Indole accumulation in sparkling wine: yeast preparation may be just as important as yeast strain selection

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INTRODUCTION

- **Indole** has been linked to '**untypical aging off-flavour' in wine** and above its aroma detection threshold (23 µg/L, Carpone *et al.* 2010) has been described to impart a '**faecal', 'plastic', 'chemical/rubber', 'mothball,' or 'styrene' character.**
- **Indole in wine was originally believed to originate from glycosidic forms in grapes** (Zoecklein *et al.* 1995). However **more recent research has linked its formation to yeast during fermentation** through the metabolism of tryptophan (Arevalo-Villena *et al.* 2010).
- **Indole accumulation is hypothesised to be linked to sub-optimal yeast vitality as it is often correlated to 'sluggish' or 'stuck' fermentation** (Coulter *et al.* 2007).
- Arevalo-Villena *et al.* 2010 showed that **different *Saccharomyces* and non-*Saccharomyces* yeasts had varying ability to produce indole during primary fermentation**, however no such studies had been conducted for secondary fermentation, despite indole off-flavours being common in sparkling wines.

➢ **The purpose of this research was to demonstrate the importance of both yeast strain selection and yeast preparation during sparkling production to limit the production of indole in sparkling wine.**

References

Arevalo-Villena, M.; Bartowsky, E.J.; Capone, D.; and Sefton, M.A. (2010) Production of indole by wine-associated microorganisms under oenological conditions. Food Microbiology 27:685-960.
Capone, D.L.; Van Leeuwen, K.; Pardon, K.H.; Daniel, M.A.; Elsey, G.A.; Coulter, A.D.; Sefton, M.A. (2010) Identification and analysis of 2-chloro-6-methylph2,6-dichlorophenol and indole - causes off taints and off-flavours in wines. Australian Journal of Grape & Wine Research 16:210-217.
Coulter, A.D.; Capone, D.L.; Baldock, G.A.; Cowey, G.D.; Francis, I.L.; Hayasaka, Y.; Holdstock, M.G.; Sefton, M.A.; Simos, C.A.; Travis, B. (2008) Taints and off-flavours in wine case studies of recent industry problems. In: Blair, R.J.; Williams, P.J.; Pretorius, I.S. (Eds.), Thirteenth Australian Wine Industry Technical Conference. Australian Wine Industry Technical Conference Inc., Adelaide, South Australia, pp.73e80.
Zoecklein, B.W.; Fugelsang, K.C.; Gump, B.H.; Nury, F.S. (1995) Wine analysis and production. Chapman and Hall, New York

MATERIAL AND METHODS

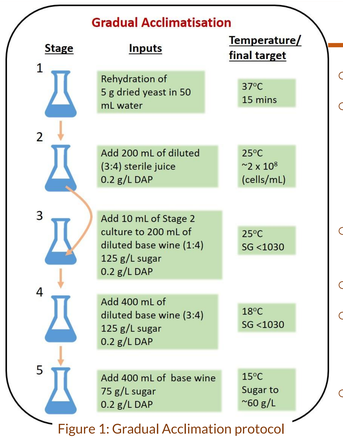

Figure 1: Gradual Acclimation protocol

PHASE 1: GRADUAL ACCLIMATATION PROTOCOL (GA)

- **Small-scale trial at AWRI** Commercial Services laboratories.
- **Comparison of 3 commercial strains**: 2 *Saccharomyces cerevisiae* strains (A & C) and 1 *Saccharomyces bayanus* strain (B). A and B are commonly used in sparkling production. Links between Strain B and indole in sparkling wine had been previously suggested by industry (personal communication). Strain C had been shown to produce indole during primary fermentation in the study of Arevalo-Villena *et al.* (2010).
- Dried yeasts were rehydrated and gradually acclimatised to wine conditions following protocol summarised in Figure 1.
- Small-scale **secondary fermentation** using the **Charmat method** in 9.5L.
- 160 mL of acclimatised yeast was pitched into 8 L of **Chardonnay base wine** (11% ABV, pH 3.3 and sugars of 22 g/L) when the sugar concentration of the propagated cultures reached approx. 60 g/L.
- Tryptophan (100 mg/L) was added to the base wine to ensure that this indole precursor was not limiting.
- Fermentations were performed in duplicate at 17°C and the pressure within each vessel was monitored daily.
- Samples were collected approximately every 4 days:
  - ✓ **Total and viable cell counts** using flow cytometry and propidium iodide staining.
  - ✓ **Indole concentrations** using GC followed by MS.
  - ✓ **Sugar** monitored by Clinitest tablets then by the Rebelein method. Fermentation complete when sugar ≤ 2 g/L.

