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Effect of hydrogen sulfide on surface pitting and related cell wall metabolism in sweet cherry during cold storage

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Summary

This study was to determine whether postharvest hydrogen sulfide (H_2S) fumigation effected surface pitting development of 'Lapins' and 'Regina' cherries after cold storage, and if so, how it minimizes surface pitting injury and its relation to cell wall metabolism. Fruit were exposed to H_2S gas released from an aqueous solution of sodium hydrosulfide (NaHS) at rates of 0.5, 1, 2, or 3 mM, then stored at 0 °C for 4 weeks. Fruit of both cultivars treated with 1 or 2 mM NaHS had a greater firmness and a lower respiration rate compared to control fruit. However, no treatments retarded losses in soluble solids content (SSC) and titratable acidity (TA). Fruit fumigated with H_2S displayed significantly suppressed stem browning, decay, and pitting incidences, but not weight loss. The positive effect of H_2S on reduced pitting might be due to the lower yields of water-soluble polysaccharides (WSP) and CDTA-soluble polysaccharides (CSP) or/and due to the lower activities of polygalacturonase (PG), pectate lyase (PL), and β-D-galactosidase (β-GAL). Overall, results demonstrated that H_2S applied to sweet cherry as a postharvest vapor has ability to control surface pitting development by stabilizing cell wall structure and regulating cell wall catabolism.

Keywords: sweet cherry; hydrogen sulfide; surface pitting; storage quality; cell wall metabolism

Abbreviations: H_2S, hydrogen sulfide; NaHS, sodium hydrosulfide; **SSC**, soluble solids content; **TA**, titratable acidity; **WSP**, water-soluble polysaccharides; **CDTA**, cyclohexanetrans-1, 2-diamine tetra acetate; **CSP**, CDTA-soluble polysaccharides; **NSP**, Na_2CO_3-soluble polysaccharides; **AIR**, alcohol insoluble residue; **PG**, polygalacturonase; **PME**, petin methylesterase; **PL**, pectate lyase; **β-GAL**, β-D-galactosidase.

Introduction

Fresh sweet cherries (*Prunus avium* L.) gain popularity among consumers worldwide due to their attractive dark mahogany color, pleasing flavor, desirable taste and high nutrients. However, they are highly perishable, non-climacteric, and available only a few weeks after storage under the optimal conditions (Serrano et al., 2005; Wani et al., 2014). Changes in fruit appearance (i.e. color, surface pitting), flavor, softening, stem quality, and dehydration are major symptoms of quality deterioration. Under current marketing conditions, pitting greatly influences consumer perception of fruit quality (Kappel et al., 2006). Fruit pulp temperature had been identified as an important factor implicated in the development of pitting during handling, storage, and shipping of sweet cherry (Lidster and Tung, 1980; Zoffoli and Rodriguez, 2009). Rapid cooling of fruit to almost 0 °C immediately after harvest is an extremely important step in postharvest handing, known to reduce pitting (Drake et al., 1988; Wade and Bain, 1980; Young and Kupferman, 1994). During storage or shipping, relatively stable low-temperature (0 °C) maintain fruit quality and low respiratory activity that retard skin darkening and loss of flavor and acidity; these quality changes, when not restricted, increase cherry susceptibility to pitting (Wang and Long, 2014). However, cherry stored at higher temperatures and lower levels of humidity developed increased pitting, likely due to rapid water loss from the fruit (Patterson, 1987; Schick and Toivonen, 2002). Additionally, pitting development may be related to the length of storage (Lidster and Tung, 1980; Drake and Elfving, 2002). It is clear that surface pitting occurs either during handling or storage and shipping; therefore improved strategies discourage development of pitting during the postharvest period will have economic benefits for growers.

Pre-harvest sprays of plant growth regulators such as $CaCl_2$, gibberellic acid, or benzyladenine increase fruit firmness and improve resistance to pitting disorder (Canli et al., 2015; Einhorn et al., 2013; Wang et al., 2015). However, sweet cherries' response to these applications is known to delay on-tree fruit maturation and fruit with lower sugar and acid levels that are likely subject to quality deterioration or mold (Kappel and Macdonald, 2002). Recently, hydrogen sulfide (H_2S), an endogenous signaling molecule similar to carbon monoxide and nitric oxide, has been reported to activate defense responses and prolong fruit life in studies on kiwi (Zhu et al., 2014), banana (Li et al., 2016), strawberry (Hu et al., 2012), and pear (Hu et al., 2014) fruit. It has been hypothesized that H_2S could up-regulate antioxidant enzymes while down regulating the expression of chlorophyll degradation-related genes and reduce membrane oxidative damage caused by reactive oxygen species (Fotopoulos et al., 2015). However, whether H_2S plays a functional role in reducing susceptibility to surface pitting injury remains unclear.

