Hydrogen Sulfide Delays Postharvest Senescence and Plays an Antioxidative Role in Fresh-cut Kiwifruit

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Abstract. Hydrogen sulfide (H₂S) was recently recognized as an endogenous gaseous molecule involved in seed germination, root organogenesis, abiotic stress tolerance, guard cell movement, and delay of senescence in plants. In the present study, we show that H₂S participates in the regulation of postharvest ripening and senescence in fresh-cut kiwifruit, *Actinidia deliciosa*. Fumigation of fresh-cut kiwifruit with the H₂S donor sodium hydrosulfide (NaHS) solution prolonged kiwifruit storage time and alleviated senescence and tissue softening in a dose-dependent manner at an optimal concentration of 1.0 mmol·L⁻¹ NaHS. H₂S treatment maintained higher levels of reducing sugars, soluble proteins, free amino acids, ascorbate, and chlorophyll and lowered carotenoid levels. H₂S treatment also significantly decreased the contents of malondialdehyde (MDA), hydrogen peroxide (H₂O₂) and superoxide anion (O₂⁻) during fruit storage compared with water controls. Furthermore, the activities of guaiacol peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) were increased by H₂S treatment, whereas the activity of lipoxygenase (LOX) was decreased compared with untreated controls. Taken together, these results suggest that H₂S is involved in prolonging postharvest shelf life and plays an antioxidative role in fresh-cut kiwifruit.

H₂S has been identified as a third gas-transmitter after nitric oxide (NO) and carbon monoxide (CO) in animals (Wang, 2002; Yang et al., 2008). Accumulating evidence now shows that H₂S plays various physiological roles in plants, including regulating seed germination, root organogenesis, abiotic stress tolerance, photosynthesis, and guard cell movement, implying that H₂S acts as an important gaseous regulator (Chen et al., 2011; García-Mata and Lamattina, 2010; Hancock et al., 2011; Rausch and Wachter, 2005; Shan et al.,

2012; Zhang et al., 2008, 2009). In our previous studies, we presented evidence supporting a role of H₂S in delaying senescence of cut flowers and prolonging vase life in a wide spectrum of botanical species including herbaceous and woody plants, suggesting that the characteristic of H₂S might be universal in plant senescence (Zhang et al., 2011). More recently, H₂S was found to play an antioxidative role in prolonging postharvest shelf life of strawberry, which is a nonclimacteric fruit (Hu et al., 2012). Much less research has been carried out on the fruits of woody plants where the physiological role of H₂S in prolonging shelf life of these fruits is poorly understood.

Kiwifruit is classified as a climacteric fruit as a result of its high sensitivity to ethylene (Pranamornkith et al., 2012). Kiwifruit has become a product of interest as a result of its high level of ascorbate (vitamin C) as well as high concentrations of many types of mineral elements. Harvested kiwifruit undergoes a rapid rise in respiration rate after a week in storage, leading to a short shelf life under ambient conditions. Growers have therefore investigated the effectiveness of treatments that potentially extend the shelf life of kiwifruit, including application of pharmacological

agents such as 1-methylcyclopropene (Jhalegar et al., 2011).

It is believed that oxidative damage is one cause of the short shelf life of postharvest fruits (Hu et al., 2012). Among the naturally occurring compounds that have been shown to protect kiwifruit from oxidative damage caused by a reactive oxygen species (ROS) burst during storage is NO (Zhu et al., 2008). We have found that H₂S plays an antioxidative role in delaying senescence in various species of cut flowers and nonclimacteric fruits such as strawberry (Hu et al., 2012; Zhang et al., 2011), leading us to investigate the possibility that H₂S has similar effects on the senescence of postharvest climacteric fruits such as kiwifruit. We demonstrate that H₂S extended the shelf life of fresh-cut kiwifruit, probably through the up-regulation of antioxidant enzymes.

