods have been proposed for nonsulfurous (Azzi-Achkouty et al. 2017; Liu et al. 2017) and sulfur-containing aromas (Fracassetti and Vigentini 2018). The sulfur-containing compounds in the latter group are usually divided into “light” (boiling point below 90°C) and “heavy” (boiling point over 90°C) compounds (Mestres et al. 2000), necessitating the application of different analytical strategies for the detection of these compounds.

Analysis of nonsulfurous volatile compounds

The analytical methods that are commonly used for separation of nonsulfurous compounds in grape and wine are based on gas chromatography (GC). Due to the complexity of wine, isolation and preconcentration of the volatiles is needed, and different sampling techniques have been proposed, including liquid-liquid extraction (LLE), simultaneous distillation liquid extraction (SDE), mobile and stationary headspace techniques, solid-phase extraction (SPE), SPME and stir bar sorptive extraction (SBSE) (Arcari et al. 2017). Other described methods involve the combination of different sampling techniques, such as static headspace and solid-phase microextraction (HS-

SPME) and SPDE (Andujar-Ortiz et al. 2009). The main differences among the static and dynamic headspace-based techniques involve the achievement of equilibrium between the gas and liquid phases, which is achieved with the static headspace. Even though relatively large amounts of volatiles pass into the dynamic headspace, increasing the sampling phase and offering high sensitivity (Lepine and Archambault 1997), the equipment is highly complex and requires larger investments than the stationary headspace. Additionally, several flaws in purge-and-trap devices have been corrected (Washall and Wampler 1990). HS-SPME is the most common used technique for qualitative and quantitative analysis of wine aromas. The fiber choice is fundamental for the analysis of volatile molecules, in addition to modification of ionic strength by means of salt treatment, the volume of the sample and the duration and temperature of incubation (Azzi-Achkouty et al. 2017). The use of a three-phase fiber (carboxen-polydimethylsiloxane-divinylbenzene; CAR-PMDS-DVB) led to greater selectivity than that of a one-phase or two-phase fiber (Versini et al. 2008). An additional LLE step can be carried out prior to HS-SPME sampling (Fracassetti et al. 2017), as well as dilution of wine with water to decrease the ethanol concentration (Torchio et al. 2016). Detection is generally performed by mass spectrometry because of the high specificity and sensitivity of this technique (Villas-Boas et al. 2005), and the use of a flame ionization detector (FID) also allows characterization of the volatile profile of wine (Arcari et al. 2017).

Analysis of glycosylated aromas

In the case of glycosylated aroma compounds, cleavage of glycosidic bonds is required prior to GC-MS analysis. However, chemical acidic hydrolysis might cause molecular readjustment. After hydrolysis, SPME fibers and solvent bar microextraction (SBME) can be used to directly collect free volatile constituents for GC analysis (Liu et al. 2017). In addition to GC-MS, Boido et al. (2013) proposed the detection of glycosylated aromas with NIR (near-infrared) spectroscopy combined with a chemometric procedure in Tannat must and seedless homogenates. However, the overlapping of the glycoside peaks did not allow the identification of individual glycosides via direct spectral examination of glycoside extracts. HPLC analysis does not require the hydrolysis of glycosylated bonds prior to analysis. Coupling with NMR (nuclear magnetic resonance) (Schievano et al. 2013) or MS/MS detectors is a promising method for the detection of glycosylated aromas. Recently, Barnaba et al. (2018) described an original nontargeted high-resolution mass spectrometry method that, via implementation in neutral loss mode, allowed the detection of 280 compounds, 130 of which were tentatively identified; few databases contain MS/MS data for glycosidic fragments because GC has been used more frequently than HPLC. However, preservation of glycosylated aroma compounds during the HPLC process makes this technique favorable, despite the improvement of MS detectors.

Analysis of sulfur-containing volatile compounds: off-flavors

HS-SPME-GC is the main methodology used for determination of the sulfur molecules responsible for aroma faults in wine. For nonsulfurous volatile compounds, the performance of the method can be improved by proper selection of the fiber, the appropriate temperature and time of incubation, and the salt used to increase the ionic strength. The fibers proposed for fermentative sulfur compounds were two-phase fibers, namely, CAR-PDMS (Mestres et al. 1999; Segurel et al. 2005) and CAR-PDMS-DVB (Fedrizzi et al. 2010); the latter showed good repeatability and reproducibility (Fedrizzi et al. 2007). The addition of magnesium sulfate allows proper optimization of ionic strength. Optimal settings for these parameters are necessary because of the diverse boiling temperatures of the sulfur compounds produced during fermentation. Nguyen et al. (2012) reported that the response is improved when the samples are incubated at 45°C for 5 minutes and the extraction is performed with agitation at 45°C for 30 minutes. The previously described analytical methodologies are appropriate for analysis of volatile compounds characterized by low boiling points (< 90°C), unlike 3SH, 3SHA and 4MSP.

