Cooperative effects of hydrogen sulfide and nitric oxide on delaying softening and decay of strawberry

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Abstract: Hydrogen sulfide (H_2S) and nitric oxide (NO) as two signal molecules play prominent roles in plant response to biotic stress. The combination effects of these two molecules on ripening and decay of postharvest strawberry were investigated. Strawberries were first dipped in distilled water, 0.8 mmol/L NaHS solution, 5 μmmol/L sodium nitroprusside (SNP) solution, and combinative solutions of 0.8 mmol/L NaHS and 5 μmmol/L SNP for 10 min, respectively, and then stored at 20 ℃. Results showed that the effects of the co-treatment of 0.8 mmol/L NaHS solution and 5 μmmol/L SNP solution were superior to that of other treatments. The former could suppress the percentage of fruit decay, inhibit respiration rate, maintain the original crust color, and slow down the decrease of firmness and the change of relative conductivity, which preserved the fruit quality. Further investigation showed that co-treatment of hydrogen sulfide and nitric oxide maintained higher activities of chitinase (CHI) and beta-1,3-glucanase (GNS), and lowered activities of pectin methylesterase (PME), polygalacturonase (PG) and endo-β-1,4-glucanase (EGase). The study indicated that the synergistic function of H_2S and NO could effectively prolong the shelf life and reduce the decay rate of harvested strawberry.

Keywords: hydrogen sulfide, nitric oxide, strawberry, enzyme activities, shelf life
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1 Introduction

Recently, a number of researches show that hydrogen sulfide (H_2S) plays various physiological roles in plants, such as seed germination, root organogenesis, abiotic stress tolerance, and senescence of fruits and cut flowers, which implies that H_2S can act as an important gaseous regulator in plants[1-4]. For instance, NaHS, a H_2S donor can increase the activity of the antioxidant enzymes while decreasing the concentration of hydrogen peroxide and lipoxygenase of wheat seeds[5]. Fumigating the strawberry fruit with NaHS prolonged the shelf life by increasing the activities of reactive oxygen species (ROS) scavenging enzymes and lowering polygalacturonase (PG) activity[3]. However, few reports explored effects of H_2S in regulating postharvest shelf life of fruits except Hu et al.[3] and Wang et al.[5].

Nitric oxide (NO) is known to play a key role in biotic and abiotic stresses in plants. NO is also reported to be effective in extending the postharvest shelf life of some fruits. Fumigation with exogenous NO or nitrogenous compounds, such as N-tertbutyl-α-phenylnitrone and 3-morpholino sydnonimine, has been employed to extend the postharvest shelf life of a wide range of fruits and vegetables[7-9]. Sodium nitroprusside (SNP), a NO donor can significantly extend the storage life of strawberry (Fengxiang variety) by inhibiting

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ethylene production, lowering respiration rate and amino-cyclopropane-carboxylate (ACC) synthase activity, and reducing the content of ACC[10].

Strawberries are susceptible to mechanical injury and fungal attacks. Its postharvest shelf life is shorter than one week even if under proper storage conditions at 0 ℃[11,12]. It is largely believed that pectin methylesterase (PME, EC 3.1.1.11), polygalacturonase (PG, EC 3.2.1.67), and endo-β-1,4-glucanase (EGase, EC 3.2.1.4) play important roles in cell wall softening after harvest[13-16]. Fruit decay, considered to be mediated by chitinase (CHI, EC 3.2.1.14) and beta-1,3-glucanase (GNS, EC 3.2.1.6), is one of the major causes for the postharvest loss[17-19].

Accumulating evidence has shown that H_2S and NO may have some potential links between each other in living organisms. For instance, Shi et al.[20] proved that NO might be the upstream signal of H_2S in bermudagrass response to cadmium stress. However, whether synergistic effects exist between H_2S and NO in postharvest fruit is still unknown. In the present study, the cooperative action of H_2S and NO in the regulation of ripening and senescence was investigated in strawberry. Exploration was conducted on activities of enzymes, including PME, PG and EGase which to some extent may be related to strawberry softening during ripening, and CHI and GNS which may have some connections with strawberry decay. In this work, the quality index of strawberry and related enzymes' activities were studied to provide a method to reduce softening and decay of the strawberry fruit.