Materials and Methods

Plant materials and treatments. Kiwifruit (*Actinidia deliciosa*) used in this work was supplied by a fruit market in Hefei, Anhui province, China. Fruit harvested in the same day with similar size and maturity (80%) were selected in the morning and transported to the laboratory within 2 h. Kiwifruit without physical damage and microbial and insect infections were selected for experiments. Fruit were cut into eight pieces of the same size and weight, and H₂S was applied through the H₂S donor NaHS (Sigma, St. Louis, MO). Aqueous NaHS solutions (150 mL) at 0, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, or 1.75 mmol·L⁻¹ were prepared in sealed containers (volume 3 L) and the eight cut pieces from eight different kiwifruit were exposed to H₂S gas released from NaHS solution in the sealed containers. The storage temperature was 20 ± 0.5 °C and the relative humidity was 85% to 90%. NaHS solutions were renewed daily and the kiwifruit were observed for every 24 h. The experiment was repeated for three fruit seasons and a similar phenomenon was observed.

Rot classification of fruits. Eight pieces of kiwifruit were used in each treatment for rot classification. Fruits were classified in four ranks according to the percentage of rotten surface area: 1 = rot surface less than 10%; 2 = rot surface between 10% and 30%; 3 = rot surface between 30% and 50%; and 4 = rot surface more than 50%. The rot classification was recorded everyday. The experiment was repeated three times.

Determination of fruit firmness. Fruit firmness was measured at the equatorial part of each fresh-cut kiwifruit pieces by a 5-mm diameter flat probe with a texture analyzer (Model TA XT plus; SMS). The penetration depth was 5 mm and the cross-head speed was 5 mm·s⁻¹. Fruit firmness values were an average of 8 kiwifruit pieces ± SD. The experiment and following ones were repeated three times.

Determination of malondialdehyde, hydrogen peroxide, and superoxide anion. The contents of MDA, H₂O₂ and O₂⁻ were determined by the procedures described by Zhang et al. (2010a). Fruit samples (5.00 ± 0.05 g)

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were ground in 3 mL of 0.1% trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 15,000 *g* for 10 min, and 0.5 mL of the supernatant fraction was mixed with 2 mL 20% TCA containing 0.5% thiobarbituric acid. The mixture was heated at 90 °C for 20 min, cooled, and centrifuged at 10,000 *g* for 5 min. Absorbance was recorded at 532 nm and the value for non-specific absorption at 600 nm was subtracted. The extinction coefficient of

155 $\text{mm}^{-1}\cdot\text{cm}^{-1}$ was used to calculate the content of MDA.

For determination of H_2O_2 , fruit samples (5.00 ± 0.05 g) were ground and extracted in 3 mL cold acetone. The homogenate was centrifuged at 10,000 *g* at 4 °C for 20 min and 0.5 mL of the supernatant fraction was mixed with 1.5 mL of CHCl_3 and CCl_4 (1:3, V/V) mixture. Subsequently, 2.5 mL of distilled water was added and the mixture centrifuged

at 1000 *g* for 1 min and the aqueous phase collected for H_2O_2 determination. The reaction system included 0.5 mL sample, 0.5 mL of buffer (phosphate-buffered saline, 200 $\text{mmol}\cdot\text{L}^{-1}$, pH 7.8), and 20 μL 0.5 unit of catalase (as controls) or inactive catalase protein (catalase inactivated by heating in boiling water for 5 min). After the mixture was incubated at 37 °C for 10 min, 0.5 mL of 200 $\text{mmol}\cdot\text{L}^{-1}$ Ti-4-(2-pyridylazo) resorcinol

Fig. 1. Effect of hydrogen sulfide (H_2S) on postharvest shelf life and rot classification in kiwifruit. (A) Photographs of kiwifruit after exposure to 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, or 1.75 $\text{mmol}\cdot\text{L}^{-1}$ H_2S donor sodium hydrosulfide (NaHS) for 0 to 5 d, respectively. Lower left rectangle indicates experimental treatments. (B) Photographs of classification standard ranging from 1 to 4 for rot classification of kiwifruit. (C) Change in rot classification of kiwifruit treated with H_2S donor NaHS at different concentrations (0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, and 1.75 $\text{mmol}\cdot\text{L}^{-1}$). (D) Effect of H_2S on changes in firmness in kiwifruit treated with H_2O (shown as CK) and 1.0 $\text{mmol}\cdot\text{L}^{-1}$ H_2S donor NaHS (shown as H_2S) at 20 °C for 5 d. 0 represents the samples from kiwifruit before treated with water or NaHS and 1, 2, 3, 4, and 5 as samples of the fruits exposed to water or 1.0 $\text{mmol}\cdot\text{L}^{-1}$ NaHS, respectively. Values are the means $\pm$ SD ($n = 3$). The symbols * and ** in this figure and following ones stand for significant difference between CK and H_2S fumigation at $P < 0.05$ and $P < 0.01$, respectively.

was added and the reaction mixture incubated at 45 °C for another 20 min. Absorbance at 508 nm was measured.

