Hydrogen sulfide: a luminous future in the postharvest preservation of fruits and vegetables

Ya-Qin Zhao, Liang-Yi Zhao, Shun-Qing Hu, Yuan-Yuan Hou, Yong-Hua Zheng, and Peng Jin*

College of Food Science and Technology, Nanjing Agricultural University, 1 Weigang, Nanjing 210095, China
* Corresponding author, E-mail: pjin@njau.edu.cn

Abstract
Hydrogen sulfide (H₂S) has emerged as a signaling molecule that plays a crucial role in the postharvest preservation of fruits and vegetables. This review summarizes the various functions of H₂S such as delaying ripening and senescence, enhancing the resistance to cold and disease, and emphasizes the underlying mechanisms. Appropriate concentrations of H₂S primarily operate through stimulating the antioxidant system, while showing positive effects on physiological metabolism relevant to storage quality and shelf life including energy, sugar, proline, phenolic, membrane lipid and cell wall metabolism. Moreover, H₂S may reduce storage loss by modulating the expression patterns of senescence-related genes, like those linked to ethylene. The coordination of H₂S and nitric oxide (NO) combats ethylene-derived negative effects during ripening and senescence. High concentrations of H₂S not only act as a regulator to induce disease resistance, but also as a fungicide to inhibit the growth and pathogenicity of fungi. The intricate crosstalk between H₂S and other molecules exists via synergistic and antagonistic roles based on protein persulfidation, which is the major signaling process of H₂S and appears to compete with other post-translational modifications (PTMs) for the same cysteine residues. This review summarizes H₂S synthesis pathways and also discusses the correlation between the signals of H₂S, Ca²⁺ and ABA, while highlighting the intrinsic mechanisms of H₂S performing its functions in postharvest preservation.


Introduction
H₂S, a toxic gas with a rotten egg odor, has recently warranted inclusion as a messenger molecule. H₂S diffuses readily through membranes as small lipophilic molecules, presenting a membrane receptor-independent signaling mechanism. H₂S signaling in mammalian systems occurs through reactions with metals and oxidants as well as protein persulfidation, which either activates or inhibits enzyme activities directly, or by forming protein adducts. Moreover, H₂S in plants induces defense systems against biotic and abiotic stress from seed germination to fruit ripening, protein persulfidation in postharvest has also obtained keen interest. After fruits and vegetables are picked, they lose their nutritional supply and gradually senescence during the storage process, so preservation has always been a relevant issue. Recent reports on H₂S application show great potential for delaying senescence in cut flowers, vegetables, climacteric and non-climacteric fruit under shelf and low temperature conditions, including enhancing the antioxidant system, mitigating chilling injury (CI) symptoms, inhibiting fungal growth, regulating senescence-related genes and interacting with other molecules. Nevertheless, the mechanisms of H₂S on postharvest physiological metabolism require further research. The residual levels of H₂S after exogenous treatment are lower than human plasma levels, and there is usually no disagreeable smell in the preparation of plant extracts. Consequently, a stimulate concentration of H₂S may have safe applications in postharvest storage and transportation. Intricate interactions between H₂S and other secondary messengers like Ca²⁺, NO, abscisic acid (ABA) and hydrogen peroxide (H₂O₂) form a cross-adaptation signaling network. The function of H₂S to activate or inhibit target proteins via persulfidation deserves more attention, while possibly contending with other PTMs for the same residue positions, especially NO-mediated S-nitrosation and nitration. Although there have been many reports, the effects of H₂S on postharvest physiology of fruits and vegetables still need comprehensive evaluation. This review focuses on the intrinsic mechanisms of H₂S signaling on senescence, CI and disease resistance, and also highlights the current progress of persulfidation with other modifications.

Biosynthesis of H₂S in plants
Transsulfuration is the major pathway for plants to produce endogenous H₂S. Wilson et al. first discovered that the emission rate of H₂S in higher plants was light-dependent. It seemed that H₂S was a crucial part of sulfur metabolism and helped plants to remove excessive inorganic sulfur anions. Since then, researchers have discovered that there are at least five pathways to generate endogenous H₂S in plants (Fig. 1). The five known pathways are as follows: (1) Plants convert the combined sulfate to the free state that is activated to form adenosine 50-phosphosulfate (APS) in the plastids. Then APS is reduced to H₂S by APS reductase (APR) and sulfite...
reductase (SIR), with reduced glutathione (GSH) as the electron donor[10]. (2) Cysteine desulphhydrase is the main pathway to generate endogenous H$_2$S, including L-cysteine desulphhydrase (L-DES), D-cysteine desulphhydrase (D-DES) and L-cysteine desulphhydrase 1 (DES1). L-DES and D-DES are not directly related, they have different substrates and subcellular localization[5,11]. (3) O-acetylserine thiol lyase (OAS-TL), also known as cysteine synthase (CS), catalyzes sulfide into O-acetylserine (OAS) to form cysteine, its reversible reactions can release H$_2$S[12]. (4) Pyridoxal 5’-phosphate-dependent L-DES catalyzes L-cysteine to generate elemental sulfur, which is further reduced to H$_2$S[13]. Two candidate genes AtNFS1 and AtNFS2 have been identified in Arabidopsis thaliana, encoding NiFs-like plastidial cysteine desulphurase, which are addressed to plastids and chloroplasts, respectively[14]. (5) β-cyanoalanine synthase (β-CAS) converts L-cysteine into H$_2$S through the detoxification of cyanide[15]. NaHS, a commonly used exogenous H$_2$S donor, gives a rapid burst of H$_2$S gas in a water solution and keeps a constant concentration, but actual physiological H$_2$S concentration triggering the defense response is yet unclear[27].

