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Development of a Method to Measure Hydrogen Sulfide in Wine Fermentation

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A hydrogen sulfide (H_2S) detecting tube was developed for the quantitative determination of H_2S produced by yeast during laboratory scale wine fermentations. The detecting tube consisted of a small transparent plastic tube packed with an H_2S-sensitive color-indicating medium. The packed medium changed color, with the color change progressing upward from the bottom of the tube, upon exposure to H_2S produced by yeast during fermentation. A calibration study using a standard H_2S gas showed that the length of the portion that darkened was directly related to the quantity of H_2S (μg) with a high correlation coefficient (r^2=0.9997). The reproducibility of the H_2S detecting tubes was determined with five repetitive measurements using a standard H_2S solution [5.6 μg/200 ml (28 ppb)], which resulted in a coefficient of variation of 3.6% at this level of H_2S. With the sulfide detecting tubes, the production of H_2S was continuously monitored and quantified from laboratory scale wine fermentations with different yeast strains and with the addition of different levels of elemental sulfur to the grape juice. This sulfide detecting tube technology may allow winemakers to quantitatively measure H_2S produced under different fermentation conditions, which will eventually lead winemakers to better understand the specific factors and conditions for the excessive production of H_2S during wine fermentation in a large production scale.

Keywords: Wine, fermentation, yeast, volatile sulfur compounds, hydrogen sulfide, sulfide detecting tube

The production of all alcoholic beverages, including wine and beer, involves the fermentation of sugar present in a raw agricultural product into alcohol. However, yeast, which is responsible for the fermentation of sugar into alcohol, also produces many other metabolic by-products. Of these, hydrogen sulfide (H_2S) is a particularly important by-product of yeast fermentation for several reasons: 1) H_2S confers a rotten egg smell at extremely low concentrations, *i.e.*, at about 1 ppb (μg/l) in wine; 2) H_2S is one of the major malodorous compounds produced by yeast during fermentation; and 3) other volatile sulfur compounds (VSC) such as mercaptans, sulfides, and disulfides responsible for potent off-odor problems in wines are mainly derived from H_2S [3, 4, 8, 11]. These secondary VSC, which have aromas described as burnt match, burnt rubber, rotten cabbage, onion, and garlic, are extremely difficult to remove once they are present in wine. Therefore, identifying the factors responsible for the overproduction of H_2S in the early stages of winemaking is important not only to reduce the production of H_2S itself, but also to prevent the formation of other secondary VSC after fermentation.

Factors affecting the formation of H_2S in wine fermentations include 1) elemental sulfur residue from vineyard spray, 2) yeast strain, 3) must nitrogen deficiency, 4) fermentation conditions, 5) micronutrients, and 6) post-fermentation treatments [6, 7, 9, 17].

Although the ability to detect and quantify H_2S formation in fermenting wines is important in order to identify and correct the causes of H_2S in wine, the quantitative analysis of H_2S produced from fermenting wines has been virtually impossible for most wineries because of the lack of availability of an easy and rapid method. At present, there are instrumental methods for the qualitative and quantitative analyses of VSC in wines, such as gas chromatography with flame photometric or sulfur chemiluminescence detection [10, 11, 14], but these methods require expensive instrumentation and skilled personnel. These instrumental methods are also better suited for the analysis of H_2S in finished wines, not for the continuous monitoring of H_2S evolved from the fermentor gas. Moreover, the analysis of H_2S using these sophisticated instruments is time consuming, and encompasses additional problems, such as the separation of H_2S from sulfur dioxide (SO_2) that interferes with the detection of H_2S produced during the course of fermentation [13].

A colorimetric method has been used for detecting and quantifying H_2S produced during fermentation and this may be the only method available for measuring total H_2S from the fermentor gas [1, 2, 6, 7, 17]. However, this method

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also has disadvantages including time-consuming set-up, labor-intensive sampling procedures, photo-oxidation of the trapping agent, interference by sulfites, and the use of toxic solutions for spectrophotometric analysis. Accordingly, a more convenient, accurate, and cost-effective method for the qualitative and quantitative measurements of H_2S during wine fermentation is still needed by the wine industry. The objective of this study was to develop a method that is easy, fast, and cost-effective, but accurate enough to quantitatively measure H_2S produced by yeast during wine fermentation.

