Effects of hydrogen sulfide on storage quality, water mobility and cell wall metabolism of strawberry fruit

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Abstract: The effects of hydrogen sulfide (H_2S) on storage quality, cellular water distribution, and cell wall metabolism of strawberry fruit after subjected to shelf or cold storage were investigated. Fruit were fumigated with a range of aqueous NaHS solution (0.4-3.2 mmol/L), then stored at 20°C for 3 d or 0°C for 9 d. H_2S-treated fruit significantly maintained higher fruit firmness (FF) and titratable acidity (TA) as well as lower decay compared to the control fruit. Furthermore, H_2S inhibited the loss in extractable juice (EJ) and improved storage quality that not only resulted from the suppressing of respiration rate, but also from the modification of water mobility and cell wall metabolism. High FF and EJ in H_2S-treated fruit were closely associated with lower exchanges of free water between vacuole and cytoplasm/free space or cell wall, water-soluble polysaccharides (WSP), and activities of cell wall-modifying enzymes. Therefore, a potential benefit of H_2S on retarding softening was that the H_2S can reinforce the hydrogen bonding in polysaccharides and reduce activities of cell wall-modifying enzymes, causing a stabilization of cell wall structure. Although approval of the use of H_2S on foods has not yet been granted, an alternative reducing agent gas based on H_2S tended to be more effective in improving strawberry quality.

Keywords: hydrogen sulfide, strawberry, softening, water mobility, cell wall metabolism
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1 Introduction

Hydrogen sulfide (H_2S), an endogenous signaling molecule such as carbon monoxide (CO) and nitric oxide (NO), has been shown to prevent fruit against senescence[1,2]. Multiple physiological roles of H_2S, i.e. maintaining antioxidant enzymes activity, down-regulating the expression of senescence-related genes, and reducing oxidative damage caused by reactive oxygen species (ROS), has been well documented with studies on kiwi[3], banana[4], sweet cherry[5], strawberry[6], and pear[7] fruit. The major function of H_2S on retarding softening is to maintain the tissue energy status and to reduce polygalacturonase activity[3-5]. However, fruit softening is characterized by a series of changes in solubilization and depolymerization of cell wall components mediated cooperatively by the wall-associated enzyme system, and whether H_2S plays an essential role in regulating cell wall metabolism remains unclear.

Strawberry is a non-climacteric fruit and preferred by consumers due to its vivid red color, desirable sweetness, juicy, full flavor, and high nutrients when eaten fresh. However, fruit is more susceptible to softening and the deterioration of skin color, sugar, acid, and flavor. As result, fruit have a short life and high rate of fungal infection during storage and marketing[8]. Free and bound water are used to represent water status in fruit. Rapid loss of free water, i.e. extractable juice, results in fruit dehydration; bound water change, when occurs, will cause the inferior of texture and eating quality[9,10]. Low-field pulsed nuclear magnetic resonance (LF-NMR) is one non-invasive and quantitative analytical method to assess water status in different water proton pools such as cell wall, cytoplasm/free space, and vacuole. Changes in spin-spin relaxation time (T_2) and relative signal intensity can easily expressed the mobility of water among these proton pools[11]. Therefore, monitoring water status may be the best approach to evaluate how the H_2S influence the softening process.

The purpose of this work was to investigate the effects of H_2S on storage quality and cellular water distribution related to the modification of cell wall components and cell wall-modifying enzymes in strawberry fruit.

2 Materials and methods

2.1 Fruit materials and treatments

Strawberry (*Fragaria* × *ananassa* Duch. cv. Sweet Charlie) were hand-harvested at commercial maturity (75%-85% red or pink color of fruit surface) from an orchard in Zhongmu County, Henan province, China (latitude 114.0°N, longitude 34.6°W, elevation 80.5 m). After being transported to the laboratory, fruit with a uniformity of weight, size, shape, and freedom from defects was selected and packed in 180 of 1 kg PET containers (about 50 fruit per container), then placed at 0°C. After 24 h, every 12 containers was loaded into a 70 L latching box, then fumigated with 0.4 mM, 0.8 mM, 1.6 mM or 3.2 mM aqueous solution of NaHS (Sigma-Aldrich Co., Shanghai, China) (Figure 1). Control fruit was fumigated with the distilled water. After being fumigated for

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30 min, the H_2S concentrations reached approximately 0.1 μmol/L, 0.6 μmol/L, 2.4 μmol/L or 4.8×10^{-4} μmol/L. Each treatment contained 3 replications and each replication represented one latching box. After being fumigated in dark at 0°C for 24 h, 6 containers from each latching box were stored at 20°C; 6 containers from each latching box were moved to cold storage at 0°C.