2 Materials and methods

2.1 Fruit materials

Strawberry fruit (*Fragaria ananassa* L. 'Fengxiang') was collected from an orchard in Shanggao Village, Shengzhuang Town, Taian City, Shandong Province, China. Only well-formed unblemished strawberry fruit was sorted and selected to eliminate physical damage and fungal infection. Over 75% of the fruit had red color surface.

2.2 Fruit treatments

NaHS was obtained from Jinan Reagent General Factory. Sodium nitroprusside (SNP), the donor of NO, was purchased from Tianjing Kaitong Chemical Reagent Ltd. Co. NaHS and SNP solutions were prepared, at 0.8 mmol/L and 5 μmmol/L, respectively, using sterile deionized water. Strawberries were divided into four groups in random, immersed in distilled water (control group), 0.8 mmol/L NaHS solution, 5 μmmol/L SNP solution, combination of 0.8 mmol/L NaHS and 5 μmmol/L SNP solution for 10 min, respectively, and air-dried naturally at ambient temperature, then placed into plastic boxes with high density polyethylene film and stored at (20±1) ℃. Each treatment contained three replicates with 2 kg per replicate, and the entire experiment was repeated three times. Decay rate was investigated on day 2 and 4. Samples were taken from 20 fruits stored at 20 ℃ on 0, 2, and 4 d after treatments. Each treatment consisted of three replicates and the experiment was repeated three times.

2.3 Fruit quality evaluation

Strawberry fruits showing surface mycelia development and over 5% rotten surface area were identified as decayed with strict standard[21]. Average firmness of 10 fruits of each treatment was measured at the surface of each strawberry fruit using a texture analyzer (TA-XT2i, Stable Micro Systems Ltd., Godalming, Surrey, UK) with a 100 mm diameter aluminum cylinder and the 1 mm/s test speed. The respiration rate was determined by CO_2 Analyzer (PBI-940437B, PBI Dansensor, Shanghai, China). Strawberry fruit (200 g) was placed in hermetically sealed 1 L chamber for 1 h. CO_2 production rate was monitored at day 0, 2 and 4, respectively. The results were expressed as milligrams of CO_2 evolved per kilogram of strawberry per hour. Strawberry fruit external color was evaluated by colorimeters (Jing Electronic Instrument co., Ltd, Shanghai, China). Ten fruits of each group were measured, and the results were averaged. Chromaticity was expressed by the values of L*(lightness), a*(-greenness to + redness), and b* (-blueness to +yellowness). L*, a*, b* are the chromaticity coordinates[22, 23]. Total soluble solids (TSS) concentration was determined at 20 ℃ on an Atago pocket refractometer (PAL-1, Atago Co., Ltd, Tokyo, Japan), and expressed as (%).

2.4 Measurement of the relative conductivity of fruit

Relative conductivity was measured with precision electric conductivity meter (DDS-307, Shanghai Weiye Instrument Factory, Shanghai, China). The same part of ten fruits from each treatment was cut to acquire the slices of 30 pieces, immersed in 40 mL of deionized water for 30 min, and then boiled for 10 min. The relative conductivity was given by the ratio of the electrical conductivity measured before and after boiling.

2.5 Determination of PME, PG and EGase activities in fruit

PME activity was determined using the method of Zhou et al.[24] with some modifications. Frozen strawberries (2.5 g) were homogenized with pre-cooled 15 mL 120 g/L polythylene glycol (PEG) solution (including 2 g/L sodium bisulfite), and then the mixture was centrifuged at 12 000×g (gravitational acceleration) for 30 min at 4℃[25]. The supernatant was used to determine the activities of PME and PG. The PME activity was expressed by the consumption of NaOH solution, and defined as mL/min g. PG activity was measured following the method of Gross [26] with some modifications. The reaction mixture, consisting of 0.2 mL enzyme extract and 0.2 mL of 40 g/L polygalacturonic acid in 0.05 mol/L sodium acetate buffer (pH 6.0) was incubated at 37℃ for 14 h[18,25]. PG activity was expressed as micromole of galacturonic acid released per hour and per gram of strawberry. EGase activity was determined using the Miller method[27] with some modifications. Enzyme activity was evaluated from extracts prepared by homogenizing 2.5 g of frozen strawberry fruit in 15 mL of 0.4 mol/L NaCl solution. Endo-β-1,4-glucanase specific activity was expressed as U/g FW, and one unit was defined as production of 1 μg glucose per mL (enzyme liquid) per minute.