MATERIALS AND METHODS

Preparation of the H_2S Detecting Tubes

The H_2S detecting tube contains reagent that changes color in the presence of H_2S produced by yeast during fermentation. The length of the color band in the tube indicates the quantity of H_2S [12]. The color-indicating packing medium was prepared by homogeneously impregnating the surface of the silica-based solid support (an average size of 210 μm) with lead acetate (Aldrich, Milwaukee, WI, U.S.A.). The impregnation ratio was varied, depending on the total amount of H_2S to be quantified: a typical ratio of solid support to lead acetate was 100 g of solid support mixed with 200 ml of 2.5% lead acetate solution. For finished wine, to detect H_2S below the sensory threshold, a much lower impregnation ratio (0.5%, w/w) was used. The slurry was then homogeneously dried for 5 h under reduced pressure, followed by subsequent drying in a desiccator. The impregnated and dried color-indicating medium was then packed uniformly into transparent plastic tubes. The size of the detecting tube was varied, depending on the desired range and quantity of H_2S to be detected. The tubes used in this experiment were two different sizes: a tube with a size of 2.5 mm i.d. with 230 mm length was used for monitoring the production of H_2S during fermentation and a size of 2.5 mm i.d. with 115 mm length was used for measuring H_2S remaining in finished wine after fermentation (Fig. 1).

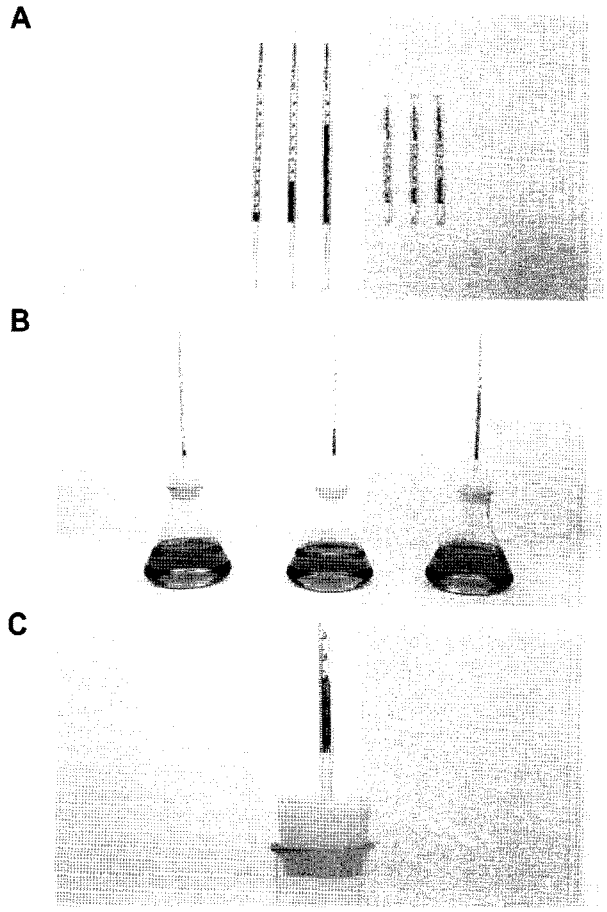

Fig. 1. A. The sulfide detecting tubes for quantifying H_2S from fermenter gas (long tube) and H_2S concentration in finished wine (short tube). **B.** The H_2S detecting tubes used with Erlenmeyer flask. **C.** The H_2S detecting tube blackened with H_2S from the fermenter gas.
The same tube was in place for the entire duration of the fermentation.

Calibration of the Detecting Tubes

The sulfide detecting tube was calibrated by a method described previously [12] and the detailed procedure is as follows. The standard H_2S gas was prepared by diluting 1 ml of pure H_2S gas (Aldrich, Milwaukee, WI, U.S.A.) contained in a lecture bottle into a 250-ml gas-sampling bulb (Supelco, Bellefonte, PA, U.S.A.), which had previously been purged with nitrogen gas at atmospheric pressure. A series of incremental volumes of H_2S gas from the gas-sampling bulb were removed by a valved, gas-tight syringe and each volume of the gas was directly injected into the sulfide detecting tube for the development of the blackened band. The calibration curve was developed by measuring the length of blackened band in the detecting tube and plotting versus the corresponding amount of H_2S gas injected. The range of detection was from 0.5 μg to 100 μg for the tube used for H_2S from the fermentor gas, and from 0.1 to 10 μg for the measurement of H_2S concentration in the finished wine.

