A method for determination of emission rates from SO2 sheets for storage of grapes

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Keywords
Botrytis cinerea; postharvest; sodium metabisulphite; storage; table grapes.

Abstract
Introduction Table grapes are very susceptible to decay caused by Botrytis cinerea during storage, and one of the common means to control decay is by packaging the fruit with a SO2-generating sheet. Typically, the sheets contain sodium metabisulphite in a granulated form that releases SO2 upon absorption of water in two phases: a fast-release phase of high SO2 level and a slow, continual release of a low level of SO2. Objective Currently, there is no published method to compare or validate the performance of different SO2 sheets. This paper describes a simple analytical method to quantify the rate of SO2 release from the sheets which does not require setting up storage experiments with grapes. Results The method was used to test the total amount of SO2, which was released under the experimental conditions. However, most of the study was focused on a relative measurement that can help with comparing different SO2 sheets or the same sheets from different production dates. Conclusion The method revealed significant differences in SO2 release pattern between different types of SO2 sheets or the same sheets from different years of production. The method can allow grape growers to have better tools that will ensure successful control of decay during storage.

Introduction
Grey mould caused by the fungus, Botrytis cinerea, and stem browning from desiccation are the two main factors that reduce table grape postharvest quality (Lichter et al., 2006). Prevention of decay during storage is achieved by the presence of SO2, either by weekly fumigation of the storage room, or the presence of a sheet containing sodium metabisulphite (SMBS). Since the late 1960s, two-stage or ‘dual release’ (DR) SO2 generators consisting of formulations that allow quick-release plus slow release of the gas have been widely used for table grape storage and transport (Nelson & Gentry, 1966; Nelson & Ahmedullah, 1976). The DR sheet contains SMBS enclosed between paper sheets of different permeability. Moisture within the package of grapes is absorbed by the sheets and reacts with the sulphite, releasing SO2. The quick-release part of the sheet gives a flush of SO2, which peaks after about 24 h and then diminishes in about a week. The slow-release part of the sheet emits a low concentration of SO2 over a long period. A level of at least 2 ppm SO2 is required to prevent the spread of Botrytis from berry to berry (‘nesting’) but a constant level of 3 ppm was not able to prevent decay of artificially inoculated ‘Redglobe’ berries (Palou et al., 2002). The DR of SO2 is achieved by using small and large SMBS particles and by proprietary formulations of the salt and the sheet. Because of this proprietary nature, sheets from different manufacturers may have different kinetics of SO2 release, even if they contain the same amount of SMBS. Typically, the SO2 concentration inside a package or pallet of stored grapes can be measured using an electrochemical sensor or colorimetric dosimeter tubes (Crisosto, 2002; Lichter et al., 2008). However, it is
impossible to infer from this method the total amount of SO2 released from the sheet.

The kinetics of SO2 release from DR sheets can be influenced by many factors: the time from when the sheets are applied to the boxes until the grapes are cooled, the rate of cooling, the temperature, the relative humidity (RH) in the storage room, the packaging material, and the amount of grape bunches and free space within the box. In addition to these external factors, there are various types of sheets from several manufacturers and it was observed that similar sheets can emit different levels of SO2 in consecutive years. Obviously, if the initial level of SO2 is too high, damage will occur to the berries, including bleaching and splitting of the grapes; on the other hand, if the amount of SO2 around the grape bunches is too low, and if the SMBS is exhausted before the end of storage, elevated decay may occur (Combrink et al., 1978; Laszlo et al., 1981). To avoid unexpected variation in the SO2 release pattern during storage, it is important to establish methodology that will allow rapid and reproducible analysis and comparison of the performance of the SO2 sheet prior to storage.

**Materials and methods**

The structure of the system to measure SO2 emission from paper sheets is described in Figure 1. It consisted of a flow meter in the range of 1 L min⁻¹ (Dwyer, Michigan, IN, USA), two gas washing bottles (Sigma Aldrich, Rehovot, IL), a 2-L glass jar with hermetic clip seal and two holes for inlet and outlet tubing, and silicon rubber tubing with internal diameter of 4 mm. The 250-mL gas washing bottles were equipped with a gas dispersion unit at the tip of the internal pipe with porosity of 40–60 μm. The system used was composed of six channels and air flow (1 L min⁻¹) was adjusted in each channel separately. The humidifier for each channel contained 100 mL of water, and 100 mL of H2O2 were added to the SO2 trap. The RH in the system was determined by placing RH sensors attached to extension cord and a data-logger (Hygroclip; Rotronic, Bassersdorf, Switzerland) and measuring RH for 16 h at 15-min intervals in duplicate channels. H2O2 was prepared fresh by diluting 30% stock kept at 4 °C to 0.03% and adjusting the pH to 7.0 with 0.01 N NaOH using an auto sampler and titrator (Metrohm). When the amount of NaOH required to titrate the H2O2 solution was expected to exceed 30 mL, the solution was diluted 10-fold. The amount of SO2 emitted from each SO2 sheet was calculated as SMBS equivalents (eq.) using the following formula:

