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Title: **"Rehydration protocols for active dry wine yeast and the search for early indicators of yeast activity"**

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The authors study which conditions are best to rehydrate dry wine yeast to recover the highest possible fermentation activity.

• Active dry yeast contains about 8% water, which is insufficient for active metabolism. So rehydration is a necessary step before inoculation into a grape must. Rehydration conditions can influence the viability and physiological state of the yeast. They can also modify their fermentation behavior, particularly the length of the lag phase. As we know, a long lag phase presents an opportunity for indigenous yeast to become predominant.

• Current rehydration procedures are over 15 years old and, in the opinion of the authors, they might not be optimal for the approximately 200 strains of yeast commercially available worldwide. In this study the authors examine the effect of 5 rehydration conditions (temperature, water hardness, sugar concentration, agitation, and rehydration duration) on the recovery of fermentation activity by various common yeast strains (K1, CEG, EC1118, V116, VL1, CSM, D254, D47, and QA23, all manufactured by Lallemand).

• The authors used a synthetic medium to simulate a standard grape juice using 1 liter fermentors. Inoculation rate was 25 g/hl (5×10^6 cells/ml). The authors looked at 3 levels (low, medium, and high) of each of the factors in their study (temperature, hardness, sugar, agitation, and duration), dividing them into two experimental designs. In the first design ("a"), low and medium levels were compared; in the second design ("b"), medium levels were compared against high levels. The standard rehydration protocol, or control, was: "**1 g of active dry yeast to 10 ml of glucose solution (50g/l) at 37°C for 30 minutes**". The authors monitored the recovery of fermentation activity by tracking *dissolved CO2 concentration* (with an ion-selective electrode, confirmed by automatic tracking of the weight loss of the fermentor).

• **Optimal rehydration parameters. (**In this part of the experiment, the authors concentrate on the first three strains: K1, CEG and EC1118). The authors found that the standard conditions (37oC, 30 min, 50 g/l glucose) were almost optimal for all these three strains. Agitation and duration made little difference, with the exception of strain EC118 for which increasing the duration from 30 to 60 minutes had a very significant positive effect. Water hardness had a negative effect on two of the three strains (K1 and CEG). Increasing the temperature from 27 to 35oC had a positive effect for all strains (but increasing the temperature from 35 to 43oC had a negative effect). Increasing sugar concentration had a positive effect for all strains.

• Thus, sensitivity to rehydration conditions appeared to be strain-specific. K1 was little affected by changes in rehydration conditions, whereas CEG and EC1118 strains were more sensitive.

• **Early indicators of yeast activity.** In the brewing industry, there are a set of parameters that have been identified as useful to estimate the physiological state of pitching yeast. But these cannot be applied to wine yeast. In an effort to develop a similar set of standards, the authors next evaluated the ability of various indicators to predict recovery of fermentation capacity. Of the indicators studied, the authors found that *CO2 production* and *medium acidification* (lowering of pH) were excellent indicators of the recovery of fermentative capacity in yeast; whereas *duration of the lag phase* and *trehalose utilization* were less useful indicators. (Trehalose is a sugar that yeast release in great quantities during rehydration).

In conclusion, dissolved CO2 concentration was a good way to quantify the ability of strains to recover their fermentation capacity after rehydration. Currently, the most frequently used method for this purpose is quantifying yeast viability under the microscope, but as the authors note, "viability and vitality are different concepts, and vitality is clearly the most relevant". The authors also found that variations in rehydration conditions had less effect than expected on the recovery of fermentative capacity. As a consequence, the authors suggest that it is unnecessary to develop optimal rehydration conditions for each commercial strain, given that "one protocol fits all". These results have practical implications for both winemakers and yeast producers.

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