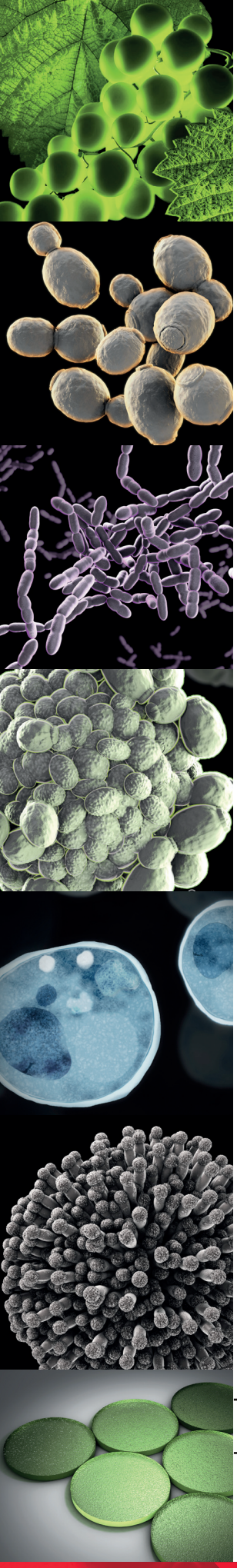

SAUVY™

For optimal expression of varietal thiol aromas

TECHNICAL ARTICLE

Introduction

Volatile thiols are a family of aroma compounds that are widely known for their contribution to the fruity notes of wines. These aromas are highly characteristic of some grape varieties, such as Sauvignon Blanc, Colombard or Verdejo and represent a huge part of their typicity. They also contribute to the complexity and fruitiness of several other varieties, even in red wines. 3MH (3-sulfanyl-hexan-1-ol, also known as 3SH), its acetate 3MHA (also known as 3SH-A) and 4MMP (4-methyl-4-sulfanylpentan-2-one, also known as 4MSP) are the three most abundant compounds; 3MH is associated with the grapefruit aroma, 3MHA of passion fruit and 4MMP is responsible for the blackcurrant and boxtree aromas. Their perception threshold is very low, especially for the 4MMP (0.8 ng/L). These compounds are present in the grape must as odourless nonvolatile precursors, cysteinylated or glutathionylated conjugates. The aromatic thiols are revealed by the enzymatic action of the yeast during the alcoholic fermentation.

The volatile thiols are revealed by wine yeasts

Saccharomyces cerevisiae is able to take up and cleave the precursors to release the free thiols, 3MH and 4MMP (Figure 1). The 3MHA is derived from 3MH by acetylation. Wine yeast have different capacities to reveal volatile thiols depending on their genetic background and their corresponding enzymatic activities.

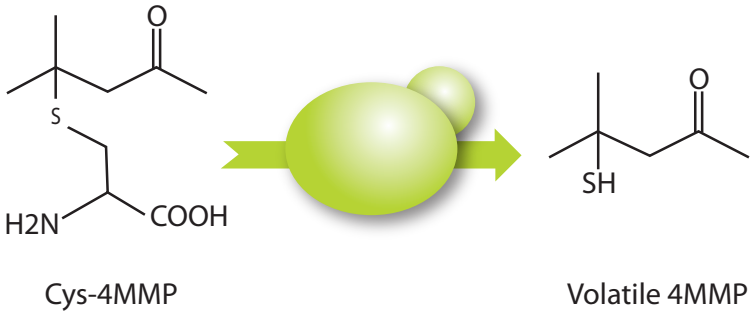

Figure 1. Conversion of odourless precursor into volatile thiol by wine yeast.