The objective of this work was to investigate (1) the effect of H_2S on fruit quality and pitting development of 'Lapins' and 'Regina' sweet cherries during cold storage and (2) to evaluate the possible mechanism of H_2S in reducing development of pitting.

Materials and methods

Plant materials

'Lapins' and 'Regina' cherries were harvested from the orchard of the Mid-Columbia Agriculture Research and Extension Center in Hood River, OR, USA (latitude 45.7°N, longitude 121.545.7°W, elevation 150 m, average annual rainfall ~800 mm). For both cultivars, trees were 15-years old and on Mazzard rootstock. They were maintained with standard cultural, fertilizer, herbicide and pesticide practices. Twenty-five boxes (~8 kg/box) of each cultivar, selected to be free of any visible damage or fungal infection and of uniformity size were picked at commercial maturity. Commercial maturity was determined as color grade 5 according to the Pacific Northwest Dark Sweet Cherry Development Index Chart developed by Oregon State University, in which 1 = Blush, 2 = Rose, 3 = Ruby, 4 = Crimson, 5 = Currant, 6 = Merlot, and 7 = Mahogany. Fruit of each cultivars with pedicels intact were divided into 5 treatments × 3 replications × 1 lot per replication = 15 lots of 10 kg/lot. Each lot was loaded

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into a 70-L latching box (650 × 430 × 340 mm) and fumigated with 0.5, 1.0, 1.5, or 3.0 mM of sodium hydrosulfide (NaHS) dissolved in 500 mL distilled water. Control fruit were fumigated with the same volume of distilled water. After fumigating, H_2S gas concentration in each lot was monitored using an H_2S gas detector (Ventis MX4, Industrial Scientific, Pasadena, TX, USA). The H_2S concentration reached approximately 0.1, 0.6 2.4, and 4.8 × 10^{-4} μM after 30 min when the fruit were exposed to 0.5, 1.0, 1.5, or 3.0 mM NaHS, respectively, then maintained at a constant concentration for 24 h during the fumigation treatment. All latching boxes were held at 0 °C for 24 h. After treatment, fruit were packed into commercial polyethylene bags (~1 kg), then stored at 0 °C and >90% relative humidity for 4 weeks. Respiration rate, storage quality, weight loss, stem browning, decay and pitting incidence, cell wall compositions, and related wall-modifying enzymes were evaluated.

Methods

Respiration rate

Thirty fruit per replicate from each treatment were equilibrated in air at 0 °C for 5 h before being place into hermetically sealed glass containers (960 mL). After 1 h incubation, the headspace CO_2 were determined using CO_2 analyzer (900161, Bridge Analyzers Inc., Alameda, CA, USA). Respiration rate was expressed as μg CO_2 kg^{-1} s^{-1}.

Fruit firmness, SSC, and TA

Before quality evaluations, fifty fruit from each treatment per replicate were randomly selected and placed in the laboratory at 20 °C for 4 h, until condensation on the surface of the fruit was dried. Fruit firmness was measured on opposite sides of each fruit, midway between the pedicel and calyx using a FirmTech 2 fruit firmness instrument (BioWorks Inc., Stillwater, OK, USA) and expressed as g mm^{-1}. Juice was prepared for SSC and TA measurements using a juicer (Acme Model 6001, Acme Juicer Manufacturing Co., Sierra Madre, CA, USA) equipped with a uniform milk filter strip (Schwartz Manufacturing Co., Two Rivers, WI). SSC was determined using a refractometer (PAL-1, Atago, Tokyo, Japan). TA was determined by titrating 10 mL of cherry juice, diluted with 40 mL distilled water to pH 8.1 with 0.1 N NaOH using a titrator (DL-15, Mettler-Toledo, Zurich, Switzerland) and expressed as the equivalent percentage of malic acid.