O_2^- samples (0.5 ± 0.05 g) were ground with 3 mL of 50 mmol·L⁻¹ Tris-HCl buffer (pH 7.5) and the homogenate was centrifuged at 5000 g at 4 °C for 10 min. The reaction mixture (1 mL) contained 50 mmol·L⁻¹ Tris-HCl buffer (pH 7.5), 0.5 mmol·L⁻¹ XTT [sodium, 3-1-(phenylamino-carbonyl)-3, 4-tetrazolium-bis(4-methoxy-6-nitro) benzene-sulfonic acid hydrate], and 50 µL of sample extracts. Corrections were made for the background absorbance in the presence of 50 U of superoxide dismutase (SOD). O_2^- content was calculated by an extinction coefficient of 2.16×10^4 M⁻¹·cm⁻¹.

Assays of POD, APX, CAT, LOX, and GR activities. Activities of CAT, and LOX were determined by procedures described in Hu et al. (2012) and Zhang et al. (2010b). Frozen fruit samples (5.00 ± 0.05 g) were homogenized in ice-cold 50 mmol·L⁻¹ phosphate buffer (pH 7.8) containing 1.0 mmol·L⁻¹ ethylenediaminetetraacetic acid. The homogenate was centrifuged at 15,000 g at 4 °C for 10 min and the supernatant was used for activity measurement. Activities of CAT were assayed spectrophotometrically by recording the decrease in absorbance of H₂O₂ at 240 nm. For the LOX assay, samples were homogenized with 50 mmol·L⁻¹ phosphate buffer (pH 6.0). The homogenate was centrifuged at 15,000 g at 4 °C for 10 min and the supernatant was used for the enzyme assay. The assay mixture in a total volume of 1.5 mL contained 200 mmol·L⁻¹ borate buffer (pH 6.0), 0.25% linoleic acid, 0.25% Tween-20, and 50 µL of enzyme extract. The reaction was carried out at 25 °C for 5 min and the activities of LOX were determined in the presence of linoleic acid by monitoring the changes in absorbance at 234 nm. POD activity was based on the oxidation of guaiacol by hydrogen peroxide. The reaction mixture contained 2.6 mL of 50 mmol·L⁻¹ phosphate buffer (pH 6.1), 1 mL of 3% H₂O₂, 1 mL of 1% guaiacol, and 100 to 200 µL of enzyme extract. The increase in absorbance at 420 nm was recorded. APX activity was determined in the presence of 0.5 mmol·L⁻¹ ascorbic acid and 0.5 mmol·L⁻¹ H₂O₂ by monitoring the decrease in absorbance at 290 nm. Activity of GR (Enzyme Commission 1.6.4.2) was determined by the method described by Knörzer et al. (1996).

Detection of reducing sugars, ascorbate, soluble protein, and free amino acids. Fruit samples (5.00 ± 0.05 g) were ground in 5 mL of phosphate buffer (pH 7.0, 200 mmol·L⁻¹). The homogenate was centrifuged at 10,000 g for 30 min, and the supernatant was used for determination of reducing sugars and soluble protein content. Reducing sugars were measured according to Miller (1959) through the dinitrosalicylic acid method. The supernatant (0.2 mL) was mixed with 1.5 mL of 3,5-dinitrosalicylic acid and 1.8 mL of distilled water; the mixture was heated at 100 °C for 5 min, cooled, and brought to 25 mL with distilled water. Reducing sugars were determined spectrophotometrically at 540 nm

and the results are expressed as mg·g⁻¹ fresh weight (FW).

The content of ascorbate was determined by the 2,6-dichloroindophenol titrimetric method according to the Association of Official Analytical Chemists method (AOAC, 1984).

For soluble protein, 0.1 mL of supernatant was mixed with 0.9 mL of distilled H₂O and 5 mL of Coomassie brilliant blue. Absorbance was recorded at 595 nm after 5 min by the method described by Bradford (1976). The results are expressed µg·g⁻¹ FW.

