ORIGINAL ARTICLE

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

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**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.

Kaplunov T, Zutahy Y, Lurie S, Lichter A (2011). A method for determination of emission rates from SO₂ sheets for storage of grapes. *Quality Assurance and Safety of Crops & Foods*, **3**, 191–197.

## 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 $SO_2$ released from the sheet.

The kinetics of $SO_2$ 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 $SO_2$ in consecutive years. Obviously, if the initial level of $SO_2$ is too high, damage will occur to the berries, including bleaching and splitting of the grapes; on the other hand, if the amount of $SO_2$ 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 $SO_2$ release pattern during storage, it is important to establish methodology that will allow rapid and reproducible analysis and comparison of the performance of the $SO_2$ sheet prior to storage.

## Materials and methods

The structure of the system to measure $SO_2$ 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 $H_2O_2$ were added to the $SO_2$ 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. $H_2O_2$ 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 of NaOH using an automatic titrator (Metrohm, Herisau, Switzerland). For generation of a standard curve, SMBS (Sigma Aldrich) was placed in 50-mL beakers in the bottom of the jar and 5 mL of water were injected into the beaker through the silicon tubing. The system was located in a cold room maintained at 10 °C ± 0.5 °C. Each point in the standard curve was repeated 6–10 times. Before use, the $SO_2$

sheets were kept sealed in polypropylene bags at ambient conditions. Three different $SO_2$ sheets were cut into quarter to half of the original size of the sheet (depending on the final size of the sheet). At each sampling time point, $H_2O_2$ was removed from the $SO_2$ trap and titrated with 0.01 N NaOH using an auto sampler and titrator (Metrohm). When the amount of NaOH required to titrate the $H_2O_2$ solution was expected to exceed 30 mL, the solution was diluted 10-fold. The amount of $SO_2$ emitted from each $SO_2$ sheet was calculated as SMBS equivalents (eq.) using the following formula:

$$\text{SMBS eq. (gr)} = \frac{(0.20V^2 + 12.26V + 7.51) \times FS}{10^3 \times PS} \quad (1)$$

where, $V$ is the volume of NaOH used to titrate undiluted $H_2O_2$ (or multiplied by 10 if diluted); $FS$ is the full size of the $SO_2$ sheet; and $PS$ the size of the $SO_2$ 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 $SO_2$ sheets, which were wrapped with polyethylene liner with 32 6 mm holes in
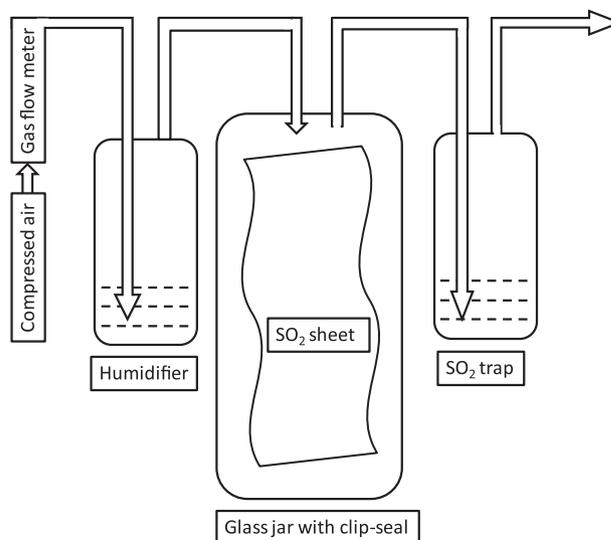


**Figure 1** A scheme that illustrates the design of the $SO_2$ analytical system. Compressed air is regulated to the minimal pressure that can generate a flow of 10 mL min⁻¹. The air flows through a humidifier, which contains 100 mL of water and a 2-L jar equipped with a clip-seal. Half a $SO_2$ sheet at typical dimension of 23 × 25 cm is placed in the jar. The air flows from the jar to the $SO_2$ trap, which contains 100 mL of hydrogen peroxide which was adjusted to pH 7.0 with NaOH. The system is maintained at 10 ± 0.5 °C.

