Postharvest Biology and Technology 147 (2019) 54–58

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Interaction of the hydrogen sulphide inhibitor, propargylglycine (PAG), with hydrogen sulphide on postharvest changes of the green leafy vegetable, pak choy

H.M.S. Al Ubeed[a,b], R.B.H. Wills[a,*], M.C. Bowyer[a], J.B. Golding[a,c]

[a] School of Environmental and Life Sciences, University of Newcastle, Ourimbah, NSW, 2558, Australia
[b] Department of Life Sciences, Faculty of Science, Al-Muthana University, Al-Muthana, Iraq
[c] New South Wales Department of Primary Industries, Ourimbah, NSW, 2258, Australia

ARTICLE INFO

Keywords:
PAG
Hydrogen sulphide
Senescence
Pak choy
Ethylene

ABSTRACT

Propargylglycine (PAG) is an inhibitor of hydrogen sulphide (H_2S) production and has been used to explore the mode of action of H_2S in prolonging storage of horticultural produce but little attention has been given to how PAG and H_2S interact when both are applied to produce. This study examined the effect of sequential application of PAG and H_2S on a range of postharvest senescence factors of the leafy vegetable pak choy (*Brassica rapa* subsp. *Chinensis)* stored at 10 °C. The results showed differential responses between factors when compared to application of PAG or H_2S alone. As expected, fumigation with H_2S reduced the rate of loss of leaf green colour, respiration rate, ethylene production, ion leakage and enhanced antioxidant activity and leaves sprayed with PAG showing converse effects. If PAG acted solely by inhibiting endogenous H_2S production then subsequent treatment with H_2S should fully negate any effect induced by PAG. However, for the combined PAG + H_2S treatment, respiration was similar leaves fumigated with H_2S, loss of green leaf colour was similar to the PAG single treatment and less than the untreated control, antioxidant activity was less than for PAG but greater than for control leaves, and ethylene production and ion leakage were similar to control leaves. Thus, the concept that PAG is exclusively an inhibitor of endogenous H_2S production was not validated, with PAG having effects on metabolism that are not linked to the action of endogenous H_2S. The additional actions of PAG could be through its inhibition of pyridoxyal-5′-phosphate (PHP) which is a coenzyme for numerous enzyme systems.

1. Introduction

Hydrogen sulphide (H_2S) was considered a toxic gas, but about 10 years ago was found to be synthesised in mammalian tissues and to have a mediating role in a wide range of cellular physiology functions (Li and Moore, 2008). It is now also known to also act as gaseous plant growth regulator, impacting a diverse range of plant physiological functions such as germination, stomatal movement, root development and flower senescence (Jin and Pei, 2015; Hancock and Whiteman, 2016). A role for H_2S in the regulation of postharvest senescence is quite recent. Zhang et al. (2011) reported delayed senescence in eight types of cut flowers and shoot explants treated with vase solutions of the H_2S donor, sodium hydrogen sulphide (NaHS). Fumigation with H_2S was subsequently extended to a range of postharvest fruit and vegetables including strawberry (Hu et al., 2012), broccoli, (Li et al., 2014, 2017a), peach (Wang et al., 2014), mulberry (Hu et al., 2014), and banana (Ge et al., 2017) with an extension in storage life achieved through inhibition of a wide range of senescence characteristics. Work examining the physiological role of H_2S in postharvest produce is limited. Li et al. (2014) and Zheng et al. (2016) showed that exogenous H_2S treatment of broccoli florets and apple slices, respectively, down regulated the expression of genes associated with ethylene biosynthesis. Ge et al. (2017) extended this understanding, showing that H_2S fumigation also upregulated ethylene receptor expression. Further, Al Ubeed et al. (2017) identified that senescence characteristics in the green leafy vegetables, pak choy, basil and kale were delayed by fumigation with H_2S and speculated that the mode of action of H_2S was through inhibition of ethylene production and action.

