Hydrogen Sulfide Alleviates Senescence of Fresh-cut Apple by Regulating Antioxidant Defense System and Senescence-related Gene Expression

Ji-Lian Zheng1, Lan-Ying Hu1, and Kang-Di Hu1
School of Biotechnology and Food Engineering, Hefei University of Technology, Hefei 230009, China

Jun Wu
College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China

Feng Yang
Xuzhou Institute of Agricultural Sciences of the Xuihuai District of Jiangsu Province, Xuzhou 221131, China

Hua Zhang2
School of Biotechnology and Food Engineering, Hefei University of Technology, Hefei 230009, PR China

Additional index words. browning, ethylene, protease, reactive oxygen species

Abstract. Hydrogen sulfide (H2S) has been identified as a multifunctional signaling molecule in plants. Here, we show that H2S delayed postharvest senescence of fresh-cut apples (Malus × pumila) in a dose-dependent manner. Exogenous H2S application maintained significantly higher levels of ascorbic acid, flavonoids, total phenolics, and lower levels of free amino acids in apple slices compared with controls. Further investigations showed that H2S significantly reduced the accumulation of superoxide radicals, hydrogen peroxide (H2O2) and malondialdehyde (MDA). Apple fruits fumigated with H2S contained significantly higher activities of antioxidant enzymes than control fruits, indicating that H2S alleviated dark-promoted senescence by enhancing the expression of defense-related genes. These results are novel and important for understanding the roles of H2S in detoxifying ROS overproduction, enhancing antioxidant defense, and regulating senescence-related gene expression.

Hydrogen sulfide, similar to nitric oxide (NO) and carbon monoxide (CO), has been shown to be an endogenous gaseous signaling molecule in animal systems with multifaceted physiological functions (Wang, 2002). In plants, H2S can be endogenously generated from cysteine and sulfite by O-acetylserine (thiol) lyase and sulfite reductase, respectively (Rausch and Wachter, 2005). Accumulating evidence indicates that H2S functions in various processes in plants, including seed germination, root organogenesis, abiotic stress tolerance, photosynthesis, guard cell movement, and postharvest senescence, suggesting that H2S acts as an important gaseous regulator in plants, as do NO and CO (Chen et al., 2015). Li et al. (2015) showed that the post-harvest application of H2S alleviated dark-promoted senescence by reducing the transcript levels of BoACS2 and BoACS3 in broccoli. Pristijono et al. (2006) indicated that NO delayed ripening and senescence of apple slices by influencing ethylene production during postharvest storage. However, there are few reports on the effects of H2S on the ethylene signaling pathway and on the antioxidant system in fresh-cut fruits. Thus, we hypothesize that H2S has similar positive effects as NO in delaying postharvest senescence of apple slices by acting as an antioxidant and by regulating senescence-related gene expression.

Materials and Methods

Plant materials and treatment. ‘Fuji’ apple (Malus × pumila) used in this work was supplied by Anhui Academy of Agricultural Sciences, Hefei, Anhui Province, China. Unwounded, healthy fruits of uniform size, color, and weight were selected for experiments. Apples were washed with tap water and sterilized with 75% ethanol. In sealed 3-L containers, 200 mL sodium hydrosulfide (NaHS) solutions at concentrations of 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 mmol-L−1 were prepared. Each apple was hand cut longitudinally into six unpeeled slices using a sharp stainless steel knife, and six cut pieces from six different apples were exposed to H2S gas released from NaHS solution in the sealed containers for 0–5 d. Storage temperature was 25 ± 0.5 °C and relative humidity ranged from 85% to 90%. NaHS solutions were renewed daily, and the apples were observed every 24 h. After treatment, apple slices were...
Assessment of rot index and external color of apple slices. Rot index was recorded using the method described by Cao et al. (2010) with minor modifications. Six apple slices were selected for rot index in each treatment. All slices were classified in five ranks according to the percentage of rotten surface area: 0, no rot; 1, rot surface less than 10%; 2, rot surface between 10% and 30%; 3, rot surface between 30% and 50%; 4, rot surface more than 50%. The rot index was calculated and recorded every 2 d using the following formula: rot index = \( \sum \frac{A_i}{A_T} \), where \( A_i \) represents the rot rank of individual slice, \( A_T \) the number of slice at that rot rank.

