Summary 75

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"Influence of different maceration techniques and microbial enzymatic activities on wine stilbene content"

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The authors quantify resveratrol levels in wines made using different red vinification methods, and study the role played by wine yeast and malolactic bacteria on final resveratrol concentrations.

• Stilbenes are a class of phenolic compounds that are produced by grape leaves and berries in response to stress, including attacks of downy mildew, powdery mildew or oidium, and bunch rot or gray rot.

• In the last years, stilbenes have gained a lot of attention due to their implication in human health, including their anticancerous properties. The most well-known stilbenes in wine are *cis-* and *trans-resveratrol.* Other stilbenes found in wine include *cis- and trans-piceid* (glucosylated derivatives of resveratrol).

• Wine stilbene concentration is influenced by many factors, including climate, grape variety, UV light, winemaking method, and microbial enzymatic activities. In this paper, the authors chose to study the last two factors.

• They harvested Merlot grapes (23°Brix, 5.4 g/l TA, 3.32 pH) and fermented them in triplicate in 30 liter plastic containers (~8 gallons), using *S. cerevisiae* Lalvin L2056, and one of the following winemaking methods:
1) **traditional**: 8-day fermentation at temperatures between 25 and 28°C (75-82°F);
2) **traditional plus enzymes**: same as above but with added Vinozyme pectolytic enzyme (2.5 g/100 kg) (0.8 oz/ton);
3) **cold maceration**: must initially held for 60 hours at 7°C followed by 25-28°C to completion; and
4) **heat treatment**: 7-day fermentation at temperatures between 25-28°C followed by 24 hours at 50°C (122°F).

• The authors analyzed the resulting wines for basic chemistry and phenolic composition. *Color intensity*, *hue*, and *total phenols* were measured by absorbance (A420+A520, A420/A520, and A280, respectively), *anthocyanins* by decoloration with sulfur dioxide, and *tannins* by acid hydrolysis. Finally, they analyzed *stilbenes* by HPLC.

• **Influence of winemaking method on wine composition**. The kinetics of alcoholic fermentation were shown to be similar for all the methods studied, except for the cold maceration. As expected, primary fermentation in the cold maceration treatment was delayed by 3 days. Juice yields were highest for the enzymatic treatment, and lowest for the heat treatment [*evaporation?*]. The kinetics of ML fermentation were consistent for all treatments. Color intensity was shown to be similar across all methods, but the authors did find differences in hue (A420/A520), which was significantly lower for the cold maceration. After an initial delay in phenolic extraction in the cold maceration treatment, this wine soon caught up with the rest, and the final phenolic extraction (as measured by A280) was the same for all treatments. The authors believe the heating process had no effect on additional phenolic extraction because the maximum potential for extraction had already been reached by the time the heat was applied, after 7 days.

• **Influence of winemaking method on stilbene concentration**. After an initial delay in piceid extraction, the cold maceration wines had the highest final concentration of both cis- and trans- piceids (8.6 mg/l for cis- and 7.8 g/l for trans-piceid). As for cis- and trans-resveratrol, their extraction also suffered a delay in the cold maceration treatment, but levels increased constantly throughout fermentation to reach a maximum at the end which was the same for all wines (6.5 mg/l). When the authors compared the extraction curves of total phenols and trans-resveratrol for each of the treatments, they noticed they had the same shape within each treatment, indicating the importance of ethanol and temperature on the extraction of both, and more importantly, that the extraction of resveratrol is subjected to the same "forces" as the extraction of anthocyanins and tannins.

• **Influence of wine microorganisms on stilbene concentration**. The authors noticed that the levels of resveratrol decreased for all wines after ML fermentation. They hypothesized that this decrease could be linked to the enzymatic activity of lactic acid bacteria. To verify this hypothesis, they incubated yeast (Y) and bacteria (B) separately and isolated "supernatant" (S) and "pellet" (P) fractions of each, to come up with 4 different preparations (which they called YS, YP, BS, and BP). After incubating a sample of red wine with each preparation, the authors examined their influence on the stilbene levels. They found that the yeast pellets (YP) lowered the concentration of both stilbenes in the wines, probably due to adsorption of these compounds to the yeast cells. But it was the bacterial pellet fraction that showed the most effect, significantly lowering the levels of both piceids, and significantly increasing the levels of both resveratrols. The authors attribute this latter effect to the ß-glucosidase activity of *Oenococcus oeni*.

In conclusion, all the winemaking methods compared yielded similar levels of resveratrol, as was the case for anthocyanin and tannin levels. The authors also demonstrate that lactic acid bacteria can impact both resveratrol and piceid levels in wines. So, in this study, it would appear that no health advantage was imparted by any particular winemaking extraction method.

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