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## Pre-storage Exogenous Application of Hydrogen Sulphide Reduces Sugar spot, Decay loss and Preserves Quality of Banana Fruit

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This research focuses on effect of hydrogen sulphide treatment (control, 0.5, 1.0, 1.5 mM) during ambient storage on sugar spot, decay loss and postharvest quality of banana fruit. Hydrogen sulphide ( $H_2S$ ) treated fruit were stored at ambient conditions ( $25\pm2^{\circ}C$  and 60-65% of relative humidity) up to 9 days. In general,  $H_2S$  treatment maintained higher values of lightness, peel firmness, reduce the respiration rate and ethylene evolution rate and extended shelf life of stored fruit by delaying progression of ripening. Moreover,  $H_2S$  (1.0 mM) was found significantly better over other treatments in reducing sugar spot or peel browning spot and maintaining the desirable overall postharvest traits of the fruit. The findings indicated that  $H_2S$  has a great potential for pre-storage application to preserve quality, reduce sugar spot and postharvest decay loss, possibly through the delayed onset of senescence, without any adverse effects on fruit quality.

Keywords: Ambient storage, Fruit quality, Postharvest, Relative humidity

## Introduction

Banana (Musa spp.) is an important fruit crop to small and medium land holding farmers in the developing countries of the tropical and sub-tropical region of the world. Banana is most liked fruit among all age groups due to its easily digestible and palatable properties. It is rich source of dietary fiber calcium, potassium, phosphorous and carbohydrate. Short shelf-life, rapid physiological deterioration, sugar or brown spot, finger drop, decay loss, chilling injury and water loss are major postharvest problems.<sup>2</sup> Among these problems, sugar spot and fruit decay mainly affect the qualitative (consumer appeal) and quantitative value of the produce. With the advancement of ripening in banana, more sugar spot appears on its yellow peel which reduces commercial value of fruit. Researchers have tried several means of controlling the postharvest problems in banana by harvesting at appropriate maturity modified storage postharvest chemical treatments<sup>3</sup>, packaging, bio-agents<sup>4</sup> and application of plant growth regulators.

H<sub>2</sub>S is a consumer-friendly low molecular weight compound, emerging as a potential tool for treatment of perishable horticultural produce.<sup>5</sup> It is reported that H<sub>2</sub>S could alleviate chilling injury and regulate

\*Author for Correspondence E-mail: ramu 211@yahoo.com postharvest senescence by reducing oxidative stress through modulating antioxidant enzymes in, mulberry<sup>6</sup>, banana<sup>7</sup>, and pak choy<sup>8</sup> fruits. Shelf-life extension is also contributed by H<sub>2</sub>S through pathogen inhibition.<sup>6</sup> These research findings on H<sub>2</sub>S application for loss reduction and quality maintenance highlight its commercial importance in postharvest management of fruit and vegetable. Therefore, this study aims to investigate the effect of H<sub>2</sub>S, on sugar spot (brown spot) reduction and postharvest decay loss minimization in bananas stored at room temperature.

## **Materials and Methods**

## **Experimental Material and Treatments**

Commercially mature banana fruit (variety Nendran) were obtained from known source at Azadpur fruit market, New Delhi (India). Uniform and healthy fruit were subjected to H<sub>2</sub>S treatments in the laboratory of Division of Food Science and Postharvest Technology, Indian Agricultural Research Institute, New Delhi. Solution of sodium hydrosulfide (NaHS·3H<sub>2</sub>O, Sigma) was used as H<sub>2</sub>S donor. Aqueous solutions of NaHS at different concentrations of 0.5, 1.0, and 1.5 mM were prepared in sealed containers (volume 30 L). Fruit were trapped in containers for 24 hours. All the treated fruit then allowed for ripening under simulated retailing conditions (room temperature 22–25°C and relative humidity 60–65%) for 9 days. The data were recorded on various physicochemical properties at an interval of 2 days. We used factorial Completely Randomized Design (CRD) with three replications each having 20 fruit.

#### Peel Color

The color of banana fruit was determined by using colour TEC PCM machine in L\*, a\* and b\* coordinates. L\* indicates the lightness co-efficient and ranges from 0 (black) to 100 (white). Positive a\* indicates a hue of red purple whereas negative a\* indicates bluish green on the horizontal axis. Similarly, on the vertical axis positive b\* indicates yellow and negative b\* represents blue. Calibration was done by using black and white tiles before evaluation. Peel color was measured by taking 2 to 3 random readings from each fruit surface. Hue angle (h°) of fruit was determined by using following equation.