Understanding the volatile thiols release by wine yeast - genetic research

The details of all the enzymes involved in the pathway to convert precursors to volatile thiols are still not fully understood. However, it is known that a family of enzymes, the carbon-sulfur lyases, is responsible for the conversion of the precursors for 3MH and 4MMP. Among them, *IRC7* is of particular importance. The gene *IRC7* encodes for an enzyme, a β-lyase that is able to cleave cysteine conjugated precursors. The role of the *IRC7* gene has been highlighted in the formation of 4MMP as *IRC7* encodes for a β-lyase that shows a definite preference for the cys-4MMP conjugate [1, 2, 3]. Most wine yeast possess a version of the gene that is truncated and produces a non-functional protein, $IRC7^{del}$ [1]. This truncated enzyme is not able to cleave the precursors of the volatile thiols. When the yeast possess a full version of the gene ($IRC7^{FL}$), it is able to cleave the precursors of the volatile thiols. Following a genetic study and a screening of our yeast collection, we identified a wine yeast that possesses one "full-length" copy of the gene, $IRC7^{FL}$. However, this gene is not fully expressed in our wine yeast due to the presence of the truncated copy as well. Through our research, we were able to obtain a wine yeast with two full length copies of the $IRC7^{FL}$ gene that can fully express all the potential of this β-lyase enzyme for the release of 4MMP. A new approach based on cellular division for diversity exploration was used where our parental yeast was induced to sporulate to produce a wide diversity of phenotypes. This resulting population was screened for full length copies of *IRC7* using allele specific PCR, allowing us to select a new wine yeast possessing the two full length alleles of the *IRC7* (Figure 2) and consequently a highly functional β-lyase with a strong affinity for the cys-4MMP.

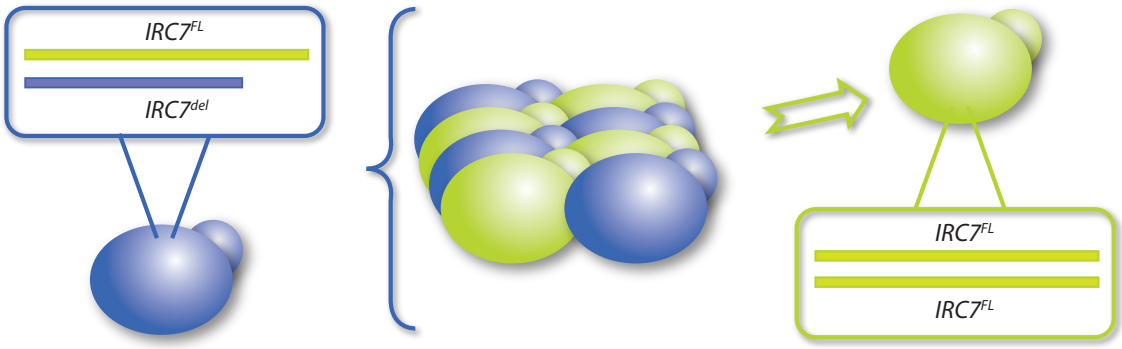

Figure 2. A new method based on cellular division for diversity exploration and selection of a yeast with two full length copies of the *IRC7* gene - Sauvy™.

Increase of 4MMP release

The selected wine yeast is named Sauvy™. The first stage of testing at laboratory scale in Sauvignon Blanc, showed its ability to release 4MMP at a much higher level compared to a control wine yeast (Figure 3). This confirmed the impact of the full length alleles of the gene *IRC7* and the great potential of this new selection.

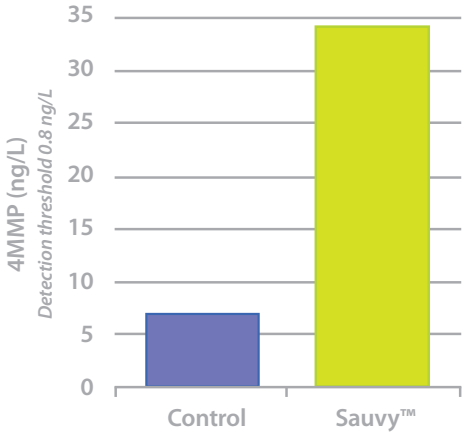

Figure 3. 4MMP concentration in final wines fermented with a control yeast or Sauvy™ (Sauvignon Blanc, 18°C, laboratory scale).

Winery trials

Three years of worldwide winery trials have confirmed its specific capacity to produce volatile thiols, especially 4MMP. Seven trials were performed in 2019 in France, Germany, United States, New Zealand and Chile on Sauvignon blanc (2019) compared to a thiolic yeast known for its ability to produce 4MMP. The final level of this thiol was up to 41% higher on average compared to the control (Figure 4).