Reproducibility and Detection Limits of the H_2S Detecting Tubes

The reproducibility of the response was measured using a standard H_2S solution prepared by dissolving 1.0 g of sodium sulfide nonahydrate (Aldrich, Milwaukee, WI, U.S.A.) in 1 l of deionized water [2]. A 100 ml aliquot was added to a beaker containing 250 ml of deionized water, 20 ml of 0.1 N iodine solution (Aldrich, Milwaukee, WI, U.S.A.), and 1 ml of dilute HCl (1+3). The excess iodine was titrated with 0.1 N sodium thiosulfate solution using starch as the indicator. One ml of 0.1 N iodine was equivalent to 1.6 mg sulfide (S^{2-}). A volume of stock solution sufficient to give the required H_2S concentration was spiked into 200 ml of deionized water to yield a final concentration of 28 ppb (5.6 μg/200 ml). The solution was carefully transferred into 500-ml Erlenmeyer flasks to determine the reproducibility of the detecting tubes. Nitrogen gas was used as the external gas source at 40 cc/min to sparge the headspace of the solution, during which time the standard solution was continuously stirred by a magnetic stirrer. The reproducibility of the tubes from the standard solution was calculated from five consecutive measurements. The limit of detection was also determined by measuring H_2S in the solutions with serial dilutions.

Interference by Sulfur Dioxide

To determine the potential interference by SO_2, which is abundant in must and wine, 1 ml of a standard SO_2 solution (5%, 5 g/100 ml

water) was spiked into the standard H_2S solution (5.6 μg/200 ml), which was used for the reproducibility test. The final concentration of SO_2 was 250 μg/ml.

Application of the H_2S Detecting Tube for Wine Fermentations

The quantity of H_2S produced by two different commercial strains of wine yeasts, Montrachet and Premier cuvée, was compared for their H_2S production by fermenting red Carignane juice in small laboratory fermentations (26 °Brix juice; sterile-filtered through an 8-μm membrane filtration system prior to inoculation; 250 ml of juice per 500-ml Erlenmeyer flask). In an additional trial assessing the effect of elemental sulfur addition on H_2S production, micronized-sulfur (Thiolux, Sandoz Crop Protection, Des Plaines, IL, U.S.A.), 2 and 4 mg/l, was added to the juice. A third trial compared the effect of six different yeast strains on H_2S production during the fermentation of 26 °Brix white juice (reconstituted from a white concentrate; 200 ml of juice with 50 mg/l SO_2 fermented in a 250-ml Erlenmeyer flask). All yeasts were obtained from Universal Foods (Red Star, Milwaukee, WI, U.S.A.) and were rehydrated with Milli-Q water (Millipore, MA, U.S.A.), followed by incubation in a warm water bath at 38°C for 30 min. Fermentations were conducted at room temperature (23°C). All fermentations were run in triplicate.

H_2S Concentration in Wines After Fermentation

For determination of residual H_2S that remained in finished wines after fermentation, a 300-ml sample of each wine was carefully transferred into an Erlenmeyer flask, and a sulfide detecting tube (2.5 mm i.d. with 115 mm length) was inserted into the hole of a silicon rubber stopper, which was then placed into the opening of the flask. To determine the amount of H_2S contained in these wines, external nitrogen gas was used at 40 cc/min to sparge H_2S from the headspace of the wines with continuous magnetic stirring.

Results and Discussion

Calibration of the Tube and the Detection Limit

To develop the calibration curve, varying amounts of a standard H_2S gas were quantitatively measured using a gas-tight syringe, and then directly injected into the detecting tube. The results showed a good linear relationship (r^2=0.9997) between the length of the colored band and the quantity of H_2S injected. The detection limit was determined from five replicate runs using standard solutions where the blackened band became difficult to measure, which was estimated at about 0.5 ng/ml H_2S in wine. The detection tube method is a colorimetric method, so visual estimation is the only way that can measure the minimum detection level.

