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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, H2S (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.¹ Short shelf-life, rapid physiological deterioration, sugar or brown spot, finger drop, decay loss, chilling injury and water loss are major postharvest problems.² 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 treatments³, packaging, bio-agents⁴ and application of plant growth regulators.

 H_2S is a consumer-friendly low molecular weight compound, emerging as a potential tool for treatment of perishable horticultural produce.⁵ It is reported that H_2S could alleviate chilling injury and regulate postharvest senescence by reducing oxidative stress through modulating antioxidant enzymes in, mulberry⁶, banana⁷, and pak choy⁸ fruits. Shelf-life extension is also contributed by H_2S through pathogen inhibition.⁶ These research findings on H_2S 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_2S , 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 H₂S treatments in the laboratory of Division of Food Science and Postharvest Technology, Indian Agricultural Research Institute, New Delhi. Solution of sodium hydrosulfide (NaHS·3H₂O, Sigma) was used as H₂S 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

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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.⁹

Hue angle $(h^{\circ}) = \arctan(b/a)$

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.¹⁰

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).⁽¹⁰⁾ Auto gas

analyzer (Model: Checkmate 9900 O_2/CO_2 , Dansensor PBI, Denmark) was used for measuring respiration rate of various treatments subjected, the results were expressed in CO_2 ml kg⁻¹h⁻¹.⁽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 $\mu l kg^{-1}h^{-1}$.⁽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.¹³

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.¹⁴ To the 100 μ l 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% Na₂CO₃ 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⁻¹) 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).⁽¹⁵⁾ 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 $(2016)^{(13)}$ 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 9th 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.¹⁶

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.¹⁷

The hue angle values were significantly affected by all the H₂S treatment over control, however negligible difference was noted among the effect of H₂S 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 3rd day while H₂S treated fruit remain green (approximately 100 hue angle). Even a 9th day of storage H₂S treatments showed that the color of bananas turned to yellow more slowly than for control. Treatments with H₂S showed higher color intensity (chroma), mainly after 7 days compared to control. Chlorophyll breakdown was reduced by H₂S by inhibiting ethylene production and action process.^{8,18}

Fruit Firmness

The fresh fruit had shown average firmness around 10.38 (N) which later on progressively decreased up to 9^{th} 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 H₂S follow by 1.0 mM of H₂S compared to the control fruit. The positive effect of H₂S for higher firmness retention may be possible as H₂S fumigation exert a negative effect on ethylene production which retards the fruit ripening process.¹⁹ It has also been reported that polygalacturonase (PG) and pectin methyl esterase (PME) activities are linked with fruit softening.²⁰ Therefore, higher fruit firmness retention in H₂S treated fruit is possible by the inhibition of PME and PG activities and hence of cell wall degradation.²¹

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 H₂S 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 H₂S (9%). In our study, disease incidence in banana was significantly higher in untreated fruit than H₂S treated fruit with least decay in 1.5 mM H₂S treated fruit. Decay loss was low in H₂S treated samples compared to control and this is consistent with the earlier work^{22,6} which showed H₂S 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 H₂S 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 H₂S specifically in downregulating the expression of senescence-related genes and reducing oxidative damage has been earlier documented in banana⁷ and sweet cherry.²³ Polyphenol oxidase (PPO) accelerates the oxidation of phenolics into a brown pigment which leads

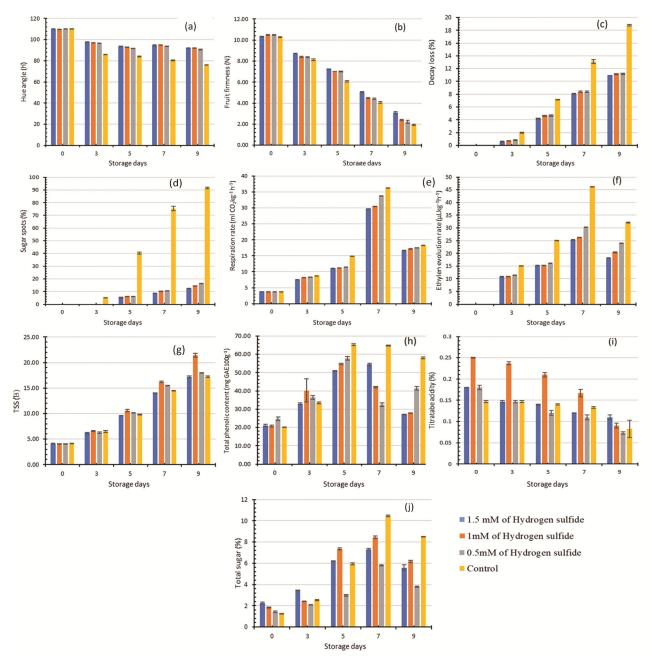


Fig. 1 — Effect of H₂S treatments and storage conditions ($25\pm2^{\circ}C$ and 60-65% RH) on (a) hue angle (h^o), (b) fruit firmness (N), (c) decay loss (%), (d) sugar spot (%), (e) respiration rate (ml CO₂ kg⁻¹ h⁻¹), (f) ethylene evolution rate (μ L kg⁻¹ h⁻¹), (g) TSS (B), (h) total sugars (%), (i) titratable acidity (%), (j) total sugar (%) in banana fruit

browning. Suppression of PPO activity of fruit and vegetables by H₂S delayes the browning in a variety of fresh as well as minimally processed products.²⁴