**Interaction of H$_2$S, Ca$^{2+}$ and ABA signaling**

The chemical properties of H$_2$S allow it to diffuse readily through membranes without the necessity of membrane channels or specific transporters, and confers its biological signaling via protein persulfidation[23]. Protein cysteine residues undergo H$_2$S-derived persulfidation to form persulphides (R-SSH), which is the mechanism by which H$_2$S performs its biological function[25]. In plant physiobiochemistry, complex crosstalk between H$_2$S and phytohormones such as salicylic acid (SA), gibberellins (GAs) and jasmonic acid (JA) have been reported. It also interacts with other messengers like NO, H$_2$O$_2$ and carbon monoxide (CO) to regulate plant growth and development[16]. This review focuses on the interconnection of Ca$^{2+}$, ABA and H$_2$S. Recent studies show that the relationship between Ca$^{2+}$ signaling and H$_2$S is not a simple upstream-downstream relationship. Under stress, intracellular Ca$^{2+}$ signaling responded to environmental stimuli to promote endogenous H$_2$S accumulation, while H$_2$S mediated Ca$^{2+}$ signaling transduction by up-regulating the transcription of calmodulin (CaM), calcineurin B-like (CBL) and calcium dependent protein kinase (CDPKs) genes. Furthermore, H$_2$S and Ca$^{2+}$ triggered ascorbic acid-glutathione (Asa-GSH) cycle against heavy metals (HMs)[17,18] and low temperature stress[19]. Fang et al.[20] confirmed how Ca$^{2+}$ promoted endogenous H$_2$S production under chromium (Cr$^{6+}$) stress in Arabidopsis. The interaction between Ca$^{2+}$/CaM2 and bZIP transcription factor (TF) TGA3 activated LCD expression by enhancing binding ability of TGA3 to the motif ‘TACGG’ of LCD promoter, forming a CaM2-TGA3-pLCD complex, resulting in H$_2$S accumulation[20]. Nevertheless, the correlative studies on Ca$^{2+}$ and H$_2$S signaling still remain in plant growth and development.

H$_2$S seems to interplay with ABA in drought responses. Shen et al. found a negative feedback loop between reactive oxygen species (ROS) and ABA signaling. ABA-triggered overproduction of ROS which further produces negative feedback to regulate respiratory burst oxidase homolog protein D (RboH-D) and DES1 activity, resulting in ABA de-sensitivity[21]. Then ABA-triggered DES1 function contributed to long hypocotyl 1 (HY1) retrograde signaling[22]. Chen et al. also reported that ABA-induced H$_2$S persulfidated the TF abscisic acid insensitive 4 (ABI4) at the residue Cys520, triggering the transactivation of mitogen-activated protein kinase kinase kinase 18 (MAPKKK18) involved in autophosphorylation or phosphorylation of the sucrose nonfermenting 1 (SNF1)-related protein kinase 2.6 (SnRK2.6)[23], while ABI4 transactivated DES1 to form a DES1-ABI4 loop[24]. Moreover, HY1 can negatively regulate ABA-induced stomatal closure by activating ABI4 expression[25]. Accordingly, there appears to be a redox-based regulatory loop between H$_2$S signaling and ABA-induced stomatal closure. Furthermore, H$_2$S mediated ABA signaling by persulfidating SnRK2.6 at Cys131 and Cys137 residues. The interaction between SnRK2.6 and downstream ABA response factor 2 (ABF2) was enhanced by persulfidation, hence promoting ABA-induced stomatal closure.

**Fig. 1** Endogenous H$_2$S biosynthesis in higher plants. SO$_4^{2-}$: sulfate ion; SO$_3^{2-}$: sulfite ion; ATPs: ATP sulfurylase; APS: adenosine 5'-phosphosulfate; APR: APS reductase; SIR: sulfite reductase; β-CAS: β-cyanoalanine synthase; L-Cys: L-cysteine; D-Cys: D-cysteine; L-DES: L-cysteine desulphhydrase; D-DES: D-cysteine desulphhydrase; DES1: L-cysteine desulphhydrase 1; Ser: serine; SAT: serine acetyltransferase; OAS: O-acetylsereine; OAS-TL: O-acetylsereine thiol lyase; GSH: reduced glutathione.
A review of hydrogen sulfide in preservation

closure,[25] Ca2+ signaling might harmonizes persulfidation and phosphorylation of SnRK2.6, and probably establishes a connection with ABA via ROS signaling.[25] However, more is pending on how this complex regulatory mechanism controls postharvest ripening and senescence.