2.6 Determination of CHI and GNS activities in fruit

Crude tissue extracts of Chitinase and β-1,3-glucanase were obtained using the method of Cao and Jiang[28] with some modifications. Frozen strawberry fruit (5 g) was homogenized in 20 mL of 0.05 mol/L sodium acetate buffer at pH 5.0. The homogenized was centrifuged at 15 000×g, 4℃ for 15 min, and the supernatant was collected. Chitinase activity in frozen strawberry was determined colourimetrically with chitinazure (sigma) as the substrate using the method of Boller et al.[29]. Reaction products were measured spectrophometrically at 585 nm. Chitinase activity was expressed as U/g FW, where one unit (U) was defined as the enzyme activity catalyzing the formation of 10^{-9} nmol N-acetyl-D-glucosamine per hour. β-1,3-glucanase was determined with laminarin (sigma) as substrate, using the method described by Ippolito et al.[30]. GNS activity was expressed as U/g FW, and the unit was defined as production of 1 mol of glucose per hour.

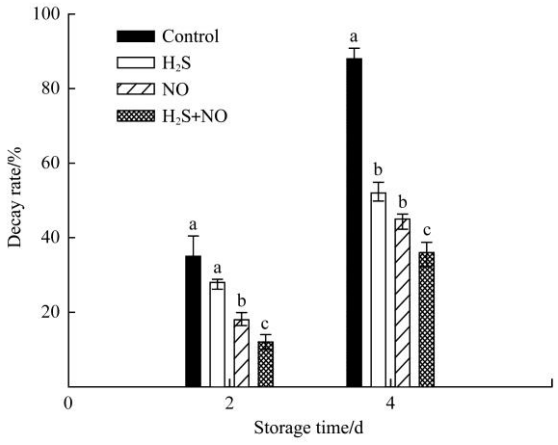
2.7 Statistical analysis

Statistical analysis was performed with SPSS version 16.0 (SPSS Inc., Chicago, USA). Treatment means were compared with Tukey test at P<0.05. The data were analyzed and graphically plotted using Sigma-plot 10.0 software (Systat Software Inc., Richmond, CA).

3 Results and discussion

3.1 Effects of different treatments on decay rate of strawberry

Decay rate increased rapidly in strawberry fruits during storage at 20℃ (Figure 1). On day 2, the percentage of decay of the control fruit could approximately reach 35%. However, the decay rates of 5 μmmol/L SNP solution and co-treatment of 0.8 mmol/L NaHS and 5 μmmol/L SNP solutions were all lower than that of control significantly (P<0.05). Even on day 4, the co-treatment fruit maintained lowest decay rate compared to NO and H_2S treatment alone. Moreover, the treatment with NO exhibited better control effects on fruit decay than that of H_2S.



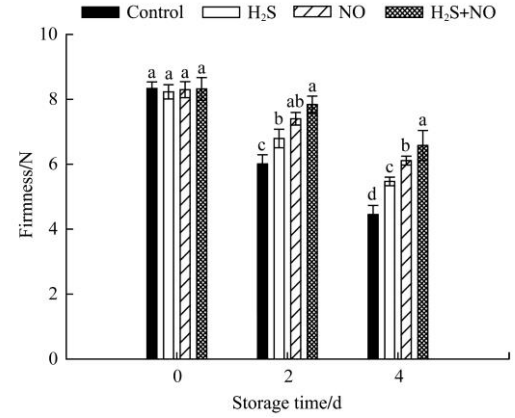
Note: Each value is presented as the mean ±SE (n=9). The same below.