\[
\text{SMBS eq. (gr)} = \left( \frac{0.20V^2 + 12.26V + 7.51}{10^2 \times PS} \right) \times FS
\]

where, \( V \) is the volume of NaOH used to titrate undiluted H2O2 (or multiplied by 10 if diluted); FS is the full size of the SO2 sheet; and PS the size of the SO2 sheet which was used in the experiment. The equation was generated by reciprocal plotting of \( V \) (x-axis) and SMBS (y-axis) in Excel® and generation of polynomial trend line and equation.

Storage of 'Redglobe' grapes was carried out as previously described (Lichter et al., 2008). In brief, 'Redglobe' grapes were harvested to 5-kg plastic boxes. Paper-based SO2 sheets, which were wrapped with polyethylene liner with 32 6 mm holes in
the bottom, were placed on the grapes and the boxes were cooled passively overnight. Stacks of three boxes (minipallets) were wrapped with linear low-density polyethylene (LLDPE) and storage was maintained at 0° ± 0.5°C and RH above 90%. SO₂ levels inside the minipallets were monitored weekly with an electrochemical sensor fitted with a small pump that operated at a flow rate of 50 mL min⁻¹ to sample air from the package into the instrument (Emproco, Ashkelon, Israel). The inlet and outlet of the sensor were connected to two silicone tubes which were introduced into the minipallets.

Experiments were carried out at least twice to validate results. In each experiment, three replicates were performed for each SO₂ sheet or concentration of SMBS (except where noted). Averages of the replication and standard deviation were calculated. Statistical analysis was carried out with the Instat software version 3.06 (GraphPad, San Diego, CA, USA) by one-way analysis of variance with the Student–Newman Keuls (SNK) post test at P < 0.05.

Results and discussion

Principle and setup

The official method for determination of sulphites in beverage and food is the Monier-Williams distillation method (Monier-Williams, 1927; Kim et al., 1990). In this method, the non-acidified or acidified solution is heated and SO₂ is released and is oxidized to sulphuric acid by hydrogen peroxide at pH 7.0. The reduction in pH is proportional to the amount of SO₂ that passes through the trap. We used the Monier-Williams trap by connecting it to a 2-L glass jar in which the SO₂ sheet was placed. The constant flow of humidified air through the jar delivers the SO₂ through the hydrogen peroxide trap and in the same time adds a constant amount of water vapour to the system that is required for wetting the SO₂ sheet and for the reaction with the metabisulphite salt.

Basic parameters of the system

The performance of the SO₂ analytical system was tested in several ways. The reproducibility was determined by adding 125 mg of SMBS to each of the six channels (Figure 1) and operation of the system for 24 h. The amount of 0.01 NaOH required to titrate the hydrogen peroxide in the SO₂ trap was 6.40 ± 0.44 mL in a single experiment. The average of 11 measurements performed in four different experiments resulted in a value of 6.50 ± 0.59 mL indicating that the system is capable of generating reproducible results at different times. From these results, it was determined that three replications are the optimal number in the current setup. The efficiency of the humidifier was tested by measuring the RH in the system without the humidifier, which yielded a value of 24.9 ± 0.4%. One humidifier increased the RH to 96.0 ± 0.5% while two humidifiers in a row generated a RH of 97.1 ± 0.2%. It was therefore decided to work with only one humidifier. The efficiency of the trap system was determined by placing a second SO₂ trap in a row after the first trap and placing 50 mg of SMBS in the 2-L jar. The results showed that the amount of NaOH required to titrate the second trap was less than 1% of that needed for the first trap. The baseline values of NaOH used to titrate hydrogen peroxide that had been connected to an empty channel were 0.09 ± 0.05 mL (n = 6 in three different experiments). Placing 1 mg of SMBS in the system gave higher average values than that of the baseline, but the difference was not significant (not shown). Placing 5 mg of SMBS in each channel resulted in a value of 0.61 ± 0.31 mL (n = 10 in four different experiments). When outliers were excluded, the average was 0.58 ± 0.13 mL (n = 8 in four different experiments). This difference was significant (P ≤ 0.01 at SNK) and it defined the current detection limit, although for such threshold, the number of replication must be greater than 3. It should be emphasized that no attempt was taken to improve the detection limit because it was not considered significant for analysis of SO₂ sheets, which typically contain more than 5 g of SMBS.