The ability to inhibit endogenous H_2S production has been a key tool for understanding the physiological actions of H_2S in living systems. A range of inhibitor compounds have been identified including L-propargylglycine which has been widely employed in both animal and plant physiology to probe H_2S activity. García-Mata and Lamattina (2010) utilised DL-propargylglycine (designated in this paper as PAG)

* Corresponding author.
E-mail address: Ron.Wills@newcastle.edu.au (R.B.H. Wills).

https://doi.org/10.1016/j.postharvbio.2018.09.011
Received 1 May 2018; Received in revised form 12 September 2018; Accepted 13 September 2018

to confirm H_2S involvement in controlling stomatal closure in *Vicia faba* and *Aridopsis thaliana*. More recently, links between H_2S homeostasis and the expression of senescence characteristics in postharvest produce has been investigated using PAG. Li et al. (2014) found the exogenous H_2S treatment of broccoli florets enhanced endogenous H_2S production leading to delayed onset of senescence characteristics and enhanced metabolic activity. They further showed PAG lowered the activity of L-cysteine desulfhydrase (LCD) and D-cysteine desulfhydrase (DCD), the enzymes that convert L- and D- cysteine, respectively, to H_2S (Jin and Pei, 2015), thereby reducing endogenous H_2S levels leading to accelerated senescence. Liu et al. (2017) identified similar effects on daylily with a range of senescence characteristics enhanced by spraying with PAG and inhibited by fumigation with H_2S.

If PAG acted solely on endogenous H_2S production, it could be expected that the addition of exogenous H_2S should negate the inhibitory effect of PAG. The only study where PAG and H_2S have been applied sequentially produce was by Hu et al. (2015) with the leafy vegetable, water spinach (*Ipomea aquatica*). They reported that fumigation with H_2S after spraying with PAG only partially negated the inhibition of H_2S synthesis induced by PAG. In this study we further examined the interaction of PAG and H_2S by measuring a range of postharvest changes of pak choy leaves sequentially treated with PAG and H_2S and comparing these effects to responses of leaves treated with a single application of PAG or H_2S.

2. Materials and methods

2.1. Produce

Pak choy plants (*Brassica rapa* subsp. *Chinensis* cv. Shanghai) (also known as bok or pok choy, choi or tsoi)) were sourced from a local farm at Mangrove Mountain, New South Wales, Australia, and transported to the laboratory within two hours of harvest. Pak choy heads were cut and a specific number of outside leaves (the number varying between different experiments) were selected and gently cleaned with tap water. The leaves from each head were randomly distributed into the required number of treatment units, with each containing 24 leaves that weighed about 0.4 kg. Each treatment unit was placed into a sealable plastic container (4 L) fitted with an inlet and outlet tubes in the lid. All experiments were replicated by obtaining batches of plants on separate occasions with at least two weeks between batches with the various experiments conducted over two seasons.

2.2. Treatments

Treatments applied to a container of pak choy leaves at 20 °C were:

1 PAG: each side of each leaf in a treatment unit was sprayed with 0.1 mL of an aqueous solution containing 2 mM PAG (Sigma-Aldrich, Australia) without addition of a wetting agent, then left to air dry at 20 °C. This concentration was shown to give optimum inhibition of LCD and DCD activity in previous studies (Cui et al., 2014; Hu et al., 2015).
2 Control treatments: leaves in a treatment unit remained untreated or were similarly sprayed with water.
3 H_2S: treatment units were fumigated for four hours with 250 $\mu L\,L^{-1}$ H_2S, the optimum concentration reported by Al Ubeed et al. (2017) to inhibit senescence of pak choy. The gas was generated *in situ* by the addition of water to solid NaHS using the method described by Zhao et al. (2014).
4 PAG + H_2S: leaves were sprayed with PAG or water and left to dry for three hours then fumigated with H_2S for four hours as per the above treatments.

