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Impact of Fermentation Rate Changes on Potential Hydrogen Sulfide Concentrations in Wine

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The correlation between alcoholic fermentation rate, measured as carbon dioxide (CO_2) evolution, and the rate of hydrogen sulfide (H_2S) formation during wine production was investigated. Both rates and the resulting concentration peaks in fermentor headspace H_2S were directly impacted by yeast assimilable nitrogenous compounds in the grape juice. A series of model fermentations was conducted in temperature-controlled and stirred fermentors using a complex model juice with defined concentrations of ammonium ions and/or amino acids. The fermentation rate was measured indirectly by noting the weight loss of the fermentor; H_2S was quantitatively trapped in real-time using a pre-calibrated H_2S detection tube which was inserted into a fermentor gas relief port. Evolution rates for CO_2 and H_2S as well as the relative ratios between them were calculated. These fermentations confirmed that total sulfide formation was strongly yeast strain-dependent, and high concentrations of yeast assimilable nitrogen did not necessarily protect against elevated H_2S formation. High initial concentrations of ammonium ions *via* addition of diammonium phosphate (DAP) caused a higher evolution of H_2S when compared with a non-supplemented but non-deficient juice. It was observed that the excess availability of a certain yeast assimilable amino acid, arginine, could result in a more sustained CO_2 production rate throughout the wine fermentation. The contribution of yeast assimilable amino acids from conventional commercial yeast foods to lowering of the H_2S formation was marginal.

Keywords: H_2S formation, CO_2 evolution, fermentation rate, wine production, DAP, *Saccharomyces*

The evolution of hydrogen sulfide and other reduced volatile sulfur components during the winemaking process has plagued vintners throughout the centuries and remains one of the most common problems of modern winemaking. Elemental sulfur residues from vineyard sprays against fungal diseases continue to be of warranted concern to winemakers as a precursor to enzymatic and non-enzymatic H_2S formation. However, the more widespread occurrence of the H_2S problem, even under cautious viticultural practices, and low (less than 2 mg/l) sulfur residues suggest that ubiquitous juice sulfates and sulfites are the main source of H_2S production [29]. Natural sulfate concentrations in juices vary widely (70 to 3,000 mg/l) [19], whereas the concentration of sulfites added at the crusher ranges commercially from 0 to about 75 mg/l depending on the quality of incoming fruit.

Exploring the causes and conditions for H_2S production rather than providing post-fermentation remedies has been the approaches over the past decade [4, 9, 21, 23–26, 29]. It has focused on juice composition and nutrient status, with special emphasis on yeast assimilable nitrogen and the role and relative ratios of individual nitrogen sources. It reconfirms the view that general recommendations for nitrogen supplementation regarding the prevention of sluggish or stuck fermentations [8, 10, 11, 17, 22, 30–32] do not automatically imply their preventative role in H_2S formation.

The role of amino and ammonium assimilable nitrogen on H_2S formation showed a high variability due to the strain of both general nitrogen requirements and H_2S production [12–16, 30]. Recently, the enzymatic pathway of H_2S formation and the genetic expression of the enzymes have been investigated [7, 16, 18, 27, 28] and concluded that the individual yeast strain's status of repression of the enzymes of the sulfate reductase sequence (sulfate transport, adenylyl sulfate, phosphoadenylyl sulfate, sulfite) or of the sulfite reductase step (sulfite, sulfide) will determine to what degree of sulfate or sulfite might be the source for H_2S formation under enological conditions. Consequently,

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juice composition has the most dramatic impact on the expression of the enzyme systems leading to H_2S formation. Nevertheless, consumption of sulfide downstream of its formation in the sulfate reduction pathway by yeast may be equally important. Specifically, the production of *S*-adenosylmethionine (SAM) and its function in the synthesis of cell membrane phospholipids as a survival factor in high sugar/high ethanol grape juice fermentations appears to be another link between H_2S and stuck fermentation problems [27, 28]. In addition, the regulatory effect of diammonium phosphate (DAP) additions were described on the expression of a variety of genes including those that are directly involved in the pathways to H_2S production [17].

