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Shelf Life Extension of Banana (*Musa* spp.) using Hexanal Formulation as a Post-harvest Dip

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Authors' contributions

This work was carried out in collaboration with all authors. Authors IM and KSS designed the study. Author PM performed the statistical analysis and wrote the protocol. Author SS wrote the first draft of the manuscript. Authors PM and IM managed the analyses of the study. Author MK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Postharvest dipping of fruits in hexanal formulation extends the shelf life by inhibiting enzyme phospholipase D (PLD) activity. To enhance the postharvest shelf-life and quality of different banana cultivars viz., Grand Naine (AAA), Ney Poovan (AB), Poovan (AAB) and Rasthali (AAB) were treated with different concentration of hexanal formulations (1% and 2% Enhanced fresh formulation (EFF)) and stored at atmospheric storage condition. The results indicate that the quality characteristics of 2% EFF treated fruits of Grand Naine (AAA), Poovan (AAB) and Rasthali (AAB) were low TSS, total sugars, reducing sugars and acidity indicating the perpetuation in quality of fruits during storage life besides extended shelf life of fruits. Banana fruits treated with hexanal, irrespective of cultivars experienced a significant delay in weight loss and higher firmness

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as compared to respective control. The high-resolution imaging had clearly shown that the application of hexanal delayed the ripening process by the structural integrity of cells on skin and fruit pulp. The PLD enzyme activity, respiration and ethylene evolution rates (more than 10%) were markedly reduced over control due to hexanal treatment. Our results revealed that the application of hexanal formulation as postharvest dip treatment in different banana cultivars significantly enhanced shelf life over control besides continuance of quality during the storage period.

Keywords: Enhanced freshness formulation; structural changes; Phospholipase D enzyme (PLD); storage.

1. INTRODUCTION

Banana (*Musa* spp.) belongs to the family Musaceae, grown in many tropical and subtropical regions at the global level. It represents the world's largest fruit crop with an annual production of 113.9 million tons in 2017 [1]. India is the largest producer of bananas with an area of 8,58,000 lakh ha and a production of 29.16 million tones [2]. Next to India, China, Philippines, and Ecuador are the largest banana producers at the global level. India occupies the 20th position in the case of export; it shares US$ 89.8 million [1]. The bulk of the produce is sold within the country to meet the local demands with an export share of only 0.5%.

Banana, like other climacteric fruits, has a distinct ripening pattern with amplified ethylene biosynthesis and respiration rates at ripening [3]. Different biochemical and physiological changes take place during the ripening process. In banana fruits, postharvest changes are important as they determine the fruit ripeness and shelf life [4]. During ripening, changes that occur in physical, chemical and mechanical properties of fruits include alterations in pigment biosynthesis and accumulation, modification of cell wall texture and ultrastructure, breakdown of starch, heightened levels of flavor and aromatic volatiles and increased susceptibility to postharvest pathogens [5].

Nearly, 30-35% of banana fruits are wasted due to poor post-harvest management techniques during harvesting, grading, packaging, transportation and storage, etc. [6]. Most of the post-harvest research has been performed to extend the green life of pre-climacteric bananas to transport them to distant markets. For example, postharvest technologies such as controlled or modified atmosphere storage, nitrogen atmosphere storage and the treatment with ethylene antagonist, 1-methyl cyclopropene (1-MCP) have all been reported to slow down the onset of the ripening process in bananas [7].

Several technologies have been developed over a period of time for improving the shelf life of banana, but recently, hexanal formulation has been emerged as one of the viable options to extend post-harvest life and quality of homestead produce [8,9,10,11,12]. Hexanal is an aldehyde, produced during the termination phase of fat oxidation in plant materials, known to extend shelf life of many horticultural commodities by inhibiting enzyme phospholipase D activity, which hydrolyzes the phospholipid to phosphatidic acid and a free head group. Phospholipase D enzyme gradually stimulated during the fruit ripening process in an autocatalytic manner, which results in membrane degradation and destabilization [13,14]. Hexanal is highly volatile and had antifungal properties against *Alternaria alternata*, *Botrytis cinerea* and *Penicillium expansum* [15]. The antimicrobial properties were supported by [16] against molds, yeast, mesophilic and psychrophilic bacteria in apple slices. The antimicrobial activity of hexanal, its aroma volatiles increased the sensory attributes of ripened fruits [17]. El Kayal et al. [18] reported that the application of hexanal formulations in strawberry reduced two PLD gene expression and other cell wall degradation enzymes. With this background, the present study was undertaken to study the effect of hexanal formulations on the physiological and biochemical changes, shelf life and quality of banana fruits under ambient storage conditions.