After seven hours, all containers in an experiment were sealed at s10 °C and ventilated through the inlet tube with air containing 0.1 $\mu L\,L^{-1}$ ethylene or ethylene-free air at 0.75 $mL\,s^{-1}$. The ventilating gas streams were humidified by bubbling through water to ensure a high humidity of 97–99 % RH was maintained to minimise water loss.

2.3. Physio-chemical assessments

Leaves in each unit were visually assessed daily for green colour and the time for each unit to develop an unacceptable colour (denoted as the market life) was determined using the scoring scale given below. Respiration rate, as evolved carbon dioxide, and ethylene production were assessed at various times during storage.

2.3.1. Visual leaf colour (market life)

The change in leaf colour from green to yellow of individual pak choy leaves was conducted daily by visual assessment using a scoring scale of 0–5 where 0 = green, 1 = 10%, 2 = 20%, 3 = 30%, 4 = 50% and 5 = > 70% loss of original green colour as proposed by Li et al. (2017b). A colour photograph was made of leaves at the various colour scores and used as a reference to maintain a uniform standard during the assessment process. Colour assessment was made by a single person but samples were coded so the observer was not aware of the treatments. The mean colour score of all leaves in a treatment unit was calculated daily. An average colour score of 3.0 was considered to be the limit of consumer acceptability and the time for leaves to reach a mean score of 3.0 was designated as the market life of that unit.

2.3.2. Respiration rate and ethylene

After various times in storage, the respiration rate was measured as carbon dioxide evolution. A container containing a treatment unit of pak choy was sealed to allow the accumulation of a measureable concentration of carbon dioxide. A gas sample (5 mL) was collected in a syringe after four hours and the concentration of carbon dioxide in the sample was determined by injecting into a thermal conductivity gas chromatograph as described by Huque et al. (2013). The respiration rate was calculated as $\mu g\,kg^{-1}\,s^{-1}$ CO_2.

The concentration of ethylene in atmosphere was determined by a collecting a gas sample (1 mL) and analysing by flame ionization gas chromatography as described by Huque et al. (2013). Samples were obtained from ventilated treatment units just before sealing the container and again three hours after sealing. The difference between readings was used to calculate the rate of ethylene production as $ng\,kg^{-1}\,s^{-1}$.

2.3.3. Ion leakage

Ion leakage was determined according to the method described by Lu (2007) after three days storage using five leaves selected from a treatment unit. This involved collecting two disks (50 mm diameter) from each leaf. The disks were immediately immersed in double distilled water (40 mL) in glass vials and incubated for two hours at 25 °C. The conductivity of the solution was then measured with a conductivity meter (Model 4071, Jenway, Staffordshire, UK). The solution was then boiled for 15 min. and after cooling to room temperature, the total conductivity was re-measured. Ion leakage was calculated as the percentage of the initial to final value.

2.3.4. Antioxidant activity

Antioxidant activity was determined after three days of storage using two samples of leaf (each about 4 g) that were cut from the top part of five leaves from a treatment unit, ground using a mortar and pestle then mixed with 50% methanol (50 mL). The mixture was placed in an ultrasonic bath (Soniclean, Australia) set at 35 °C and 100 W for 30 min. before being filtered through filter paper (Whatman Grade 5). The filtrate was stored at –20 °C until analysed. Antioxidant activity was determined with 2,2-diphenyl-picrylhydrazine (DPPH), using the method described by Vuong et al. (2013). Briefly, a stock solution of DPPH (0.24 $g\,L^{-1}$ methanol) was prepared and stored at −20 °C until

required. The working solution was freshly prepared by diluting 10 mL stock solution with 45 mL methanol to obtain an absorbance (λ = 515 nm) of 1.1 ± 0.02. Pak choy extracts (0.15 mL) were mixed with DPPH solution (2.85 mL) and incubated at room temperature in the dark for one hour. The solution absorbance was then recorded and quantified as mg kg^{-1} Trolox equivalent (TE) using a standard curve of Trolox against absorbance

2.4. Statistical analysis

Data for respiration rate and ethylene production were analysed by two-way analysis of variance (ANOVA) with time as the other factor. Data for market life, ion leakage and antioxidant activity was analysed by one-way ANOVA. Where a significant difference between treatments was found, the least significant difference (LSD) of the mean values at P = 0.05 was calculated. Statistical procedures were performed using SPSS for Windows, version 22.0 software package (SPSS Chicago, IL).