Roles of H2S in postharvest senescence

Antagonism with ethylene

It has been well-established that H2S modulates ripening and senescence processes by mediating ethylene biosynthesis and signal transduction, and H2S plays a positive role in both climacteric and non-climacteric fruit (Table 1). After fumigating three green leafy vegetables with exogenous H2S, then using 0.1 µL L−1 ethylene to ventilate, respiration rate and chlorophyll loss were decreased.[26] Compared with ethylene treatment, H2S reduced the accumulation of reactive oxygen species (ROS) by enhancing the antioxidant system, and obviously delayed the color change of tomato fruit[27]. Consequently, H2S antagonizes ethylene-induced fruit ripening, which was associated with the antioxidant system. At the transcriptional level, Li et al. found that exogenous H2S attenuated the expression of ethylene synthesis-related genes, such as AdSAM, AdACO1, AdACS2, AdACO2 and AdACO3.[28]. In addition, H2S also affects the expression patterns of genes linked to ethylene synthesis and signaling, including ethylene receptor genes and ethylene response factors (ERFs). Apple slices treated with 0.4 mM NaHS showed lower expression of MdACO1, MdERS1 and MdETR.[29]. Similarly, H2S (1.0 mM NaHS) was combined with ethylene (1.0 g L−1 ethephon) to fumigate banana, the released H2S significantly down-regulated ethylene biosynthesis genes expression (MaACS1, MaACS2 and MaACO1) and up-regulated ethylene receptor genes expression (MaETR, MaERS1 and MaERS2).[30]. In this case, ethylene receptors may act as a negative regulator to inactivate by ethylene binding.[31] Lin et al. reported that H2S treatment reduced the expression of ethylene receptor 2 (ETR2) and ERF genes (ERF003, ERF5 and ERF016), but increased the expression of ERF4 and ERF113.[32]. Generally, H2S counteracts the quality deterioration caused by ethylene via regulating ethylene-related genes, which is essential to postpone postharvest senescence.

There appears to be a cooperative relationship between H2S and NO against ethylene. The combination of H2S and NO delayed ripening and decay of strawberry[33] and peach fruit.[34]. Combinatorial treatment (20 µL L−1 H2S gas and 15 µL L−1 of NO gas) further attenuated ACC synthase (ACS) and oxidase (ACO) activities, and significantly decreased ACC and MACC content.[34]. Munoz-Vargas et al. also found that H2S levels were increased during sweet pepper fruit ripening, while NO levels were reduced.[33]. NO binding with ACO to form a ternary complex reduces ethylene generation during the fruit ripening process.[36]. However, it remains uncertain how the intricate crosstalk between H2S and NO co-regulates ethylene-induced ripening and senescence.

Regulation of senescence-related genes

The genes associated with ethylene mentioned above are also senescence-related genes.[35]. Co-treatment (0.9 mM NaHS and 1.0 g L−1 etephon) decreased protease activity and sustained a high level of bioactive compounds which were essential for tomato postharvest quality by principal component analysis (PCA), including soluble protein, starch, titratable acids (TA) and ascorbic acid (AsA). Meanwhile, H2S reduced the transcription of ripening-related TFs (ERF003 and DOF22), and changed the expression pattern of genes encoding beta-aminase and UDP-glycosyltransferase including BAM3, UFGT73 and UFGT5.[37]. Consistent results were obtained from studies on fresh-cut pears. Reducing sugar, soluble protein and total amino acids content of pear slices under H2S fumigation were higher.[38]. After harvest, chloro-