Bely *et al*. [5] investigated the importance of initial assimilable nitrogen content on the kinetics of alcoholic fermentation and the effectiveness of ammonium additions, but the impacts of juice nitrogen composition on fermentation kinetics, gas evolution, and H_2S production have not yet been explored to their fullest extent. Two or more peaks of H_2S concentrations above sensory threshold in the fermentor headspace can be observed under commercial and experimental conditions during the course of many fermentations [29, 30]. The first peak and its disappearance after DAP additions can be explained by yeast's satisfied demand necessary for the cell to synthesize the sulfur-containing essential amino acids, cysteine and methionine [4, 15]. However, subsequent H_2S peaks are more relevant to residual sulfides in the eventual wine and cannot be easily explained, or corrected by DAP additions [26, 30]. Commercial yeast foods are often added by winemakers to prevent fermentation problems. However, their specific composition and contribution with respect to yeast assimilable amino nitrogen, especially free amino acids, is usually not provided to the user. During the course of standard grape juice fermentation starting at around 24 Brix, the amount of CO_2 leaving the fermentor is equivalent to roughly 60 times the juice volume. This provides a significant stripping effect for the most volatile odor components such as H_2S, which has a boiling point of −60°C with a sensory threshold of 1 mg/l. For winemakers, the important concentration of H_2S is the one left in the wine at the end of fermentation, not the total of all H_2S that was produced during different stages of a particular fermentation. Linear regression of published data (Fig. 1 and 2) showed a general negative correlation between juice arginine concentrations and total H_2S formation as well as residual H_2S concentration in wine [22]. This study investigated the relationship between fermentation rate changes and rate of H_2S evolution and subsequently the final H_2S concentration in wine based on the initial juice assimilable nitrogen composition.

Materials and Methods

Model Grape Juice

The fermentation medium was based on the Australian Wine Research Institute's synthetic model juice [12] but with a defined combination of nitrogenous compounds added. The model juices used in the experiments presented here contained either 210 mg N/l from DAP (equivalent to 960 mg/l of DAP, the legal limit for DAP additions in the USA), or 105 mg N/l as DAP plus 105 mg N/l as arginine (equivalent to 1,305 mg arginine/l, conservatively assuming the utilization of one primary amino group per arginine molecule). The standard concentration of sulfate was 480 mg/l, and 50 mg/l sulfur dioxide was added prior to inoculation.

Commercial Yeast Foods

To assess their contribution of yeast assimilable nitrogen, preparations were dissolved in a base model juice at a recommended rate of 2 lb/1,000 gal, which was equivalent to 240 mg/l. NOPA [11] analysis for yeast assimilable amino acids, and an enzymatic ammonia analysis (Ammonia Assay Kit; Sigma-Aldrich, St. Louis, MO, USA) were performed in triplicate on all samples. To verify complete extraction of all yeast assimilable nitrogen, the suspended preparations were

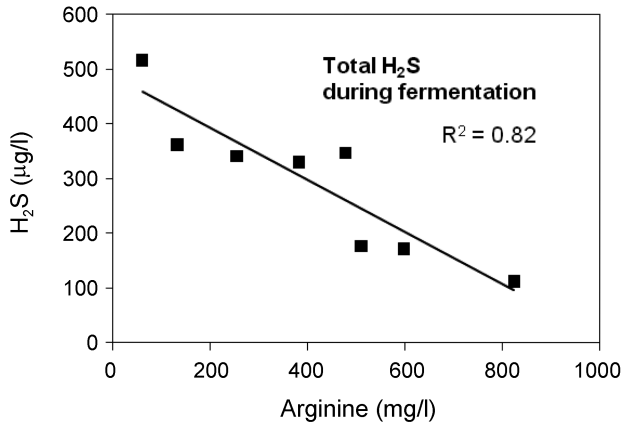

Fig. 1. Total H_2S formation (µg/l juice) during fermentation and juice arginine concentration.