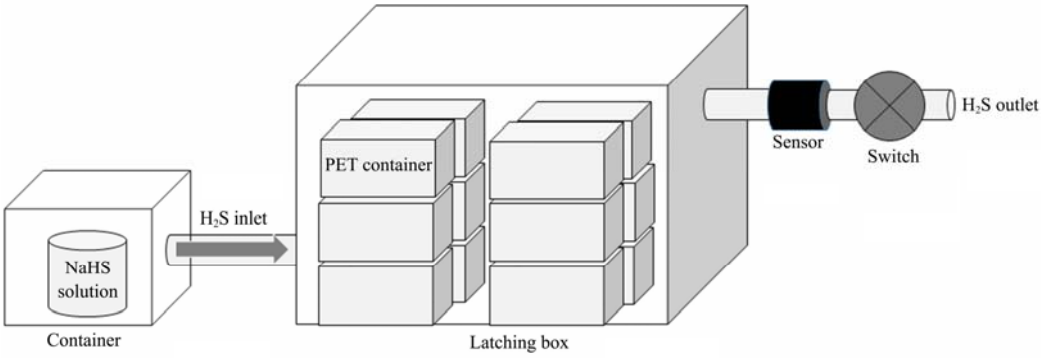


Figure 1 Schematic diagram of the H_2S fumigation

2.2 Respiration rate (RR), fruit firmness (FF), soluble solids content (SSC), titratable acidity (TA), and extractable juice (EJ)

RR was measured from 30 fruit per replicate from each treatment at the initial and after 3 d at 20°C and 9 d at 0°C, respectively. Fruit was sealed in a 3.25 L air-tight container at 20°C for 1 h. The headspace gas was withdrawn using a 10 mL air-tight syringe and injected into a CO_2 analyzer (HWF-1A, Kexi Instruments Co., Jiangsu, China). FF was determined on opposite sides of the equator of each fruit using a digital hardness meter (GY-4, Yueqing Handpi Instrument Co., Zhejiang, China) equipped with a 3.5 mm probe and penetration was 5 mm. After FF determination, flesh tissue samples (30 g) were squeezed with a double layer of nylon-gauze filter to get fruit juice. The SSC of juice was measured using a digital refractometer (PAL-1, ATAGO, Tokyo, Japan). TA was determined by titrating 5 mL of juice with 0.1 M NaOH to pH 8.1 and expressed as percentage malic acid. EJ was measured by a centrifuge method[12]. A 10 mL centrifuge tube with an absorbent cotton on the bottom was weighed as $W1$. One flesh disc (9 mm diameter and 10 mm long) was removed from the central region of fruit with a cork borer, then weighed and loaded into tube. After centrifuging at 1500×g for 10 min, flesh disc was removed and weighed as $W2$. EJ (%) was calculated as follows:

$$\text{EJ}(\%) = (W2 - W1) / \text{Total weight of pulp disc} \times 100 \quad (1)$$

2.3 Evaluation of decay incidence

Decay was counted on day 3 at 20°C and day 9 at 0°C, and any pathological lesion was considered as decay and expressed as percentage of incidence.

2.4 Measurement of cellular water distribution

One flesh disc (9 mm diameter and 20 mm long) was removed and loaded into a glass sample tube, then analyzed using a NMI20-15 LF-NMR (Shanghai Niumag Analytical Instrument Co., Shanghai, China) operated at a magnetic field frequency of 21.3 MHz[13]. Spin-spin relaxation time (T_2) and signal intensity were measured using Carr–Purcell–Meiboom–Gill (CPMG) sequences, performed by the magnetic field frequency at 18 MHz and 32°C. The maximum point of every second echo was accumulated using a 90° pulse of 13 μs and 180° pulse of 25 μs. The dwell time was 1 μs. Echo time was 160 μs and total echo count was 10 000 points. Ten fruit from each treatment of each replicate was evaluated at each sampling time.