Calibration curve

A calibration curve was determined by placing different amounts of SMBS (5, 25, 125, 625 and 1250 mg) in the system and measuring the amount of NaOH required to titrate hydrogen peroxide in the SO₂ trap after 24 h of flow. The correlation coefficient R was equal to 1 at the range of 0–625 mg of SO₂ (Figure 2A). The slope of the line was 53.07, which is the amount of 0.01 NaOH in mL which is required to titrate SO₂ emitted from 1 g of SMBS. When the amount of SMBS in the system was doubled from 625 mg, the curve line lost its linear course (Figure 2B), suggesting that the amount of SMBS exceeded the capacity of the hydrogen peroxide trap to oxidize the gaseous SO₂ to water-soluble acid. However, a second-order equation (Eq. 1) could give accurate description of the kinetics of the curve. Therefore, when the amount of NaOH used for titration is higher than 33 mL, it is possible to use Eq. 1 to determine the amounts of SO₂ emitted from SO₂ sheets. To improve the capacity and linearity of the system without changing its configuration, it should be feasible to increase the amount of hydrogen peroxide in the SO₂ trap.
The kinetics of SO2 emission in the flow system

The kinetics of SO2 emission from a DR SO2 sheet were determined by trapping the SO2 emitted from half sheets enclosed in glass clip-seal jars (Figure 1) and sampling once a week over a period of 8 weeks until the SO2 was exhausted (Figure 3). The sampling was also performed twice a week to ensure that the capacity of the H2O2 trap (not shown). The area under the curve was calculated to be 10.7 g SMBS equivalents, while the sheet was rated as having 9 g of slow-release and most likely 0.5 g of SMBS in the fast-release formulation. These results suggest that the sheets released all their SO2 during the 8 weeks and also that during the weekly sampling all the SO2 was absorbed by the hydrogen peroxide traps. It also illustrates the kinetics of SO2 released from the sheet under the experimental conditions. However, it also shows that the system may overestimate the amount of SMBS by a factor of approximately 10%. Interestingly, in bi-weekly sampling, the calculated amount of SMBS in the sheet yielded a total of 8.9 ± 0.3 g, which is closer to the expected yield of 9.5 g. However, it also supports the fact that the capacity of the trap was not the limiting factor because otherwise, it is expected that the measured amount in bi-weekly sampling will exceed that of weekly sampling. Possibly, opening the system twice a week instead of once a week introduced errors into the measurements that resulted in lower absolute amount. While the results discussed previously do show that practically all the SMBS was converted to SO2, it is not known how much of the SMBS can be oxidized to sulphate ions and to what extent this factor may account to variations between manufacturers and batches. This type of measurement allows the determination of how long the sheet will continue to produce SO2 and what is the absolute level of SO2 that will be released from the sheets. However, from a practical standpoint, it is required to measure the capacity of the sheet during a shorter period of about 1 week.

Short-term SO2 emission rates

To test initial SO2 emission rates, half sheets from two manufacturers were placed in the glass clip-seal jars in three replicates. SMBS equivalents were determined after 1, 2, 3, 7 and 8 days in the system (Figure 4). Each time point consisted of 24 h sampling and in between 3 and 6 days, the system was running without daily sampling and without trapping the SO2. The results show that SMBSeq were relatively high in the first 2 days of sampling and were reduced by more than 30% in the third day of sampling. The amounts of SMBSeq at days 7 and 8 were less than a third of the amount in the first 2 days for manufacturer A and fifth for manufacturer B. This pattern was consistent in several experiments and in differ-
ent time frames and demonstrates the DR mode of the SO₂ sheets. The DR mode was formulated in order to generate an initial burst that will kill fungi that are present on the berry and is followed by a lower SO₂ emission rate, which inhibits the development and spread of *B. cinerea* during the cold storage (Laszlo et al., 1981; Palou et al., 2002). This reduction in SO₂ emission during cold storage is important, because it was shown that it was not necessary to maintain high doses of SO₂ during storage for fungal control, while high doses had a negative impact in terms of bleaching, cracking and compromised taste (Nelson, 1985; Opperman et al., 1999). From these data and other similar experiments, it was decided to use a framework of 1 week of measurements that will consist of a first phase of 72 h of continuous sampling followed by 48 h of flow without sampling followed by 48 h of sampling in the second phase. This format allowed analysis of two sheet types in three replicates during 1 week. Ideally, these two phases would correspond to the fast- and slow-release portion of the sheets; however, it is shown from Figure 3 that most of the SO₂ is emitted during the first 3 weeks. However, as stated before, rapid analysis of SO₂ sheets is necessary in order to meet practical demands.