Table 1. H2S counteracts the effects of ethylene in postharvest fruits and vegetables.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Treatment</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pak choy (Shanghai) Green curly kale</td>
<td>NaHS fumigation for 4 h (0, 50, 100 and 250 µL L−1 H2S)</td>
<td>Decreased ethylene production, chlorophyll loss and respiration rate</td>
<td>[26]</td>
</tr>
<tr>
<td>Sabellica) Sweet Italian basil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Ocimum basilicum)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-climacteric fruit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strawberry (Fengxiang)</td>
<td>0.8 mM NaHS and 5 µM SNP immersion for 10 min</td>
<td>Inhibited respiration rate and maintained crust color</td>
<td>[33]</td>
</tr>
<tr>
<td>Climacteric fruit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peach (Dahong)</td>
<td>15 µL L−1 NO gas and 20 µL L−1 H2S gas fumigation for 20 min</td>
<td>Reduced ACS and ACO activities and inhibited respiration rate</td>
<td>[34]</td>
</tr>
<tr>
<td>Kiwifruit (Qinmei)</td>
<td>1.0 mM NaHS fumigation for 8 d</td>
<td>Down-regulated the expression of ethylene synthesis-related genes like AdSAM, AdACS1, AdACO2 and AdACO3</td>
<td>[28]</td>
</tr>
<tr>
<td>Kiwifruit (Jinkui)</td>
<td>20 µL L−1 H2S gas fumigation for 0.5 h</td>
<td>Down-regulated the expression of ethylene receptor 2 (ETR2), ERF003, ERF5 and ERF016; up-regulated the expression of ERF4 and ERF113</td>
<td>[32]</td>
</tr>
<tr>
<td>Fresh-cut apple (Fuji)</td>
<td>0.4 mM NaHS fumigation for 5 d</td>
<td>Up-regulated MdDHAR expression and down-regulated the expression of MdLOX2, MdPG1, MdPPO, MdACO1, MdERS1 and MdETR</td>
<td>[29]</td>
</tr>
<tr>
<td>Banana (Brazil)</td>
<td>1.0 mM NaHS and 1.0 g L−1 ethephon solution fumigation for 6 d</td>
<td>Inhibited chlorophyll loss; up-regulated the expression of ethylene receptor genes MaETR, MdERS1 and MaERS2; down-regulated the expression of ethylene synthesis-related genes like MaACS1, MaACS2 and MaACO1</td>
<td>[30]</td>
</tr>
<tr>
<td>Tomato (Micro Tom)</td>
<td>0.9 mM NaHS and 1.0 g L−1 ethephon solution fumigation for 24 h</td>
<td>Maintained better appearance and postharvest quality and down-regulated the expression of ERF003 and DOF22</td>
<td>[37]</td>
</tr>
</tbody>
</table>
Cumulative reports demonstrate that H$_2$S positively to improve surface pitting symptoms in cold-stored sweet cherry (S)_2 and lower yields of WSP and β-galactosidase (β-gal). Besides, H$_2$S participates in cell wall metabolism and provides the integrity of the cell wall at the transcription level [32].

The genes linked to cell wall metabolism are controlled by ethylene. Zhu et al. [39,40] reported that H$_2$S regulates senescence-related genes to ameliorate ethylene-induced quality deterioration, as well as higher nutritional compounds.

**Regulation of cell wall metabolism**

Softening and textural changes are modified by several enzymes, and this process maybe different in various types of fruits [44]. It is well known that cell wall modifying enzymes take part in fruit ripening are controlled by ethylene. Zhu et al. [34] found that H$_2$S treatment (20 µL L$^{-1}$ H$_2$S gas) attenuated ethylene production and avoided cell wall loosening, while markedly decreasing the activities of cell wall modifying enzymes, such as polygalacturonase (PG), pectin methylesterase (PME) and endo-β-1,4-glucanase (EGase). H$_2$S-NO co-treatment (15 µL L$^{-1}$ NO gas and 20 µL L$^{-1}$ H$_2$S gas) further reduced the increases of water-soluble polysaccharides (WSP) and CDTA-soluble polysaccharides (CSP) in peach fruit, as well as the breakdown of Na$_2$CO$_3$-soluble polysaccharides (NSP) [34]. Similarly, in postharvest strawberry, synergistic interactions between H$_2$S and NO (0.8 mM NaHS and 5 μM SNP) reduced cell wall modifying enzyme activity, and further improved softening and shelf life [33]. The genes linked to cell wall degradation (EGase, PME and β-galactosidase) were down-regulated under H$_2$S treatment (20 µL L$^{-1}$ H$_2$S gas), while the expression pattern of ethylene responsive genes were also changed in kiwifruit, indicating that H$_2$S modifies the integrity of the cell wall at the transcription level [32]. Besides, H$_2$S participates in cell wall metabolism and provides tolerance for cold stress. Lower activities of PG, pectate lyase (PL) and β-galactosidase (β-gal) and lower yields of WSP and CSP under H$_2$S treatment (1.0 or 2.0 mM NaHS) contributed positively to improve surface pitting symptoms in cold-stored sweet cherry [45]. Cumulative reports demonstrate that H$_2$S inhibits the depolymerization and solubilization of cell wall polysaccharides. In addition, Forlani et al. proposed that ABA is also a key ripening-associated regulator, which can promote ethylene biosynthesis [44]. Whether the synergistic or antagonistic effects of H$_2$S and other molecules in fruit softening and senescence regulate cell wall metabolism directly or indirectly needs to be explored.