Weight loss, stem browning, decay, and surface pitting incidence

The bags of fruit were weighed initially and after 4 weeks of storage. Weight loss was expressed as percentage loss of original weight. Stem browning was recorded for those pedicels exhibiting >30% of the entire surface browned. Fruit decay was assessed as a percent of fruit from one hundred fruit samples showing any type of decay, however, decay organisms were not identified. Surface pitting was evaluated and recorded as a percentage of one hundred fruit samples showing visible pitting. One hundred fruit from each treatment of each replicate were randomly selected and were scored for stem browning and surface pitting.

Isolation and measurement of cell wall polysaccharides

Cell wall polysaccharides were isolated and measured as described by Salato et al. (2013). Flesh tissue samples (10 g) were homogenized in 20 mL of 80% ethanol, then boiled for 30 min, and centrifuged at 9,000 g for 10 min at 20 °C. The supernatant was decanted and the residue twice suspended with ethanol, then twice with acetone, and dried at 75 °C for 6 h. The samples were AIR. AIR (100 mg) was treated with distilled water twice, centrifuged, and two supernatants were collected as WSP. Next, the residue was treated with 50 mM sodium acetate buffer (pH 6.5) containing 50 mM CDTA twice, centrifuged, and two supernatants were collected as CSP. Finally, the residue was treated with 50 mM Na_2CO_3 containing 10 mM $NaBH_4$ twice, centrifuged, and two supernatants were collected as NSP.

Extract polysaccharides solution (0.2 mL) was added to 1 mL of sulfuric acid containing 75 mM sodium borate. After boiling for 10 min, 20 μL of 0.5% NaOH containing 0.15% m-phenylphenol was added to the mixture and loaded in a 96-well plate. Absorbance at 520 nm was monitored in a plate reader (Elx800, Bio-Tek Instruments Co., Winooski, VT, USA). A standard calibration curve was constructed using galacturonic acid and the data were expressed as AIR mg g^{-1}.

Cell wall-modifying enzymes

PG activity was performed as described by Gross (1982). Flesh tissue samples (0.25 g) were homogenized in 5 mL of 0.3 M NaCl. After centrifugation at 12,000 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 mM sodium acetate buffer (pH 4.5), and 0.15 mL of 0.3 M NaCl, then incubated at 37 °C for 2 h. After adding 4 mL of 0.1 M borate buffer (pH 9.0) and 0.6 mL of 1% 2-cyanoacetamide, the mixture was boiled in a water bath for 10 min. One unit of PG activity was defined as the release of 1 μg of galacturonic acid at 276 nm absorbance per min.

PME activity was performed as described by Basak and Ramaswamy (1996). All solutions, including distilled water, NaCl, pectin, and supernatant were adjusted to pH 7.5 with 0.1 M NaOH. Flesh tissue samples (4 g) were homogenized in 4 mL of 0.3 M NaCl. After centrifugation, the supernatant (2 mL) was added to 25 mL of 0.5% pectin and then incubated for 1 h at 37 °C. The mixture was titrated to pH 8.1 with 0.1 M NaOH and the volume of NaOH solution was recorded. One unit of PME activity was defined as the release of 1 mM of ester hydrolyzed per min.

PL activity was performed as described by Belge et al. (2015). Flesh tissue samples (0.25 g) were homogenized in 5 mL of 50 mM Tris-HCl (pH 8.5) containing 0.6 mM $CaCl_2$, 5 mM EDTA, and 0.05% Triton X-100. After centrifugation, the supernatant (0.4 mL) was added to 3.1 mL of 50 mM Tris-HCl (pH 8.5) containing 0.6 mM $CaCl_2$ and 0.24% polygalacturonic acid, then incubated at 37 °C for 30 min. After boiling in a water bath for 10 min, one unit of PL activity was defined as the formation of 1 μM of 4,5-unsaturated product at 232 nm absorbance per min.

β-GAL was performed as described by Belge et al. (2015). Flesh tissue samples (0.5 g) were homogenized in 5 mL of 0.3 M NaCl. After centrifugation, the supernatant (0.1 mL) was added to 2 mL of 0.04% *p*-nitrophenyl-β-D-galactoside and 0.3 mL of 0.3 M NaCl, then incubated at 37 °C for 30 min. After adding 2 mL of 0.2 M Na_2CO_3, one unit of β-GAL activity was defined as the release of 1 mg of *p*-nitrophenol at 400 nm absorbance per min.