Reproducibility of Detection

The reproducibility of the H_2S detecting tubes was determined using a standard H_2S solution prepared by dissolving 1.0 g of sodium sulfide nonahydrate (Aldrich, Milwaukee, WI, U.S.A.) in 1 l of deionized water [2]. The final concentration of the standard solution for the reproducibility test was 5.6 μg/200 ml (28 ppb). The length of blackened band from the reaction of H_2S with the color-indicating medium in the tubes was measured. The five repetitive measurements were 5.3, 5.6, 5.9, 5.6, and 5.6 μg with a coefficient of variation of 3.6% at this level of H_2S. The high degree of reproducibility was primarily due to the homogeneous impregnation of the tubes and the controlled condition of the test.

Interference by Sulfur Dioxide

Wines contain much higher levels of SO_2 than the levels of H_2S and the highest level could be up to 250 ppm. The presence of 250 μg/ml SO_2 was not found to cause any interference in the determination of H_2S. Since the color-indicating reaction depends solely upon the presence of H_2S, the possibility of interference by other sulfur compounds is unlikely. Previous methods such as zinc acetate trapping followed by colorimetric analysis, which were predominantly used for the quantitative determination of H_2S from fermentation gas, showed many interferences due to the presence of sulfite and thiols [17].

Difference in H_2S by Yeasts and Sulfur Addition

As yeast produced H_2S during fermentation, the produced H_2S immediately passed through the tube with CO_2, which changed the color of the packing medium progressively from the bottom of the tube. By reading off the blackened band distance imprinted on the tube at 24-h intervals, we were able to continuously monitor the production of H_2S through the entire course of fermentation. Fig. 2 illustrates the H_2S production during juice fermentation by two different yeast strains, Premier cuvée (PC) and Montrachet (Mont). Each strain was used for three fermentations: the control juice, the control juice with 2 mg/l micronized sulfur addition, and the control juice with 4 mg/l micronized sulfur addition. These levels of elemental sulfur residues were normally found in grapes harvested in California [18]. Differences in H_2S produced in sulfur-spiked juice samples relative to the control are, thus, due to the presence of the added sulfur. The amount of H_2S was measured daily by reading the blackened band distance of the detecting

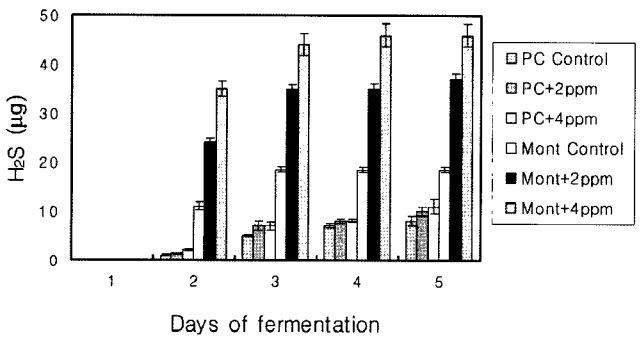

Fig. 2. Differences in H_2S levels produced during fermentation by Montrachet (Mont) and Premier cuvée (PC) spiked with a 2 and 4 mg/l (ppm) micronized sulfur to the control juices.
The production levels were expressed as cumulative value from each fermentation day.

tube and expressed as the cumulative amount of H_2S evolved during the fermentation.

Montrachet (*Saccharomyces cerevisiae*), which is known as a high H_2S producer [5, 9, 17], produced more H_2S in the juices that contained more sulfur: twice as much H_2S was produced by the 2 mg/l sulfur-spiked juice relative to the control, and 2.5 times as much was produced by the 4 mg/l sulfur-spiked juice relative to the control. In contrast, Premier cuvée (formerly named Prise de Mousse), which is generally known as a low H_2S producer [9, 17], not only produced much lower levels of H_2S, but also no significant differences were observed in the amount of H_2S produced by the two sulfur-spiked juices (2 and 4 mg/l) in these laboratory-scale fermentations. Premier cuvée has been widely used in wine industries, probably owing to its low production of H_2S. However, a large production-scale fermentation use of Premier cuvée could also result in significant differences due to the lower redox potential, fermentation vigor, and increased head pressure in the tank that increases the solubility of H_2S in fermenting wine [13, 16]. These observations suggest that the amount of H_2S that would be produced during large production-scale fermentation, due to elemental sulfur residues left on grapes at harvest, could easily be estimated in advance by small-scale fermentation in the laboratory, which will take only less than a week to complete the fermentation.