Figure 1 Effects of different treatments on decay rate of strawberry fruit stored at (20±1)℃

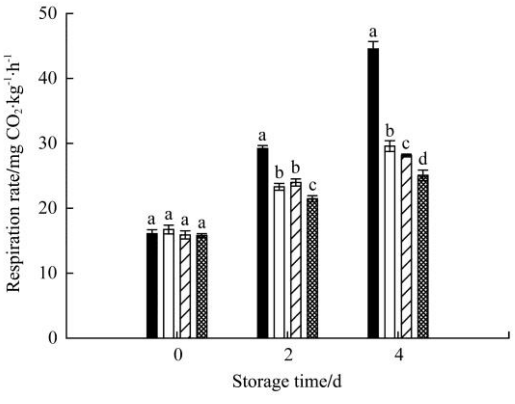
Softening and decay of strawberry fruit are always accompanied after harvest, and are the main factors in inhibiting its shelf life. The results are in accordance with those of previous studies[3,9]. In this study, it was found that co-treatment of H_2S and NO significantly (P<0.05) inhibited the decay rate (Figure 1).

3.2 Effects of different treatments on firmness and respiration rate

The firmness and respiration of strawberries in different treatments are shown in Figure 2. The firmness in strawberry fruit obviously decreased during the storage. Nevertheless, co-treatments of H_2S and NO solution significantly (P<0.05) dropped slower than other treatments (Figure 2a). The firmness of control fruit dropped the fastest. Either H_2S or NO separate treatment could reduce the production of CO_2 of strawberry during storage at 20 ℃, however, co-treatment of these two solutions could more effectively inhibit respiration rate of fruit (Figure 2b).



a. Firmness



b. Respiration rate

Figure 2 Effects of different treatments on firmness and respiration rate of strawberry fruit stored at (20±1) ℃

Firmness loss of strawberry has been largely associated with solubilization and depolymerization of cell wall polysaccharides[31,32]. In the present study, firmness obviously decreased during strawberry storage at 20 ℃, which might be related to cell wall degradation.

As shown in Figure 2b, the co-treatment of H_2S and NO solution maintained a lower respiration rate for strawberry than other treatment groups during the entire storage period. The CO_2 production of the co-treatment group was significantly lower than that of other treatment groups (P<0.05). Respiration rate, which to some extent reflects storage time, showed a rising trend during the whole storage period, and may be associated with fruit ripening or some pathogens' attack.

3.3 Effects of different treatments on color of fruit and TSS

Color is an important factor that reflects strawberry fruit quality. Figures 3a-c show the chromaticity changes of strawberry fruit during storage at 20 ℃, as given by the values of L*, a* and b*. As shown in Figure 3a, L* values decreased in each treatment of fruit in four days. During storage at 20 ℃, fruits in treatment groups showed higher L* values than those in the control group, especially the group co-treated with H_2S and NO. On day 4, L* decreased approximately by 66.25% for the control group and approximately by 61.86%, 56.53%, and 48.24% for fruit treated with NaHS, SNP, and the combination of NaHS and SNP, respectively. The changes of a* and b* values in the co-treated fruit increased slower than samples treated with H_2S or NO separately, and were significantly (P<0.05) lower than that of control on days 2 and 4 (Figure 3b and Figure 3c).

External color of strawberry is a main factor which contributes to the consumer acceptability and the product value, and usually influenced by storage temperature and humidity[22,33]. Compared with the control, H_2S and NO separate or cooperative treatments, maintained the lightness and color stability of fruit (Figure 3). Especially, the co-treatment of H_2S and NO played an important role in protecting the original color, which might be related to the delayed ripening and senescence of fruit.

Figure 3d shows fewer remarkable changes of TSS in strawberry during the storage time. On day 2, the TSS of four treatments experienced a very slight increase. A

very small difference was observed between the control and the combined treatment of H_2S and NO during storage. TSS of the four treatments had a slight decrease after 2 days. It can be expected that the co-treatment of H_2S and NO results in a less apparent decreasing trend than the control due to lower enzyme activities related to the solubilization of the cell wall, which might contribute to the increase in TSS.