Analysis of sulfur-containing volatile compounds: varietal thiols

Varietal thiols are high-boiling volatiles that are highly reactive, and the concentrations of these thiols in wine are at the ng/L level. Consequently, the analytical method needs to overcome both the chemical properties and concentrations of these thiols in wine. Derivatization prior to LLE, followed by evaporation of the organic solvent, has been the most promising methodology for detection of varietal thiols. Moreover, the use of deuterated analogs as internal standards allows compensation for possible loss during sample preparation (Schneider et al. 2003). Among the molecules used for derivatization, Tominaga et al. (1998c) first suggested the use of p-hydroxymercuribenzoate (pHMB), and the analysis was carried out by GC-MS. In addition to 4MSP, 3SH and 3SHA, the methodology allows the quantification and identification of other sulfur-containing aroma compounds, such as 2-furanmethanethiol (Tominaga et al. 1998b) and benzenemethanethiol (Tominaga et al. 2003), in wines. Although very effective, this method is time consuming; moreover, the organomercury salt formed is a harmful, toxic substance, which is the main disadvantage this methodology. Alternative analytical techniques are based on the use of pentafluorobenzyl bromide (Mateo-Vivaracho et al. 2008) and ethyl propiolate (Herbst-Johnstone et al. 2013) as derivatization agents, and analysis of the derivatized thiols was carried out by GC-MS. Piano et al. (2015) suggested an analytical methodology in which varietal thiols are identified by ultrahigh-performance liquid chromatography (UPLC) combined with MS/MS. Varietal thiols were derivatized with o-phthaldialdehyde (OPA). Sample preparation required several steps for protection of the thiol aroma compounds against oxidation, and LLE was used to determine the levels of the analytes. This methodology allowed us to quantify 3SH and 3MSA; however, derivatization of 4MSP did not occur. The hydrogen bond between the thiolic group and the carbonyl moiety within the compound and the steric hindrance probably prevented the formation of the 4MSP-OPA derivative.

Analysis of varietal thiol precursors

Two different analytical approaches were described for the varietal thiol precursors, including indirect and direct methods (Peña-Gallego et al. 2012). The indirect method requires transformation of the precursors of volatile compounds, while the direct approach requires only a purification step prior to analysis (Table 4). The GC technique is generally used for the indirect method coupled with different flame photometric detectors (FDPs) (Tominaga et al. 1995). A derivatization procedure has also been proposed, and detection has been carried out by MS (Tominaga et al. 1998a), atomic emission detection (AED) (Howell et al. 2004) or detection-capture mass spectrometry (DCMS) (Subileau et al. 2008b). For the latter two methods, propyl thioacetate was used as an internal standard and ethylchloroformate was used as a derivatization agent. Direct determination of thiol precursors has been carried out by both LC and GC-MS. Derivatization is required for GC-MS analysis, and different derivatization agents have been proposed (Shinkaruk et al. 2008; Thibon et al. 2008b; Thibon et al. 2010). In the case of LC, SPE was performed to achieve sample purification before both HPLC-MS and HPLC-MS/MS. Measurement was performed based on the patterns of labeled compounds (Capone et al. 2010; Luisier et al. 2008; Roland et al. 2010b) as well as without labeling (Fedrizzi et al. 2009; Fracassetti et al. 2018). Measurement by liquid secondary ionization mass spectrometry (LSIMS) has also been described (Des Gachons et al. 2002) and, recently, by UPLC-MS/MS and stable isotope dilution assays (Bonnaffoux et al. 2017).

CONCLUSIONS

Wine aroma is a complex matrix of hundreds of chemical substances from different origins (varietal, microbial, wood barrels, etc.), which, depending on chemical structure and concentration, could have varying effects on the distinctive characteristics of a wine. Detailed chemical characterization and elucidation of the sensory relevance of these compounds in the complex wine nonodorant matrix have been conducted, and considering the increasing difficulty of this task, it is likely that this work will continue in the future.

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Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Figure captions

Fig. 1 Biogenesis of varietal monoterpenes. Yeasts and bacteria can release free monoterpenes from the corresponding sugar-bound nonodorant precursors, found in musts, by two-step enzymatic hydrolysis. Linalool monoterpene is used as an example.

Fig. 2 Biogenesis of varietal thiols. Yeasts, mainly *Saccharomyces cerevisiae*, are involved in thiol production. Nonodorant cysteinylated and glutathionylated precursors, among others, are found in musts and grapes, and these compounds are converted to aroma compounds (3SH: 3-sulfanylhexan-1-ol, 3SHA: 3-sulfanylhexyl acetate, 4MSP: 4-methyl-4-sulfanylpentan-2-one) via the activity of different enzymes, mainly β-lyases (Tominaga et al., 1998; Peyrot Des Gachons, et al., 2002; Fedrizzi et al., 2009).

Fig. 3 Yeast metabolic pathways involved in the production of varietal thiols. Uptake of the precursors is mediated by general amino acid transporters (Gap1p and Opt1p). Once inside the cell, the cysteinylated precursors (red pathway) are cleaved by a carbon-sulfur-β-lyase enzyme. Glutathionylated precursors (green pathway), which enter the cell through Opt1p, are not cleaved directly but are degraded to the cysteinylated form as an intermediate in a multistep pathway (Cordente, et al., 2015).

Fig. 4 Alternative pathways for 4MSP (A) and 3SH (B) thiol release proposed by Schneider et al. (2006) and Duhamel et al. (2011). The reduction step (indicated by the green arrow) might be carried out by yeasts during alcoholic fermentation.

Table 1

Table 1: Odors descriptors found in wine and their associated molecule.