Hue angle  $(h^o)$  = arctan (b/a)

#### **Fruit Firmness**

Fruit firmness was recorded individually by using a Texture Analyzer (model: TA + Di, Stable micro systems, UK) coupled with cylindrical probe of 2 mm diameter, under compression test. This probe was advanced at a pre test speed of 2 mm/s and test speed of 0.5 mm/s. Measurements were taken at three points (mid, bottom and top) of each unpeeled whole fruit. First peak force (N) in the force deformation curve was taken as firmness of the sample and the results were expressed in Newton. <sup>10</sup>

## **Decay Loss**

Decay loss was observed and recorded as per methodology described by (Bazie *et al.*, 2014). (11) The percentage decay was calculated by using the formula:

$$\% \ Decay = \frac{Number \ of \ decayed \ fruits}{Total \ number \ of fruits} \times 100$$

### **Sugar Spots**

Sugar spots in stored banana fruit were visually observed developing on the peel according to a subjective scale (0%, 1–25, 26–50, 51–75, 76–100) as described by (Baez-Sanudo *et al.*, 2009). (12)

#### **Respiration Rate**

Respiration rate was determined by the method followed by Barman et al. (2016). Auto gas

analyzer (Model: Checkmate 9900 O<sub>2</sub>/CO<sub>2</sub>, Dansensor PBI, Denmark) was used for measuring respiration rate of various treatments subjected, the results were expressed in CO<sub>2</sub> ml kg<sup>-1</sup>h<sup>-1</sup>. (10)

#### **Ethylene Evolution Rate**

A Hewlett Packard (HP) gas chromatograph (Model 5890 series II) equipped with a flame ionization detector (FID), Porapak-N 80/100 mesh packed stainless steel column and a HP integrator was used for determination of ethylene at 85°C. Five fruit were trapped in a 2.0 L airtight container for 1 h at 20°C. One ml of head space gas was withdrawn using Hamilton gas tight micro syringe and injected into the Gas Chromatograph. Finally, data were expressed as μl kg<sup>-1</sup>h<sup>-1</sup>. (10)

#### **Total Soluble Solids**

The total soluble solids of banana fruit pulp samples were estimated using FISHER Hand Refractometer (range 0 to 50), and expressed in °B. The best results calculated was at the room temperature i.e. 18–28°C. <sup>13</sup>

### **Total Phenolics Content**

The total phenolics content was measured with the help of spectrophotometer (Double beam UV-VIS Spectrophotometer, UV 5704SS, ECIL, India), using Folin-Ciocalteu reagent and gallic acid as a standard. To the 100 μl of the sample extract (extracted in 80% ethanol), 2.9 ml of deionized water, 0.5 ml of Folin-Ciocalteu reagent and 2.0 ml of 20% Na<sub>2</sub>CO<sub>3</sub> solutions were added. Thereafter, mixture was allowed to stand for 90 minutes in dark condition. The absorbance of the mixture was then measured at 760 nm. Gallic acid (0–800 mg L<sup>-1</sup>) was used to produce standard calibration curve. The amount of total phenolics content was expressed as Gallic Acid Equivalents (GAE) in mg/100 g fresh weight.

#### **Titratable Acidity (TA)**

Titratable acidity was determined by the method described by Ranganna (1999). For this 5 g of fruit sample was weighed and put to 50 ml water. It was thoroughly mixed and then filtered. The filtered sample was titrated against 0.1 N NaOH using a few drops of 1% phenolphthalein solution as indicator. The observed titratable value was used for calculating the values as % malic acid (predominant acid in banana).

## **Total Sugars**

Total sugars were determined by the method described by AOAC (2016)<sup>(13)</sup> by taking a known

quantity of fruit pulp, using lead acetate to remove excess of lead. Lead free aliquot were examined by titrating against boiling Fehling's solution, using methylene blue as an indicator till brick red color appears. The data were expressed in percentage.

#### **Sensory Score**

Sensory score of the treated and untreated banana fruit was performed on 9<sup>th</sup> day using 9-point hedonic ranking scale: where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = like extremely. Estimated scores of 6 and more than 6 was considered as consumer acceptable. Fruit peel color, aroma, firmness, taste and overall acceptability were taken for scoring. <sup>16</sup>

### **Analysis of Data**

Data from different treatments with respect to various physical, physiological, biochemical and functional parameters were pooled and subjected to analysis of variance using SAS 9.3 software (2) and significant effects (p< 0.05) were noted.