3. Results

3.1. Leaf colour (market life)

The effect of PAG on the change in leaf colour from green to yellow of pak choy, assessed as the time to a fixed colour score (3.0) which is designated as the market life, is shown in Table 1. Spraying leaves with PAG significantly decreased the market life relative to the respective control leaves (water spray or untreated). Fumigation with H_2S significantly increased market life relative to control leaves. When leaves were treated with both PAG and H_2S, market life was significantly greater than in leaves sprayed with PAG alone but lower than control leaves. Thus, fumigation with H_2S did not fully negate the inhibitory effect of PAG. Since there was no significant difference between water-sprayed and untreated leaves, in all subsequent experiments, the control treatment was sprayed with water.

3.2. Respiration rate

The respiration rate of pak choy leaves was significantly affected by treatment and storage time but there was also a significant interaction between treatment and storage time ($P < 0.001$). The interaction between treatment and storage time is presented in Fig. 1 and shows that spraying with PAG resulted in a significantly higher respiration than control (water sprayed) leaves from two days of storage. However, fumigation with H_2S resulted in a lower respiration rate than control leaves at four days. The respiration rate of the combined PAG-H_2S treatment was not significantly different to H_2S alone. Thus, fumigation with H_2S fully reversed the stimulatory effect of PAG on respiration.

Table 1
Market life of pak choy sprayed with PAG and/or fumigated with H_2S during storage at 10 °C and ventilation with air containing 0.1 μL L^{-1} ethylene.

Treatment	Market life (days)
PAG	7.2[a]
PAG + H_2S	7.8[b]
Water	8.4[c]
Untreated	8.6[c]
Water + H_2S	10.3[d]
LSD	*0.44*

Each value is the mean of 30 assessments (10 batches of produce x 3 units). LSD values are the least significant difference between means at P = 0.05.

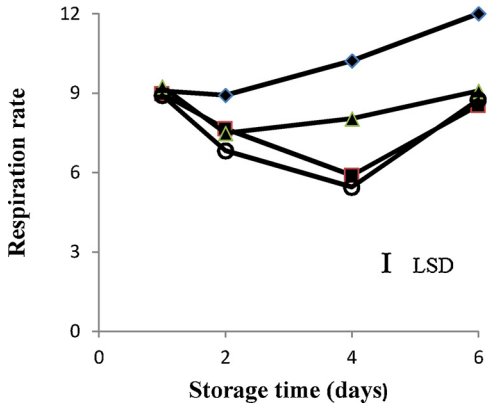

Fig. 1. Respiration rate (μg. kg^{-1} s^{-1} CO_2) of pak choy sprayed with PAG and/or fumigated with H_2S during storage at 10 °C and ventilation with air containing 0.1 μL L^{-1} ethylene.
Treatments: ♦ PAG, ▲ control (water spray), ○ H_2S, ■ PAG + H_2S.
Each value is the mean of 15 readings (5 batches of produce x 3 units). LSD between means was 1.15 at P = 0.05.

3.3. Ethylene production

Ethylene production by pak choy leaves was significantly affected by both treatment and storage time and also showed a significant interaction between treatment and storage time ($P < 0.001$), but the interaction differed to that observed for respiration. Fig. 2 shows that ethylene production of control leaves ventilated with ethylene-free air decreased during storage. Spraying leaves with PAG significantly increased ethylene production at three and six days storage while fumigation with H_2S significantly decreased ethylene production from day one in storage. The combined PAG-H_2S treatment showed a similar reduction in ethylene production as H_2S alone at day one but at three and six days, ethylene production was similar to control leaves. The data thus indicate that the effect of PAG on ethylene production did not materialise until three days storage whereas H_2S had impacted on ethylene production early in storage.