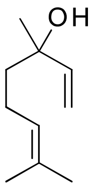
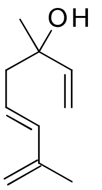
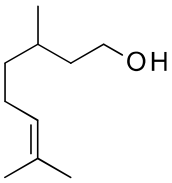
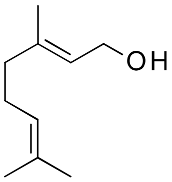
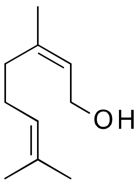
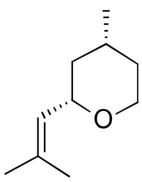
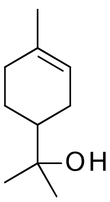
Odor quality	Associated molecule	CAS registry number
Albariño		
Lemon	Citral	5392-40-5
Grapefruit	3-Mercaptohexyl acetate	136954-20-6
Nectarine	---	---
Melon	(Z)-6-Nonen-1-yl acetate	76238-22-7
Wet gravel	---	---
Sauvignon Blanc		
Gooseberry	Furfuryl butyrate	623-21-2
Green melon	(Z)-6-Nonen-1-yl acetate	76238-22-7
Grapefruit	3-Mercaptohexyl	136954-20-6
White peach	γ-Nonalactone	104-61-0
Passion fruit	(Z)-Buchu mercaptan	33284-96-7
Chardonnay		
Yellow apple	Ethyl butyrate	105-54-4
Starfruit	Butyl heptanoate	5454-28-4
Pineapple	Ethyl acetate	141-78-6
Butter	Diacetyl	431-03-8
Chalk	---	---
Viognier		
Tangerine	4,7-Decadienal	934534-30-2
Peach	γ-Decalactone	104-61-0
Mango	2,6-dipropyl-5,6-dihydro-2H-Thiopyran-3-carbaldehyde	61407-00-9
Honeysuckle	Phenyl-acetaldehyde	122-78-1
Rose	Phenethyl alcohol	60-12-8
Gewürztraminer		
Lychee	(Z)-Rose oxide	16409-43-1
Rose	Phenethyl alcohol	60-12-8
Pink grapefruit	3-Mercaptohexyl	136954-20-6
Tangerine	4,7-Decadienal	934534-30-2
Guava	Ethyl (E)-3-hexenoate	26553-46-8
Muscat		
Meyer lemon	Citral	5392-40-5
Mandarin orange	Octanal	124-13-0
Pear	Hexyl acetate	142-92-7
Orange blossom	Acetyl tetralin	774-55-0
Honeysuckle	Phenyl-acetaldehyde	122-78-1
Riesling		
Lime	Wine lactone	182699-77-0
Green apple	Ethyl butyrate	105-54-4
Beeswax	Ethyl phenyl acetate	101-97-3
Jasmine	2-hexylidene Cyclopentanone	17373-89-6
Petroleum	Isobutyl methyl ketone	108-10-1

Pinot noir		
Cranberry	2-Methyl-3-pentenoic acid	37674-63-8
Cherry	Benzaldehyde	100-52-7
Raspberry	β-Ionone	14901-07-6
Clove	4-Vinylguaiacol	7786-61-0
Mushroom	allyl glycol	111-45-5
Cabernet franc		
Strawberry	Ethyl hexanoate	123-66-0
Roasted pepper	3-Isobutyl-2-methoxypyrazine	24683-00-9
Red plum	Plum crotonate	68039-73-6
Crushed gravel	---	---
Chili pepper	3-Isobutyl-2-methoxypyrazine	24683-00-9
Carmenere		
Raspberry	β-Ionone	14901-07-6
Green bell pepper	3-Isobutyl-2-methoxypyrazine	24683-00-9
Black plum	Plum crotonate	68039-73-6
Blackberry	Ethyl 2-hydroxy-4-methyl valerate	10348-47-7
Vanilla	Vanillin	121-33-5
Garnacha		
Dried strawberry	Isobutyl-3-(methyl thio) butyrate	127931-21-9
Grilled plum	Plum crotonate	68039-73-6
Ruby red grapefruit	3-Mercaptohexyl	136954-20-6
Leather	4-Ethyl phenol	123-07-9
Licorice	(E)-anethol	4180-23-8
Merlot		
Raspberry	β-Ionone	14901-07-6
Black cherry	Benzaldehyde	100-52-7
Sugar plum	Plum crotonate	68039-73-6
Chocolate	2-Isobutyl-3,5-(and 3,6)-dimethyl Pyrazine	38888-81-2
Cedar	Cedrenol	28231-03-0
Sangiovese		
Red currant	---	---
Roasted tomato	Methional	3268-49-3
Raspberry	β-Ionone	14901-07-6
Potpourri	---	---
Clay pot	---	---
Zinfandel		
Blackberry	Ethyl 2-hydroxy-4-methyl valerate	10348-47-7
Strawberry	Ethyl hexanoate	123-66-0
Peach preserves	γ-Decalactone	104-61-0
5-Spice powder	---	---
Sweet tobacco	Phenethyl acetate	103-45-7
Cabernet sauvignon		
Black cherry	Benzaldehyde	100-52-7
Black currant	Mercaptomethyl pentanone	75832-79-0