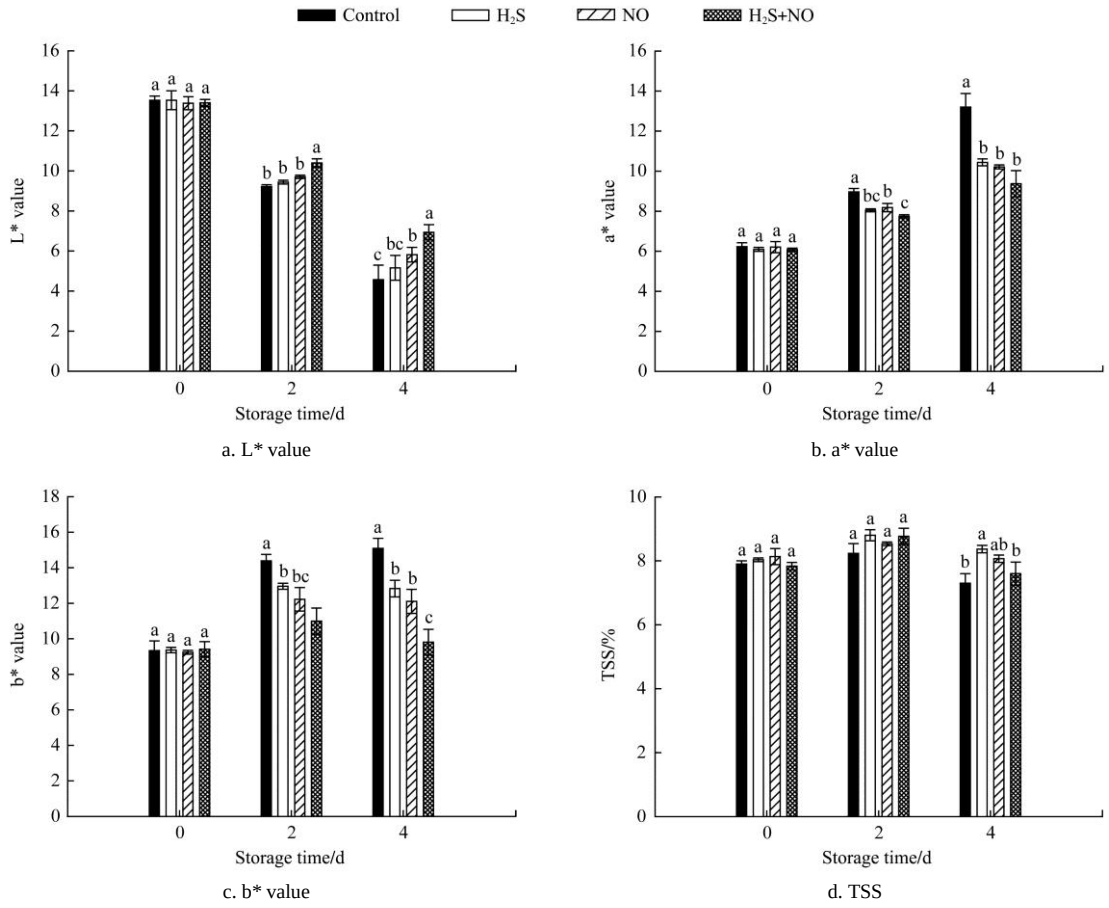


Figure 3 Effects of different treatments on skin L*, a*, b* value, TSS of strawberry fruit during storage at (20±1)℃

3.4 Effects of different treatments on relative conductivity of fruit

The treatment with the combination of H_2S and NO significantly reduced the rate of increase on relative conductivity compared to the control sample. H_2S or NO separate treatments had no significant (P>0.05) effects on relative conductivity of strawberries on day 4. On day 2, the relative conductivity in fruit treated with H_2S had no significant (P>0.05) difference compared with that of the control.

Relative conductivity is another important factor that can influence the softening of strawberry fruit. To some extent, it reflects the damage of cell membrane. Through the changes of the quality indexes, conclusions were acquired that H_2S and NO could effectively protect the cell membrane structure, and they played synergistic roles in delaying senescence of strawberry.

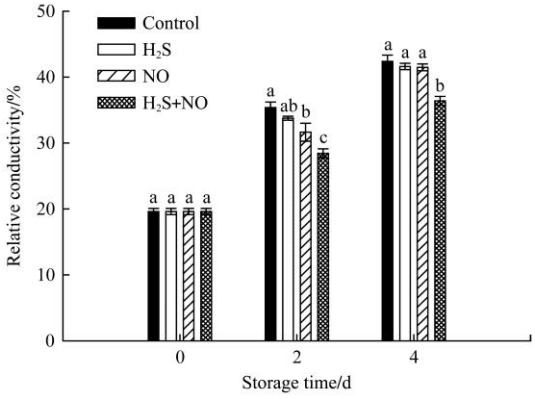


Figure 4 Effects of different treatments on relative conductivity of strawberry fruit stored at (20±1)℃