**Roles of H$_2$S in antioxidant system during postharvest**

The ROS scavenging system is divided into non-enzymatic and enzymatic. The enzymatic antioxidant system is composed of antioxidant enzymes like glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR) and glutathione S-transferase (GST), and the non-enzymatic antioxidant system consists of antioxidants, such as GSH, AsA, polyphenols, and carotenoids [46]. Abiotic stresses can destroy the dynamic balance of reactive oxygen species and reactive nitrogen species (ROS/RNS), which may ultimately cause irreversible nitro-oxidative damage [3]. Accumulating evidence verifies that H$_2$S counteracts ROS accumulation to reduce oxidative damage. Ni et al. [47] reported that high concentrations of H$_2$S (NaHS as donor) had toxic effects on Kyoho grape, described as severe decay and threshing rate. The optimal concentration of H$_2$S (1.0 mM NaHS) increased antioxidant content like AsA, flavonoid and total phenolics, as well as higher APX and CAT activity, while inhibiting H$_2$O$_2$, superoxide anion (O$_2^−$) and malondialdehyde (MDA) accumulation due to its higher antioxidant capacity [47]. Furthermore, it was reported that H$_2$S treatment (0.1 mM NaHS) showed higher relative expression of genes encoding SOD, CAT and GST, which ameliorated surface browning of fresh-cut apple by stimulating non-enzymatic and enzymatic antioxidant systems [48]. Similarly, Yao et al. found that additional H$_2$S (0.9 mM NaHS) enhanced the activities of POD, APX, CAT and SOD, and also up-regulated the relative expression of SIAPX2, SICAT1, SIPOD12 and SICuZnSOD-27. In nutshell, this is a possible mechanism for H$_2$S to delay postharvest senescence by sustaining the redox balance.

Like senescence, CI also induces ROS burst [46]. Application of H$_2$S induced chilling tolerance by enhancing antioxidant capacity as reported in banana [49], hawthorn [50] and kiwifruit [51] during postharvest (Table 1). It is indicated that H$_2$S alleviates CI-induced quality deterioration during postharvest, which is associated with the antioxidant system. Interestingly, water spinach treated with NaHS enhanced L-DES and D-DES activity that generated more endogenous H$_2$S. However, it was recovered by using DL-proparglyglycine (PAG, L-DES inhibitor), while the hypotaurine (HT, H$_2$S-scavenger) group was unaffected. Hence, Hu et al. deduced that endogenous H$_2$S was involved in the regulation of senescence [43]. During cold storage, NaHS application on hawthorn fruit [52] and mulberry fruit [52] also exhibited higher levels of endogenous H$_2$S, as well as H$_2$S-generating enzyme activity (L-DES and D-DES). Moreover, low temperature induced endogenous H$_2$S accumulation and higher expression levels of H$_2$S synthesis-related genes like Csa1G574800.1 (AdCP3) and CsaDES1 (CsaG034800.1) and CsaDE51
A review of hydrogen sulfide in preservation

Zhao et al. Food Materials Research 2022, 2: 3
tase (PSCS) and proline dehydrogenase (PDH) activity that synthesized more proline\(^{49}\). Proline, a major osmolyte, can sustain membrane permeability\(^{61}\), and stabilize antioxidant enzymes or stimulate additional ROS scavenging pathways in response to abiotic and biotic stresses\(^{62}\). Thus, it is implied that \(H_2S\) mediates the production of ROS and proline to mitigate CI. Soluble protein and soluble sugar are also important osmotic substances. As mentioned earlier, they can be improved by \(H_2S\). Besides, \(H_2S\) up-regulated most differentially expressed unigenes (DEGs) associated with antioxidant and energy, while down-regulating the expression of genes encoding membrane-degrading enzymes like PLD and LOX\(^{48}\). Thus, \(H_2S\) postpones membrane lipid metabolism to reduce the damage of lipid peroxidation at the transcriptional level. In combination with the above, the roles of \(H_2S\) in CI may be achieved by regulating physiological metabolism including (i) ROS, (ii) energy, (iii) membrane lipid, (iv) proline and (v) cell wall metabolism, there are still vast areas to be studied.

### Roles of \(H_2S\) in disease resistance

#### Inhibition of fungal growth

Fruits and vegetables often rot and deteriorate due to fungal infection during the process of transportation and storage. Recent studies have verified that \(H_2S\)-induced reduction in postharvest decay primarily operates through inhibiting fungal growth (Table 3). For example, Tang et al. reported that \(H_2S\) fumigation (1.0−2.5 mM NaHS) effectively inhibited the growth of fungal pathogens on medium and sweet potato slices in a dose-dependent manner, while reducing the incidence of black rot or soft rot\(^{63}\). Additionally, low doses of \(H_2S\) also controlled the mycelium growth on medium and pears\(^{38}\). How does \(H_2S\) rely on dosage to combat fungi? This study seems to explain, 0.5 mM NaHS treatment inhibited the growth of Aspergillus niger and Penicillium italicum on medium and inoculated fruits, and this facilitation may be attributed to lower CAT and SOD activities and down-regulation of relative genes in A. niger. \(H_2S\) in high concentrations had a bactericidal effect on the growth of some foodborne pathogens, among which Staphylococcus aureus was more sensitive to \(H_2S\)\(^{64}\). Taken together, the potential mechanism by which \(H_2S\), in low doses, exert antibacterial effects is to induce ROS overproduction in fungi by attenuating antioxidant capacity, and high concentrations of \(H_2S\) principally act as a fungicide to inhibit spore germination, germ tube elongation and mycelial growth (Table 3).