PHASE 2: DIRECT REHYDRATION PROTOCOL (DR)

- The previously reported link between Strain B and indole 'off-flavours' in sparkling wine were using a much more rapid yeast propagation protocol compared to that used in Phase 1 of this study.
- A more 'Direct Rehydration' protocol described in Figure 2, was adopted in Part 2 to compare with Part 1 results on yeast total and viable cells.

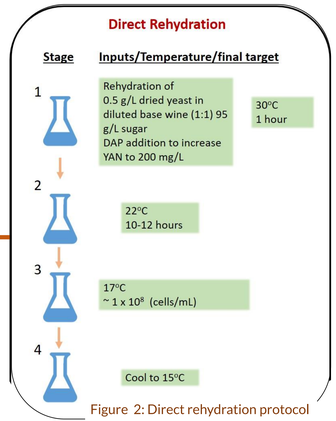

Figure 2: Direct rehydration protocol

RESULTS

PHASE 1 : YEAST VARIATION IN THE PRODUCTION OF INDOLE DURING SECONDARY FERMENTATION

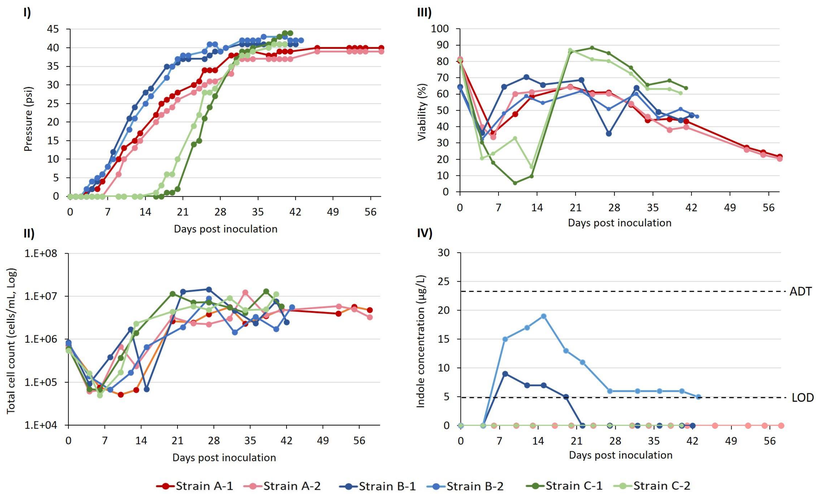

Figure 3: Comparison of duplicated secondary fermentations and indole production using the three Saccharomyces strains A, B and C with GA

- **CELL COUNT & VIABILITY :**
  - All cell populations had large dips in viability between 0-5 dpi (days post inoculation).
  - Strain A and B populations increased quickly after this and reached 58 – 70% by 8-13 dpi
  - The viability of Strain C continued to decrease after 4 dpi and did not recover until after 13 dpi. The long lag phase of this yeast may suggest that it is not as robust as other strains under these conditions (secondary fermentation).
- **INDOLE PRODUCTION**:
  - Despite the clear stress exerted on Strain C yeast populations, and the known ability for this strain to produce indole during primary fermentation, this compound was not detected in these wines.
  - Only Strain B ferments contained indole above the Limit of Detection (LOD, ≥5 µg/L). These levels spiked between 5-20 dpi, following the dip in the culture viability at 5 dpi and accompanying the regrowth. It should be noted, however, that indole levels produced by Strain B were below the aroma detection threshold (23 µg/L) in finished wines and did not correlate to tryptophan consumption (data not shown).