Red bell pepper	3-Isobutyl-2-methoxypyrazine	24683-00-9
Baking spices	---	---
Cedar	Cedrenol	28231-03-0
Malbec		
Red plum	Plum crotonate	68039-73-6
Blueberry	1-Ethoxyethyl acetate	1608-72-6
Vanilla	Ethyl vanillin	121-32-4
Sweet tobacco	Phenethyl acetate	103-45-7
Cocoa	2-Isobutyl-3,5-(and 3,6)-dimethyl Pyrazine	38888-81-2
Nebbiolo		
Rose	Phenethyl alcohol	60-12-8
Cherry	Benzaldehyde	100-52-7
Leather	4-Ethyl phenol	123-07-9
Clay pot	---	---
Anise	Para-anisaldehyde	123-11-5
Petit verdot		
Black cherry	Penzaldehyde	100-52-7
Plum	Plum crotonate	68039-73-6
Violet	β-Ionone	14901-07-6
Lilac	(±)-Lilac aldehyde	67920-63-2
Sage	Clary propyl acetate	131766-73-9
Syrah		
Blueberry	1-Ethoxyethyl acetate	1608-72-6
Plum	Plum crotonate	68039-73-6
Milk chocolate	3(2)-Hydroxy-5-methyl-2(3)-hexanone	163038-04-8
Tobacco	Phenethyl acetate	103-45-7
Green peppercorn	3-Isobutyl-2-methoxypyrazine	24683-00-9
Tempranillo		
Cherry	Benzaldehyde	100-52-7
Dried fig	Fig crotonate	68039-69-0
Cedar	Cedrenol	28231-03-0
Tobacco	Phenethyl acetate	103-45-7
Dill	2,3-Octane dione	585-25-1
Touriga nacional		
Violet	β-Ionone	14901-07-6
Blueberry	1-Ethoxyethyl acetate	1608-72-6
Plum	Plum crotonate	68039-73-6
Mint	Tert-butyl methyl ether	1634-04-4
Wet slate	---	---

Table 2

Table 2: Structure, aroma descriptors, concentration and perception thresholds of main monoterpenes in wine.

Name	Structure	Aromas	Concentration range regard wine variety (ng/L)	Perception threshold (ng/L)
Linalool		Flowery, fruity, muscat [a]	White varieties: nd – 307 [b], [c] Red varieties: nd – 16.4 [b], [d], [e]	6 [1], [f] 15 [2], [c]
(*E*)-Hotrienol		Faint flowery, elder flower [g]	Riesling renano: 2.8 – 116.6 [h], [i]	110 [3], [j]
Citronellol		Green lemon [a]	White varieties: nd – 31.4 [b] Red varieties: nd – 5.5 [b], [e]	8 [1], [k] 100 [2], [c]
Geraniol		Roses, geranium [l]	White varieties: nd – 221 [b], [c] Red varieties: nd - 44.4 [d], [m]	32 [1], [f] 30 [2], [c]
Nerol		Citrus, floral [n]	White varieties: 16.6 – 49 [b] Red varieties: nd – 100.3 [b], [p]	300 [1], [o] 300 [2], [p]
(−)-*cis*-Rose oxide		Geranium oil [n] Floral green [q]	White varieties: 0.1 – 9.1 [r]	0.5 [1], [q] 0.2 [2], [p]
α-Terpineol		Floral, woody [s]	White varieties: nd -123.8 [b] Red varieties: nd – 33 [b], [d], [e]	350 [1], [f] 250 [4], [d]

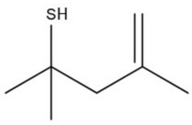
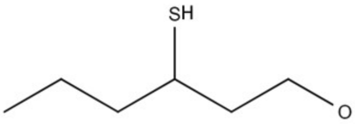
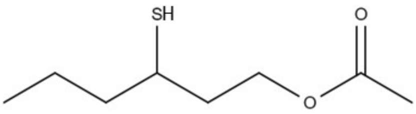
*Threshold determined in: [1] water, [2] water/ethanol (90 + 10, w/w), [3] sugar-water solution [4] synthetic wine (11% v/v ethanol, 7 g/L glycerin, 5 g/L tartaric acid, pH adjusted to 3.4 with 1 M NaOH). (nd: not detected).

Literature: [a] Peng et al. 2013, [b] Piñeiro et al. 2006, [c] Guth 1997, [d] Ferreira et al. 2000, [e] López et al. 2002, [f] Buttery et al. 1971, [g] Jørgensen et al. 2000, [h] Versini et al. 1981, [i] Rapp and Mandery 1986,

[j] Simpson 1979, [k] Ong and Acree 1999, [l] García-Carpintero et al. 2011, [m] Sabon et al. 2002, [n] Styger et al. 2011, [o] Ohloff 1978, [p] Zhao et al. 2017a, [q] Yamamoto et al. 2002, [r] Koslitz et al. 2008, [s] Zhao et al. 2017b.

Table 3

Table 3: Structures, odor descriptors, concentration and perception thresholds of the main varietal thiols in wines.