2. MATERIALS AND METHODS

Laboratory experiments were carried out at the Department of Fruit Crops, Horticultural College and Research Institute, Periyakulam during 2017-18 to evaluate the influence of different concentrations of hexanal formulation (1% and 2% Enhanced Fresh Formulation) on the shelf life of different economically important banana cultivars (Grand Naine (AAA), Ney poovan (AB), Poovan (AAB) and Rasthali (AAB)) harvested at 85% maturity were treated with or without in different hexanal formulations. Besides hexanal

formulation, alum (0.2%) and carbendazim (400 ppm) were given as postharvest dip treatment at 5 min to improve the shelf-life of banana fruits. To study physiological, biochemical and structural changes during storage of the fruits each treatment consists of three replications and each replication, had 10 kg of fruits. From each replicate, samples were analyzed at three days interval.

2.1 Shelf-life Studies

Banana fruits harvested at 85% maturity, Grand Naine (AAA) (98 days after inflorescence emergence), Ney Poovan (AB) (93 Days after inflorescence emergence), Poovan (AAB) (95 days after inflorescence emergence) and Rasthali (AAB) (97 days after inflorescence emergence) were rope harvested (Export standard). Harvested fruits were kept separately as upside down under shade conditions to reduce the field heat and de-sapping. The fruits were properly packed by cushion sheets with the crates (60 cm × 40 cm × 20 cm) and transported from the experimental site to the laboratory by air-conditioned vehicle. After reaching into the laboratory, the whole banana hand was dipped for 5 min regardless of different hexanal formulations, shade dried on the blotting paper to remove the excess wet water solutions and placed in the plastic crates (holding capacity is 10kg). The total weight of 5 kg was stored in each replication under each treatment. Treated and untreated fruits containing plastic crates were kept under room temperature at 27 ± 2°C with the RH of 60%, and the shelf-life was estimated by calculating days are taken from harvest to reach the optimal edible stage (ripened).

2.2 Physical Parameters

2.2.1 Physiological loss in weight (PLW)

Fruit weight was taken at the time of imposing treatment which considered as the initial weight of the fruit. Loss in weight was measured at every three days and expressed in percent. PLW (%) = (Initial fruit weight – Final fruit weight) / (Initial fruit weight) X 100.

2.2.2 Firmness (N)

The firmness of the fruits was measured using handheld penetrometer. The firmness was measured at three different places (proximal, distal and middle according to [19]. and values are expressed in Newton (N).

2.3 Quality parameters

Total soluble solids (TSS) were measured using a Hand-held refractometer from the clear juice filtrate at three days interval and expressed as ^{o}Brix. Titratable acidity in percent was determined (percent of citric acid) according to [20]. Ascorbic acid content was estimated as per the [21] and expressed in percent. Total sugar was estimated by the Anthrone method [22] and expressed in percent. Reducing sugar was calculated by Nelson Somogyi's method [23] and expressed in percent. The pH of the fruit pulp was estimated by macerating the fruit pulp (5 g) and made up to 250 mL with distilled water. From which, 100 mL of banana fruit juice was taken and pH was measured using pH meter (Model − Global digital pH meter).

2.4 Phospholipase D (PLD) Enzyme Assay

PLD (EC 3.1.4.4.) assay kit (MAK 137, Sigma Aldrich, St. Louis, MO, USA) was used to estimate PLD activity [14]. PLD activity expressed as µ mol of choline produced kg^{-1} on a fresh weight basis.