2.5 Isolation and measurement of cell wall polysaccharides

Flesh tissue samples (10 g) were homogenized in 30 mL of 80% ethanol, and then boiled for 20 min. After centrifuging at 9000×g for 10 min at 20°C, the supernatant was decanted and residue was suspended twice with ethanol, then twice with acetone, and dried at 75°C for 12 h. The samples were collected as alcohol insoluble residue (AIR). AIR (100 mg) was treated twice with 10 mL of distilled water, centrifuged, and the two supernatants were collected as water-soluble polysaccharides (WSP). Next, residue was treated twice with 10 mL of 50 mmol/L cyclohexanetrans-1, 2-diamine tetra acetate (CDTA), centrifuged, and the two supernatants were collected as CDTA-soluble polysaccharides (CSP). Finally, residue was treated twice with 10 mL of 50 mmol/L Na_2CO_3 containing 10 mmol/L $NaBH_4$, centrifuged, and the two supernatants were collected as Na_2CO_3-soluble polysaccharides (NSP). Each extract polysaccharides solution (0.2 mL) was added to 1 mL of H_2SO_4 containing 75 mmol/L $Na_2B_4O_7$. After boiling for 10 min, 20 μL of 0.5% NaOH containing 0.15% *m*-phenylphenol was added to the mixture and loaded into a 96-well plate. Absorbance at 520 nm was monitored in a DNM-9602 microtiter reader (Beijing Perlong Technology Co., Beijing, China). A standard calibration curve of galacturonic acid was plotted and results were expressed as mg/kg AIR[14].

2.6 Determination of cell wall-modifying enzymes

2.6.1 Determination of polygalacturonase (PG)

Flesh tissue samples (0.3 g) were homogenized in 5 mL of 0.3 mol/L NaCl. After centrifuging at 10 000×g for 15 min at 4°C, the supernatant (50 μL) was added to 0.6 mL of 0.1% polygalacturonic acid, 0.6 mL of 50 mmol/L NaAc-HAc (pH 4.5), and 0.15 mL of 0.3 mol/L NaCl, then incubated at 37°C for 2 h. After adding 4 mL of 0.1 mol/L borate buffer (pH 9.0) and 0.6 mL of 1% 2-cyanoacetamide, the mixture was boiled for 10 min. PG activity was defined as the release of 1 mg of galacturonic acid per min at 276 nm[15].

2.6.2 Determination of petin methylesterase (PME)

All solutions, including NaCl solution, pectin, and supernatant were adjusted to pH 7.5 with 0.1 mol/L NaOH. Flesh tissue samples (3 g) were homogenized in 4 mL of 0.3 mol/L NaCl. After centrifuging, the supernatant (3 mL) was added to 37 mL of 0.5% pectin and incubated at 37°C for 1 h. The mixture was titrated by 0.1 mol/L NaOH solution. PME activity was defined as the release of μmol of ester hydrolyzed per min[16].

2.6.3 Determination of pectate lyase (PL)

Flesh tissue samples (1 g) were homogenized in 5 mL of 50 mmol/L Tris-HCl (pH 8.5) containing 0.6 mmol/L $CaCl_2$, 5 mmol/L EDTA and 0.05% Triton X-100. After centrifuging, the supernatant (1 mL) was added to 5 mL of 50 mmol/L Tris-HCl

(pH 8.5) containing 0.6 mmol/L $CaCl_2$ and 0.24% polygalacturonic acid and incubated at 37°C for 30 min. After boiling for 10 min and ice cooling, PL activity was defined as the formation of 1 μmol of 4, 5-unsaturated product per min at 232 nm[17].

2.6.4 Determination of β-galactosidase (β-GAL)

Flesh tissue samples (4 g) were homogenized in 4 mL of 0.3 mol/L NaCl. After centrifuging, the supernatant (0.2 mL) was added to 2 mL of 0.04% *p*-nitrophenyl-β-D-galactoside, 0.3 mL of 0.3 mol/L NaCl, and 0.5 mL of distilled water. After incubating at 37°C for 30 min, the mixture was added to 2 mL of 0.2 mol/L Na_2CO_3. β-GAL activity was defined as the release of mg of *p*-nitrophenol per min at 400 nm[18].

2.7 Statistical analysis

All experiments were performed using a completely randomized design. Analysis of variance (ANOVA) was carried out to determine the significance of differences according to Fisher's protected least significant difference (LSD) test at $p<0.05$. Statistical analysis was carried out using IBM SPSS Statistics (version 19.0, IBM Co., Armonk, NY, USA).

3 Results

3.1 Storage quality

RR in control fruit increased from 5.48 mL CO_2/kg/h to 33.45 mL CO_2/kg/h and to 9.90 mL CO_2/kg/h after shelf and cold storage, respectively. Compared with control, H_2S significantly inhibited RR under both storage conditions, especially in 1.6 mmol/L NaHS treatment. Regardless of storage temperature, FF decreased in all treatments. This decreased firmness may due to the moisture loss[19]. However, H_2S treatments showed relatively higher FF than the control. No treatments affected SSC during storage. TA and EJ in all treatments declined from the initial to the end of storage at 20°C or 0°C, but fruit treated with H_2S had higher TA and EJ than the control fruit. Compared to cold storage, control fruit stored at 20°C led to a faster decay. However, H_2S reduced decay under both storage conditions, and 0.8 mmol/L or 1.6 mmol/L NaHS treatment showed no decay incidence over the whole storage periods (Table 1).

