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Co-fermentation with *Pichia kluyveri* increases varietal thiol concentrations in Sauvignon Blanc

By: N. Anfang, M. Brajkovich and M. Goddard

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• Volatile thiols are responsible for the characteristic aroma of Sauvignon Blanc. There are 4 main volatile thiols:
- 4-mercapto-4-methylpentan-2-one (4MMP): *box tree, broom*
- 3-mercaptohexyl acetate (3MHA): *passion fruit*
- 4-mercapto-4-methylpentan-2-ol (4MMPOH): *citrus zest*
- 3-mercaptohexan-1-ol (3MH): *grapefruit*

A comparative study of Sauvignon Blancs from around the world showed that those from Marlborough, New Zealand had significantly higher concentrations of 3MH and 3MHA. These two volatile compounds are considered, therefore, to significantly contribute to the "Marlborough style" Sauvignon blanc.

• Volatile thiols are not present in the grape. Instead, they are released from odorless S-cysteine precursors through the action of the yeast during fermentation. Several strains of the common wine yeast *Saccharomyces cerevisiae* have already been assessed for their ability to produce volatile thiols. However, it is the contention of the current authors that many non-*Saccharamyces* genera (and there are at least 15 involved in winemaking) might also possess this ability.

• To evaluate that possibility, the authors isolated yeast from the early stages of various Chardonnay juices sampled from wineries in West Auckland and North Island, New Zealand, and identified them through DNA sequencing (11 non-*Saccharomyces* isolates, 8 *Saccharomyces* isolates) *[Why not from Sauvignon blanc juices?]*. They also used 7 commercially available *Saccharomyces* strains. Fermentations were conducted in 250 ml Erlenmeyer flasks containing 2005 Sauvignon Blanc juice, kept at either 25°C (to encourage yeast growth) or at 14°C (to better mimic the actual commercial situation). These micro-fermentations consisted of either **pure cultures** (inoculated at 2.5 x 10^6 cells/ml with either a *Saccharomyces* or a non-*Sacharomyces* strain), or **co-ferments** (inoculated with a *Saccharomyces*: non-*Saccharomyces mixture* at either 1:1, 9:1, or 1:9 ratios.

• At the end on fermentation (7 days) the flasks were centrifuged and the supernatants were collected for volatile thiol analysis (dichloromethane extraction, followed by gas chromatograph analysis).
The authors also sampled the flasks at various times during fermentation to track the yeast population dynamics.

• **Thiol production of pure cultures**. All the New Zealand *S. cerevisiae* isolates were able to produce 3MH and 3MHA. In contrast, thiol production was low for most of the non-*Saccharomyces* isolates. However, two non-*Saccharomyces* species were able to produce noticeable amounts of both 3MHA and 3MH: ***Pichia kluyveri*** and ***Candida zemplinina***.

• **Thiol production of co-ferments**. VL3 is a commercial *S. cerevisiae* strain known to be a "good" thiol producer. When the authors co-inoculated VL3 and a non-*Saccharomyces* strain (at about the same ratio, or at a ratio initially favoring the non-*Saccharomyces* strain), they observed significantly higher 3MH production than with VL3 alone. More specifically, **when they inoculated with VL3:*P. kluyveri* at a 1:9 ratio, they found the greatest production of 3MHA.**

• Given these striking results, the authors decided to test *P. kluyveri* with other commercial *S. cerevisiae* strains besides VL3 (VIN7, X5, EC1118, QA23, SVG). They found that the "combos" VIN7:*P. kluyveri* and X5:*P. kluyveri* increased 3MHA production (but not 3MH). In contrast, the "combos" EC1118:*P. kluyveri*, QA23:*P. kluyveri* and SVG:*P. kluyveri* did not increase 3MHA (but they increased 3MH). The detection threshold for 3MHA is 15 times lower than that for 3MH, and therefore, 3MHA exhibits the greatest potential to impact Sauvignon blanc aroma. As we can see, the **thiol production of co-ferments of *P. kluyveri* and *S. cerevisiae* differed according to the *S. cerevisiae* strain.**

• **Population dynamics of co-ferments**. Population sizes for each species were depressed in the co-ferments compared to the single ferments. Yet, the thiol concentration was as much as seven times higher in the co-ferments than in the single ferments. This suggested to the authors that the observed changes in thiol concentration were not solely due to a change in yeast cell numbers. There had to be some other mechanism, even though it was not clear what that was.

In conclusion, the authors showed a significant increase in the Sauvignon Blanc aromatic compound 3MHA in co-ferments of *Pichia kluyveri* and VL3 initiated at a 9:1 ratio compared to VL3 single ferments. Quoting the authors, "if these laboratory observations hold at commercial scale, these co-ferments may provide a tool for winemakers to increase 3MHA in Sauvignon Blanc without using genetically modified organisms". The authors do admit that many questions remain to be answered, such as the nature of these inter-strain interactions (not all strains were effective), as well as the impact on other aroma compounds (there is more to SB aroma than 3MH and 3MHA).

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