In mammals and yeast, cystathionine-\(\beta\)-synthase (CBS) and cystathionine-\(\gamma\)-lyase (CSE) are key enzymes involved in \(H_2S\) synthesis from sulfur metabolism. As shown in Fig. 2a, CBS catalyzes the dehydration condensation of homocysteine (Hcy) with Ser or Cys to form \(H_2O\) or \(H_2S\), respectively. Additionally, CBS also converts Cys to \(H_2S\) through the \(\beta\)-replacement reaction of Cys with thiols, and CSE produces \(H_2S\) via the \(\gamma\)-replacement reaction between Hcy molecules and the \(\alpha\beta\)-elimination reaction with cystine (Fig. 2b)\(^{65,66}\). Interesting results suggest that \(H_2S\) in fungi is also produced through the transsulfuration pathway. Guo et al.\(^{67}\) reported that the deletion of the Ano5g00160 gene (encoding CBS, named cbsA) in A. niger promoted Cys and GSH levels while decreasing endogenous \(H_2S\) content, which is responsible for enhancing cadmium (Cd\(^{2+}\)) tolerance and pathogenicity. Pear fruits infected with the cbsA mutant brought about an excessive production of ROS. In accordance with these results, it is suggested that CBS mainly decomposed Cys to produce \(H_2S\), and cbsA deletion harmonized redox homeostasis in A. niger to resist ROS burst of the host\(^{67}\). This maybe the reason why fungi can still grow on fruits, the detailed mechanisms require further research.

### Regulation of defense-related proteins

Under abiotic and biotic stresses, pathogenesis-related proteins (PRs) are triggered through the signaling network...

---

**Table 3. Effects of \(H_2S\) on disease resistance in postharvest fruits and vegetables.**

<table>
<thead>
<tr>
<th>Materials</th>
<th>Pathogens</th>
<th>Treatment</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pear (Dangshan)</td>
<td>Aspergillus niger</td>
<td>NaHS fumigation for 6d (2.0 mM)</td>
<td>Enhanced antioxidant capacity of fresh-cut pear and inhibited ROS accumulation; increased POD activity; reduced PAL and PPO activities; inhibited mycelium growth and lesion diameter on pears</td>
<td>[38]</td>
</tr>
<tr>
<td>Fresh-cut sweet potato (Kushu 18)</td>
<td>Mucor rouxianus Geotrichum candidum</td>
<td>NaHS fumigation for 4d (2.0 mM)</td>
<td>Inhibited spore germination, germ tube elongation, mycelial growth; promoted ROS accumulation by reducing SOD and CAT activities in A. niger, as well as higher expression of SOD and CAT</td>
<td>[64]</td>
</tr>
<tr>
<td>Apple (Malus domestica), mandarin (Citrus reticulata), sweet orange (Citrus sinensis), kiwi-fruits (Actinidia delicosa), pear (Pyrus bretschneideri Rehdi), tomato (Lyccopersicon esculentum)</td>
<td>Aspergillus niger Penicillium italicum</td>
<td>NaHS fumigation for 9d (0.5 mM)</td>
<td>Deletion of the cbsA gene in A. niger</td>
<td>niger MA: induced higher resistance to cadmium stress and stronger infectivity to pears; increased Cys and GSH levels; decreased endogenous (H_2S) content</td>
</tr>
<tr>
<td>Chinese white pear (Pyrus bretschneideri)</td>
<td>Aspergillus niger</td>
<td>Deletion of the cbsA gene in A. niger</td>
<td>Infection with above mutant</td>
<td>Pear fruit: increased the accumulation of (H_2O_2), (O_2^-), and MDA; induced larger lesion diameter</td>
</tr>
<tr>
<td>Peach (Mengyin)</td>
<td>Monilinia fructicola</td>
<td>NaHS fumigation for 2h (50 mM)</td>
<td>(M. fructicola): inhibited spore germination, mycelial growth and pathogenicity Peach fruit: enhanced CHT, GLU and PAL activities; while up-regulated the expression of related genes; reduced disease incidence and lesion diameter</td>
<td>[70]</td>
</tr>
</tbody>
</table>
It is worth noting [48] that  is not and the reduction of enzymatic browning by −1 −1  activity and relative expression in fresh-cut apple. Moreover, Chen et al. found that  showed posi−1 