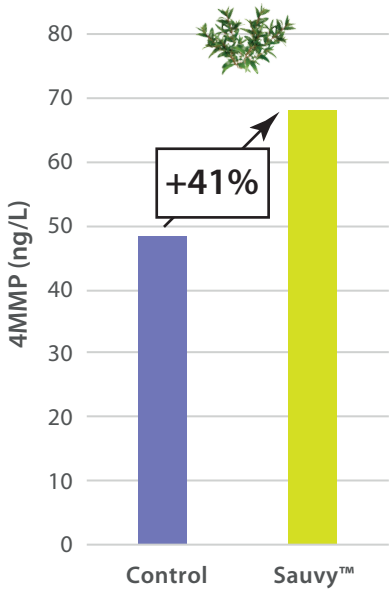

Figure 4. 4MMP released by the thiolic yeast Sauvy™ compared to a reference thiolic yeast (average of 7 trials performed in Sauvignon blanc 2019 from France, Germany, United States, New Zealand and Chile).

In addition to its unique ability to enable optimal release of 4MMP, Sauvy™ showed in the majority of the trials a full and intense thiolic profile. For example, the aromas index based on Odor Activity Value (Figure 5) in a Sauvignon blanc from Gers, France, 2019, showed that Sauvy™ presents a higher vegetal thiolic perception as well as a complex aromatic profile with citrus, tropical and yellow fruits aromas compared to the control. Not only is Sauvy™ showing higher boxtree aromas but also other thiolic aromas perceptions.

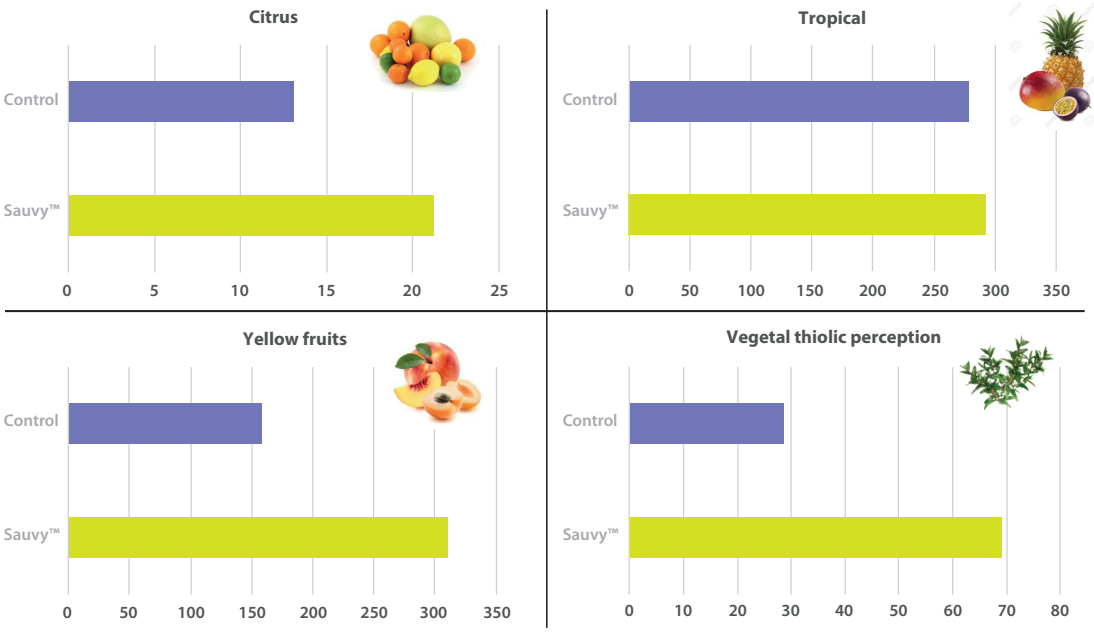

Figure 5. Aromas index based on Odor Activity Value. Comparative trial on a Sauvignon blanc wine (Gers, France, 2019) control vs Sauvy™.

A sensory analysis was conducted by a professional tasting panel (36 tasters) on a Sauvignon Blanc, Bordeaux, France, 2018 and showed a high complexity with a marked "vegetal thiol perception" and "citrus" notes for Sauvy™ compared to the control (Figure 6). The flavor was perceived as fresher with significantly more acidity, and 26 out of 36 tasters preferred the wine fermented with Sauvy™.