External color of cut surface of apple slices (a total of 12 cut surfaces of 6 apple slices for each treatment) was directly measured with a color difference meter (model \( \text{L}^* \)). The number of slice at that rot rank.

The homogenate was centrifuged at 10,000 \( g \) for 30 min, and the supernatant was used for the activity assay. Activity of PAL (EC 4.3.1.13) was determined by procedures described by Beaudoin-Eagan and Thorpe (1985). One unit of PAL activity was defined as a change of 0.01 OD value in absorbance at 500 nm per minute. The results were expressed on an FW basis as U \( g \). Activity of PPO (EC 1.10.3.1) was assayed according to the method by Benjamin and Montgomery (1973). Apple samples (5.00 ± 0.05 g) were homogenized with 3.0 mL of 10% (w/v) polyvinyl pyrrolidone, centrifuged at 10,000 \( g \), at 4 °C for 30 min, and the supernatant was divided into aliquots, frozen in liquid nitrogen, and stored at –80 °C for further activity measurement.

Activity of APX, CAT, and PPO was defined as an increase or decrease of 0.01 in absorbance per minute under the assay conditions. The activities of APX, CAT, and PPO were expressed on an FW basis as U \( g \). SOD activity was assayed from the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) in the presence of riboflavin. One unit of SOD was defined as the amount of enzyme that inhibits the rate of NBT reduction by 50% under the above assay conditions. The activity was expressed on an FW basis as U \( g \). Activities of GR (EC 1.6.4.2) and LOX (EC 1.13.11.12) were detected by procedures described by Garcia-Limones et al. (2002) and Surrey (1964), respectively. One unit of GR and LOX was defined as a decrease of 0.01 OD value in absorbance per minute, and the results were expressed on an FW basis as U \( g \).
transcription kit (Prime Script™ RT Master Mix; Takara) from 2.5 μg total RNA. cDNA fragments were amplified by reversed transcript polymerase chain reaction (RT-PCR) with Easy Taq™ DNA Polymerase (Trans-Gen, Beijing, China). Primers used for RT-PCR are shown in Table 1. PCR conditions were initial denaturation at 94 °C for 5 min, followed by appropriate cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s. Ml18S RNA was used as a control.

Statistical analysis. The data in the manuscript are based on three replicates in each experiment, and the experiments were repeated independently for three times and similar change pattern was observed. Statistical significance was tested by one-way analysis of variance (ANOVA) using IBM SPSS Statistics (SPSS version 20.0; Armonk, NY), and the results were expressed as the means ± SD. Least significant difference test was performed on all data following ANOVA tests to test for significant (P < 0.05 or P < 0.01) differences between treatments.

Results

H2S alleviates postharvest senescence of apple slices. Fresh-cut apple slices were fumigated with H2S released from different concentrations of NaHS with slices exposed to water as controls. The positive effect of H2S on delaying senescence of apple slices is presented in Fig. 1A. Rot index of water control apple slices increased quickly after 2 d of storage and reached a maximum value on Day 6, while different NaHS treatments alleviated postharvest rot in a dose-dependent manner (Fig. 1B). However, there was no additional protection against browning, senescence, and rot after exposure to concentrations of NaHS above 0.4 mmol·L−1. Thus, 0.4 mmol·L−1 NaHS was regarded as optimal and was the concentration used in all subsequent experiments. The change in external color of cut surfaces was evaluated, and the results showed the decrease in lightness and hue angle values in control fruits (Fig. 2A and C), while H2S treatment significantly delayed the darkening and browning of fruit slices compared with those of controls. Besides, chroma of control slices increased significantly with the storage time, while the increase was attenuated in H2S-fumigated fruit (Fig. 2B).