3.4. Ion leakage and antioxidant activity

Ion leakage was determined after three days storage and Table 2 shows that ion leakage was significantly increased by PAG and decreased by H_2S compared to control leaves. Application of both PAG and H_2S resulted in an ion leakage that was not significantly different to

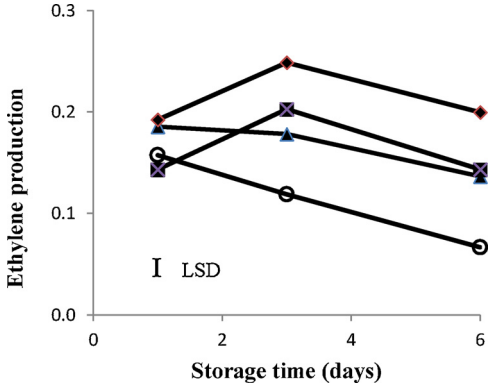

Fig. 2. Ethylene production (ng kg^{-1} s^{-1}) of pak choy sprayed with PAG and/or fumigated with H_2S during storage at 10 °C and ventilation with ethylene-free air.
Treatments: ♦ PAG, ▲ control (water spray), ○ H_2S, ■ PAG + H_2S.
Each value is the mean of 9 readings (3 batches of produce x 3 units). LSD between means was 0.025 at P = 0.05.

Table 2
Ion leakage and antioxidant activity of pak choy leaves that had been sprayed with PAG and/or fumigated with H_2S after three days storage at 10 °C and ventilated with air containing 0.1 μL L^{-1} ethylene.

Treatment	Ion leakage (%)	Antioxidant activity (mg kg^{-1} TE)
PAG	73.6[c]	0.578[a]
Water	44.5[b]	0.583[a]
PAG + H_2S	37.5[b]	0.653[b]
H_2S	28.8[a]	0.704[c]
LSD	*8.7*	*0.050*

Each value is the mean of 12 assessments (4 batches of produce x 3 units). LSD values are the least significant difference between means at $P = 0.05$.

that of control leaves.

Leaves from the same treatment units used for assessment of ion leakage were also used to determine antioxidant activity. Table 2 shows that there was no significant difference in antioxidant activity between control and PAG-treated leaves but leaves fumigated with H_2S had a significantly higher antioxidant activity. Treatment of leaves with both PAG and H_2S resulted in an antioxidant activity that was significantly lower than H_2S alone but higher than control leaves.

4. Discussion

The results show that fumigation of pak choy with H_2S inhibited senescence, as expressed by a reduced rate of loss of leaf green colour (i.e. extended market life), respiration, ethylene production, ion leakage and enhanced antioxidant activity. These effects confirm the findings of Al Ubeed et al. (2017) that postharvest application of H_2S inhibited the senescence of pak choy. This study also showed the converse effect when pak choy leaves sprayed with PAG, accelerated senescence through enhanced green colour loss, respiration, ethylene production and ion leakage, although there was no statistically significant effect on antioxidant activity. These effects are consistent with findings of Hu et al. (2015); Liu et al. (2017) and Li et al. (2014) who found PAG enhanced senescence of water spinach, daylily flowers and broccoli florets, respectively.

The synthesis of H_2S in both animals and plants occurs by a variety of enzyme systems which are all pyridoxyal-5′-phosphate (PHP) dependent (Kamoun, 2004; Jin and Pei, 2015). The mode of action of PAG in both animals and plants is attributed to its ability to irreversibly bind to PHP to yield an inactive product (Abeles and Walsh, 1973). This then results in inhibition of H_2S synthesis by blocking the action of the associated PHP cofactor (Guo et al., 2017; Romero et al., 2014). However, PHP is also involved in all cysteine pathways in plants, with many unrelated to H_2S synthesis (Eliot and Kirsch, 2004) with more than 140 enzyme systems identified as being PHP dependent (Richard et al., 2009). If PAG acted solely by inhibiting endogenous H_2S production, it could be expected that treatment with PAG followed by exogenous H_2S would fully negate the diminished endogenous H_2S production induced by PAG. However, respiration was the only senescence-related factor where the PAG + H_2S treatment was not significantly different to that in pak choy fumigated with H_2S alone.