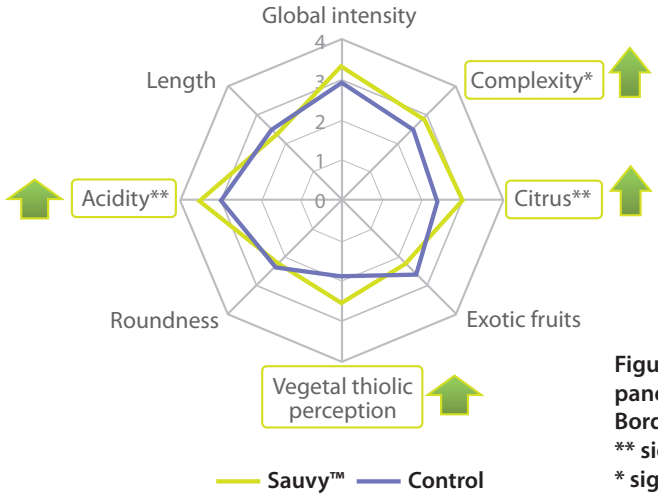

Figure 6. Sensory analysis by a professional panel (36 tasters) on a Sauvignon Blanc, Bordeaux, France, 2018. Average scores:
**** significant at Friedman test at 5%**
*** significant at Friedman test at 10%.**

Furthermore, in all the trials, the volatile acidity was measured at very low levels for the Sauvy™. There was also a very good production of the major esters and 2-phenyl ethyl ethanol, bringing stronger fruity notes to the wines.

The 29 trials conducted at pilot and winery scales under various conditions and in different countries confirmed the good fermentation performance and 4MMP production, especially in high initial YAN conditions and with a good yeast nutrition management.

Different pilot trials were undertaken to have a better understanding of the impact of specific environmental parameters, especially temperature on Sauvy™. Fermenting at low temperature (14°C) or high temperature (18°C) did not significantly affect the fermentation kinetics nor the thiols production, as shown in Figure 7.

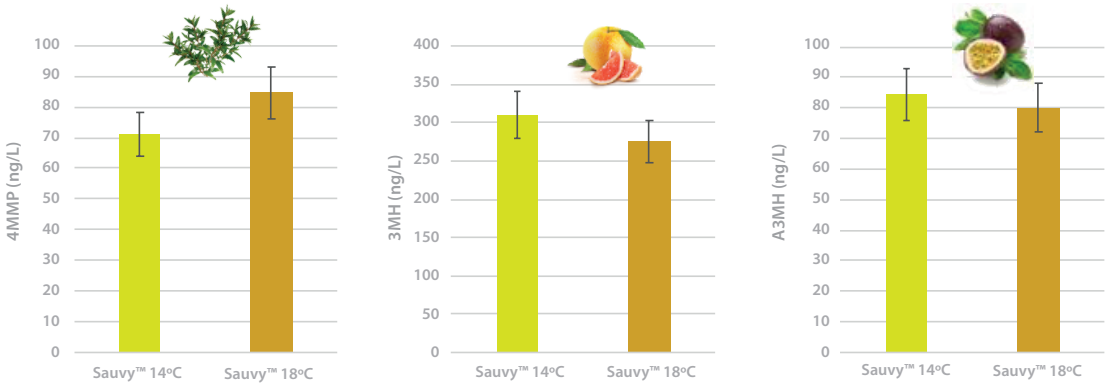

Figure 7. Thiols production for Sauvy™ at two different fermentation temperatures (Sauvignon blanc, Val de Loire France, 2019)

Conclusion

Genetic understanding of volatile thiols release and our expertise in selection and characterization of wine yeasts enabled us to successfully develop a new wine yeast with an optimal release of 4MMP. Sauvy™ shows an exceptional potential for the production of intense and fresh aromatic white wines. Wines fermented with Sauvy™ exhibit typical flavor profiles described as boxwood, gooseberry, tomato leaf, passion fruit, citrus and blackcurrants. Sauvy™ also brings refreshing and crisp mouthfeel.

References

[1] Roncoroni M, Santiago M, Hooks DO, Moroney S, Harsch MJ, Lee SA, Richards KD, Nicolau L, Gardner RC (2011) **The yeast *IRC7* gene encodes a β-lyase responsible for production of the varietal thiol 4-mercapto-4-methylpentan-2-one in wine**. *Food Microbiol*. 28(5):926-935

[2] Thibon C, Marullo P, Claisse O, Cullin C, Dubourdieu D, Tominaga T (2008) **Nitrogen catabolic repression controls the release of volatile thiols by *Saccharomyces cerevisiae* during wine fermentation**. *FEMS Yeast Res* 8: 1076–1086

[3] Santiago M, Gardner RC (2015) **Yeast genes required for conversion of grape precursors to varietal thiols in wine**. *FEMS Yeast Res*. 15(5):fov034