

RUBY[™] – expressing thiols in red wine through an innovative yeast selection

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Volatile thiols are a family of aroma compounds widely known for their contribution to the fruity notes of wines, especially in whites. These aromas are highly characteristic of some grape varieties, such as Sauvignon blanc, Colombard and Verdejo, and represent a significant part of their typicity. 4-mercapto-4-methylpentan-2-one (4MMP or 4MSP), 3-mercaptohexan-1-ol (3MH or 3SH) and 3-mercaptohexylacetate (3MHA or A3SH) are the three most abundant thiol molecules. The importance of thiols in whites is well described, but their role in red wine aromatic profile is largely underestimated, but seems to be an important contributor of the intensity and diversity. Fermentation management plays a key role in the revelation of these compounds in wines, the yeast being responsible for transforming precursors to volatile thiols.

The new wine yeast, RUBY[™], is a major innovation in the red winemaking process. This is the first yeast specifically selected for revealing volatile thiols in red wines, combining exceptional potential to release those compounds and other characteristics regarding robustness, versatility and taste perceptions.

TABLE 1. Varietal thiol concentrations in various red varieties.

Varieties	Number of wines	Country	Min - max (ng/L) 3MH-3MHA-4MMP		
Cabernet Sauvignon and Merlot blends	12	France	10 - 5 000	1 - 200	nq
Negrette	5	France	909 - 1 617	8 - 22	2 - 4
Carmenère	6	Chile	667	373	nq
Blends with a majority of Syrah, Grenache and/or Mourvèdre	10	France	678 - 3 423 [11 487]*	5 - 26 [154]*	5 - 54
Pinot noir	34	Australia	250 - 1 250	0-16	0 - 16
Cabernet Sauvignon	20	United States	299 - 1 161	39 - 91	nq
Cabernet Sauvignon and Cabernet franc blends Merlot blends	24	France	100 - 634	nq	3 - 20
Carmenère	2	Chile	422 - 760	8 - 22	nq
Shiraz	16	South Africa	73 - 363	5 - 8	0 - 3
Cabernet Sauvignon Pinotage	16 16		77 - 147 127 - 311	23 - 24 7 - 12	3 - 3 0 - 2
Shiraz	46	Australia	100 - 470	na	na
Cabernet Sauvignon	24		130 - 440 [605]*	nq	nq
Merlot	9		145 - 360	nq	nq
Pinot noir	8		295 - 760	nq	nq
Durif	3		195 - 290	nq	nq
Grenache	3		295 - 360	nq	nq
Malbec	3		180 - 320	nq	nq
Mataro	3		200 - 220	nq	nq
Petit Verdot	3		100 - 110	nq	nq
Tempranillo	3		265 - 320	nq	nq

Thiols in red wines – occurrence and findings

Bouchilloux and other authors (1998) were the first to demonstrate the presence of 3MH and 3MHA in Cabernet Sauvignon and Merlot. In 2004, the aroma of six premiumquality Spanish red wines was studied by Culleré and other authors (2004). Among other aromatic compounds, 3MH, 3MHA and 4MMP were identified as responsible for the sensory difference between the wines. 3MH and 3MHA were also identified in Carmenère by Dominguez and Agosin (2010). Different analytical methods were used in these various studies, so comparing the final concentration found between studies and varieties is difficult. Interestingly other red grape varieties were identified as containing varietal thiols (3MH, 3MHA and/or 4MMP): Syrah/Shiraz, Pinot noir, Tempranillo, Malbec, Grenache, etcetera. (Table 1) here below.

The sensory impact of thiols in red wines

In white wines, the sensory contribution of 3MH, 3MHA and 4MMP is well identified with descriptors such as grapefruit, passionfruit and box tree aromas often used to describe these thiolic compounds. However, ascribing specific attributes to these thiols in reds seems more challenging, because of a more complex matrix, higher interactions and less research on this topic.

Rigou and other authors (2014) demonstrated the correlation between thiol content and specific sensory

impact in French red-blended wines for the first time. The 4MMP concentration correlated to the blackcurrant aroma, while 3MHA and 3MH acted as enhancers of this perception. The impact of 3MH and 3MHA was also investigated by Sibert and other authors (2019) in 10 grape varieties. More intense red fruit aromas were linked with these compounds. More recently, Panzeri and other authors (2020) confirmed the aromatic contribution of 3MH, 3MHA and 4MMP in different grape varieties. 4MMP was linked with red and black fruits, such as raspberries and blackberries. 3MH, particularly 3MHA, increased this aroma perception, also adding some plum and berry jam aromas. Depending on the grape variety, 3MH was also associated with spices, while 3MHA and 4MMP could bring herbal notes.