Table 1 Changes in respiration rate (RR), fruit firmness (FF), soluble solids content (SSC), titratable acidity (TA), extractable juice (EJ), and decay incidence of strawberry fruit influenced by H_2S after 3 and 9 d of storage at 20°C and 0°C, respectively

Storage periods	NaHS rate /mmol·L^{-1}	RR/mL CO_2·(kg h)$^{-1}$	FF/N	SSC/%	TA/%	EJ/%	Decay/%
At harvest		5.48±1.12	52.8±3.15	7.8±0.2	0.72±0.10	41.0±2.9	0
3 d at 20°C	0	33.45±2.30a	28.2±1.13d	7.7±0.2a	0.37±0.08b	20.1±2.2c	23.3±5.8a
	0.4	29.55±2.15b	31.2±2.45c	7.9±0.3a	0.40±0.05b	22.1±2.4c	6.7±2.8b
	0.8	27.60±1.05c	36.2±1.01b	7.6±0.3a	0.49±0.09a	28.5±2.2b	0d
	1.6	25.70±0.75d	40.5±2.16a	7.7±0.3a	0.47±0.10a	31.5±3.2a	0d
	3.2	27.31±1.07c	39.8±1.51a	7.6±0.3a	0.48±0.05a	32.3±2.1a	3.3±1.6c
$LSD_{0.05}$		1.24	2.19	0.3	0.05	2.36	3.2
9 d at 0°C	0	9.90±0.37a	47.0±1.27b	7.9±0.2a	0.48±0.04c	28.7±2.7c	3.3±1.6a
	0.4	7.18±0.09b	49.1±0.96b	7.6±0.3a	0.53±0.03b	32.8±1.9b	0b
	0.8	6.46±0.06c	50.7±1.10a	7.8±0.2a	0.63±0.04a	34.6±2.7b	0b
	1.6	6.38±0.09c	51.2±0.57a	7.7±0.1a	0.65±0.01a	38.8±3.4a	0b
	3.2	6.34±0.10c	50.8±0.13a	7.5±0.4a	0.61±0.06a	36.7±2.9ab	0b
$LSD_{0.05}$		0.55	0.89	0.3	0.05	2.6	0.9

Note: Values are presented as the means±standard deviation (SD), n=3. Different letters indicate significant differences between treatments at each storage condition according to Fisher's protected lease significant difference (LSD) test at $p<0.05$.