Effects of H2S on the contents of reducing sugars, soluble protein, free amino acids, ascorbic acid, total phenolics, and flavonoids in apple slices. As shown in Fig. 3A, reducing sugar content in water controls accumulated slightly to a maximum on Day 3, and then declined, whereas the content of reducing sugars in H2S-fumigated fruit increased gradually to Day 3 of storage then plateaued (Fig. 3A). Soluble protein content of apple slices continually decreased during storage in water controls, but the content significantly increased in H2S-treated apple slices in the first 2 d of storage (Fig. 3B).

Ascorbic acid content in apple slices declined steadily regardless of treatments (Fig. 3C). However, apple slices in 0.4 mmol·L−1 NaHS treatment sustained significantly higher levels of ascorbic acid compared with water controls after 2 d of storage (Fig. 3C). Results in Fig. 3D show that H2S application induced an accumulation of total phenolics, whereas they were significantly lower in controls during the whole storage period (Fig. 3D). Similarly, Fig. 3E shows that apple slices exposure to H2S contained significantly higher level of flavonoids in comparison with controls.

Table 1 illustrates the changes in selected amino acids and total amino acids in control and NaHS-treated apple slices during 3 d of incubation. H2S application sustained significantly lower levels of total free amino acids, dropping by ≈33% relative to water control after 3 d of storage. Both cysteine (Cys) and histidine (His) content in H2S-treated tissue were higher than those of water control. His content increased significantly on Day 3 in both control and NaHS treatment compared with those on Day 0. An increase in alanine (Ala), aspartic acid (Asp), glutamic acid (Glu), and serine (Ser) was observed in water controls on Day 3 relative to Day 0, and the increase was attenuated in H2S-fumigated apple slices.

Table 1. Primers used for reversed transcript polymerase chain reaction amplification of senescence-related gene expression.

| Gene name | Forward primer (5′–3′) | Reverse primer (5′–3′) |
|---|---|---|
| MloidHAR | ATCAATCTCAGGGCACAAACC | ACAATCACATCGAAATCCAGG |
| MlidPAL | GAGAAACACGGTAAGAAGACA | AAACATACCTCTATGCAACAC |
| MlidPO | CGTACATAGGGAAGGTGTO | ACTGCTCTTTCTGCTGTC |
| MlloX2 | TCACACCGGCCTCCCTTTC | GCTCAAATCGCTTCCTCA |
| Mlpg1 | TCCCTGCTGACCCTTCCTTTT | TGACCCATTCCTTATAGC |
| Mlacs1 | GGGCACCACAAATGACCGA | CCAACACGAAACAGCAGA |
| Mlacs3 | AGCAACCCATCTGGATCTACATC | TTTGGCTCTTCTCCTTAAAT |
| Mlacs01 | TCAGGATGGTGAATGGGTGA | AATGAGCTCTGCTGATGTT |
| Mlacs02 | CAGGGAGAGTGGTGTAGT | TACGGTGTTGTGCTGTTGAG |
| MledTR1 | CTTAATTTCTTCATCCGCTA | CCAATGCCAAAGTCTACCT |
| MledRS1 | TGATTTCATTCTCCTTCTT | TCAGCATTCAAACATTCAG |
| MledRS2 | CTGCTTTTCGCCCCTCACT | TCCGACGCAAATCCTAGC |
| MledEF3 | GAAGAGAGAGAGAGAGAGA | GAAGGGTTGGAGAGTTGGGTTG |
| MledEF4 | CACCTCCCTCAACACCCAA | GCGAGGAAAGTTCCTCCCTA |
| MledEF5 | AATGTTGGTTGGCGGAGATTC | ATGAGGCGGAGAGGCGG |
| Mlid18SRNA | CTGCCGTTGCTCTGA | CTGCCCTTCTTGATGTT |