At the other end of the effectiveness scale was loss of green leaf colour, where pak choy treated with PAG + H_2S had a market life that was close to that of the PAG single treatment and was less than the untreated control. This indicates that the exogenous H_2S only weakly nullified the effect of PAG. This is consistent with results presented by Hu et al. (2015) showing that sequential treatment of water spinach with PAG followed by H_2S resulted in only a partial restoration of endogenous H_2S production. Assuming that the loss of green leaf colour was associated with loss of chlorophyll, the effect of PAG on chlorophyll degradation would thus seem to be largely on systems that are independent of H_2S synthesis. Our findings would appear to question conclusions drawn by Li et al. (2014) for broccoli that chlorophyll retention is predominantly influenced by endogenous H_2S production. It is therefore plausible to assume that as yet unmapped PAG influences outside endogenous H_2S production exist.

The effect on antioxidant activity, as measured by the free radical scavenger DPPH (Okawa et al., 2001), differed again. While PAG alone had no significant effect on antioxidant activity, the PAG + H_2S treatment significantly reduced the increase in antioxidant activity effected by H_2S alone but it was only a partial neutralisation of H_2S as the level was greater than for control leaves. This suggests that for antioxidant activity, the activity of PAG is not solely related to H_2S synthesis. The result is supported by Yu et al. (2013) who speculated that alleviation of inhibited antioxidant capacity in salt stressed cucumber hypocotyls and radicles by exogenous H_2S may be linked to H_2S-induced stimulation of glutathione synthesis, a known antioxidant metabolite in plants (Riemenschneider et al., 2005).

The effect of the PAG + H_2S treatment had a different effect on the rate of ethylene production during storage. After one day of storage, ethylene production of the combined treatment was not significantly different to that generated by H_2S alone but at three and six days, the rate of production was not significantly different to control leaves. This could be explained by H_2S having a rapid effect on ethylene production which is seen at one day but the effect of PAG on ethylene production is not generated until three days when it negates the effect of H_2S and thus has a similar production rate to control leaves. Ion leakage showed a similar response with the PAG + H_2S treatment being not significantly different to control leaves but was greater than H_2S and lower than PAG. Thus, the beneficial effect of H_2S would seem to largely neutralise the detrimental action of PAG on ethylene production and ion leakage.

Thus, the concept that PAG is exclusively an inhibitor of endogenous H_2S production would seem to be an oversimplification, with PAG potentially having effects on pak choy metabolism that are not linked to the action of endogenous H_2S. The additional actions of PAG would seem to be linked to its inhibition of PHP which is a coenzyme for numerous enzyme systems. It can therefore be misleading to conclude that effects from the application of PAG are entirely due to effect on endogenous H_2S production. However, it is recognised that we applied a single transient exposure to H_2S and continuous exposure to exogenous H_2S may generate different responses. Notwithstanding, it could be expected that leaves absorbed sufficient H_2S during the four hour exposure to maintain its effect beyond the exposure period.

5. Conclusions

The concept that the action of PAG is solely through inhibition of endogenous H_2S production was not validated as PAG was found to have effects on metabolism that were not linked to the action of endogenous H_2S. The additional actions of PAG could be through its known inhibition of pyridoxyal-5′-phosphate (PHP) as this is a coenzyme for numerous enzyme systems.

Acknowledgements

HMSAU wishes to thank the Ministry of Higher Education and Scientific Research of Iraq for the scholarship that enabled her to undertake postgraduate studies.