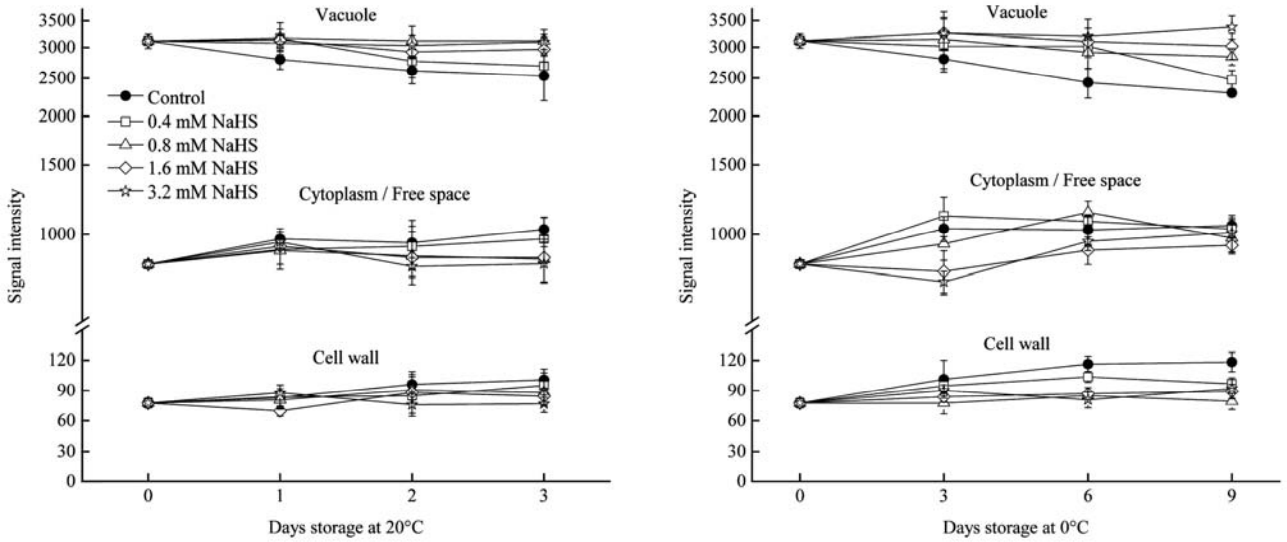
3.2 Cellular water mobility

In order to describe spin relaxation in different cell compartments[20], three main signal intensities were observed when T_2 at 2-8 ms, 150-300 ms, and 1000-1500 ms, which were recognized as water in the cell wall, cytoplasm/free space and vacuole, respectively. In cell wall and cytoplasm/free space, T_2 and signal intensities in control fruit increased from the initial to the end of storage at 20°C or 0°C (Table 2 and Figure 2). Low temperature slowed down the increase of T_2 and maintained higher signal intensities compared to shelf storage (Figure 2b). Under both storage conditions, H_2S significantly delayed the increases of T_2 and signal intensities, especially in 0.8 or 1.6 mmol/L NaHS treatment. In vacuole, although T_2 in control fruit increased to 1072 ms and 1178 ms after 3 d and 9 d at 20°C and 0°C, respectively, signal intensities dramatically decreased. Both 1.6 mmol/L and 3.2 mmol/L NaHS treatments under both storage conditions had higher T_2 and signal intensities than control and other NaHS treatments. The 0.4 mmol/L and 0.8 mmol/L NaHS treatment slightly affected water mobility, and T_2 and signal intensities were lower than those of 1.6 mmol/L or 3.2 mmol/L NaHS treatment.

Table 2 Changes in spin-spin relaxation time (T_2) in different cell compartments of strawberry fruit influenced by H_2S after 3 d and 9 d of storage at 20°C and 0°C, respectively

Storage periods	NaHS rate /mmol·L^{-1}	Cell wall /ms	Cytoplasm/Free space /ms	Vacuole /ms
At harvest		2.2±0.2	175±11	960±35
3 d at 20°C	0	3.7±0.3a	287±9a	1072±51c
	0.4	3.2±0.4b	231±11b	1232±39b
	0.8	2.4±0.2cd	183±6c	1355±62a
	1.6	2.1±0.2d	182±8c	1359±45a
	3.2	2.6±0.3c	188±10c	1378±59a
$LSD_{0.05}$		0.4	11	53
9 d at 0°C	0	2.7±0.3a	221±4a	1178±43c
	0.4	2.5±0.1ab	210±9b	1232±11b
	0.8	2.1±0.2b	176±11c	1232±34b
	1.6	2.3±0.2b	175±8c	1332±17a
	3.2	2.6±0.3a	210±9b	1355±31a
$LSD_{0.05}$		0.3	10	31

Note: Values are presented as the means±SD, n=10. Different letters indicate significant differences between treatments at each storage condition according to Fisher's protected LSD test at $p<0.05$.