Fig. 1. Effects of hydrogen sulfide (H2S) on (A) postharvest shelf life and (B) rot index of fresh-cut apples. Fresh-cut apples were respectively fumigated with different concentrations (0, 0.2, 0.4, 0.6, 0.8, and 1.0 mmol·L−1) of aqueous solutions of sodium hydrosulfide (NaHS) for 0–14 d as shown in lower right part of A. Photographs (A) were taken from Day 0 to Day 5, and the rot index (B) of apple slices was recorded every 2 d from Day 0 to Day 14.
contents of Ala, Asp, Glu, and Ser in NaHS treatment were lower than those of control on Day 3. Cys and Valine were detected only in untreated and treated samples on Day 3, respectively, while other amino acids were not detectable (Table 2).

H$_2$S decreases the production rate of $\cdot$O$_2^-$ and the contents of H$_2$O$_2$ and MDA. The production rate of $\cdot$O$_2^-$ and the contents of H$_2$O$_2$ and MDA in H$_2$S-fumigated fruits and controls are shown in Fig. 4. The generation of $\cdot$O$_2^-$ in control apple slices increased steadily over 5 d, whereas H$_2$S fumigation significantly inhibited $\cdot$O$_2^-$ production rate (Fig. 4A). As shown in Fig. 4B, H$_2$O$_2$ content increased both in control and NaHS-treated apple slices during the whole storage period. However, a lower level of H$_2$O$_2$ was maintained by H$_2$S in comparison with controls (Fig. 4B). MDA content showed a quick increase during the entire storage in control apple slices, whereas the content in H$_2$S-treated apple was maintained at a significantly lower level (Fig. 4C).

$H_2S$ upregulates the activities of APX, CAT, GR, POD, SOD and downregulates the activities of LOX, PAL, PPO, and protease in apple slices. To further understand the role of H$_2$S in ROS metabolism in apple slices, we measured the activities of enzymes involved in oxidative metabolism in plants, such as APX, CAT, GR, LOX, PAL, PPO, POD, and SOD in apple slices exposed to 0.4 mmol L$^{-1}$ NaHS or water (Fig. 5). As shown in Fig. 5A, H$_2$S fumigation induced a rapid increase of CAT activity until 2 d followed by a decline on Day 3. In contrast, CAT activity in water control decreased immediately on 1 d of storage then slightly increased on Day 2 followed by a relative lower level compared with H$_2$S treatment. Figure 5B shows that APX activities increased in both control and NaHS treatment during 5 d of incubation. H$_2$S fumigation induced a burst of APX activity on 1 d, and its activity was maintained at a significantly higher level compared with water control (Fig. 5B). Figure 5C illustrates a similar pattern of POD activity in both water and NaHS treatments. POD activity increased steadily in both treatments, whereas significantly higher activities were observed in fruits exposed to H$_2$S compared with controls (Fig. 5C). As shown in Fig. 5D, H$_2$S fumigation maintained significantly higher SOD activity compared with controls during the entire storage. SOD activity increased gradually in H$_2$S-treated apple until 4 d, thereafter it was maintained at a constant level, while SOD activity in control slices increased and peaked on Day 2 followed by a decline (Fig. 5D). Figure 5E shows that GR activities were enhanced in NaHS treatment in comparison with water control. GR activities in H$_2$S-treated fruits increased steadily, while in water control increased after 2 d of storage and peaked on Day 4 (Fig. 5E).