Respiration Rate

Initial respiration rate of the fresh mature green fruit was recorded 5.0 mL $CO_2 \ kg^{-1}h^{-1}$ (Fig. 1e). Climacteric peak of $\approx 38.07 \ mL \ CO_2 kg^{-1}h^{-1}$ was recorded on 7th day of storage in control and 30.0 mL $CO_2 \ kg^{-1}h^{-1}$ in H₂S (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.²⁵ Therefore, it is expected that PG and PME activities in H₂S treated fruit got suppressed which in turn reduced the respiration rate. The above findings are well supported by earlier work on storage life of H_2S treated strawberry fruit.²⁶

Ethylene Evolution Rate

The presented data showed that the highest climacteric peak of ethylene evolution was occurred in the control fruit $(45.7\mu Lkg^{-1}h^{-1})$ and the lowest $(21.89 \ \mu Lkg^{-1}h^{-1})$ in 1.5 mM H₂S treated fruit on 7th day of storage (Fig. 1f). The ethylene evolution rate of fruit had shown increasing trend up to 7th day but on 9^{th} 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, H₂S is potent ethylene inhibitor which inhibits ethylene production by suppressing ACS enzyme activities.^{2,27} The higher dose of H_2S could have been effective in suppressing the ACS activities over other lower doses. These findings are consistent with the work of Lu *et al.* $(2015)^{(19)}$ which showed suppression of ethylene production in H₂S treated banana fruit.

Total Soluble Solids

Total soluble solids (TSS) were significantly affected by the treatments; however, the difference was more pronounced after 5th 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 H₂S treated fruit due to ethylene biosynthesis inhibition properties of H₂S.⁷ It might also be involved in the regulation of postharvest shelf life of respiratory climacteric or non-respiratory climacteric fruit. Zhu *et al.* (2013)⁽²⁸⁾ also reported similar findings while working on kiwi fruit.

Total Phenolics Content

The fresh fruit had the average total phenolics content (TPC) content ≈ 21.69 mg GAE 100 g⁻¹, which later on showed a fluctuating trend throughout the storage period (Fig. 1h). H₂S treatments have significantly suppressed the evolution of TPC over control. The highest average value (≈ 60 mg GAE 100 g⁻¹) was recorded in control and lowest (≈ 28 mg GAE 100 g⁻¹) in H₂S (1.0–1.5 mM) treated fruit on 9th 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.²⁹ Besides, Giovanelli *et al.* (1999)⁽³⁰⁾ reported different TPC trends under different ripening ambience. Being a ripening linked photochemical; the anti-senescence role of H₂S might have decreased the TPC level in treated fruit over control.³¹

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.²⁹ As described above, the ripening process (respiration, ethylene production) was effectively suppressed by H_2S (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_2S treated mulberry fruit.⁶

Total Sugars

Irrespective of treatment, initially the total sugars content increased up to 7th 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 H₂S treated fruit. Fruit ripening and senescence delaying properties of H₂S has been earlier documented by other workers^{2,28} while working on postharvest physiological response of banana and kiwifruit.

Sensory Score

The sensory score was adjudged by tasters on 9^{th} day of storage with respect to peel colour, aroma, firmness, taste and overall acceptability. H₂S @1.0 mM treatment had given better score over other treatments and control (Table 1).

Fruit treated with H_2S (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_2S . The finding revealed that, overall acceptability of fruit after 9 days of storage was highly influenced by H_2S treatment

Table 1 — Sensory score of banana fruit on 9 th day of storage at 25°C \pm 2 temperature in H ₂ 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±2.2b	7.0±2.1a	7.9±2.1ab	8.7±2.2a	8.5±2.2a
Hydrogen sulphide (1.5mM)	8.0±2.1b	6.8± 2.5ab	8.5±2.1ab	6.0±2.4b	6.9±2.5b
Control	5.4±1.7a	5.2±2.0a	4.1±2.3a	4.8±2.2c	4.6±2.3 c

and its concentration. Among the treatments, H₂S 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 H₂S inhibits ethylene production by suppressing ACS enzyme activities.^{2,27} Therefore, characteristic rise in treated fruit might have been delayed and reduced by H_2S .

Conclusions

Exogenous application of H_2S (*a*) 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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