Name	Structure	Aromas	Concentration range regard wine variety (ng/L)	Perception threshold (ng/L)
4-methyl-4-sulfanylpentan-2-one (4MSP)		Blackcurrant box-tree, broom, passion fruit	Sauvignon Blanc: 0.6 - 88 Gewurztraminer: nd - 15 Reisling: nd - 7.6	0.8
3-sulfanylhexan-1-ol (3SH)		Grape fruit, citrus peel, passion fruit	Sauvignon Blanc: 26 - 18.700 Gewurztraminer: 1340 - 3280 Reisling: 407 – 562	60
3-sulfanylhexyl acetate (3SHA)		Passion fruit, box tree, box wood	Sauvignon Blanc: 29 - 2510 Gewurztraminer: 0.5 - 5.7 Reisling: nd - 6.4	4

*Information summarized from: Roland et al, 2011; Waterhouse et al, 2016; Jeffery, 2016. (nd: not detected).

Table 4

Table 4: Analytical methods for the determination of varietal thiol precursors.

Compounds detected	Analytical technique	Sample preparation	Reference
Cys-4MSP, Glu-4MSP	GC-FDP	Cell free-extract with cysteine β-lyase activity	Tominaga et al. 1995
Cys-3SH, Cys-4MSP, Cys-4MSPOH	GC-MS	Cell free-extract with cysteine β-lyase activity	Tominaga et al. 1998a
Cys-4MSP	GC-AED	Trimethylsilylation derivatization	Howell et al. 2004
Cys-3SH	GC-DCMS	Purification with cation exchange resin and derivatization with ethyl-chloroformate	Subileau et al. 2008b
Cys-4-MSP	GC-MS	Trimethylsilylation derivatization	Shinkaruk et al. 2008
Cys-3SH (R and S diasteroisomers)	GC-MS	Purification with chelating Sepharose column; trimethylsilylation derivatization	Thibon et al. 2008
Cys-3SP, Cys-SH, Cys-3SHp, Cys-2M3SB	GC-MS	Purification with chelating Sepharose column; derivatization with *tert*-butyldimethylsilyl	Thibon et al. 2010
GSH-3SH	HPLC-MS	Purification with chelating Sepharose column	Des Gachons et al. 2002
Cys-3SH	HPLC-MS	SPE purification	Luisier et al. 2008
GSH-4MSP	HPLC-MS/MS	C18-packed sintered funnel; C18 sorbent with low pressure column chromatography	Fedrizzi et al. 2009
Cys-3SH, GSH-3SH (R and S diasteroisomers)	HPLC-MS	SPE purification	Capone et al. 2010

Cys-3SH, GSH-3SH, Cys-4MSP, GSH-4MSP	Nano LC-MS/MS	Purification with ion exchange resin	Roland et al. 2010
GSH-3SH-Al, GSH-3SH-SO_3	HUPLC-FTMS	Extraction of targeted compounds with MPLC	Thibon et al. 2016
CysGly-3SH, CysGly-4MSP, cGluCys-3SH, cGluCys-4MSP	UPLC-MS/MS and stable isotope dilution assay	No must purification	Bonnaffoux et al. 2017
Cys-3SH, GSH-3SH, CysGly-3SH, GluCys-3SH, GSH-4MSP, Cys-4MSP, CysGly-4MSP, GSH-3MSA	UPLC-HRMS	SPE purification	Fracassetti et al. 2018a

*Abbreviations: **Cys-3SH**, S-3-(hexan-1-ol)-cysteine; **GSH-3SH**, S-3-(hexan-1-ol)-glutathione; **CysGly-3SH**, S-3-(hexan-1-ol)-cysteine-glycine; **GluCys-3SH**, S-3-(hexan-1-ol)-glutamyl-cysteine; **GSH-4MSP**, S-3-(4-mercapto-4-methylpentan-2-one)-glutathione; **Cys-4MSP**, S-3-(4-mercapto-4-methylpentan-2-one)-cysteine; **CysGly-4MSP**, S-3-(4-mercapto-4-methylpentan-2-one)-cysteine-glycine; **GSH-3SHA**, S-3-(hexanal)-glutathione; **Cys-4MSPOH**, S-3-(4-mercapto-4-methylpentan-2-ol)-1-cysteine; **GSH-3MH-SO_3**, bisulfite S-3-(hexanal)-glutathione; **Cys-3SP**, S-3-(pentan-1-ol)-l-cysteine; **Cys-SH**, S-3-(hexan-1-ol)-l-cysteine; **Cys-3SHp**, S-3-(heptan-1-ol)-l-cysteine; **Cys-2M3SB**, S-3-(2-methylbutan-1-ol)-l-cysteine.