Figure 5F shows the changes in the activity of LOX, an index of lipid peroxidation, in apple slices. In water controls, LOX activity was induced on the first day of storage and then declined on Day 2 followed by a steady increase. In contrast, LOX activity was induced on the first day of storage and then declined on Day 2 followed by a steady increase. In contrast, LOX activity was induced on the first day of storage and then declined on Day 2 followed by a steady increase.
H$_2$S-treated tissue than in water controls (Fig. 5F). As shown in Fig. 5G, NaH$_2$S treatment maintained lower activities of PPO compared with control. PPO activity in control apples increased rapidly and was about 1.4-fold higher than H$_2$S-treated fruit on 1 d, then declined till Day 3 followed by a relatively stable level, whereas PPO activity in NaH$_2$S treatment was maintained at a significantly lower level in comparison with control (Fig. 5G). Figure 5H illustrates similar patterns in PAL activities both in H$_2$S treatments and water controls. In both treatments, PAL activities declined immediately and were lowest on Day 2 of storage, then increased more on Day 3 followed by a slight decrease until the end of storage. However, H$_2$S application maintained significantly lower levels of PAL activities relative to controls except the first day of storage (Fig. 5H).

The changes in protease activities were shown in Fig. 5I. Consistent with the higher level of soluble protein and lower level of total free amino acids in H$_2$S-fumigated fruits (Fig. 3B; Table 2), H$_2$S fumigation significantly inhibited the enhancement of protease activity compared with that of controls since the second day of storage.

Effect of H$_2$S on the relative expressions of senescence-related genes. Figure 6A shows the effects of H$_2$S on the relative expression of MdDHAR (dehydroascorbate reductase), MdLOX2, MdPAL, MdPPO, and MdPG1 (polygalacturonase). After fresh-cut MdPG1 expressions of on Day 2 compared with water control. The expression levels of these genes on Day 1 significantly suppressed MdDHAR expression of senescence-related genes. Figure 6B shows that the expressions of MdACS1 (1-aminocyclopropane-1-carboxylic acid synthase 1), MdETR1 (ethylene receptor 1), and MdERF1 and MdERF5 (ethylene-responsive factor 3) decreased slightly on Day 1 then increased on Day 2 in control samples, while H$_2$S fumigation inhibited the expressions of MdACS1 (1-aminocyclopropane-1-carboxylic acid oxidase 1), MdETR1, and MdERF1 (on Day 2) and induced the expression of MdERF3 and MdERF5. However, H$_2$S had no significant influence on the expressions of MdACS3, MdetR1, and MdERS2 were not detectable regardless of treatments (Fig. 6B).

Discussion

In the present study, fresh-cut apple slices were used as a model to study the physiological function of H$_2$S during fruit storage (Fig. 1A). An important goal for retailers and food service sectors is to delay fruit rot and enhance storability to deliver benefits to consumers. In this paper, we demonstrate that H$_2$S fumigation extended the storage of fresh-cut apples by retarding the browning and decay of fruit at 25 °C (Figs. 1 and 2), implying that H$_2$S acts as a alleviating regulator in the senescence of postharvest fruit. Protein degradation is one of the significant characteristics of plant senescence, and protease is responsible for protein mobilization from dying cells into actively growing tissues (Foyer et al., 1994). In our work, H$_2$S treatment reduced the level of protease (Fig. 5I) and total free amino acids (Table 2), and a concomitantly higher content of soluble protein (Fig. 3B) compared with those of control was observed. All these data suggested a protective role of H$_2$S in maintaining the postharvest quality and delaying proteolysis by promoting higher contents of reduces sugar and soluble protein and lower level of protease and total free amino acids during fresh-cut apple storage.