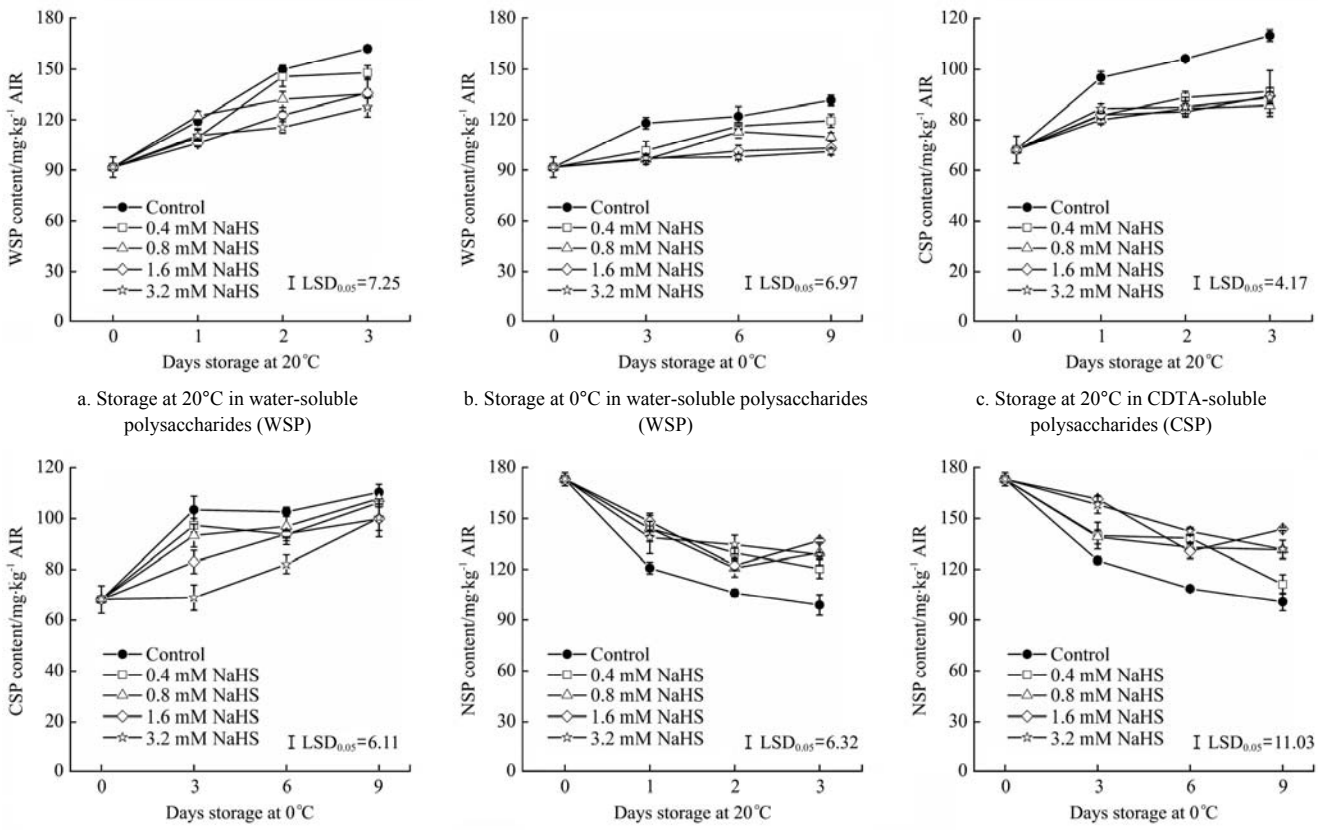
a. Storage at 20°C b. Storage at 0°C

Note: Values are presented as means±SD (n=10).

Figure 2 Changes in signal intensities of water in cell wall, cytoplasm/free space, or vacuole of strawberry fruit influenced by H_2S following storage at 20°C for 3 d or at 0°C for 9 d

3.3 Cell wall polysaccharides

In this study, three type of polysaccharides were extracted by water, CDTA, and sodium carbonate, which represented loosely-, ionically-, and tightly-bound polysaccharides[21]. High solubility of loosely-bound WSP along with ionically-bound CSP increased in all treatments under both storage temperatures (Figures 3a-3d); however, the low solubility of tightly-bound NSP decreased (Figures 3e and 3f). H_2S remarkably delayed the increases in WSP and CSP and maintained relatively higher NSP compared to the control, especially in high-rate application.



a. Storage at 20°C in water-soluble polysaccharides (WSP)

b. Storage at 0°C in water-soluble polysaccharides (WSP)

c. Storage at 20°C in CDTA-soluble polysaccharides (CSP)

d. Storage at 0°C in CDTA-soluble polysaccharides (CSP)

e. Storage at 20°C in Na_2CO_3-soluble polysaccharides (NSP)

f. Storage at 0°C in Na_2CO_3-soluble polysaccharides (NSP)