Figure 1

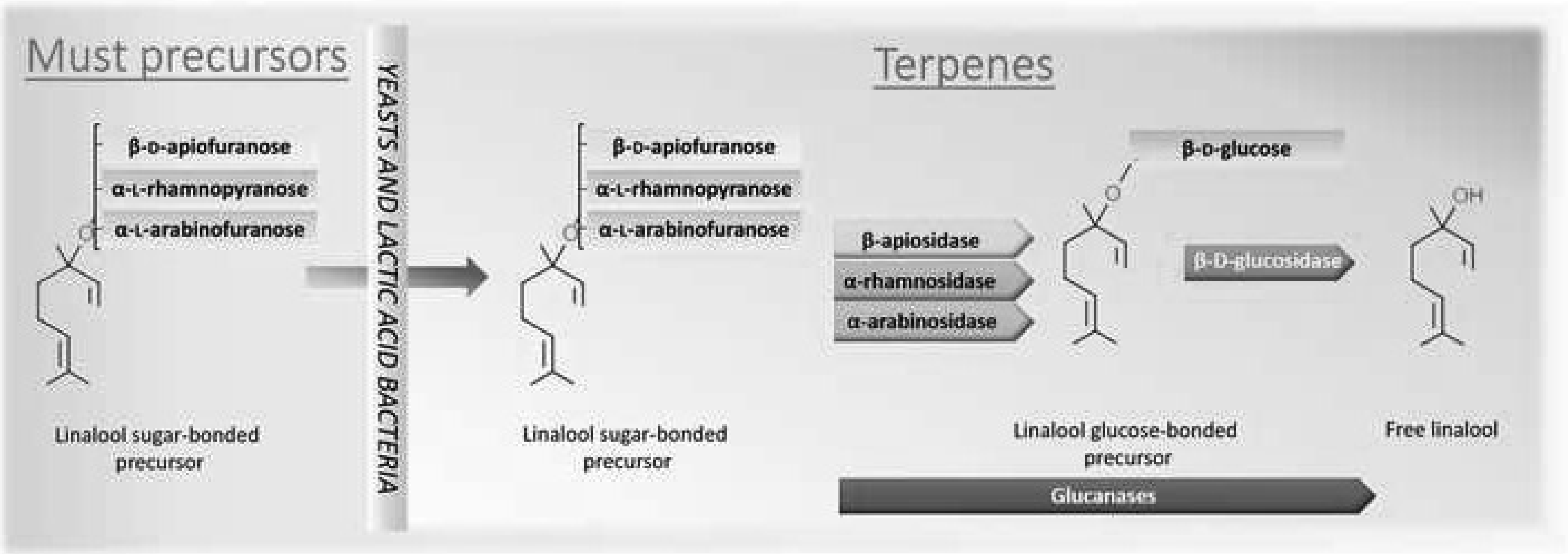

Must precursors
β-D-apiofuranose
α-L-rhamnopyranose
α-L-arabinofuranose
Linalool sugar-bonded precursor
YEASTS AND LACTIC ACID BACTERIA
Terpenes
β-D-apiofuranose
α-L-rhamnopyranose
α-L-arabinofuranose
Linalool sugar-bonded precursor
β-apiosidase
α-rhamnosidase
α-arabinosidase
β-D-glucose
Linalool glucose-bonded precursor
β-D-glucosidase
OH
Free linalool
Glucanases