For assay of free amino acid content, samples (5.00 ± 0.05 g) were ground in 5 mL of 80% ethanol, the homogenate was centrifuged at 10,000 g for 5 min, and the supernatant was used for free amino acid measurement. Free amino acid content was measured according to Hu et al. (2012) on an automatic amino acid analyzer (Model S433D, Syknam; GER).

Chlorophyll and carotenoid determination. Chlorophyll was measured according to Lichtenthaler and Wellburn (1983) with minor modifications. Fresh samples of kiwifruit (5.00 ± 0.05 g) were homogenized using a pestle and mortar in the presence of ice. One hundred milliliters of pure ethanol and 80% acetone (1:1) were added to the fruit sample to 25 mL. After 24 h incubation, the two

phases separated, and an aliquot was sampled from the upper solution for O.D. value measurement at 663, 645, and 440 nm in a spectrophotometer. Chlorophyll and carotenoid contents were calculated with the following equations: Car = $A_{440}V/W$; Chla = $(12.7A_{663} - 2.69A_{645})V/W$; Chlb = $(22.9A_{663} - 4.68A_{645})V/W$; Chl = Chla + Chlb. Chlorophyll and carotenoid contents were expressed as mg·g⁻¹ FW.

H₂S treatment on some other fruits. Apples and cantaloupes were used as materials in this experiment, kindly supplied by the orchard of Anhui Academy of Agricultural Sciences, Anhui, China. Aqueous NaHS solutions (150 mL) at different concentrations were prepared in sealed containers (volume 3 L). Then six pieces from six different fruits are fumigated with H₂S gas released from NaHS in the sealed containers. The storage temperature was 20 ± 0.5 °C and the relative humidity was 85% to 90%. Treatment solutions were renewed daily and the apples and cantaloupes were observed every 12 h.

Statistics. Statistical significance was tested by one-way or two-way analysis of variance, and the results are expressed as the mean values $\pm$ SD of three independent experiments. Fisher's least significant differences were calculated following a significant ($P < 0.01$ or $P < 0.05$) *t* test. The symbols * and ** in the

Fig. 2. Effect of hydrogen sulfide [1.0 mmol·L⁻¹ sodium hydrosulfide (NaHS)] on contents of (A) ascorbate, (B) chlorophyll, and (C) carotenoid in kiwifruit during storage at 20 °C. Values are the means $\pm$ SD ($n = 3$).

figures stand for significant difference between treatments at $P < 0.05$ and $P < 0.01$, respectively.

Results

The H₂S donor NaHS prolongs postharvest shelf life of fresh-cut kiwifruit. In our experiments, fresh-cut kiwifruit were fumigated with different NaHS concentrations (0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 mmol·L⁻¹) and exposure to deionized water served as controls. H₂S fumigation alleviated postharvest ripening and senescence of kiwifruit in a dose-dependent manner (Fig. 1A–C). Kiwifruit exposed to water vapor matured and rotted quickly after 1 d storage, whereas kiwifruit treated with a range of NaHS concentrations had significantly prolonged storage life (Fig. 1A) according to the four ranks of rotting classification (Fig. 1B). The maximum post-harvest shelf life of a kiwifruit slice was obtained by exposed to 1.0 mmol·L⁻¹ NaHS, and this optimal concentration was therefore used for subsequent experiments (Fig. 1C). Treatment with higher concentrations of NaHS (above 1.0 mmol·L⁻¹ to 1.75 mmol·L⁻¹) showed no additional protection against kiwifruit senescence. Kiwifruit showed a decrease in firmness over time during storage in both controls and H₂S treatment (Fig. 1D). However, fruits treated with 1.0 mmol·L⁻¹ NaHS were significantly firmer than controls during the storage period.

Effects of H₂S on ascorbate, chlorophyll, and carotenoid content of kiwifruit. Ascorbate exists in kiwifruit as a natural antioxidant buffer, whereas chlorophylls and carotenoids are two pigments that affect the color of kiwifruit and are good measures of fruit senescence (Nishiyama et al., 2005). To test the effect of H₂S fumigation on delayed ripening and senescence in kiwifruit, contents of ascorbate, chlorophyll, and carotenoid in kiwifruit were determined. The ascorbate content of kiwifruit slices exposed to water vapor declined rapidly, whereas 1 mmol·L⁻¹ NaHS treatment maintained a significantly higher level of ascorbate after 1 d storage (Fig. 2A). H₂S fumigation significantly alleviated the drop in ascorbate content in fruits (Fig. 2A).