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%. SO$_2$ 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 SO$_2$ 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 Keules (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 SO$_2$ 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 SO$_2$ that passes through the trap. We used the Monier-Williams trap by connecting it to a 2-L glass jar in which the SO$_2$ sheet was placed. The constant flow of humidified air through the jar delivers the SO$_2$ 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 SO$_2$ sheet and for the reaction with the metabisulphite salt.

### Basic parameters of the system

The performance of the SO$_2$ 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 SO$_2$ 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 SO$_2$ 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 ($P \leq 0.01$ 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 SO$_2$ 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 SO$_2$ trap after 24 h of flow. The correlation coefficient $R$ was equal to 1 at the range of 0–625 mg of SO$_2$ (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 SO$_2$ 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 SO$_2$ 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 SO$_2$ emitted from SO$_2$ 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 SO$_2$ trap.

**(a)**

$y = 53.38x$
$R^2 = 1.00$

NaOH (mL)

Sodium metabisulfite (g)

**(b)**

$y = -15.52x^2 + 62.71x - 0.10$
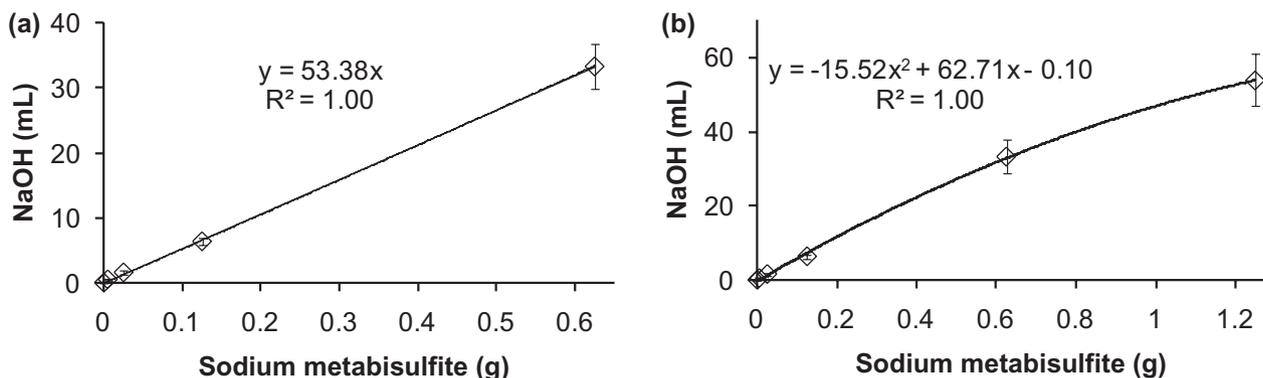$R^2 = 1.00$

NaOH (mL)

Sodium metabisulfite (g)

**Figure 2** Calibration curves for sodium metabisulphite. The curves plot between the amount of sodium metabisulphite and the amount of 0.01 N NaOH, which was used to titrate hydrogen peroxide to pH of 7.0. (a) Trend line and equation for the range of 0–0.625 g of sodium metabisulfite. (b) Trend line and equation for the range of 0–1.250 g of sodium metabisulphite.
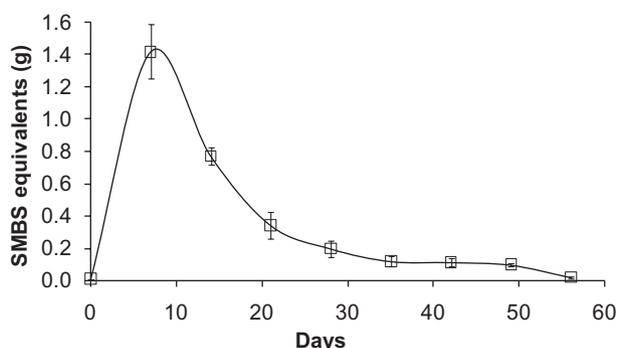
SMBS equivalents (g)

Days

**Figure 3** The amount of sodium metabisulphite (SMBS) equivalents released from an SO₂ sheet. Sampling was done once a week.