Several researchers reported that over-production of ROS such as the ·O$_2$- and H$_2$O$_2$ contributed to accelerated senescence of fresh-cut fruit (Hu et al., 2014). The reactive nature of ROS is potentially harmful to all cellular components, including proteins, lipids, and nucleotides (Apel and Hirt, 2004) and oxidative damage caused by ROS has been universally observed during post-harvest storage of vegetables and fruits (Gao et al., 2013; Li et al., 2014). Plants possess two principal ROS-scavenging systems (Mittler et al., 2004), one involving antioxidant enzymes including APX, CAT, DHAR, GR, POD, and SOD and the other natural antioxidants such as ascorbic acid, total phenolics, and flavonoids. APX, DHAR, and GR are the key enzymes that maintain ascorbate-glutathione cycle and play vital roles in maintaining ascorbate and glutathione homeostasis in plants (Mittler et al., 2004). In the present study, we showed there were significantly decreased production of ·O$_2^-$, H$_2$O$_2$; elevated levels of APX, CAT, GR, POD, and SOD activity; enhanced MdDHAR expression; and higher contents of antioxidants (ascorbic acid, total phenolics, and flavonoids) in H$_2$S-treated apple slices (Figs. 3C–E, 4A–E, and 6A), suggesting that H$_2$S might function in alleviating ROS damage via promoting the antioxidants content and the activities of antioxidant enzymes during fruit storage.
The senescence of fresh-cut apple is a complex and highly regulated process accompanied with lipid peroxidation. Thus, MDA content in apple slices was measured to provide an index of the structural integrity of membranes (Hu et al., 2014). LOX activity is positively correlated with lipid peroxidation in plant tissues (Hu et al., 2014). We found that LOX activity, as well as MDA content, were lower after H2S fumigation of apple slices (Figs. 4C, 5F, and 6A), suggesting that H2S reduced lipid peroxidation, thereby maintaining membrane integrity and contributing to a delay in fruit tissue senescence. Enzymatic browning is an important indicator of quality deterioration that limits the storage life of fresh-cut fruit. Data in Figs. 1A and 2 show that fresh-cut apple slices browned quickly, while H2S application sustained good visual appearance. PAL and PPO are involved in the synthesis of free phenolics and catalyze the oxidation of phenolics into brown pigments, and finally contribute to surface browning of fresh-cut fruit (Nguyen et al., 2003). As shown in Figs. 5G–H and 6A, the decreased PAL and PPO activities and gene expression were observed in H2S-fumigated apple slices compared with water control, implying that H2S fumigation delayed enzymatic browning of fresh-cut apple surface. However, the higher level of phenolic compounds in H2S-fumigated slices (Fig. 3D) and lower PAL activity (Fig. 5H) seem like a contradiction, which was also observed in the study of banana peel (Nguyen et al., 2003).

Ethylene plays a crucial role in the ripening and senescence of apple. To preliminarily understand the effect of H2S on ethylene pathway, we studied the expression patterns of genes that are involved in ethylene bio-synthesis (MdACS1, MdACS3, MdACO1, and MdACO2) and signal transduction (MdETR1, MdERS1, MdERS2, MdERF3, MdERF4, and MdERF5) in ‘Fuji’ apple. Endopolygalacturonase is a key enzyme involved in pectin dissolution, fruit softening and maturation...
fresh-cut apple. Quality and delay postharvest senescence has the potential to maintain higher fruit safety of H2S application in postharvest fruit storage (Hu et al., 2012). In conclusion, this study shows that H2S treatment not only helps to eliminate ROS overproduction, improve the antioxidant capacity, but also affects the expression of senescence-related genes in apple slices. Taken together, H2S has the potential to maintain higher fruit quality and delay postharvest senescence of fresh-cut apple.

Literature Cited

AOAC. 1984. Vitamin C (ascorbic acid) in vitamin preparations and juices: 2,6-dichloroindophenol titrimetric method, p. 844–845. Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC.

Apel, K. and H. Hirt. 2004. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55:373–399.

Beaudoin-Eagan, L.D. and T.A. Thorpe. 1985. Tyrosine and phenylalanine ammonia lyase activities during shoot initiation in tobacco callus cultures. Plant Physiol. 78:438–441.

Benjamin, N.D. and M.W. Montgomery. 1973. Polyphenol oxidase of Royal Ann cherries: Purification and characterization. J. Food Sci. 38:799–806.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254.

Cao, S.F., Z.C. Hu, and B. Pang. 2010. Optimization of postharvest ultrasonic treatment of strawberry fruit. Postharvest Biol. Technol. 55:150–153.