As shown in Figure 2B, chlorophyll content increased gradually in control and NaHS-treated fruit slices during the first 3 d and increased dramatically between Days 4 and 5. However, chlorophyll content in NaHS-treated fruit was significantly higher than in the control. Carotenoid content of NaHS-treated fruit remained at the initial level until 2 d and then increased gradually, whereas carotenoid content in water controls increased during the whole storage and was always significantly higher than that of water controls. For instance, carotenoid level in water controls on Day 5 was 25% higher than in NaHS-treated fruit (Fig. 2C).

Contents of malondialdehyde, superoxide anion, and hydrogen peroxide in kiwifruit. To investigate whether the alleviating effect of H₂S fumigation on kiwifruit senescence is

associated with lipid peroxidation and oxidative stress, MDA, 'O₂⁻, and H₂O₂ contents were evaluated. As shown in Figure 3A, MDA content increased steadily in kiwifruit treated with NaHS or water and peaked on Day 4 d followed by a decrease on Day 5. However, H₂S fumigation maintained a significantly lower level of MDA compared with water controls.

The content of 'O₂⁻ in both treatments increased during storage but the content in H₂S-fumigated kiwifruit was significantly lower than in water controls (Fig. 3B). For example, on Day 3, 'O₂⁻ content in water controls was nearly 2-fold that of NaHS-treated tissue. H₂O₂ content also increased dramatically in control kiwifruit on 1 d and then fell until 3 d followed by a rapid increase (Fig. 3C). Although a gradual increase in H₂O₂ content was observed, NaHS treatment maintained a significantly lower level of H₂O₂ in kiwifruit.

Effects of NaHS treatment on POD, CAT, APX, GR, and LOX activities in kiwifruit. To further understand the role of H₂S on ROS metabolism in kiwifruit slices during storage, we analyzed the activities of antioxidant enzymes. As shown in Figure 4A, POD activity in kiwifruit increased in both treatments but H₂S fumigation maintained a significantly higher level of POD activity than in water controls, leading to levels of POD that were

almost twice that in water controls. Figure 4B shows the changes of CAT activity in both treatments. CAT activity increased rapidly in NaHS-treated kiwifruit on Day 1 followed by a gradual decrease until 5 d. In contrast, CAT activity in water controls decreased during the whole treatment time and was always markedly lower than that of H₂S-fumigated fruit.

APX activity in kiwifruit exposed to H₂S increased gradually on Day 1 and maintained a stable level from 1 to 3 d followed by a sharp decrease until 5 d (Fig. 4C). Maximum APX activity occurred at 1 d of storage in water controls and thereafter the activity dropped dramatically. During the entire 5-d storage period, H₂S fumigation sustained a higher level of APX in fresh-cut kiwifruit than water controls.

Glutathione reductase, which catalyzes the regeneration of reduced glutathione, was measured as another indicator of ROS. GR activity in both NaHS and water treatments increased dramatically on Day 1, but the level of GR activity in NaHS-treated tissue was ≈30% higher than in controls (Fig. 4D). Thereafter, GR activity in NaHS treatment decreased sharply to 4 d and maintained a stable level to 5 d. In contrast, in water controls, GR activity decreased rapidly after 2 d of storage. On Day 5 of storage, GR activity in water controls was only ≈50% of that in H₂S-fumigated tissue.

Fig. 3. Effect of hydrogen sulfide (H₂S) [1.0 mmol·L⁻¹ sodium hydrosulfide (NaHS)] on the contents of (A) malondialdehyde (MDA), (B) superoxide anion ('O₂⁻), and (C) hydrogen peroxide (H₂O₂) in kiwifruit during storage at 20 °C. Values are the means ± SD (n = 3).

Fig. 4. Effect of hydrogen sulfide (H_2S) [$1.0 \text{ mmol} \cdot \text{L}^{-1}$ sodium hydrosulfide (NaHS)] on the activities of (A) guaiacol peroxidase (POD), (B) catalase (CAT), (C) ascorbate peroxidase (APX), (D) glutathione reductase (GR), and (E) lipoxygenase (LOX) in kiwifruit during storage at 20°C . Values are the means $\pm$ SD ($n = 3$).

LOX is a marker of lipid peroxidation. Figure 4E shows that LOX activities increased gradually during storage under both

treatment conditions. However, NaHS treatment always maintained a significant lower level of LOX than in water controls.