➢ Both Strains A and C had overall lower viability than Strain B during this part of the ferment, however no indole was detected in any of their wines. This suggests that while culture vitality may contribute to the production of indole in wine, there might also be predispositions in some yeast strains, including Strain B, which increases their indole production potential.

PHASE 2 : EFFECT OF YEAST PROPAGATION METHODS ON CULTURE VIABILITY

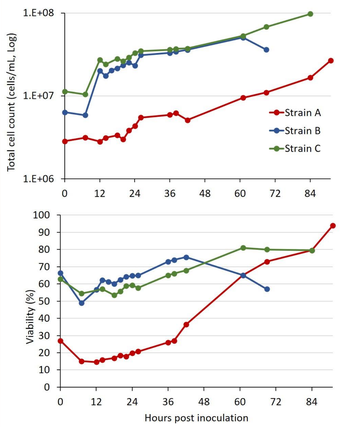

Figure 4: total cell count and viability of the 3 strains with DR method

- We hypothesised that the viability of a culture would be reduced with a less robust rehydration and acclimatisation and therefore could increase indole accumulation risks.
- **Each strain responded differently to the DR protocol** (Figure 4). Max cell viability was reached at different times. Strain A began with the lowest viability and took the longest time to reach its maximum, but once reached this figure was better then B&C. The highest viability of B&C were reached earlier during fermentation and before maximum cell counts. Their viability then regressed. Target cell density was almost reached for C but not at all for B.
- **Final cell counts, viability and total propagation lengths were then compared between yeast preparation protocols from Phase 1&2** (Figure 5). For all strains, the GA method took significantly longer compared to DR (approx. +1 week). For Strain A, final cell counts were slightly higher at the end of GA but final cell viability was significantly greater after DR. Strain C also performed better on all fronts using this method. In contrast, Strain B had both higher final cell counts and significantly higher viability when prepared using the GA protocol.

➢ **This data illustrates that a 'one-protocol fits all' approach to yeast propagation for secondary fermentation is not appropriate.** A rapid protocol such as DR is time and cost effective and so optimal for robust yeast strains. Other strains may require a gentler strategy to obtain the highest level of vitality.

➢ **Many wineries rely on cell counts alone to assess propagation cultures. This study shows that max cell counts do not necessarily correlate to optimal culture vitality. Understanding how each selected yeast strain behaves during propagation will be important to ensure appropriate pitching targets are set.**

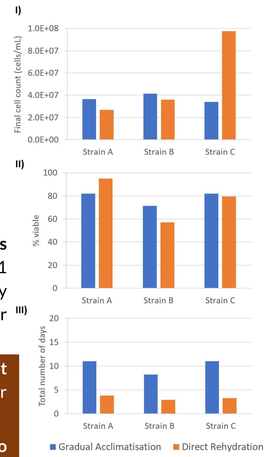

Figure 5: Comparison of total cell count, viability and propagation time for the 3 strains and 2 methods (GA & DR)

CONCLUSION

- This **study agrees with previous bodies of work** that suggests that **some yeast strains may have natural predispositions to indole production during fermentation** compared to others.
- **Our data also shows links to increased indole accumulation when yeast population viability decreased, further supporting claims that indole production is linked to culture vitality**. It is not surprising, therefore, that indole off-taints in sparkling wine can often arise considering the additional stresses imparted on yeast during secondary fermentation (i.e. high alcohol, increasing barometric pressure, low pH and low nutrient levels).

➢ **Both yeast strain selection and the methods used to propagate and acclimatise the yeast are equally important considerations.**

➢ **Some strains of yeast may be suitable for sparkling wine production** and impart unique and desirable characters to the wine **but may also require a more robust preparation method to ensure optimal performance.**