Chen, J., F.H. Wu, W.H. Wang, C.J. Zheng, G.H. Lin, X.J. Dong, J.X. He, Z.M. Pei, and H.L. Zheng. 2011. Hydrogen sulfide enhances photosynthesis through promoting chloroplast biogenesis, photosynthetic enzyme expression, and thiol redox modification in Spinacia oleracea seedlings. J. Expt. Bot. 62:4481–4493.

Foyer, C.H., P. Descourcières, and K.J. Kunert. 1994. Protection against oxygen radicals: An important defence mechanism studied in transgenic plants. Plant Cell Environ. 17:507–523.

Gao, S.P., K.D. Hu, L.Y. Hu, Y.H. Li, Y. Han, H.L. Wang, K.L. Yu, V.S. Liu, and H. Zhang. 2013. Hydrogen sulfide delays postharvest senescence and plays an antioxidative role in fresh-cut kiwifruit. HortScience 48:1385–1392.

García-Limones, C., A. Hervás, J.A. Navas-Cortés, R.M. Jiménez-Diaz, and M. Ten. 2002. Induction of an antioxidant enzyme system and other oxidative stress markers associated with compatible and incompatible interactions between chickpea (Cicer arietinum L.) and Fusarium oxysporum f. sp. ciceris. Physiol. Mol. Plant Pathol. 61:325–337.

Garcia-Mata, C. and L. Lamantia. 2010. Hydrogen sulfide, a novel gasotransmitter involved in guard cell signalling. New Phytol. 188:977–984.

Gil, M.I., D.M. Holcroft, and A.A. Kader. 1997. Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. J. Agr. Food Chem. 45:1662–1667.

Hadfield, K.A. and A.B. Bennett. 1998. Polygalacturonases: Many genes in search of a function. Plant Physiol. 117:337–343.

Hu, H.L., W.B. Shen, and P.X. Li. 2013. Effects of hydrogen sulfide on quality and antioxidant capacity of mulberry fruit. Intl. J. Food Sci. Technol. 49:399–409.

Hu, L.Y., S.L. Hu, J.W. Yu, H.C. Li, R.L. Zheng, Z.J. Wei, L.L. Liu, H.L. Wang, Y.S. Liu, and H. Zhang. 2012. Hydrogen sulfide prolongs postharvest shelf life of strawberry and plays an antioxidative role in fruits. J. Agr. Food Chem. 60:8684–8693.

Hu, K.D., Q. Wang, L.Y. Hu, S.P. Gao, J.W. Yu, Y.H. Li, J.L. Zheng, Y. Han, Y.S. Liu, and H. Zhang. 2014. Hydrogen sulfide prolongs postharvest storage of fresh-cut pears (Pyrus pyrifolia) by alleviation of oxidative damage and inhibition of fungal growth. PLoS One 9:e85524.

Jia, Z.S., M.C. Tang, and J.M. Wu. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64:555–559.

Johnston, J.W., E.W. Hewett, and M.L. Hertog. 2002. Postharvest softening of apple (Malus domestica) fruit: A review. N. Z. J. Crop Hort. Sci. 30:145–160.

Kays, S.J. 1991. Metabolic processes in harvested products, p. 75–142. In: S.J. Kays (ed.). Postharvest physiology of perishable plant products. Van Nostrand Reinhold, New York, NY.

Li, S.P., K.D. Hu, L.Y. Hu, Y.H. Li, A.M. Jiang, F. Xiao, Y. Han, Y.S. Liu, and H. Zhang. 2014. Hydrogen sulfide alleviates postharvest senescence of broccoli by modulating antioxidant defense and senescence-related gene expression. J. Agr. Food Chem. 62:1119–1129.