Effects of H_2S on content of reducing sugar, soluble protein and free amino acid. Figure 5A shows that NaHS counteracted the

decrease in reducing sugar content in kiwifruit during postharvest storage. During early stages of storage (0 to 2 d), reducing sugar content of NaHS-treated fruits increased dramatically and peaked at 2 d and then fell rapidly until the end of the experiment. The content of reducing sugars in control fruits maintained the initial level to 2 d and then decreased sharply.

H₂S fumigation prevented the decline in soluble protein in fresh-cut kiwifruit. As shown in Figure 5B, soluble protein content decreased in both treatments during the first day of storage and then increased gradually until 3 d. Thereafter, the content of soluble protein in NaHS treatment maintained a stable level from 3 d to 5 d, whereas that of the water control decreased rapidly. At Day 5 of storage, soluble protein in H₂S-treated tissue was reduced by only ≈15% of initial values, whereas in water controls, it was ≈50% of the initial value.

The effects of H₂S fumigation on the contents of free amino acids in kiwifruit after 2 and 3 d of storage are shown in Table 1. With the exceptions of aspartic acid, glutamic acid, and histidine, the contents of all free amino acids in NaHS-treated fruit were higher than those of fresh fruit. In contrast, with the exception of Val, the contents of free amino

acids in control fruits were lower than those in fresh fruit. NaHS treatment maintained a higher level of total free amino acids than water control after 2 and 3 d of storage.

Effect of H₂S on shelf life in other fruits. To establish whether the effect of H₂S on the shelf life of a range of commercially important fruits is universal, we exposed slices of these fruits to H₂S vapors. Fresh-cut apple and cantaloupe were fumigated with different concentrations of NaHS. Similar to that observed in kiwifruit, NaHS treatment maintained the freshness and extended the shelf life of fresh-cut fruit in a dose-dependent manner (Fig. 6A–B).

Discussion

The present study shows that H₂S fumigation extends postharvest shelf life of kiwifruit by counteracting fruit senescence, fruit softening, and decay. Our data show that the effects of H₂S are likely exerted by reducing the impact of oxidative stress in stored tissue. We also show that the effects of H₂S on postharvest shelf life are also applicable to other fruits because we show that senescence of cantaloupe and apple can be alleviated by exposure to H₂S.

In our experiments, H₂S was generated from the H₂S donor NaHS. NaHS has been widely used as a H₂S donor in both animal and plant systems (Hosoki et al., 1997; Zhang et al., 2008). NaHS has been shown to dissociate into Na⁺ and HS⁻ in aqueous solution and HS⁻ associates with H⁺ to produce H₂S. Aqueous NaHS solution (200 mL) of 1.00 mmol·L⁻¹ in sealed containers (volume: 3 L) could steadily release 1.50 × 10⁻¹⁰ mol·L⁻¹ H₂S gas since 30 min after NaHS solution is prepared and almost kept at the same level until 1440 min (24 h). The concentrations of the applied H₂S gas are quite low. Besides, our previous report shows that the levels of endogenous H₂S in fruits treated with exogenous H₂S gas are ≈10% to 20% higher than those of the water control, further suggesting its safe use in postharvest storage. Fresh-cut fruit are wounded, causing accelerated consumption of sugars, lipids, organic acids, and senescence. Thus, fresh-cut fruits are widely used as models to study the decay and senescence during storage (Oms-Oliu et al., 2008). In the present study, fresh-cut kiwifruit was used to as a fresh-cut fruit model to study the role H₂S on fruit senescence.

Although H₂S has been widely regarded as a toxic gas, it has been demonstrated as a novel gaseous signaling molecule along with other gases such as NO and CO (Wang, 2002; Yang et al., 2008; Zhang et al., 2008). H₂S has been shown to be involved in various processes in plants including seed germination, root organogenesis, abiotic stress tolerance, photosynthesis, and guard cell movement (Chen et al., 2011; García-Mata and Lamattina, 2010; Hancock et al., 2011; Rausch and Wachter, 2005; Shan et al., 2012; Zhang et al., 2008). Recently, H₂S were found to delay the senescence of cut flowers and nonclimacteric fruit (Hu et al., 2012; Zhang et al., 2011).