References

Abeles, R.H., Walsh, C.T., 1973. Acetylenic enzyme inactivators. Inactivation of gamma-cystathionase, in vitro and in vivo by propargylglycine. J. Am. Chem. Soc. 95, 6124–6125.
Al Ubeed, H.M.S., Wills, R.B.H., Bowyer, M.C., Vuong, Q.V., Golding, J.B., 2017. Interaction of exogenous hydrogen sulphide and ethylene on senescence of green leafy vegetables. Postharvest Biol. Technol. 133, 81–87.
Cui, W.T., Chen, H.P., Zhu, K.K., Jin, Q.J., Xie, Y.J., Cui, J., Xia, Y., Zhang, J., Shen, W.B., 2014. Cadmium-induced hydrogen sulfide synthesis is involved in cadmium tolerance

in *Medicago sativa* by reestablishment of reduced (Homo) glutathione and reactive oxygen species homeostasis. PLoS One 9, e109669.
Eliot, A.C., Kirsch, J.F., 2004. Pyridoxal phosphate enzymes: mechanistic, structural, and evolutionary considerations. Annu. Rev. Biochem. 73, 383–415.
García-Mata, C., Lamattina, L., 2010. Hydrogen sulphide, a novel gasotransmitter involved in guard cell signalling. New Phytol. 188, 977–984.
Ge, Y., Hu, K.D., Wang, S.S., Hu, L.Y., Chen, X.Y., Li, Y.H., Yang, Y., Yang, F., Zhang, H., 2017. Hydrogen sulfide alleviates postharvest ripening and senescence of banana by antagonizing the effect of ethylene. PloS One 12, e0180113.
Guo, H., Zhou, H., Zhang, J., Guan, W., Xu, S., Shen, W., Xu, G., Xie, Y., Foyer, C.H., 2017. L-cysteine desulfhydrase-related H_2S production is involved in OsSE5-promoted ammonium tolerance in roots of *Oryza sativa*. Plant Cell Environ. 40, 1777–1790.
Hancock, J.T., Whiteman, M., 2016. Hydrogen sulfide signaling: interactions with nitric oxide and reactive oxygen species. Ann. New York Acad. Sci. 1365, 5–14.
Hu, L.Y., Hu, S.L., Wu, J., Li, Y.H., Zheng, J.L., Wei, Z.J., Liu, J., Wang, H.L., Liu, Y.S., Zhang, H., 2012. Hydrogen sulfide prolongs postharvest shelf life of strawberry and plays an antioxidative role in fruits. J. Agric. Food Chem. 60, 8684–8693.
Hu, H., Shen, W., Li, P., 2014. Effects of hydrogen sulphide on quality and antioxidant capacity of mulberry fruit. Intern. J. Food Sci. Technol. 49, 399–409.
Hu, H., Liu, D., Li, P., Shen, W., 2015. Hydrogen sulfide delays leaf yellowing of stored water spinach (*Ipomoea aquatica*) during dark-induced senescence by delaying chlorophyll breakdown, maintaining energy status and increasing antioxidative capacity. Postharvest Biol. Technol. 108, 8–20.
Huque, R., Wills, R.B.H., Pristijono, P., Golding, J.B., 2013. Effect of nitric oxide (NO) and associated control treatments on the metabolism of fresh-cut apple slices in relation to development of surface browning. Postharvest Biol. Technol. 78, 16–23.
Jin, Z., Pei, Y., 2015. Physiological implications of hydrogen sulfide in plants: pleasant exploration behind its unpleasant odour. Oxid. Med. Cell. Longev. 2015, 1–6. https://doi.org/10.1155/2015/397502.
Kamoun, P., 2004. Endogenous hydrogen sulfide production in mammals. Amino Acids 26, 243–254.
Li, L., Moore, P.K., 2008. Putative biological roles of hydrogen sulfide in health and disease: a breath of not so fresh air? Trends Pharmacol. Sci. 29, 84–90.