## **Result and Discussion**

## **Development of Peel Color**

Synthesis of pigments and degradation reactions could be correlated in a better way to hue angle values, which simulates to color receptor molecules of the retina in human eyes.<sup>17</sup>

The hue angle values were significantly affected by all the H<sub>2</sub>S treatment over control, however negligible difference was noted among the effect of H<sub>2</sub>S treatments (Fig.1a). A sharp decline in green color was noticed during first 3 days which later on decreases with a slow pace. The yellow color was appeared in control fruit (at 87 hue angle) on 3<sup>rd</sup> day while H<sub>2</sub>S treated fruit remain green (approximately 100 hue angle). Even a 9<sup>th</sup> day of storage H<sub>2</sub>S treatments showed that the color of bananas turned to yellow more slowly than for control. Treatments with H<sub>2</sub>S showed higher color intensity (chroma), mainly after 7 days compared to control. Chlorophyll breakdown was reduced by H<sub>2</sub>S by inhibiting ethylene production and action process.<sup>8,18</sup>

### **Fruit Firmness**

The fresh fruit had shown average firmness around 10.38 (N) which later on progressively decreased up to 9<sup>th</sup> day of storage (Fig.1b). Irrespective of storage

days all the treatments have influenced the fruit firmness. After 9 days of storage at market simulated conditions (room temperature 22–25°C) fruit firmness was significantly higher in hydrogen sulphide treated fruit and best treatment being 1.5 mM of H<sub>2</sub>S follow by 1.0 mM of H<sub>2</sub>S compared to the control fruit. The positive effect of H<sub>2</sub>S for higher firmness retention may be possible as H<sub>2</sub>S fumigation exert a negative effect on ethylene production which retards the fruit ripening process.<sup>19</sup> It has also been reported that polygalacturonase (PG) and pectin methyl esterase (PME) activities are linked with fruit softening.<sup>20</sup> Therefore, higher fruit firmness retention in H<sub>2</sub>S treated fruit is possible by the inhibition of PME and PG activities and hence of cell wall degradation.<sup>21</sup>

## **Decay Loss**

Higher decay incidence was observed in untreated fruit during storage (Fig. 1c). The decay incidence, which is strongly influenced with acceleration of senescence, was significantly suppressed by H<sub>2</sub>S treatments. By day 9, decay loss was 2-fold more in control fruit (18.77%) compare to fruit which were treated with 1.5 mM of H<sub>2</sub>S (9%). In our study, disease incidence in banana was significantly higher in untreated fruit than H<sub>2</sub>S treated fruit with least decay in 1.5 mM H<sub>2</sub>S treated fruit. Decay loss was low in H<sub>2</sub>S treated samples compared to control and this is consistent with the earlier work<sup>22,6</sup> which showed H<sub>2</sub>S has higher efficacies against spore germination by inhibiting germ tube elongation and cytoplasm fragmentation.

## **Sugar Spot**

Peel browning or sugar spot of banana limit the shelf-life of the banana fruit and reduce its commercial value. Peel browning or sugar spot is a ripening and senescence related disorder which appears at advance stage of banana finger ripening. Sugar spots were significantly reduced by H<sub>2</sub>S treatments, while control showed already about 8% incidence at even third day of storage, by day 8 it reached 90% coverage of fruit skin (Fig. 1d). In treated fruit, sugar spot incidence reached upto 10.5% only, even after 9 days of storage. The antisenescence role of H<sub>2</sub>S specifically in downregulating the expression of senescence-related genes and reducing oxidative damage has been earlier documented in banana<sup>7</sup> and sweet cherry.<sup>23</sup> Polyphenol oxidase (PPO) accelerates the oxidation of phenolics into a brown pigment which leads