Ripening and senescence in fruit are complex processes, which involve the overproduction of ROS such as the superoxide anion (O²⁻) and H₂O₂ (Vicente et al., 2006). The accumulation of ROS resulting from an altered balance between ROS production and ROS scavenging capacity causes oxidative damage to plant cells (Apel and Hirt, 2004), including protein breakdown and lipid peroxidation. We show that NaHS treatment of a

Fig. 5. Effect of hydrogen sulfide (H₂S) [1.0 mmol·L⁻¹ sodium hydrosulfide (NaHS)] on the contents of (A) reducing sugar and (B) soluble protein in kiwifruit during storage at 20 °C. Values are the means ± SD (n = 3).

Table 1. Effect of hydrogen sulfide (H₂S) on free amino acid content of kiwifruit during storage at 20 °C.^a

	Asp	Ser	Glu	Gly	Ala	Met	Val	Ile
0	292.04 ± 3.18 a ^y	774.94 ± 6.49 a	160.21 ± 1.51 a	22.63 ± 1.93 a	220.82 ± 3.03 a	9.80 ± 0.23 a	ND	9.02 ± 1.29 a
CK2	148.22 ± 3.09 b	462.46 ± 13.25 b	126.11 ± 3.49 b	17.70 ± 1.02 b	136.07 ± 4.28 b	6.26 ± 0.53 b	ND	6.63 ± 0.74 a
T2	50.31 ± 0.20 c	800.22 ± 35.22 a	58.61 ± 14.38 c	119.94 ± 2.90 c	802.95 ± 19.07 c	11.98 ± 0.32 c	44.63 ± 2.54 a	31.20 ± 2.53 b
CK3	131.14 ± 2.35 d	247.14 ± 7.01 c	103.23 ± 2.79 d	29.13 ± 2.25 d	77.41 ± 2.58 d	7.30 ± 0.35 b	11.66 ± 8.44 b	7.01 ± 0.43 c
T3	25.52 ± 1.63 e	463.13 ± 0.68 b	30.38 ± 0.36 e	109.44 ± 1.27 e	742.45 ± 2.29 e	9.43 ± 0.24 a	55.47 ± 6.49 c	37.82 ± 0.47 d
	Leu	Tyr	Phe	His	Lys	Arg	Total AA	
0	30.89 ± 0.38 a	8.70 ± 0.10 a	8.52 ± 0.34 a	103.03 ± 4.90 a	27.38 ± 0.51 a	513.86 ± 34.65 a	2181.82 ± 33.36 a	
CK2	22.71 ± 0.82 b	9.04 ± 0.42 a	8.08 ± 0.03 a	76.44 ± 3.60 b	17.41 ± 0.26 b	358.13 ± 11.03 b	1395.27 ± 41.09 b	
T2	57.73 ± 3.06 c	18.80 ± 2.04 b	14.64 ± 1.13 b	102.79 ± 3.20 a	47.84 ± 1.12 c	605.69 ± 19.68 c	2767.34 ± 106.99 c	
CK3	24.21 ± 0.81 b	8.87 ± 0.63 a	5.49 ± 1.24 c	71.63 ± 1.02 b	18.23 ± 0.79 b	208.85 ± 5.55 d	951.29 ± 15.19 d	
T3	59.38 ± 0.58 c	15.63 ± 2.30 b	15.40 ± 1.64 b	87.26 ± 0.17 c	44.59 ± 3.47 c	338.23 ± 3.03 b	2034.12 ± 3.05 e	

^aSlices of kiwifruit were fumigated with H₂O (shown as CK2, CK3) or 1 mmol·L⁻¹ sodium hydrosulfide (NaHS) solution (shown as T2, T3) for 2 or 3 d and prepared for amino acid determination. 0 represents freshly harvested fruit; CK represents H₂O control, and T represents treatment with NaHS.

^yDifferent letters mean significant difference between treatments at *P* < 0.01.

Asp = aspartic acid; Ser = serine; Glu = glutamic acid; Gly = glycine; Ala = alanine; Met = methionine; Val = valine; Ile = isoleucine; Leu = leucine; Tyr = tyrosine; Phe = phenylalanine; His = histidine; Lys = lysine; Arg = arginine; AA = amino acid; ND = not detected.

