VITICULTURE & ENOLOGY
UNIVERSITY OF CALIFORNIA DAVIS

"Formation of hydrogen sulfide and glutathione during fermentation of white grape musts"

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In: Am. J. Enol. Vit. 51(2)91-97. 2000

Funded by: American Vineyard Foundation

In a previous article, the authors studied the impact of juice nitrogen status on the production of *sulfites* (SO_2) by yeast (*Summary 70*). The current authors study the influence of nitrogen on another important – and undesirable - component of wine: *sulfides* (H_2S).

• Hydrogen sulfide is the main volatile sulfur compound responsible for the all-too-familiar smell of "rotten eggs" in wines. Factors that affect the formation of hydrogen sulfide during fermentation include: 1) elemental sulfur, 2) yeast strain, 3) free amino nitrogen level, and 4) fermentation conditions such as temperature and redox potential Another potential factor is 5) the tri-peptide *glutathione*, since one of its amino acids –cysteine- is a precursor of H_2S.

• Currently, sulfur additions in the vineyard are carefully monitored to avoid late applications; fermentation nitrogen deficiencies are routinely corrected with diammonium phosphate or equivalent nutrients; and low H_2S-producing yeast strains are carefully selected. Still, we get the headache of H_2S problems during wine fermentation more often than we'd like.

• So the authors decided to evaluate the formation of volatile and non-volatile sulfur compounds during the fermentation of 8 different lots of white grape musts. The main **volatile sulfur compounds** are *H_2S*, *SO_2*, *ethanethiol*, and *dimethylsulfide*. The main **non-volatile sulfur compound** studied was *glutathione*. The grape types studied included: Thompson Seedless, Palomino, Chenin blanc, Sauvignon blanc, and Chardonnay.

• Musts (650 ml) were inoculated with *S. cerevisiae* Montrachet and allowed to ferment at 23^{o}C (73^{o}F), which is on the warm side for a white. For each of the 8 fermentations, the authors removed 3ml samples daily for analysis. Non-volatile sulfur compounds were analyzed by high pressure liquid chromatography (HPLC). Volatile sulfur compounds were analyzed by HPLC/mass spectrometry using the headspace above the fermentations as their sample, and identifying the compounds by comparing their retention times with commercial standards.

• To be able to correlate sulfur-compound formation with nitrogen status, the authors measured the following, besides other standard parameters of the original juice: 1) glucose and fructose (HPLC), 2) amino acid content (HPLC/fluorometry), 3) total nitrogen (Carlson ammonia analyzer), and 4) yeast biomass (absorbance). Finally, by adding up the amounts of those amino acids readily utilized by the yeast, the authors measured a last useful parameter, 5) "easily assimilable amino acids" or EAA. *[In case you wonder about the relationship between EAA and YAN (Yeast Assimilable Nitrogen), this later measurement also includes ammonia (NH_4^+)].*

• **Production of H2S**. Even though H_2S, SO_2, ethanethiol and dimethylsulfide were all detected during the fermentations, only H2S was produced throughout the fermentation. **Final H_2S was highest in those juices lowest in assimilable amino acids**. This was due to the fact that high-EAA musts (musts with sufficient assimilable amino acids) reached a high H_2S concentration during rapid fermentation, and then decreased towards the end. In contrast, low-EAA musts (musts deficient in assimilable amino acids) also produced H_2S in high concentrations during rapid fermentation, but production continued to increase until the end.

• **Production of glutathione**. Glutathione was the main non-volatile S-compound found both during fermentation and in the finished wine. Must and wine glutathione were highest in those musts with the highest EAA and highest total nitrogen. The authors believe that, to fully understand the production of hydrogen sulfide, glutathione deserves further study. This is because of the potential of one of its components (cysteine) to form H_2S, as well as the potential ability of glutathione to reduce elemental sulfur to H_2S.

• **Classification of juices.** Based on assimilable amino acid levels, the authors classified juices in 4 groups: extremely high (>2000 mg/l), high, intermediate, and low (<500 mg/l). Of the juices studied, Palomino fell in the first class; one of the Chardonnays fell in the last class; and the remaining juices (Thompson Seedless, Chenin blanc, both Sauvignon blancs, and the second Chardonnay) anywhere in between (with EAA levels from 500-1400 mg/l). **Musts low in EAA took longer to ferment and had higher H_2S levels**, both during and at the end of fermentation. The authors also found that **"easily assimilable amino acid" is a better predictor of H_2S production than "total nitrogen".**

• Surprisingly, even though the authors were able to measure H_2S in all of the fermentations, they could not detect an associated sulfide odor in any of them at the end of the trial! The authors attribute this to the fact that the small-volume fermentations were rather warm (23°C) and continuously stirred, which would have emphasized evaporation and loss of any volatile compounds formed.

In summary, total production of H_2S in eight white grape juices was inversely related to the levels of assimilable amino acids. Deficiency in assimilable amino acids may be a major factor in the formation of H_2S, and juices with low levels (<1000 mg/l) should be corrected.

Author: Bibiana Guerra, Editor: Kay Bogart. This summary series funded by J. Lohr Vineyards & Wines.