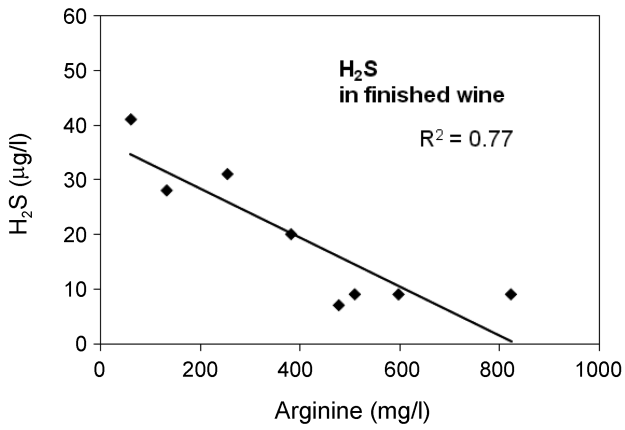

Fig. 2. Residual H_2S (µg/l wine) after fermentation and juice arginine concentration.

kept refrigerated at 8°C, with 50 mg/l SO_2 added for five days, and then re-analyzed.

Yeast Strains

Saccharomyces cerevisiae (strain UCD522 Montrachet) and *Saccharomyces bayanus* (strain UCD819 Premier Cuvée, Prise de Mousse, PdM) were evaluated since they were well characterized as high and low producers for H_2S during fermentation [18, 29].

Fermentors

Model fermentations were conducted in concurrent duplicate in stirred (100 rpm), temperature-controlled (18°C) 15 L Nalgene-Lightnin Biomixer fermentors (SPX Corp., Charlotte, NC, USA) to simulate the fermentation kinetics of commercial-scale fermentation vessels.

CO_2/H_2S Determination

Fermentation rate was measured by weighing the fermentors on a laboratory scale, assuming that once CO_2 saturation of the juice is reached, weight loss corresponds directly to CO_2 evolution, which in return is proportional to ethanol formation [5]. H_2S production was followed using a calibrated transparent tube packed with metal acetate, which changes color upon reaction with H_2S produced during fermentation [20]. The tubes were inserted into one of the fermentor ports instead of a regular gas lock, and the length of blackening from metal sulfide inside the tube provided a quantitative reading for the amount of H_2S formed.

Data Analysis

In a statistical evaluation following the fermentations, the ratios between the CO_2 and H_2S production rates as well as the ratio between them were calculated.

RESULTS AND DISCUSSION

The fermentations confirmed the previous observations that high concentrations of yeast assimilable nitrogen do not necessarily protect against elevated H_2S formation [24, 30]. High initial concentrations of ammonium ions (*e.g.*, through addition of DAP) caused a higher production of total H_2S than in a non-supplemented yet adequate juice (Fig. 3). As methionine was not added to either one of the model juices, thus methionine-induced repression of the sulfate reduction pathway was excluded, and sulfite reductase expression was maximized by the high concentration of ammonium ions [15, 28]. Under these circumstances, our observations indicated that juice sulfate concentration was proportional to H_2S formation in commercial grape juices [23]. Since ammonium to amino nitrogen ratios vary greatly between grape juices [10], the main source of sulfur for the yeast will depend on the individual juice amino acid and sulfite/sulfate composition.

We observed that the excess availability of arginine caused a higher CO_2 production rate throughout the alcoholic fermentation (Fig. 4.). The juice with equal amounts of nitrogen from DAP and arginine had a 11% lower maximum fermentation rate than the juice with DAP as sole nitrogen source. However, it sustained a 87% higher fermentation rate in the days following the maximum rate until the end of fermentation. Arginine has a consumption pattern of a Phase I and II amino acid [3]; that is both its uptake and its utilization after storage in intracellular vacuoles can be delayed by the yeast. Consequently, the H_2S evolution rate reached its relative maximum earlier in the fermentation with more than twice the relative amount of CO_2 (*ca.* 60 *vs.* 30 juice volumes) to be produced during the remainder of the fermentation. Although this study did not quantify the amounts of residual H_2S in the wine, the more than 30 extra volumes of CO_2 allow for a far more sustained stripping of H_2S (Fig. 4). However, high concentrations (more than 1,000 mg/l) of arginine in a juice are undesirable as they may increase the risk of urea excretion by the yeast into the wine and subsequent formation of ethyl carbamate [2, 3]. Moreover, despite that this study did not investigate the composition of the finished model wines, it is certain that any unused or excreted yeast nutrients may encourage growth of secondary desirable or undesirable wine organisms.