Li, S.P., Hu, K.D., Hu, L.Y., Li, Y.H., Jiang, A.M., Xiao, F., Han, Y., Liu, Y.S., Zhang, H., 2014. Hydrogen sulfide alleviates postharvest senescence of broccoli by modulating antioxidant defense and senescence-related gene expression. J. Agric. Food Chem. 62, 1119–1129.
Li, D., Li, L., Ge, Z., Limwachiranon, J., Ban, Z., Yang, D., Luo, Z., 2017a. Effects of hydrogen sulfide on yellowing and energy metabolism in broccoli. Postharvest Biol. Technol. 129, 136–142.
Li, Y., Wills, R.B.H., Golding, J.B., 2017b. Interaction of ethylene concentration and storage temperature on postharvest life of the green vegetables, pak choi, broccoli, mint and green bean. J. Hortic. Sci. Biotechnol. 92, 288–293.
Liu, D., Xu, S., Hu, H., Pan, J., Li, P., Shen, W., 2017. Endogenous hydrogen sulfide homeostasis is responsible for the alleviation of senescence of postharvest daylily flower via increasing antioxidant capacity and maintained energy status. J. Agric. Food Chem. 65, 718–726.
Lu, S., 2007. Effect of packaging on shelf-life of minimally processed bok choy (*Brassica chinensis* L.). LWT-Food Sci. Technol. 40, 460–464.
Okawa, M., Kinjo, J., Nohara, T., Ono, M., 2001. DPPH (1,1-Diphenyl-2-Picrylhydrazyl) radical scavenging activity of flavonoids obtained from some medicinal plants. Biol. Pharmacol. Bull. 24, 1202–1205.
Richard, J.P., Amyes, T.L., Crugeiras, J., Rios, A., 2009. Pyridoxal 5'-phosphate: electrophilic catalyst extraordinaire. Curr. Opin. Chem. Biol. 13, 468–476.
Riemenschneider, A., Nikiforova, V., Hoefgen, R., de Kok, L., Papenbrock, J., 2005. Impact of elevated H_2S on metabolite levels, activity of enzymes and expression of genes involved in cysteine metabolism. Plant Physiol. Biochem. 43, 473–483.
Romero, L.C., Aroca, A.M., Laureano-Marín, A.M., Moreno, I., García, I., Gotor, C., 2014. Cysteine and cysteine-related signaling pathways in Arabidopsis thaliana. Mol. Plant 7, 264–276.
Vuong, Q.V., Hirun, S., Roach, P.D., Bowyer, M.C., Phillips, P.A., Scarlett, C.J., 2013. Effect of extraction conditions on total phenolic compounds and antioxidant activities of *Carica papaya* leaf aqueous extracts. J. Herb. Med. 3, 104–111.
Wang, W., Shen, Y.G., Zhu, L.Q., Zhang, W., Wu, S.F., Du, H.Y., 2014. Effects of exogenous H2S on preservation of peaches and reactive oxygen metabolism. J. Fruit Sci. 31, 302–307.
Yu, L., Zhang, C., Shang, H., Wang, X., Wei, M., Yang, F., Shi, Q., 2013. Exogenous hydrogen sulfide enhanced antioxidant capacity, amylase activities and salt tolerance of cucumber hypocotyls and radicles. J. Intern. Agric. 12, 445–456.
Zhang, H., Hu, S.L., Zhang, Z.J., Hu, L.Y., Jiang, C.X., Wei, Z.J., Liu, J., Wang, H.L., Jiang, S.T., 2011. Hydrogen sulfide acts as a regulator of flower senescence in plants. Postharvest Biol. Technol. 60, 251–257.
Zhao, Y., Biggs, T.D., Xian, M., 2014. Hydrogen sulfide (H_2S) releasing agents: chemistry and biological applications. Chem. Commun. 50, 11788–11805.
Zheng, J.L., Hu, L.Y., Hu, K.D., Wu, J., Yang, F., Zhang, H., 2016. Hydrogen sulfide alleviates senescence of fresh-cut apple by regulating antioxidant defense system and senescence-related gene expression. HortScience 51, 152–158.