Likewise, in fresh-cut lotus root, the activity of PAL, POD and PPO were higher than control at the later stages [73]. Interestingly, POD and PPO activity are also major drivers of the fungicidal substance against fungal infection. Zhang et al. observed that  and NO in association, caused an overall reduction in the decay incidence of strawberry, associated with an increase in GLU and CHT activity [33]. Analogous results were obtained on peach fruit, where  and HT application both effectively controlled lesion development diameter by enhancing GLU, CHT and PAL activities, with accompanying higher gene expression (U49454, AF20663 and KC757351). But HT treatment had no obvious impact on the incidence of brown rot compared with control [70]. HT function usually acts as a scavenger, but the assumptions of how  and HT promote PRs responsible in disease resistance remains unclear. Related proteins triggering in the absence of fungal infection were considered as 'PR-like' proteins such as PPO, POD and PAL [69], also known as PRs. The metabolites lignin and phenols produced through phenylpropanoid metabolism are correlated with disease resistance and the metabolic network composed of PAL, POD, PPO, 4-coumarate coenzyme A ligase (4CL), cinnamate-4-hydroxylase (C4H) and chalcone isomerase (CHI) co-regulate their synthesis [71,72]. POD and PPO activity are also major drivers of the fungicidal substance quinone. Vacuum infiltrated  increased total phenols, diminished quinone accumulation and further mitigated pericarp browning in cold-stored Litchi by altering the activity of PAL, POD and PPO. Interestingly, POD and PPO activity were higher than control at the later stages [73]. Likewise, in fresh-cut lotus root,  showed positive effects on reducing browning degree, which corresponded to higher phenolic content and antioxidant capacity [54]. Moreover, Chen et al. found that  incompletely suppressed POD activity and relative expression in fresh-cut apple, and the reduction of enzymatic browning by  is not realized by reducing phenolic substrates [48]. It is worth noting that phenols are not only regulators of disease resistance, but also antioxidants to delay postharvest senescence. However, how  induces phenolic synthesis involved in the equilibrium between antioxidant and disease resistance is vague, less is known about whether  regulates other key enzymes of phenolic metabolism.

**H₂S-derived protein persulfidation**

  H₂S triggers antioxidant system and controls ROS/RNS to harmonize the redox homeostasis, which may be pivotal to its functions [3]. It occurs primarily through mediating PTMs of proteins that convert cysteine (Cy-SH) into R-SSH [74]. This process was initially called S-sulfidation which can not signify hydration, but persulfidation is more suitable [3]. Cysteine residues are the ‘switch’ of redox and undergo other PTMs such as S-nitrosation (NO), S-glutathionylation (GSH), S-cyanlation (cyanide) and S-acylation (fatty acids) during different stress periods, which compete with  for thiol (-SH) groups [75]. Persulfidation modifies the structure, function and subcellular localization of target proteins. Proteomic analysis showed that the number of targets identified in Arabidopsis was in the following order: persulfidation > S-nitrosation > S-glutathionylation [8]. This implies that  derived persulfidation plays a crucial role in biology. Two bHLH TFs, CsaSG156220 and CsaSG157230, regulated the expression of the gene cluster involved in cucurbitacin C biochemistry [76].  derived persulfidation enhanced binding ability of two TFs with bitter leaf (Bo) promoter, further resulting in CuC accumulation and helping cucumber to withstand biotic and cold stress [53]. Likewise,  persulfidated mitogen-activated protein kinase MPK4 to resist cold stress in Arabidopsis [77]. Jia et al. also proposed that ethylene-induced  feedback regulated ethylene generation by reducing ACO activities via persulfidation under osmotic stress, and the residue Cys60 was a potential binding site in LeACO1 [78]. As mentioned previously,  forms a regulatory loop with ABA and redox signaling based on protein persulfidation. There is growing evidence that the interconnection between  and ROS signaling operates through persulfidation.
also called NADPH oxidase (NOX), takes part in ROS/RNS metabolism. During sweet pepper fruit ripening, NOX activity is suppressed by S-nitrosation, nitration and S-glutathionylation\(^{[79]}\). NADPH is a key cofactor in ROS/RNS metabolism, where some enzymes like GR, NOX and NO synthase-like (NOS) are NADPH-dependent to perform their functions\(^{[80]}\). H\(_2\)S and NO regulate NADPH-generating enzymes based on PTMs to harmonize cellular redox homeostasis\(^{[81]}\). G6PDH, 6PGDH, NADP-malic enzyme (NADP-ME) and NADP-dependent isocitrate dehydrogenase (NADP-ICDH) enzymes are responsible for NADPH supply. NADP-ICDH, a potential H\(_2\)S target, was probably suppressed by S-nitrosylation at Cys133 and nitration at Tyr450 during the ripening phase\(^{[35]}\). Moreover, further studies showed that higher H\(_2\)S and NO levels were designed to diminish NADP-ME activity to modulate pepper fruit ripening, while 6PGDH activity was unaffected\(^{[82]}\). Cumulative reports extrapolate that NADPH generation involved in fruit ripening is a good example of the association