Statistical Analysis

The experiment design was completely randomized with three replicates for each treatment since the trees were uniform in the orchard. Homogeneity tests were performed for two continuous years and the data were homogeneous for each parameter tested. The data were combined and subjected to analysis of variance using SPSS software (SPSS Inc., Chicago, IL, USA). When appropriate, means were separated by Fisher's protected least significant difference test at $P < 0.05$.

Results and discussion

Effect of H_2S on fruit respiration

Postharvest application of H_2S has been shown to reduce fruit quality deterioration, including appearance and texture, to resist patho-

gen infection, and to extend fruit storability (Hu et al., 2012; Hu et al., 2014; Li et al., 2016; Zhu et al., 2014). Respiration rate is a major indicator of physiological and biochemical activity, and it increases as fruit deterioration. In this study, respiration rates were inhibited by H_2S in both cultivars, especially in 1 and 2 mM NaHS treatments, compared to the control (Tab. 1). This demonstrated that H_2S may lower respiration and lower the loss of quality for stored fruit.

Tab. 1: Effect of H_2S on respiration rate, fruit firmness, soluble solids content (SSC), and titratable acidity (TA) of 'Lapins' and 'Regina' cherries at harvest and after 4 weeks of storage at 0 °C.

Treatments	Respiration rate (μg CO_2 kg^{-1} s^{-1})	Fruit firmness (g mm^{-1})	SSC (%)	TA (%)
'Lapins'				
At harvest	3.21 d[a]	315.01 e	17.01 a	0.59 a
Control	4.55 a	335.12 c	16.00 b	0.47 b
0.5 mM NaHS	4.39 a	328.49 d	16.01 b	0.45 b
1 mM NaHS	3.51 c	347.30 a	15.93 b	0.46 b
2 mM NaHS	3.44 c	346.07 a	15.87 b	0.46 b
3 mM NaHS	3.87 b	342.10 b	15.64 b	0.47 b
'Regina'				
At harvest	2.98 d	361.14 d	18.10 a	0.45 a
Control	3.98 a	370.11 c	18.01 a	0.38 b
0.5 mM NaHS	3.85 ab	371.08 c	17.83 a	0.41 b
1 mM NaHS	3.34 c	391.10 a	17.93 a	0.41 b
2 mM NaHS	3.31 c	390.08 a	18.06 a	0.41 b
3 mM NaHS	3.76 b	379.33 b	18.03 a	0.38 b

[a]Means within a column in each cultivar followed by different letters indicate significant differences according to the Fisher's protected least significant difference test $P < 0.05$.

Effect of H_2S on fruit quality

'Lapins' and 'Regina' cherries were picked at typical commercial harvest maturity; fruit firmness was 315.01 and 361.14 g mm^{-1}, SSC was 17.01% and 18.10%, and TA was 0.59% and 0.45%, respectively. After 4 weeks of storage at 0 °C, firmness increased in all treatments. This increased firmness may be related to moisture loss (Drake and Elfving, 2002), although no significant weight losses were detected between the control and H_2S-treated fruit after 4 weeks (Tab. 2). In both cultivars, H_2S significantly increased fruit firmness, while losses in SSC and TA were not delayed by the treatment. Both 1 and 2 mM NaHS treatments in both cultivars led to significantly higher firmness than control and other NaHS treatments. The 0.5 mM NaHS treatment did not affect storage quality. The effect of the 3 mM NaHS treatment were similar to the 1 or 2 mM NaHS treatment for firmness, but fruit firmness was lower than that in 1 or 2 mM NaHS treatment (Tab. 1), indicating that high H_2S concentration might increase the levels of malondialdahyde, and oxidative damage to cell membrane systems (Zhang et al., 2011). These results indicated that the effect of H_2S in both cherry cultivars was dose-dependent, the greatest positive effect exerted by the 1 to 2 mM dose. Facteau (1982) reported that fruit firmness was negatively related to pitting development in 'Bing' cherries; our finding in H_2S-treated fruit confirmed his observations.

As expected, consumers favor sweet cherries with less surface pitting (Chauvin et al., 2009). In both cultivars, fruit treated with H_2S expressed delayed deterioration of fruit indicators stem browning, decay, and surface pitting (Tab. 2). Hu et al. (2002) reported reduced physiological disorders as a response to greater antioxidant enzymes (i.e. catalase, guaiacol peroxidase, ascorbate peroxidase, and glutathione reductase) activity which might be activated by H_2S. Other reports indicated that the effect of H_2S on quality deterioration disorders was probably due to fruit higher energy status (Li et al., 2016). However, susceptibility to pitting is an intrinsic characteristic of cherry skin. Therefore, cell wall metabolism seems to be implicated in the development of pitting.