In different red grape varieties, a higher content of 3MH, 3MHA and/or 4MMP can enhance the aromatic intensity and complexity of the wines.

A specific yeast selection to optimise thiols revelation in reds

Thiols 3MH, 3MHA and 4MMP are present in the grape must as non-aromatic and non-volatile precursors. They can be cysteinylated or glutathionylated conjugates. Some viticulture techniques can favour thiol precursor accumulation. Fermentation conditions greatly influence the thiol release. Temperature and nutrition are essential for



FIGURE 1. Pathway of thiol precursors uptake into the wine yeast cell and transformation into volatile thiols.



FIGURE 2. Thiol concentrations in final wines fermented with RUBY^M or a control yeast in Merlot with added thiols precursors (226 g/L sugar, 13.5% ν/ν potential alcohol, 138 mg/L YAN, 24°C).

thiol management. However, the wine yeast strain plays a key role, as shown in Figure 1.

Through their cleaving enzymes, wine yeasts can release the aromatic volatile thiols from their non-aromatic conjugated precursors. IRC7 is the gene involved in the formation of the enzyme. Different forms of IRC7 and several combinations of IRC7 alleles exist naturally in yeast. Most of those combinations lead to reduced activity due to truncated and/or mutated forms of the gene. A comprehensive screening of our collection of wine yeasts highlighted this diversity. Using an innovative non-GMO strategy of cellular division followed by an allelic study, we selected a new wine yeast with fully active IRC7 alleles. This new yeast strain RUBY[™] combines excellent characteristics essential in red winemaking and in red wine conditions with the full capacity to reveal volatile thiols.



FIGURE 3. Thiol concentrations at the end of alcoholic fermentation (AF) with RUBYTM compared to Yeast A and Yeast B in Syrah, South of France (238 g/L sugar, 14% v/v potential alcohol, 136 mg/L YAN, 27°C). Addition of 20 g/hL Fermaid O at the beginning of AF + 20 g/hL Fermaid E at 1/3rd.





FIGURE 4. Thiols concentration in bottled wines fermented with $RUBY^{TM}$ or Yeast A in Tempranillo, La Rioja, Spain (231 g/L sugar, 14% v/v potential alcohol, 108 mg/L YAN, 25°C). Addition of 40 g/hL Stimula Syrah at the beginning of AF + 20 g/hL Fermaid E^{TM} at 1/3rd.

Revealing thiols and varietal complexity in red wines

The selected wine yeast, RUBY[™], was tested at a laboratory scale in Merlot to determine its ability to release 4MMP, 3MH and 3MHA. It showed a much higher thiol concentration than a control wine yeast (Figure 2). This confirmed the efficiency and robustness of the selection method and the great potential of this new strain.

During the 2022 vintage, several trials were done on a pilot scale in France, Italy, Spain and Germany on different grape varieties (Cabernet Sauvignon, Merlot, Syrah, Tempranillo, Lemberger and Dolcetto). Thiols were detected in various grape varieties, and RUBY[™] confirmed its exceptional ability to reveal volatile thiols Sensory evaluation of wines fermented with RUBY[™] often revealed red berries and complexity. In Tempranillo, wild berries and spicy were also mentioned. In Dolcetto, wines were described as intense, fresh and fruity.

Sensory analysis conducted by a professional international panel on a Merlot (Germany) revealed significant black and red fruits, with more aromatic freshness and volume in the wine fermented with RUBYTM (Figure 6).



FIGURE 5. Thiol concentrations in bottled wines fermented with $RUBY^{\text{TM}}$ compared to Yeast D and Yeast E in Cabernet Sauvignon, IFV Bordeaux, France (265 g/L sugar, 15.7% v/v potential alcohol, 51 mg/L YAN, 25°C). Addition of 30 g/hL Fermaid O at the beginning of AF + 40 g/hL Fermaid E at 1/3rd.

Conclusion

There is an increasing interest in volatile thiols in red wines' aromatic profiles. The three main thiols (3MH, 3MHA and 4MMP) can be found in many red grape varieties and have a very important role in red wine aromatic complexity and intensity.

The increasing understanding of volatile thiols and our expertise in yeast selection and characterisation enabled us to select the first wine yeast dedicated to revealing thiols in red wines. Wines fermented with RUBY[™] show intense and complex flavour profiles described as blackcurrant, gooseberry, plums, spices and some refreshing herbal notes. RUBY[™] also favours a nice and fine tannin structure with a long aftertaste. RUBY[™] is a breakthrough in wine microbiology and opens new exciting perspectives in the red winemaking approach. ■



FIGURE 6. Sensory analysis by an international professional panel (13 tasters) in Merlot, Germany.

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