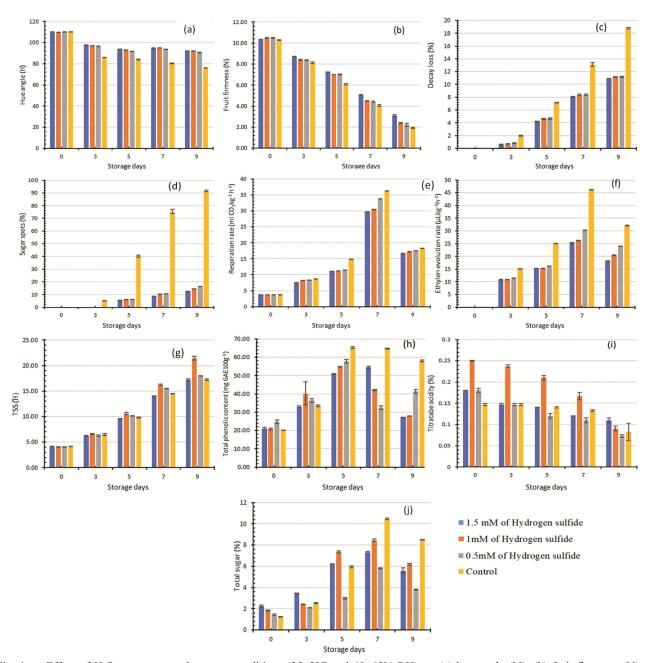


Fig. 1 — Effect of  $H_2S$  treatments and storage conditions (25±2°C and 60–65% RH) on (a) hue angle (h°), (b) fruit firmness (N), (c) decay loss (%), (d) sugar spot (%), (e) respiration rate (ml  $CO_2$  kg<sup>-1</sup> h<sup>-1</sup>), (f) ethylene evolution rate ( $\mu L$  kg<sup>-1</sup> h<sup>-1</sup>), (g) TSS (B), (h) total sugars (%), (i) titratable acidity (%), (j) total sugar (%) in banana fruit

browning. Suppression of PPO activity of fruit and vegetables by H<sub>2</sub>S delayes the browning in a variety of fresh as well as minimally processed products.<sup>24</sup>

## **Respiration Rate**

Initial respiration rate of the fresh mature green fruit was recorded 5.0 mL  $CO_2$  kg<sup>-1</sup>h<sup>-1</sup> (Fig. 1e). Climacteric peak of  $\approx 38.07$  mL  $CO_2$ kg<sup>-1</sup>h<sup>-1</sup> was recorded on  $7^{th}$  day of storage in control and 30.0 mL  $CO_2$  kg<sup>-1</sup>h<sup>-1</sup> in  $H_2S$  (1.0 – 1.5 mM) treated fruit and

thereafter a sharp decline until end of storage. Respiration is a catabolic process which reduces reserve carbohydrate into simple sugars during onset of climacteric peak at fruit ripening. Respiration rate is regulated by several physiological factors but enzymes like polygalucturonase (PG) and pectinmethylestrase (PME) play a key role in the regulation of respiration process. Therefore, it is expected that PG and PME activities in H<sub>2</sub>S treated

fruit got suppressed which in turn reduced the respiration rate. The above findings are well supported by earlier work on storage life of  $\rm H_2S$  treated strawberry fruit.<sup>26</sup>

#### **Ethylene Evolution Rate**

The presented data showed that the highest climacteric peak of ethylene evolution was occurred in the control fruit (45.7µLkg<sup>-1</sup>h<sup>-1</sup>) and the lowest  $(21.89 \mu \text{Lkg}^{-1}\text{h}^{-1})$  in 1.5 mM H<sub>2</sub>S treated fruit on 7<sup>th</sup> day of storage (Fig. 1f). The ethylene evolution rate of fruit had shown increasing trend up to 7th day but on 9<sup>th</sup> days of storage it was found to be decreased. Banana is a climacteric fruit where exogenous use of ethylene is commercially exploited for ripening process. Ethylene plays both desired and undesired role in banana fruit ripening. After triggering of ripening, higher level of ethylene inside or around the fruit could quickly spoil the bananas. As described by earlier workers, H<sub>2</sub>S is potent ethylene inhibitor which inhibits ethylene production by suppressing ACS enzyme activities.<sup>2,27</sup> The higher dose of H<sub>2</sub>S could have been effective in suppressing the ACS activities over other lower doses. These findings are consistent with the work of Lu et al. (2015)<sup>(19)</sup> which showed suppression of ethylene production in H<sub>2</sub>S treated banana fruit.

## **Total Soluble Solids**

Total soluble solids (TSS) were significantly affected by the treatments; however, the difference was more pronounced after 5<sup>th</sup> day of storage (Fig. 1g). By the day 9, untreated fruit evolved 22% TSS compared to hydrogen sulphide (1.0 mM–1.5 mM) treatments (16%). Therefore, it is presumed that there was less sugar accumulation in H<sub>2</sub>S treated fruit due to ethylene biosynthesis inhibition properties of H<sub>2</sub>S. It might also be involved in the regulation of postharvest shelf life of respiratory climacteric or non-respiratory climacteric fruit. Zhu *et al.* (2013)<sup>(28)</sup> also reported similar findings while working on kiwi fruit.