H_2S Production by Six Yeast Strains

Fig. 3 shows the H_2S production during the fermentation of 200 ml of reconstituted white juice by six different wine yeast strains. Montrachet began fermentation earliest and produced the highest level of H_2S. Vigorous fermenting yeasts such as Montrachet, Pasteur champagne, and Flor sherry are valued because of the short fermentation time, but all produced higher levels of H_2S than the less vigorous fermentors. Such vigorous fermentors have been observed in many previous studies to be associated with higher levels of H_2S production under any fermentation conditions [1, 15, 17]. Accordingly, less vigorous fermentors such as

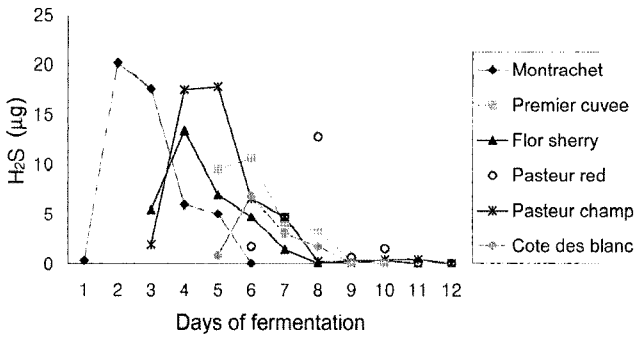

Fig. 3. Daily production of H_2S during fermentation from six yeast strains.
H_2S production was measured at 24-h intervals by reading the blackened scale imprinted on the H_2S detecting tube.

Table 1. Total amount of H_2S produced by six different yeast strains during fermentation, and H_2S concentration at the end of fermentation.

Yeasts	Total H_2S (µg/l juice)	in finished wines (µg/l wine, ppb)
Montrachet	49.9 (±3.5)	0.6
Premier cuvée	27.0 (±2.2)	<0.5
Flor sherry	31.7 (±0.3)	<0.5
Pasteur red	21.2 (±7.7)	0.9
Pasteur champagne	48.8 (±1.2)	1.9
Cote des blanc	12.5 (±1.5)	<0.5

Numbers in the parentheses are standard deviations.

Premier cuvée, Pasteur red, and Cote des blanc may appear preferable because they produce less H_2S, but wine fermented by such strains may also retain problematic levels of H_2S, because the H_2S produced toward the end of the fermentation is less likely to be entrained by CO_2. Nevertheless, high H_2S producers had high concentrations of H_2S remaining in the wines at the end of fermentation (Table 1). The wine fermented by Pasteur champagne was the highest in H_2S (1.9 µg/l), followed by the wine fermented by Pasteur red (0.9 µg/l) and Montrachet (0.6 µg/l). Low H_2S producers had relatively lower levels of H_2S (<0.5 µg/l) in the finished wines. In commercial-scale fermentation, however, the amount of H_2S remaining in the finished wine would depend on several factors, including yeast strain, juice or must nutrition, type and quantity of vineyard sulfur residues, juice clarity, temperature, fermenter size, agitation, and punch-down or pump-over frequency and methodology.

In conclusion, the ability to analyze H_2S is important for understanding the sources and factors that are responsible for the overproduction of H_2S in winemaking. However, there have been many difficulties and problems involved in the analysis of H_2S during fermentation and in the determination of residual H_2S after fermentation. Accordingly, a new method was developed using the H_2S detecting tube technology, which can be used easily and conveniently to measure the quantity of H_2S produced by yeast during fermentation, and also to determine the residual concentration of H_2S remaining in finished wines.

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