Tab. 2: Effect of H_2S on weight loss, stem browning, decay, and surface pitting of 'Lapins' and 'Regina' cherries after 4 weeks of storage at 0 °C.

Treatments	Weight loss (%)	Stem browning (%)	Decay (%)	Surface pitting (%)
'Lapins'				
Control	0.87 a[a]	34.51 a	6.71 a	13.34 a
0.5 mM NaHS	0.85 a	33.93 a	5.11 b	12.19 a
1 mM NaHS	0.87 a	23.66 c	3.52 d	8.76 c
2 mM NaHS	0.86 a	23.71 c	3.30 e	8.97 c
3 mM NaHS	0.86 a	26.87 b	4.01 b	10.34 b
'Regina'				
Control	0.84 a	23.51 a	5.87 a	9.97 a
0.5 mM NaHS	0.83 a	23.32 a	5.57 ab	9.35 ab
1 mM NaHS	0.83 a	13.21 b	3.41 b	6.13 d
2 mM NaHS	0.84 a	13.17 b	3.67 b	7.07 c
3 mM NaHS	0.83 a	23.50 a	5.14 b	8.64 b

[a]Means within a column in each cultivar followed by different letters indicate significant differences according to the Fisher's protected least significant difference test $P < 0.05$.

Effects of H_2S on cell wall composition and metabolism

Pitting appears as one or more irregular depressions around the fruit surface and can occur anywhere without particular location (Porritt et al., 1971). Examinations of the ultrastructure of fruit skin tissue has shown that a single layer of epidermal cells covers the surface of fruit, 4-5 layers of hypodermal cells with thicker walls are found below the epidermal layer, underneath the hypodermal layers are composed of radially elongated and rounded parenchymatic mesocarp cells (Lane et al., 2000; Param and Zoffoli, 2016). Pitting originating from mechanical damage is caused by the collaps of injured cells in the epidermis and hypodermis or/and parenchymatic mesocarp. Anatomical differences among epidermal, hypodermal and mesocarp cells across cultivars affects cell layout, cell number, cell size, and susceptibility to pitting (Porritt et al., 1971; Param and Zoffoli, 2016). 'Regina' had a great uniformity in cell size and arrangement with less susceptibility to pitting, while 'Lapins' had smaller and less-uniform cells and greater susceptible to pitting (Param and Zoffoli, 2016). In this study, even though H_2S did not affect the initiation of mechanical damage in either cultivars, it postponed the development of macroscopically visible dents that might alter the structural composition of the cell wall in fruit skin tissue. Compared to the control, H_2S-treated cherries of both cultivars had lower WSP and CSP and higher NSP (Tab. 3), especially in 1 mM NaHS treatments. Previous work failed to show a relationship between the depolymerization of cell wall polysaccharides and pitting development (Facteau et al., 1982; Kappel et al., 2006; Salato et al., 2013). Our results indicated that H_2S has the ability to reinforce the connection between cell wall polysaccharides and the cell

Tab. 3: Effect of H_2S on water-soluble polysaccharides (WSP), CDTA-soluble polysaccharides (CSP), and Na_2CO_3-soluble polysaccharides (NSP) of 'Lapins' and 'Regina' cherries after 4 weeks of storage at 0 °C.

Treatments	WSP (AIR[a] mg g^{-1})	CSP (AIR mg g^{-1})	NSP (AIR mg g^{-1})
'Lapins'			
Control	11.72 a[b]	8.96 a	13.44 c
0.5 mM NaHS	10.86 b	8.89 a	14.47 b
1 mM NaHS	7.00 e	6.17 d	17.71 a
2 mM NaHS	7.72 d	6.64 c	17.33 a
3 mM NaHS	8.53 c	7.16 b	17.04 a
'Regina'			
Control	8.89 a	6.44 a	15.41 c
0.5 mM NaHS	7.96 b	5.98 a	15.39 c
1 mM NaHS	5.43 c	3.98 b	18.46 a
2 mM NaHS	5.19 c	4.17 b	17.41 b
3 mM NaHS	7.79 b	6.35 a	14.97 c

[a]AIR = alcohol insoluble residue
[b]Means within a column in each cultivar followed by different letters indicate significant differences according to the Fisher's protected least significant difference test $P < 0.05$.