Fig. 6. Effect of hydrogen sulfide (H_2S) on postharvest shelf life in other fruits. Photographs of apples (A) and cantaloupes (B) after exposure to different concentrations of H_2S donor sodium hydrosulfide (NaHS) for storage time. Lower left rectangle indicates experimental treatments.

kiwifruit slice alleviates senescence-induced oxidative stress by maintaining lower levels of O_2^- and H_2O_2 (Fig. 3B–C), suggesting that H_2S fumigation functions in part by decreasing ROS production, consequently reducing oxidative stress in fresh-cut kiwifruit.

MDA, a secondary end product of polyunsaturated fatty acid oxidation, is an index of lipid peroxidation (Imahori et al., 2008). In the present study, kiwifruit senescence was accompanied by an increase in the content of MDA, whereas H_2S fumigation prevented MDA accumulation (Fig. 3A). LOXs are a family of enzymes that catalyze the oxygenation of polyunsaturated fatty acids and the formation of lipid hydroperoxides during responses to various environmental stresses (Porta and Rocha-Sosa, 2002; Shibata and Axelrod, 1995). The decreased level of MDA in H_2S -treated kiwifruit tissue is associated with lowered LOX activity compared with water

controls (Figs. 3A and 4E), suggesting that H_2S reduced lipid peroxidation, maintaining a balanced redox state in postharvest kiwifruit.

Plants have developed a scavenging system to mitigate and repair oxidative damages triggered by ROS accumulation. An antioxidant defense system is composed of ROS scavenging enzymes such as SOD, CAT, POD, and APX (Apel and Hirt, 2004) and also some antioxidants such as glutathione (Rausch and Wachter, 2005). SODs catalyze the dismutation of O_2^- and H^+ into H_2O_2 (Gallie, 2013), and H_2O_2 is reduced to water by enzymes such as POD, CAT, or APX (Gill and Tuteja, 2010; Korniyev et al., 2001). It is widely accepted that APX, CAT, and POD constitute the primary antioxidant defense system that provides protection against ROS in plants. APX and GR are key enzymes in the ascorbate–glutathione cycle and play important roles in the homeostasis of ascorbate and

glutathione in plants (Noctor and Foyer, 1998). In the present study, H_2S fumigation enhanced the activities of POD, CAT, APX, and GR in kiwifruit compared with water controls (Fig. 4A–D), and we propose that the enhanced antioxidant system contributes to reducing the levels of O_2^- and H_2O_2 (Fig. 3B–C). In agreement with the present study, we previously reported on the role of H_2S in regulating antioxidant enzymes in cut flowers and strawberry (Hu et al., 2012; Zhang et al., 2011), suggesting that the up-regulation of ROS scavenging enzyme activities triggered by H_2S may be a general mechanism for delaying senescence in plants.

During storage of a kiwifruit slice, the contents of soluble protein and ascorbate were decreased in water controls, whereas H_2S fumigation maintained relatively stable levels of these compounds (Figs. 2A and 5B). Free amino acids are a nitrogen pool, containing many intermediates involved in nitrogen metabolism. H_2S fumigation promoted the accumulation of most free amino acids in comparison with water treatment, except for aspartic acid, glutamic acid, and histidine. The content of carotenoid was reduced in H_2S -fumigated kiwifruit (Fig. 2C). Besides, H_2S fumigation maintained a higher level of chlorophyll than the water control (Fig. 2B). Surprisingly, we observed that chlorophyll in kiwifruit increased with storage time even in water controls (Fig. 2B).

H_2S can be formed endogenously from sulfite or cysteine by the actions of sulfite reductase and desulfhydrases, respectively (Rausch and Wachter, 2005). Plants have been shown to respond to pathogen attack by releasing H_2S , implying a signaling role of endogenous H_2S (Bloem et al., 2004). Previous work has also reported the antioxidative role of H_2S against various abiotic stresses in plant (Zhang et al., 2008, 2009). In this work, exogenous-applied H_2S is able to delay the senescence of postharvest kiwifruit through the enhancement of antioxidant enzymes, suggesting a ubiquitous function of H_2S in the prevention of oxidative stress. Meanwhile, there is growing concern by consumers about the safety of synthetic chemicals on postharvest fruits. Research has therefore focused on developing alternative treatments of postharvest fruits that are more acceptable to consumers. In this work, exogenous-applied H_2S was found to be able to delay the senescence of postharvest kiwifruit, suggesting its considerable commercial significance in fruit storage.

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