---

**Fig. 3** Exogenous H\(_2\)S regulates numerous physiological processes to improve postharvest quality of fruits and vegetables. (a) A simple model showing a cascade of events after exogenous H\(_2\)S, which may stimulate various components of enzyme systems to maintain cellular homeostasis, but it is still not well understood how H\(_2\)S works with other molecules like ABA, NO, Ca\(^{2+}\) and ROS, etc. Broken red arrows represent processes triggered by H\(_2\)S, and broken blue arrows indicate abiotic stress-induced. (b) Various functions of H\(_2\)S in metabolic enzyme systems. (b-i) H\(_2\)S regulates related genes to delay senescence, especially ethylene (ET) biosynthesis and signaling transduction. (b-ii) The roles of H\(_2\)S in the antioxidant system, which is essential for H\(_2\)S-derived beneficial effects. (b-iii) H\(_2\)S sustains the integrity of cell membrane. (b-iv) H\(_2\)S facilitates ATP supply. (b-v) Inhibitory effect of H\(_2\)S on cell wall loosening. (b-vi) H\(_2\)S shows different mechanisms of redox homeostasis in hosts and fungi. Red arrows indicate that exogenous H\(_2\)S enhanced the enzyme activity. The orange font implies that different results are obtained after H\(_2\)S treatment, which correlates with whether the analyses are performed under cellular/subcellular or cell-free conditions (purified proteins)\(^{[81]}\).
between H$_2$S and NO via PTMs. Similarly, APX and CAT are targets of both persulfidation and S-nitrosylation at the same position, where H$_2$S acts upstream or downstream of NO in response to different stimuli.$^{[83,84]}$. H$_2$S and NO co-regulate root development, stomatal closure, and programmed cell death (PCD) in plants, where ABA and ROS signaling play an integral role.$^{[85]}$. These incorporating reports mean that synergistic or antagonistic properties of H$_2$S and NO under various stresses are based on PTMs, which is in agreement with previous studies. Nonetheless, the precise molecular mechanism of how they work remains ambiguous.

Conclusion and perspective

There is growing proof that exogenous H$_2$S promotes endogenous H$_2$S directly or by synthetic enzymes to further oppose metabolic disorders in fruits and vegetables caused by abiotic stress, which appears to affect enzyme activity via persulfidation (Fig. 3a). However, large numbers of reports suggest that H$_2$S can regulate various metabolic enzyme systems to improve postharvest quality (Fig. 3b), but fewer persulfidated target proteins have been identified. Many issues have emerged that require urgent attention. H$_2$S-induced chilling tolerance remains an extensive area of research, and it is necessary to investigate the effects of H$_2$S application on GABA and PAs catabolism. Whether H$_2$S stimulates the expression of cold responsive proteins at low temperature is also a research interest. Multiple studies have corroborated that H$_2$S can delay ripening and senescence and corroborated that H$_2$S promotes 

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31972125,32172265)

Conflict of interest

The authors declare that they have no conflict of interest.

Dates

Received 23 November 2021; Accepted 30 December 2021; Published online 14 February 2022

REFERENCES


erization of ethylene in tomato fruit ripening. Journal of Agricultural and Food Chemistry 66:10380−7


tant defense system and senescence-related gene expression. HorticScience 51:152−58


35. Muñoz-Vargas MA, González-Gordo S, Cañas A, López-Jaramillo J, Palma JM, et al. 2018. Endogenous hydrogen sulfide (H$_{2}$S) is up-regulated during sweet pepper (Capsicum annum L.) fruit ripening. In vitro analysis shows that NADP-dependent isocitrate dehydrogenase (ICDH) activity is inhibited by H$_{2}$S and NO. Nitric Oxide 81:36−45


ance of fruit quality of kiwifruits during low-temperature storage. Transactions of the Chinese Society of Agricultural Engineering 31:367−72


A review of hydrogen sulfide in preservation


56. Li D, Limwachiranon J, Li L, Du R, Luo Z. 2016. Involvement of energy metabolism to chilling tolerance induced by hydrogen sulfide in cold-stored banana fruit. *Food Chemistry* 208:272−78


79. Chu-Puga Á, González-Gordo S, Rodríguez-Ruiz M, Palma JM, Corpors FJ. 2019. NADPH Oxidase (Rboh) activity is up regulated during sweet pepper (*Capsicum annuum L.*) fruit ripening. *Antioxidants* 8:9

80. Aghdam MS, Palma JM, Corpors FJ. 2020. NADPH as a quality footprinting in horticultural crops marketability. *Trends in Food Science & Technology* 103:152−61


Copyright: © 2022 by the author(s). Exclusive Licensee Maximum Academic Press, Fayetteville, GA. This article is an open access article distributed under Creative Commons Attribution License (CC BY 4.0), visit https://creativecommons.org/licenses/by/4.0/.