Tab. 4: Effect of H_2S on activities of polygalacturonase (PG), petin methylesterase (PME), pectin lyase (PL), and β-D-galactosidase (β-GAL) at harvest and after 4 weeks of storage at 0 °C.

Treatments	PG (U g^{-1})	PME (U g^{-1})	PL (U g^{-1})	β-GAL (U g^{-1})
'Lapins'				
At harvest	118.96 b[a]	0.44 a	229.20 e	437.64 e
Control	125.69 a	0.43 a	420.59 a	950.22 a
0.5 mM NaHS	123.34 a	0.44 a	367.33 c	828.87 b
1 mM NaHS	117.50 b	0.45 a	289.76 d	685.89 d
2 mM NaHS	121.36 a	0.43 a	294.20 d	722.88 c
3 mM NaHS	127.62 a	0.44 a	409.60 b	841.30 b
'Regina'				
At harvest	84.79 c	0.35 a	187.63 e	227.67 e
Control	99.87 a	0.36 a	338.46 a	556.34 a
0.5 mM NaHS	102.23 a	0.34 a	348.54 a	413.60 b
1 mM NaHS	88.46 b	0.35 a	223.39 d	399.85 b
2 mM NaHS	85.35 bc	0.36 a	249.98 c	427.66 b
3 mM NaHS	96.98 a	0.38 a	290.30 b	405.57 b

[a]Means within a column in each cultivar followed by different letters indicate significant differences according to the Fisher's protected least significant difference test $P < 0.05$.

wall. Firmer fruit was more resistant to mechanical damage, and displayed reduced susceptibility to surface pitting (Toivonen et al., 2004; Kappel et al., 2006).

Andrews and Li (1995) reported that the cell wall-modifying enzymes including PG, PME, and carboxymethylcellulase increased in sweet cherry during development and reached to maximum activity when the fruit skin color turned to dark-red. After fruit were harvested and subjected to cold storage, the activity of PG and β-GAL continued to increase, but PME activity was not affected by cold temperatures (Belge et al., 2015). In this study, the activity of cell wall-modifying enzymes in 'Lapins' and 'Regina' cherries at harvest were PG of 118.96 and 84.79 U g^{-1}, PME of 0.44 and 0.35 U g^{-1}, PL of 229.20 and 187.63 U g^{-1}, and β-GAL of 437.64 and 227.67 U g^{-1}, respectively. After 4 weeks of storage, the activity of PG and β-GAL in control fruit of both cultivars increased, while no significant differences were observed in PME activity (Tab. 4). Fruit treated with 1 mM NaHS in both cultivars displayed a significant reduction in PG activity over the control as reported in strawberry (Hu et al., 2002). Furthermore, PG and β-GAL activity were delayed by the 1 mM NaHS treatment, while PME activity was not effected by H_2S (Tab. 4). These results indicate that PG and β-GAL may play important roles in the development of surface pitting, and that H_2S could inhibit their activity, and result in reduced cell wall disassembly and improved resistance to pitting injury. Belge et al. (2015) reported that PL did not show any distinct changes in 'Celeste' or 'Somerset' cherries when fruit were held at 0 °C for 14 days. By contrast, PL in the control of both varieties studies here increased after 4 weeks of storage; the H_2S-treated fruit displayed significantly inhibited PL activity. Further studies should be conducted to see if the low activity of β-GAL in cherries is protects fruit against the development of surface pitting.

Conclusion

This study demonstrated that postharvest application of H_2S gas (1-2 mM NaHS) increased fruit firmness and reduced respiration rate, stem browning, decay, and surface pitting in 'Lapins' and 'Regina' cherries after 4 weeks of cold storage. The potential benefit of H_2S in minimizing pitting development was associated closely with reduction of the degradation of cell wall polysaccharides and suppression of cell wall-modifying enzymes (PG, PL, and β-GAL) activities. These results are the first report on the effect of H_2S on surface pitting development in 'Lapins' and 'Regina' cherries. Although approval of the use of H_2S gas on food has not yet been granted, an alternative reducing agent gas based on H_2S tended to be effective in controlling physiological disorders and improving fruit quality in sweet cherry.

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