Figure 2

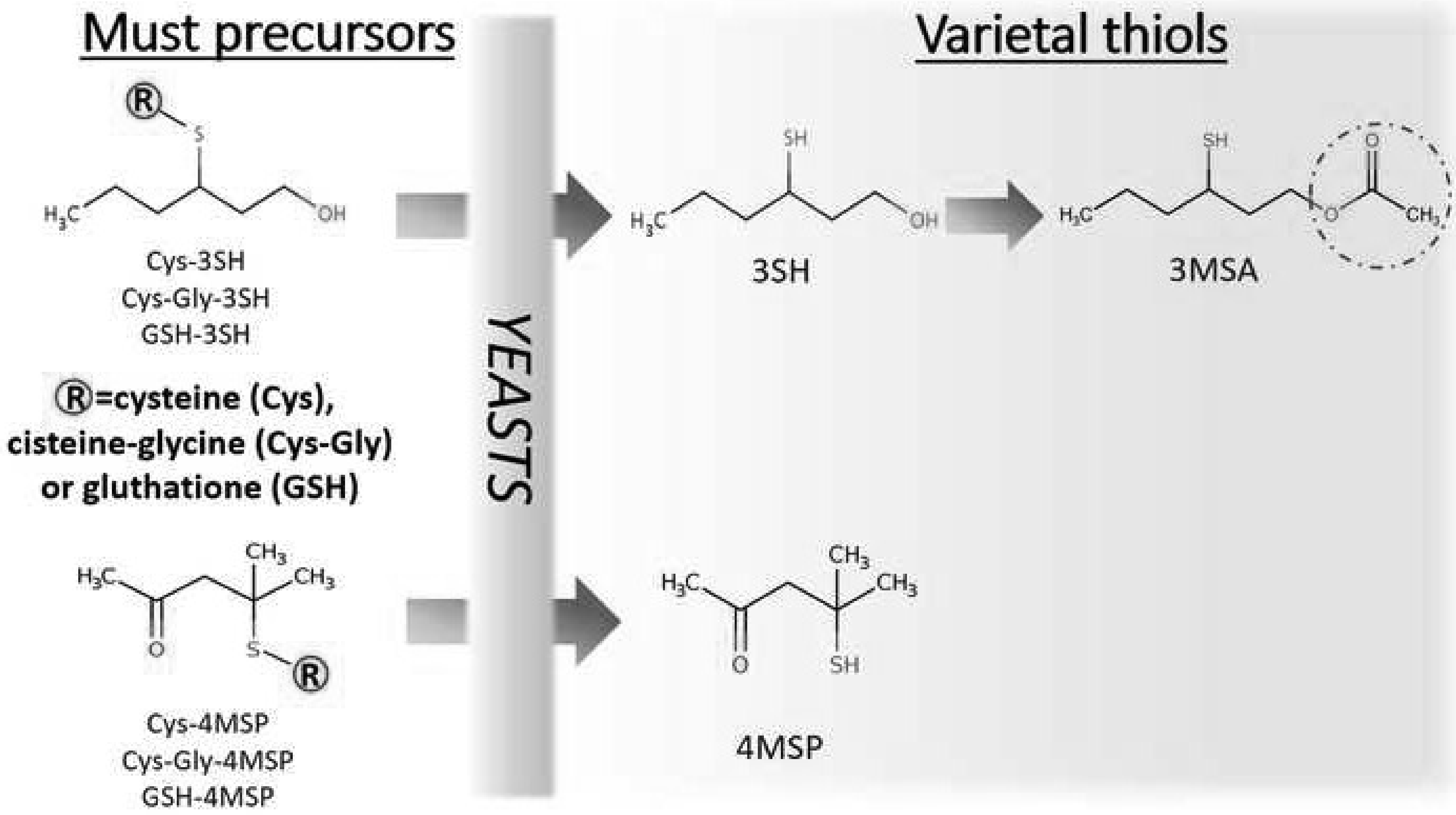
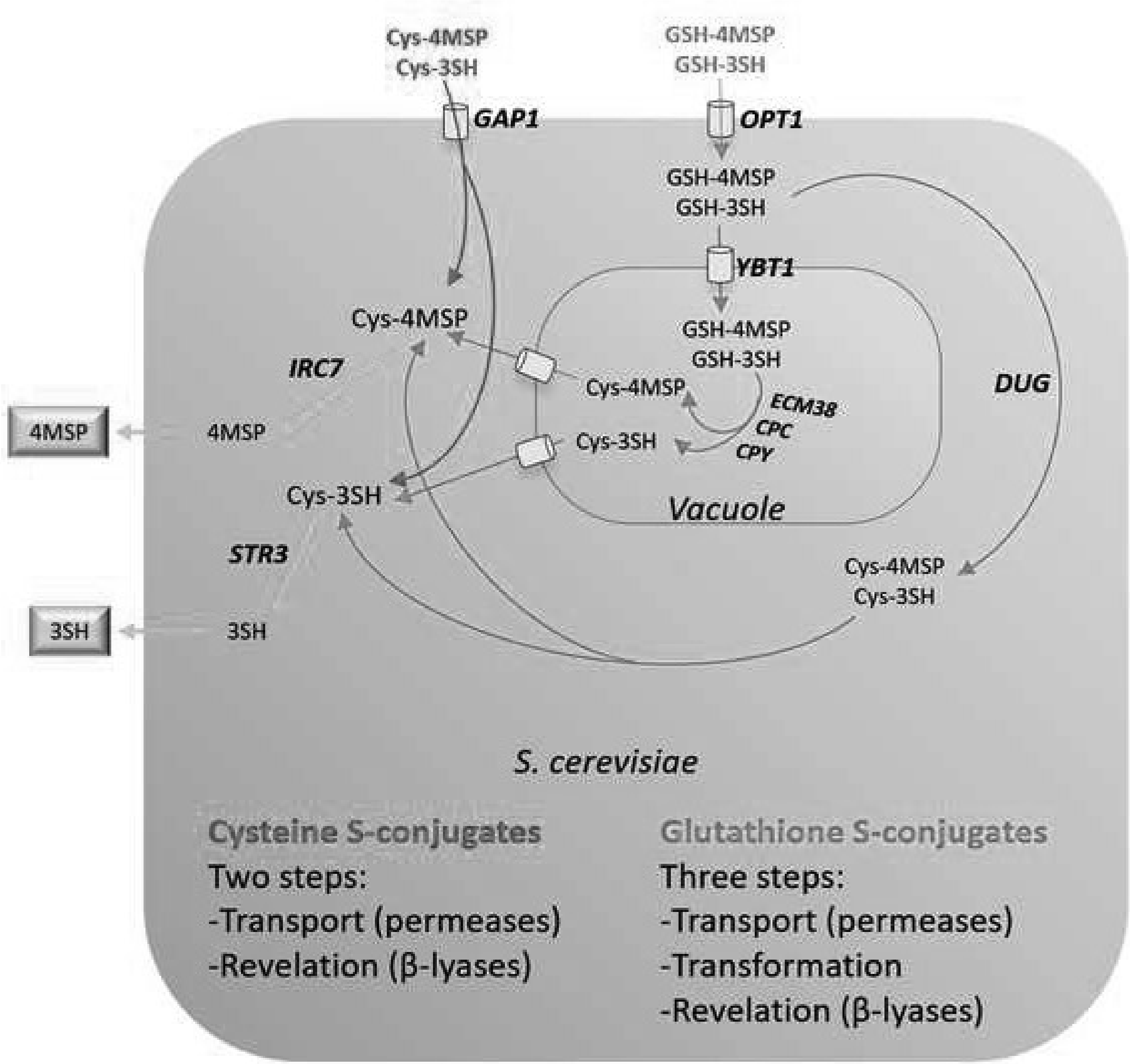
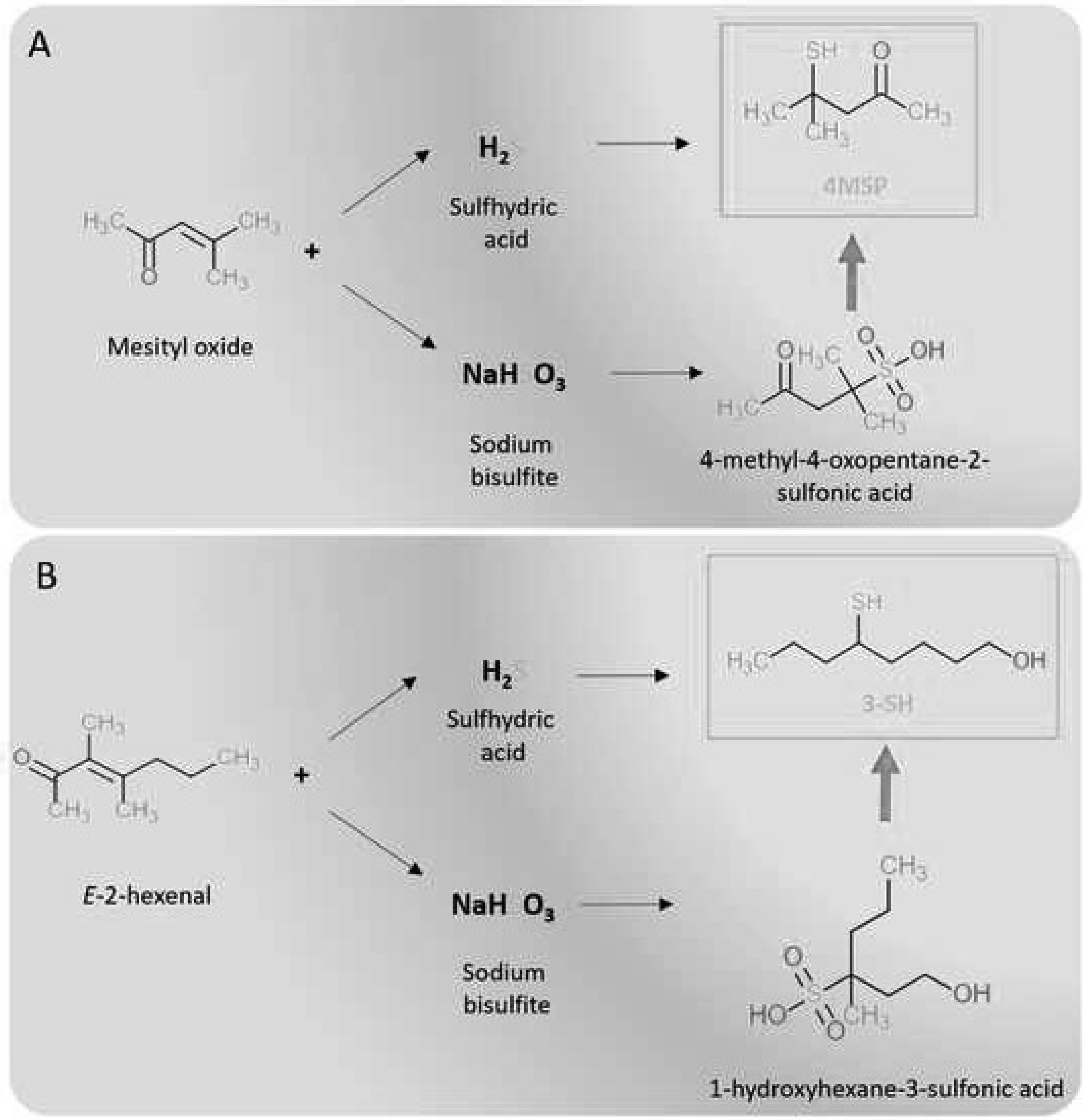