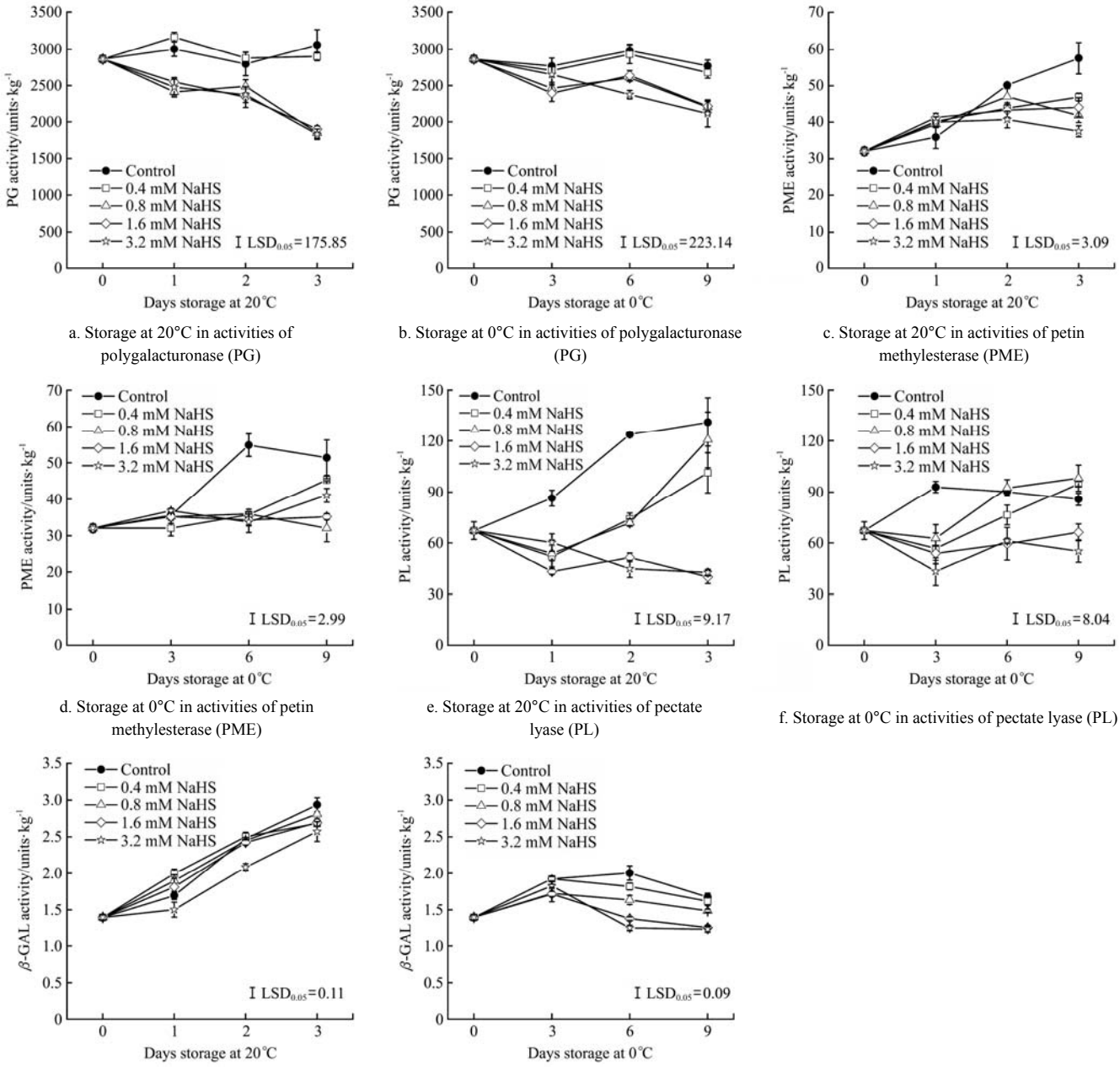
Note: Values are presented as means±SD (n=3).

Figure 3 Changes in water-soluble polysaccharides (WSP), CDTA-soluble polysaccharides (CSP), and Na_2CO_3-soluble polysaccharides (NSP) of strawberry fruit influenced by H_2S following storage at 20°C for 3 d or at 0°C for 9 d

3.4 Cell wall-modifying enzymes

The control and 0.4 mmol/L NaHS-treated fruit maintained high PG activity levels during shelf or cold storage, while 0.8 mmol/L, 1.6 mmol/L and 3.2 mmol/L NaHS treatments

reduced PG activities with no significant difference among the 3 treatments (Figures 4a and 4b). The activities of PME and PL in control fruit increased under both storage conditions. Fruit treated with 0.8 mmol/L or 1.6 mmol/L NaHS showed a significant inhibition in PME activity and a reduction in PL activity over the control (Figures 4c-4f). *β*-GAL activity in all treatments increased rapidly when fruit were held at 20°C for up to 3 d. However, low temperature retarded the increase of *β*-GAL activity and decreased it after 6 d of storage at 0°C. Regardless of storage temperature, *β*-GAL activity was significantly inhibited by the 1.6 mmol/L or 3.2 mmol/L NaHS treatment (Figures 4g and 4h).



a. Storage at 20°C in activities of polygalacturonase (PG)

b. Storage at 0°C in activities of polygalacturonase (PG)

c. Storage at 20°C in activities of petin methylesterase (PME)

d. Storage at 0°C in activities of petin methylesterase (PME)

e. Storage at 20°C in activities of pectate lyase (PL)

f. Storage at 0°C in activities of pectate lyase (PL)

g. Storage at 20°C in activities of *β*-galactosidase (β-GAL)

h. Storage at 0°C in activities of *β*-galactosidase (*β*-GAL)

Note: Values are presented as means±SD (n=3).

