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"Inhibition of malolactic fermentation by *Saccharomyces* during alcoholic fermentation under low- and high-nitrogen conditions: a study in synthetic media"

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This paper brings our attention to the fact that the nutrient level of a juice or wine has an impact on the inhibition of malolactic fermentation by *Saccharomyces*. The mechanism seems to be the production by *Saccharomyces* of varying amounts of SO_2, depending on whether the nitrogen in the medium is high or low.

- Malolactic fermentation (MLF), performed by the bacteria *Oenococcus oeni*, can become sluggish due to the presence of several factors: 1) low pH, 2) high ethanol, 3) inadequate temperature, 4) inadequate nutrients and/or, 5) competition from the wine yeast *Saccharomyces*. Originally, researchers believed that the mechanism behind *Saccharomyces* interference with *Oenococcus* was the removal by the faster-growing yeast of nutrients important for the bacteria. But, according to this study, this may not be the case, and a more acceptable explanation is that the yeast produce metabolites toxic to the bacteria.

- Some of the yeast metabolites that are toxic to the bacteria include: 1) ethanol, 2) SO_2, 3) medium-chain fatty acids, and 4) antibacterial peptides. Among these, **SO_2**, a well-known antimicrobial, is the most MLF-inhibiting factor of all. It is interesting to note that most strains of *Saccharomyces cerevisiae* are able to produce between 10 and 30 mg/l SO_2 during alcoholic fermentation. Some are even able to produce as much as 100 mg/l ! This fluctuation is often accounted for by variations in the concentration of sulfate, cysteine and methionine, oxygen, sulfite-binding compounds, insoluble solids, and assimilable nitrogen in the juice, as well as genetic factors.

- Because much is known about the effect of **assimilable nitrogen** on *sulfide* production, but little about its effect on *sulfate* production (despite the fact that sulfides and sulfates are in the same metabolic pathway), the authors decided to investigate 1) the influence of nitrogen on SO_2 production, and 2) the effect of the SO_2 produced by *Saccharomyces* on malolactic fermentation.

- Alcoholic fermentations (5 liters) using a synthetic grape juice were set-up, in triplicate, to explore the influence of 2 levels of yeast assimilable nitrogen (60 and 250 mg/l) and 6 yeast strains (Saint Georges S101, UCD 522, EC1118, RUBY.ferm, UCLM S325, and V 1116). This is called a 2 x 6 factorial design. For each fermentation, the authors measured: 1) yeast viability by plating (wort agar), and 2) SO_2 production by titration with iodine.

- For the malolactic fermentations, the authors took 100 ml samples from the alcoholic fermentors at gradual intervals, and after sterile-filtration, inoculated them with *Oenococcus oeni* to see the effect that each yeast strain/nitrogen combination might have had on the bacteria growth. Once again, for each fermentation the authors measured: 1) bacterial viability by plating (Man, Rogosa and Sharpe agar), and 2) MLF progression by determining malic acid enzymatically.

- **Effect of yeast strain on SO_2 production.** Despite no difference in yeast growth curves throughout the fermentation (all strains achieved large populations ~10^7 CFU/ml~ on Day 2), yeast strains produced varying amounts of SO_2. V1116 produced the highest amount. Saint Georges S101 produced the lowest.

- **Effect of nutrient level on SO_2 production**. All but one of the yeast strains produced significantly higher SO_2 under high nitrogen conditions than under low nitrogen conditions. (Saint Georges also produced higher SO_2 at high nitrogen levels, but the difference was not significant).

- **Effect of yeast strain on MLF**. Strains UCD 522, RUBY.ferm, UCLM S325, and V1116 inhibited MLF, while strains Saint Georges S101 and EC1118 did not. This is consistent with previous studies, which lead to the description of wine yeast as "malolactic-friendly" (the latter group above), or "malolactic-unfriendly" (the former group).

- **Effect of nutrient level on MLF**. Besides the strain, the inhibition of MLF was also influenced by the nitrogen status of the grape juice. This is because some yeast strains (UCD 522, RUBY.ferm, and UCLM S325) inhibited MLF *only* during fermentation in high-nitrogen grape juice. V1116 was the only yeast strain showing MLF inhibition at both low and high nitrogen levels (very MLF unfriendly!). All of the above results led the authors to believe that the influence of high nitrogen concentration on the inhibition of MLF was linked to the production of high SO_2 under the high nitrogen conditions.

- To further explore this point, the researchers did something interesting. For two representatives of the "unfriendly" yeasts, they took low-nitrogen fermentations (which had produced lower SO_2 than the corresponding high-nitrogen ones), and added exogenous SO_2 to the same level of SO_2 present in the high-nitrogen fermentations. Then they inoculated with *O. oenis* and monitored malate consumption as before. What they found was that **MLF was also inhibited by yeast growing under low nitrogen conditions provided that exogenous SO_2 had been added to a specific level** So the SO_2, not the assimilable nitrogen, was the main culprit.

- Even though SO_2 production by the yeast, and the influence of nitrogen on its production, were able to account for most of the bacterial inhibition observed, **a correlation between SO_2 production and *O. oeni* inhibition was not always present**. This proved to the authors that unknown mechanisms other than SO_2 must also exist. Finally, very low free-SO_2 was measured at any stage, suggesting that the bound form of SO_2 was responsible for the inhibitions.

In summary, some *Saccharomyces* strains (like UCD 522, RUBY.ferm, and UCLM S325) can produce sufficient SO_2 to inhibit malolactic fermentation by *Oenococcus oeni*, particularly under nutrient-rich conditions. The inhibitions observed in this study happened even when the bacterial inoculations took place late in the primary fermentations, after yeast had been completely eliminated by filtration, so the results would still be relevant when MLF is timed after completion of alcoholic fermentation. The message to the winemaker is that, if MLF is to be encouraged, both the yeast strain and the nitrogen content of the juice should be considered.

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