## **Total Phenolics Content**

The fresh fruit had the average total phenolics content (TPC) content  $\approx 21.69$  mg GAE 100 g<sup>-1</sup>, which later on showed a fluctuating trend throughout the storage period (Fig. 1h). H<sub>2</sub>S treatments have significantly suppressed the evolution of TPC over control. The highest average value ( $\approx 60$  mg GAE 100 g<sup>-1</sup>) was recorded in control and lowest ( $\approx 28$  mg GAE 100 g<sup>-1</sup>) in H<sub>2</sub>S (1.0-1.5 mM) treated fruit on  $9^{th}$ 

day of storage. Phenolics are important to maintain the radical scavenging activity and thus constitute the non-enzymatic antioxidant system in plant tissues. In our study we found higher total phenolics compound in the control fruit compared to the hydrogen sulphide treatments. In general, TPC concentration increase during ripening and reach the highest level at half ripe stage and then start declining with a slow pace.<sup>29</sup> Besides, Giovanelli *et al.* (1999)<sup>(30)</sup> reported different TPC trends under different ripening ambience. Being a ripening linked photochemical; the anti-senescence role of H<sub>2</sub>S might have decreased the TPC level in treated fruit over control.<sup>31</sup>

#### **Titratable Acidity**

All the treatment showed a decreasing trend in titratable acidity (TA) with progression of ripening and advancement in storage period (Fig. 1i). The changes in the TA of stored fruit are ripening dependent in banana. There is a high demand of energy during ripening and organic acids along with other metabolites are used to meet out the energy requirements. As described above, the ripening process (respiration, ethylene production) was effectively suppressed by H<sub>2</sub>S (1.5 mM) and thus might have helped in higher retention of TA over other treatments. These findings got support of earlier work on qualitative changes in H<sub>2</sub>S treated mulberry fruit.

## **Total Sugars**

Irrespective of treatment, initially the total sugars content increased up to 7<sup>th</sup> day of storage and later on showed a declining trend (Fig. 1j). The data showed that control fruit has recorded the highest level of total sugars content over H<sub>2</sub>S treated fruit. Fruit ripening and senescence delaying properties of H<sub>2</sub>S has been earlier documented by other workers<sup>2,28</sup> while working on postharvest physiological response of banana and kiwifruit.

#### **Sensory Score**

The sensory score was adjudged by tasters on 9<sup>th</sup> day of storage with respect to peel colour, aroma, firmness, taste and overall acceptability. H<sub>2</sub>S @1.0 mM treatment had given better score over other treatments and control (Table 1).

Fruit treated with H<sub>2</sub>S (1.0 mM) showed highest overall acceptance score (8.5) at the end of storage period followed by 1.5 and 0.5 mM H<sub>2</sub>S. The finding revealed that, overall acceptability of fruit after 9 days of storage was highly influenced by H<sub>2</sub>S treatment

Table 1 — Sensory score of banana fruit on 9 <sup>th</sup> day of storage at 25°C ±2 temperature in H <sub>2</sub> S treated fruit					
Treatment	Peel color	Aroma	Firmness	Taste	Overall acceptability
Hydrogen sulphide (0.5mM)	5.5±1.5a	5.5±2.3a	5.8±2.0a	5.3±2.2b	5.2±2.4bc
Hydrogen sulphide (0.1mM)	$7.5\pm 2.2b$	$7.0\pm 2.1a$	7.9±2.1ab	$8.7 \pm 2.2a$	8.5±2.2a
Hydrogen sulphide (1.5mM)	$8.0\pm2.1b$	$6.8 \pm 2.5 ab$	8.5±2.1ab	$6.0\pm 2.4b$	6.9±2.5b
Control	5.4±1.7a	$5.2\pm2.0a$	4.1±2.3a	4.8±2.2c	4.6±2.3 c

and its concentration. Among the treatments, H<sub>2</sub>S 1.0 mM had higher overall acceptability score mainly because these fruits had appealing aroma, color, texture and taste. In case of untreated samples, texture, color and appearance were highly affected due to faster ageing. The sensory quality in ripe banana fruit is mainly attributed to pulp taste (TSS, acidity, TSS: acid and total phenol) and visual appearance — presence and absence of spot on peel and its color. Excess presence of ethylene accelerates the softening and breakdown of fruits besides other factors. As a potent ethylene biosynthesis inhibitor H<sub>2</sub>S inhibits ethylene production by suppressing ACS enzyme activities.<sup>2,27</sup> Therefore, characteristic rise in treated fruit might have been delayed and reduced by  $H_2S$ .

## **Conclusions**

Exogenous application of  $H_2S$  @ 1.0 mM maintained higher sensory score, avoided abrupt peel yellowing, sugar spot occurrence and fruit firmness loss at ambient storage. Besides,  $H_2S$  treatments were also found helpful in maintaining the desirable postharvest traits of banana fruit during storage. These results can be gainfully utilized by the industry in efficient postharvest management of banana fruit. Acknowledgements

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