Must precursors
Varietal thiols
YEASTS
Cys-3SH
Cys-Gly-3SH
GSH-3SH
Ⓡ=cysteine (Cys),
cisteine-glycine (Cys-Gly)
or gluthatione (GSH)
Cys-4MSP
Cys-Gly-4MSP
GSH-4MSP
3SH
3MSA
4MSP

Figure 3


Cys-4MSP
Cys-3SH
GAP1
GSH-4MSP
GSH-3SH
OPT1
GSH-4MSP
GSH-3SH
YBT1
Cys-4MSP
GSH-4MSP
GSH-3SH
IRC7
DUG
Cys-4MSP
ECM38
CPC
CPY
4MSP
4MSP
Cys-3SH
Cys-3SH
Vacuole
STR3
Cys-4MSP
Cys-3SH
3SH
3SH
S. cerevisiae
Cysteine S-conjugates
Two steps:
-Transport (permeases)
-Revelation (β-lyases)
Glutathione S-conjugates
Three steps:
-Transport (permeases)
-Transformation
-Revelation (β-lyases)


A
H₂
Sulfhydric acid
Mesityl oxide
NaH O₃
Sodium bisulfite
4MSP
4-methyl-4-oxopentane-2-sulfonic acid
B
H₂
Sulfhydric acid
E-2-hexenal
NaH O₃
Sodium bisulfite
3-SH
1-hydroxyhexane-3-sulfonic acid

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