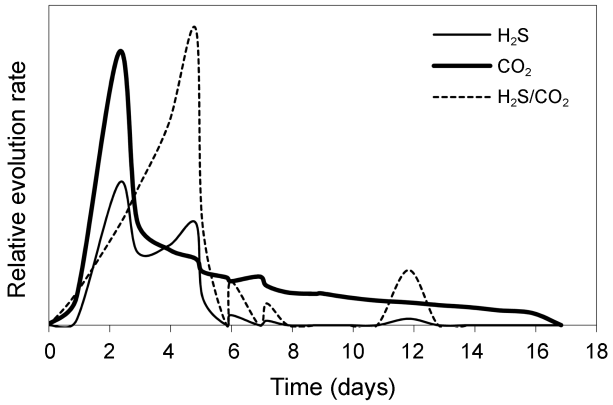

Fig. 3. *S. cerevisiae* UCD522: Gas evolution rates in juice with 210 mg N/l as DAP only.

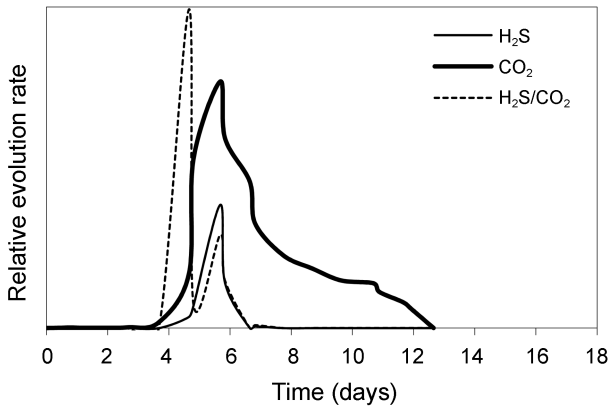

Fig. 4. *S. cerevisiae* UCD522: Gas evolution rates in juice with 105 mg N/l as DAP and 105 mg N/l as arginine.

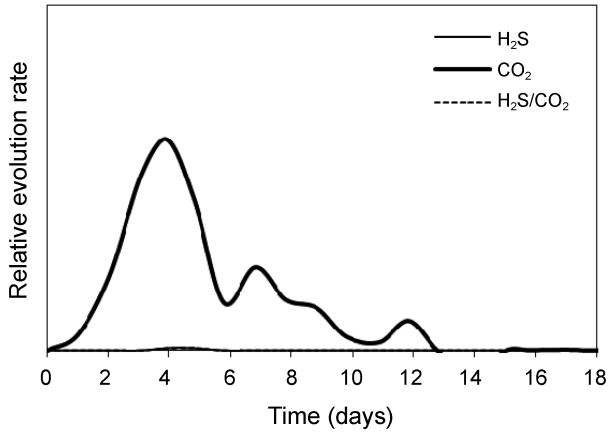

Fig. 5. *S. bayanus* UCD819: Gas evolution rates in juice with 210 mg N/l as DAP and 35 mg N/l as arginine.