**Comparison of SO₂ sheets**

Table 1 is a compilation of measurements of a number of sheets from three manufacturers, from different years of the same manufacturer and also sheets with external lamination. The largest collection of sheets was from Manufacturer A, including outdated batches from 2003.

<table>
<thead>
<tr>
<th>Type</th>
<th>Manufacturer</th>
<th>Year</th>
<th>Phase I – 72 h</th>
<th>Phase II – 48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>A</td>
<td>2003</td>
<td>3.01 ± 0.30</td>
<td>1.32 ± 0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2007</td>
<td>2.81 ± 0.05</td>
<td>0.52 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2008</td>
<td>3.32 ± 0.10</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>2005</td>
<td>5.96 ± 0.08</td>
<td>0.75 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>2007</td>
<td>2.06 ± 0.12</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>II</td>
<td>A</td>
<td>2008</td>
<td>1.88 ± 0.09</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>2005</td>
<td>4.40 ± 0.33</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>2006</td>
<td>0.27 ± 0.04</td>
<td>0.06 ± 0.01</td>
</tr>
</tbody>
</table>

Type I are paper-based SO₂ sheets and type II are SO₂ sheets with external plastic lamination.

Interestingly, it was found that in 2003, the second phase of sampling was considerably higher than subsequent years, while in 2008, the first phase of sampling was higher than in previous years. These data fit our experimental measurements from ‘in package’ SO₂ measurements as well as growers’ observations in 2003 of increased SO₂ damage on their grapes. In general, it can be concluded that large variations can be measured for SO₂ emission among similar SO₂ sheets from the same manufacturer and also variations between manufacturers can be significant, especially within sampling in phase II. Also, the SO₂ levels in sheets of Manufacturer A seem to be higher except for sample II in one of the 2007 batches. It should be emphasized that all the paper sheets in this category have similar practical indications in terms of SMBS, format and recommended usage. Because the formulation of the sheets is proprietary, it is difficult to dissect the causes of the differences found in Table 1. All the paper sheets were rated as having the same amount of SMBS. The size of the granules and the ratio of fine to coarse granules may have contributed to differences in release rate. However, the differences in release rate of different batches from the same manufacturer indicate an issue of standardization. The laminated sheets differed substantially from each other mainly in phase I, and the amount of SMBS was lower in these sheets than in the paper sheets. However they were rated for the same length of storage of grapes as the paper sheets.

**Performance of two types of SO₂ sheets in storage of grapes**

Two paper sheets from different manufacturers were compared for their performance during storage of ‘Redglobe’ grapes. The SO₂ sheets were wrapped in polyethylene liners...
as previously described (Lichter et al., 2008). The wrapped sheets were placed over 5 kg of ‘Redglobe’ grapes in plastic boxes and three boxes composed minipallets that were wrapped with low-density polyethylene. The minipallets were stored at 0 ± 0.5 °C and SO₂ was monitored over 60 days in three replications with the electrochemical sensor. The data show clear differences in the kinetics of SO₂ release between the two SO₂ sheets (Figure 5). The sheets from Manufacturer A emitted a large burst at the beginning of the measurement period, which declined to a stable level after less than 30 days. This level did not decrease further for the entire period of the experiment (60 days). On the other hand, the sheet of Manufacturer B did not produce the initial peak and SO₂ level reached a maximal level of 7 ppm after 14 days, which remained rather constant during storage. Interestingly, both manufacturers rated their sheets as having the same amount of SMBS of 9.5 g. Comparison of the performance of the same sheets in the analytical system revealed an initial level of 2.81 or 2.06 SMBSeq and in the second phase a level of 0.52 and 0.11 SMBSeq for sheet A and B, respectively. These results show a fivefold difference between the sheets in the slow-release phase.

Summary

The method described here can determine either the absolute level of SO₂ that can be emitted from an SO₂ sheet or the cumulative amount at any time point, which seems appropriate to compare the performance of different SO₂ sheets. These time points can match the functional role of the SO₂ sheet in terms of the fast- or slow-release phases but as seen previously (Figure 3), there is no distinct separation between the phases. The data presented in Table 1 demonstrates the importance of knowing the rate of SO₂ release rather than the total amount of SMBS in the fast or slow phase. The exact kinetics of SO₂ emission are expected to be slower when the sheets are in boxes with grapes at 0 °C, but 10 °C was chosen as a compromise to allow rapid analysis of SO₂. It is likely that higher temperature and air flow will enable faster analysis of the sheets. Understanding of the kinetics of SO₂ release can allow matching the type of sheet to specific routes of storage and export. The method can also be employed to determine if a specific batch is in good shape and if it is similar in performance to what was obtained from manufacturers in the past. This method can also be used by manufacturers for quality control and for development of better SO₂ sheets.

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References