Figure 4 Changes in activities of polygalacturonase (PG), petin methylesterase (PME), pectate lyase (PL), and *β*-galactosidase (*β*-GAL) of strawberry fruit influenced by H_2S following storage at 20°C for 3 d or at 0°C for 9 d

4 Discussion

So far there is no standard procedure for H_2S application in industry up to our knowledge. Based on the previous report[6], an equipment for fumigation was designed to treat the fruit as shown in Figure 1. In this study, H_2S significantly maintained FF, reduced TA loss, and inhibited the increases of RR and decay during shelf or cold storage (Table 1). Above results expanded the previous finding[6, 22] that H_2S could improve strawberry quality and minimize disease incidence even in cold storage. After 3 d of storage at 20°C, decay increased in 3.2 mmol/L NaHS treatment. This increased decay might be related to the high H_2S rate that caused oxidative damage to cell membrane[23].

Extractable juice is a reliable indicator in reflecting the quality change during postharvest storage. An elevated EJ loss in fruit is associated with fruit susceptibility to softening[24]. Under both storage conditions, H_2S protected fruit against EJ loss, probably due to the relative low RR (Table 1). However, by analyzing

cellular water distribution using LF-NMR, it is noticed that H_2S-treated fruit had low T_2 and signal intensities in cell wall and cytoplasm/free space, but very high in vacuole, indicating that the effects of H_2S on reduction of EJ loss was probably related to the changes in water content and its mobility among different water proton pools. In fruit, water is compartmentalized into the cell wall, cytoplasm/free space or vacuole. The exchange proportion between each water proton pools is resulted from the dynamic modification of hydrogen bonding between water and biopolymers[25]. T_2 and signal intensity are used to describe the state and concentration of water in different cell compartments. A short T_2 and lower intensity represent a low water mobility and content[26]. In this study, the water mobility in cell wall and cytoplasm/free space was inhibited by H_2S. Moreover, some amount of water was restricted in the vacuole. These results indicated that H_2S might increase the hydrogen linkage between water and biopolymers including starches, lipids, proteins or other polymers in vacuole, thus prevent water inflow into the cytoplasm/free space or cell wall. As a result, lower losses of quality and EJ were observed in H_2S-treated fruit. In addition, the reduction of the quality deterioration and softening induced by H_2S might be due to the greater ROS scavenger antioxidant enzymes which can protect the integrity of cell membrane[3,6,7].

Softening is a part of the ripening syndrome. During fruit softening, cell wall polysaccharides undergo a continuous depolymerization and solubilization, and this change contributes to cell wall loosening and disintegration[27]. In this study, WSP and CSP increased in all treatments with different storage durations. H_2S significantly reduced the depolymerization/solubilization process, suggesting that H_2S has an ability to maintain tight bonds among the cell wall polysaccharides, and specifically, H_2S plays an important role in minimizing the loosely- or ionically-bound polysaccharides and stabilizing the tightly-bound polysaccharides. On the other hand, Ben-Arie and Lavee[28] found that more low molecular-mass WSP were able to form gel and to absorb free water from intracellular space into their gel structure. This water could not be removed from the gel structure of WSP using centrifugation method, which explained why a significant reduction in EJ was observed in control fruit. The H_2S-treated fruit had an intact cell membrane and less WSP; therefore, relative higher EJ was observed. The depolymerization and solubilization of cell wall polysaccharides is due to the action of cell wall-modifying enzymes[27]. Hu et al.[6] reported that the down-regulating of PG activity delayed the softening process in H_2S-treated fruit. In this study, cell wall-modifying enzymes were found to be inhibited by H_2S not only in PG, but also in PME, PL, and β-GAL as well, indicating that H_2S could decrease the activities of multiple cell wall-modifying enzymes, and result in reducing cell wall disassembly and maintain high storage quality. However, due to the approval issues for using H_2S, it was unable to find a source that refers to how much H_2S was allowable in food. But, an alternative fumigation agent based on H_2S would be investigated in a further study.

5 Conclusions

Postharvest fumigation of H_2S (0.8 mmol/L and 1.6 mmol/L NaHS) significantly reduced RR, delayed fruit softening, and also reduced losses in TA and EJ and decay in strawberry fruit during shelf or cold storage. H_2S provided the potential in improving fruit quality and minimizing juice loss by restricting the exchanges between the vacuole and cell wall or cytoplasm/free space, reducing the solubilization of cell wall polysaccharides, and suppressing the activities of PG, PME, PL, and β-GAL. H_2S has been proved as the third endogenous gaseous signaling molecule in animal and plant systems after NO and CO. While approval of the use of H_2S gas on foods has not yet been granted, this work provided valuable insights that explained why H_2S acted on the retarding of strawberry softening. Based on the toxicity of H_2S, the direct use of H_2S is not recommended in commercial applications. However, an alternative of H_2S agent without toxicity will be a new way to improve fruit quality in the future.

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