## 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 SO₂ emitted from the sheet did not exceed the capacity of the H₂O₂ trap (not shown). The area under the curve was calculated to be $10.7 \pm 0.7$ 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 $8.9 \pm 0.3$ 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 $SMBS_{Eq}$ 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 $SMBS_{Eq}$ 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-
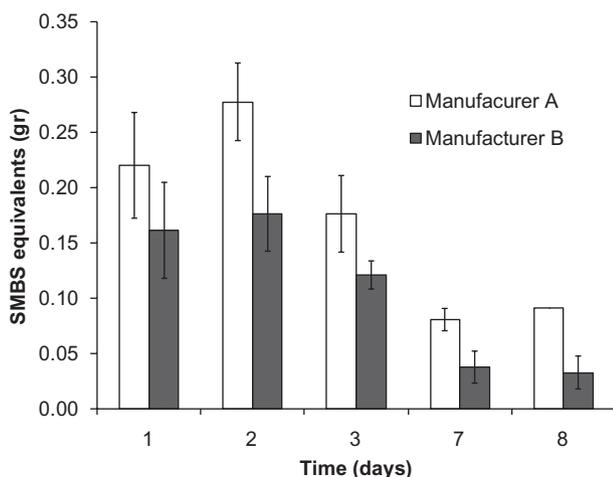
**Figure 4** The amounts of sodium metabisulphite (SMBS) equivalents released from two different SO₂ sheets. Each sampling was performed after 24 h from closing the system at the specified time points.

**Table 1** The amount of sodium metabisulphite equivalents released from different SO₂ sheets after 72 and 48 h of sampling with a 48-h interval between the two samplings

| Type | Manufacturer | Year | Phase I – 72 h | Phase II – 48 h |
|------|--------------|------|----------------|-----------------|
| I | A | 2003 | $3.01 \pm 0.30$ | $1.32 \pm 0.50$ |
| | | 2007 | $2.81 \pm 0.05$ | $0.52 \pm 0.04$ |
| | | 2007 | $3.32 \pm 0.10$ | $0.10 \pm 0.02$ |
| | | 2008 | $5.96 \pm 0.08$ | $0.75 \pm 0.08$ |
| | B | 2005 | $1.41 \pm 0.03$ | $0.14 \pm 0.03$ |
| | C | 2007 | $2.06 \pm 0.12$ | $0.11 \pm 0.03$ |
| II | A | 2008 | $1.88 \pm 0.09$ | $0.15 \pm 0.01$ |
| | B | 2005 | $4.40 \pm 0.33$ | $0.06 \pm 0.02$ |
| | C | 2006 | $0.27 \pm 0.04$ | $0.06 \pm 0.01$ |

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

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.

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
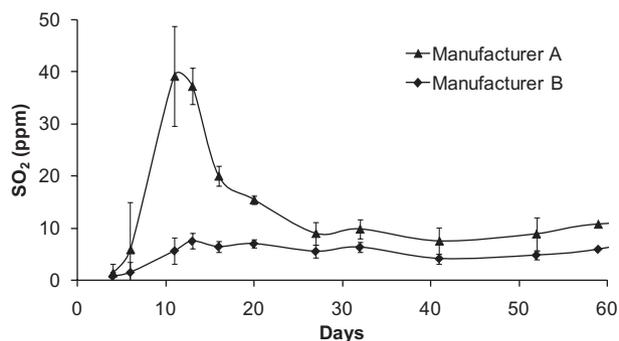
**Figure 5** The amount of SO₂ measured during storage of 'Redglobe' grapes. Two paper sheets from different manufacturers were wrapped in macro-perforated polyethylene liners. The sheets were placed over 5 kg of 'Redglobe' grapes in plastic boxes and three boxes composed minipallets that were wrapped with low-density polyethylene. The minipallets were stored at $0 \pm 0.5$ °C and SO₂ was monitored over 60 days in three replications with the elechtrochemical sensor.

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 LLEDP. SO₂ was monitored over 60 days in three replications with the elechtrochemical 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 SMBS$_{eq}$ and in the second phase a level of 0.52 and 0.11 SMBS$_{eq}$ 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.

## Acknowledgements

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