3.5 Effects of different treatments on activities of PME, PG and EGase in fruit

The activities of PME, PG and EGase of strawberry fruit are shown in Figure 5. During storage, NO combined with H_2S treated fruit showed significant (P<0.05) lower activities of PME than the control and NO

or H_2S-treated fruit (Figure 5a). In comparison with the control fruit, NO and H_2S significantly (P<0.05) inhibited PG activity from 2 to 4 d, however, the PG activity of co-treated fruit was obviously lower than that of other treatments on day 2 and 4 (Figure 5b). The EGase activity increased during the storage in both control and other treated fruit (Figure 5c). The enzyme activity of co-treated fruit decreased approximately 31.7% at day 2 and 25% at day 4 in comparison with the corresponding control.

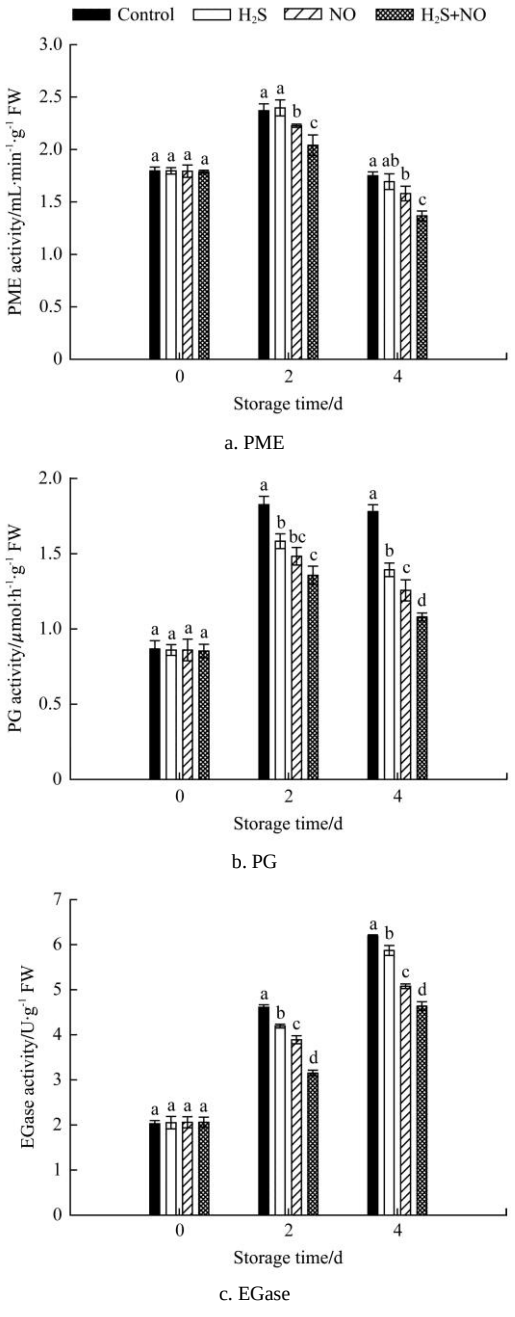


Figure 5 Effects of different treatments on activities of PME, PG and EGase of strawberry fruit during storage at (20±1)℃

It is generally agreed that fruit texture softening as a consequence of associated changes which occurs at level of the cell walls, and that different enzymes, such as cell wall hydrolases, acting on the different parietal components, are responsible for the cell wall weakening which takes place during the softening[32, 34, 35]. PME, PG and EGase are ubiquitous plant enzymes, and have a certain connection with the softening of fruits. The inhibition of the PME, PG and EGase activities demonstrated here suggests that application of combination of H_2S and NO plays an effective role in delaying senescence and maintaining quality of harvested strawberry fruit. Studies shown on strawberry have suggested that PG could be one of the enzymes that determine the different softening rates between cultivars[36,37]. In addition, both activities of PME and PG reached a peak on day 2, and then decreased (Figure 5a, 5b). The similar trend during the storage may raise the credibility of hypothesis that both PME and PG play important roles in pectin degradation on cell walls of strawberry fruit[38]. Some experiments demonstrated that EGase had a high expression during ripening process, and the high amounts of EGase that largely destructed the structure of cell wall had a steep increase throughout the ripening process[35,39]. The present results showed that EGase activity of strawberry increased and maintained a high level after strawberries were picked and became overripe (Figure 5c), which suggested that EGase played an important role in softening of fruit during storage.