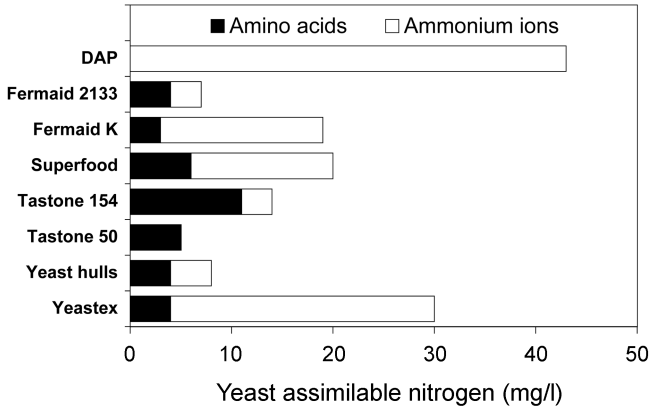

Fig. 7. Yeast food contribution to yeast assimilable nitrogen (at 240 mg/l).

The strain differences regarding H_2S release were dominant, with Montrachet producing large quantities of H_2S even with high quantities of DAP and no elemental sulfur present. The *Saccharomyces bayanus* strain (Fig. 5 and 6) produced much smaller absolute quantities of H_2S during fermentation, and displayed a more sustained though slightly sluggish fermentation rate in the model juice. Besides the high strain dependence of H_2S formation profiles, equal proportions of ammonium to amino nitrogen and moderate initial concentrations of DAP (100 to 150 mg N/l, equal to 48% to 71% the legal limit) resulted in the lowest sulfide formation after that maximum fermentation rate. However, the winemaker's means to achieve a 1-to-1 ratio between ammonium and amino nitrogen in a juice by supplementation are limited, as conventional commercial yeast foods tend to provide insignificant amounts of yeast assimilable amino acids (Fig. 7). We have conducted initial fermentation experiments with natural grape juices and chemically defined nutrient additions at a rate of 1,200 mg/l and contributing yeast assimilable nitrogen (600 mg/l DAP, 600 mg/l arginine) as well as essential vitamins (250 μg/l Ca-pantothenate, 250 μg/l thiamin a HCl, 25 μg/l pyridoxine, 2 μg/l biotin). These trials have indicated similar fermentation kinetics to what we observed in these model juices. However, the delicate relationship between arginine and ethyl carbamate in wine requires that accurate knowledge of juice nitrogen status is established before commercial application could proceed.

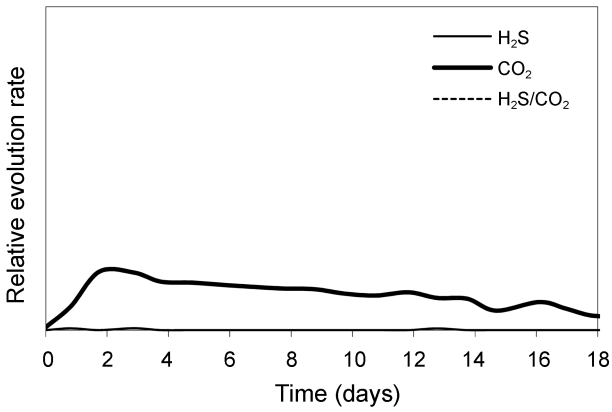

Fig. 6. *S. bayanus* UCD819: Gas evolution rates in juice with 105 mg N/l as DAP and 105 mg N/l as arginine.

In conclusion, our research provided winemakers with additional information on how yeast nutrition and strains directly impact the formation of undesirable H_2S during winemaking. A better understanding of the relationship between yeast assimilable amino acids and ammonium ion concentrations will eventually lead to specially designed yeast foods, resulting in fewer problem fermentations and improved wine quality. Precise control over the amino acid composition of nutritional supplements added to deficient grape juices was important to meet the nitrogen requirements for individual yeast strains, and to avoid excessive formation of H_2S. Current regulatory limits on approved materials for use as yeast nutrients should be adjusted to facilitate desirable fermentation profiles. Eventually, and in combination with the use of the appropriate nitrogen assays for yeast assimilable amino acids and ARGOPA for arginine, the winemaker would be able to prevent a potential H_2S problem prior to fermentation by adjusting the juice nutrient status rather than by chemical fining of the wine.

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