Li, Z.R., K.D. Hu, F.Q. Zhang, S.P. Li, L.Y. Hu, Y.H. Li, S.H. Wang, and H. Zhang. 2015. Hydrogen sulfide alleviates dark-promoted senescence in postharvest broccoli. HortScience 50:416–420.

Lin, Z.F., S.L. Zhong, and D. Grieron. 2009. Recent advances in ethylene research. J. Expt. Bot. 60:3311–3336.

Manjunatha, G., K.J. Gupta, V. Lokesh, L.A. Mur, and B. Neelwarne. 2012. Nitric oxide counters ethylene effects on ripening fruits. Plant Signal. Behav. 7:476–483.

McGuire, R.G. 1992. Reporting of objective color measurements. HortScience 27:1254–1255.

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31:426–428.

Mittler, R., S. Vanderauwera, M. Gollery, and F.V. Breusegem. 2004. Reactive oxygen gene network of plants. Trends Plant Sci. 9:490–498.

Nguyen, T.B.T., S. Ketsa, and W.G. van Doorn. 2003. Relationship between browning and the activities of polyphenoloxidase and phenylalanine ammonia lyase in banana peel during low temperature storage. Postharvest Biol. Technol. 30:187–193.

Pirie, A. and M.G. Mullins. 1976. Changes in anthocyanin and phenolics content of grapevine leaf and fruit tissues treated with sucrose, nitrate and asbiscic acid. Plant Physiol. 58:468–472.

Pristijono, P., R.B.H. Wills, and J.B. Goldberg. 2006. Inhibition of browning on the surface of apple slices by short term exposure to nitric oxide (NO) gas. Postharvest Biol. Technol. 42:256–259.

Raseetha, S., S.Y. Leong, D.J. Burritt, and I. Oey. 2013. Understanding the degradation of ascorbic acid and glutathione in relation to the levels of oxidative stress biomarkers in broccoli (Brassica oleracea L. italica cv. Bellstar) during storage and mechanical processing. Food Chem. 138:1360–1369.

Rausch, T. and A. Wachter. 2005. Sulfur metabolism: A versatile platform for launching defence operations. Trends Plant Sci. 10:503–509.

Reimerdes, E.H. and H. Klostermeyer. 1976. Determination of proteolytic activities on casein substrates. Methods Enzymol. 45:26–28.

Rico, D., A.B. Martín-Diana, J.M. Barat, and C. Barry-Ryan. 2007. Extending and measuring the quality of fresh-cut fruit and vegetables: A review. Trends Food Sci. Technol. 18:373–386.

Simpson, R.J., M.R. Neuberger, and T.Y. Liu. 1976. Complete amino acid analysis of proteins from a single hydrolysate. J. Biol. Chem. 251:1936–1940.

Surrey, K. 1964. Spectrophotometric method for determination of lipoxidase activity. Plant Physiol. 39:65–70.

Wang, R. 2002. Two company’s three’s a crowd: Can H2S be the third endogenous gaseous transmitter? FASEB J. 16:1792–1798.

Zhang, H.L., Y.H. Hu, K.D. Hu, Y.D. He, S.H. Wang, and J.P. Luo. 2008. Hydrogen sulfide promotes wheat seed germination and alleviates the oxidative damage against copper stress. J. Integr. Plant Biol. 50:1518–1529.

Zhang, H., S.L. Hu, Z.J. Zhang, L.Y. Hu, C.X. Jiang, Z.I. Wei, J. Liu, H.L. Wang, and S.T. Jiang. 2011. Hydrogen sulfide acts as a regulator of flower senescence in plants. Postharvest Biol. Technol. 60:251–257.

Zhang, H., J. Tang, X.P. Liu, Y. Wang, W.Y. Yu, W.Y. Peng, F. Fang, D.F. Ma, Z.J. Wei, and L.Y. Hu. 2009. Hydrogen sulfide promotes root organogenesis in Ipomoea batatas, Salix matsuana, and Glycine max. J. Integr. Plant Biol. 51:1084–1094.