3.6 Effects of different treatments on activities of CHI and GNS in fruit

It was clear that the activities of CHI and GNS in co-treated fruit were obviously higher than those in the control and H_2S and NO separately treated fruit on day 2 and 4 (Figure 6). In comparison with the control, H_2S, NO and combination of solutions enhanced CHI activity in fruit on day 2 (Figure 6a). NO-treated fruit had higher CHI activity than that by H_2S on days 2 and 4. The changes in GNS activity showed a similar trend in four treatments, but the activity had no significant (P>0.05) difference between NO and H_2S separately treated fruit (Figure 6b).

Chitin and β-1,3-glucans are two major components of the fungal cell wall. CHI catalyzes the hydrolysis of the β-1-4-linkage of chitin's N-acetylglucosamine

polymer. GNS decomposes β-1,3-glucans[40,41]. CHI and GNS are the main enzymes for protecting the pathogen-caused damages in plants, and proved the existence of the synergistic effects in transgenic tomato plants[18,42,43-44]. The present data showed that the co-treatment of NO and H_2S and separate treatments with NO or H_2S alone caused the accumulation of CHI and GNS in strawberry fruit with low decay rate in treated fruit (Figure 1 and Figure 6). These results implied that the higher activities of CHI and GNS may play important roles in weakening fungal cells, consequently enhancing disease resistance and reducing decay loss of strawberry fruit. It is therefore suggested that NO and H_2S, the two important gaseous molecules may be involved in inducing disease resistance and further delaying disease development in postharvest fruits.

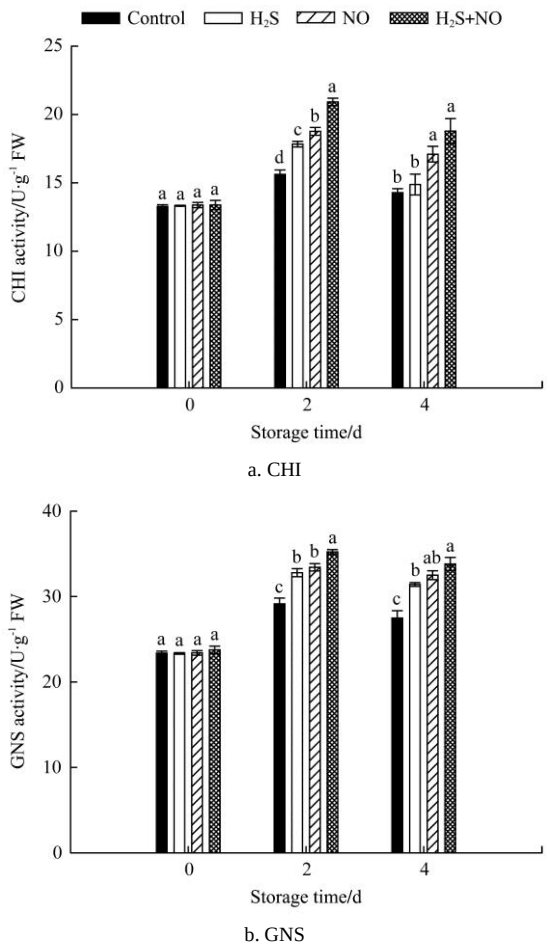


Figure 6 Effects of different treatments on enzyme activities of CHI and GNS in strawberry fruit

4 Conclusions

The co-treatment with H_2S and NO maintained fruit quality by lowering decay rate, respiration rate, relative conductivity and enzyme activities of PME, PG and EGase, and enhancing CHI and GNS activities which were related to delay of decay, and therefore extended shelf life of strawberry fruit after harvest. The effects of the co-treatment are greater than those of separate treatments with H_2S or NO alone. Therefore, it is fair to presume that H_2S and NO have synergistic effects to prolong the postharvest shelf life of strawberry fruit. This suggests that the co-treatment of H_2S and NO is a promising measure for extending shelf life of strawberry on a commercial scale.

Acknowledgments

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