2.5 Ethylene and Respiration Rate

Ethylene evolution and respiration rate of control and treated fruits were studied under atmospheric storage conditions and different time periods with the following procedure. Plastic containers of 5.6 liters and the acrylic chamber of 27-liter capacity with airtight lid were used. A known weight of fruits each from all sub-plots (treated and untreated) was used. Plastic lids with rubber septa were inserted at the center of the lids to enable the withdrawal of gas samples. Changes in ethylene, CO_2 and O_2 concentrations in the chamber were recorded every third day using F-950 Three Gas Analyzer. The ethylene and respiration rates were calculated based on [24].

2.6 High-resolution Imaging of Fruit Peel and Pulp

The stored frozen samples (peel along with pulp) were cut into 2-3 mm long segments with the help of a surgical blade. After lyophilizing the segments, they were observed under SEM (SEM; Quanta 250, FEI, Hillsboro, OR, USA) and

the machine was set at a vacuum 2 kV, with a spot size of 3 and pressure of 20 Pa. The high-resolution imaging was taken at ×500 and ×2000 magnifications.

2.7 Statistical Analysis

The experiment was conducted with Factorial Completely Randomized Design (FCRD) which was employed to understand the main effects of treatments, cultivars and their interactions for the different parameters examined in fruit samples in the laboratory and mean comparisons were made after computing Least Significant Difference (LSD) values and ANOVA with P< 0.01 level. All the statistical analyses were achieved utilizing the statistical analysis software AGRES.

3. RESULTS AND DISCUSSION

3.1 Effect of Hexanal Formulation on Physiological Parameters

3.1.1 Changes in the PLW

A significant increase in weight loss was observed during ripening under ambient storage conditions irrespective of the cultivars and treatments. The lowest PLW was recorded in 2% EFF treated Grand Naine (11.36%) than control (17.27%) fruits at the end of the storage period (18 days) (Fig. 1a). The PLW in fruits can lead to shriveling, which reduces both the market value and consumer acceptability due to the loss of water through transpiration [25]. It was noted that a positive correlation exists between moisture loss and PLW of fruits which indicates that an increase in water loss is directly proportional to the weight loss of the fruits. Excess energy produced from the respiration process in the form of heat is released from the fruits by evaporation of water causing a weight loss [26,27]. The present data are in close agreement with the observations in banana cv. Grand Naine (AAA) [28], mango [10], Guava [11], sweet cherry [8] and papaya [29,30].

3.1.2 Firmness

Fruit firmness decreased over time in treated and untreated fruits, but treated fruits maintained its firmness significantly higher. The 2% EFF treated fruits of AAB group cultivars showed increased firmness till the fruits attained edible stage (complete ripening). Among the other cultivars, Ney Poovan (AB) registered the highest firmness in 1% EFF treated fruits compared with other treatments (Fig. 1b).

External firmness, a major quality attribute that often dictates the shelf life of fruits. The decrease in fruit firmness is an indication of the level of softening of the fruit. Fruit softening a major aspect of the ripening process and considered to be a consequence of compositional and structural changes in the cell wall that involves pectin solubilization and depolymerization and accompanied by increasing hydrolyses enzyme activity such as β-galactosidase, pectin methylesterase (PME) and α-galactosidase [31,30]. All the cultivars treated with 2% EFF had a delayed loss in firmness when compared to other treatments except Ney Poovan (AB) in which 1% EFF registered the highest delay in loss of fruit firmness. [18] reported that hexanal treated cherry fruit had significantly higher firmness during storage compared to that of control. Similar results have also been observed in tomato [32], guava [33], mango [10], banana [34], cherry [8] and apples [13].

3.2 Effect of Hexanal Formulation on Quality Parameters

3.2.1 Total Soluble Solids (TSS)

TSS content gradually increased towards the ripening process, a significant difference (P = 0.05) was observed between hexanal treated fruits and non-treated fruits of both AAB as well as the AB group banana cultivars (Fig. 2a). Among the cultivars, Poovan (AAB) showed the highest level of TSS reduction in the 2% EFF treated fruits compared to its respective control. TSS indicates the level of acids and sugars in the fruit; the biochemical pathways that produce these components are stimulated by climacteric ethylene [35]. The increase of TSS is an important trait of hydrolysis of starch into soluble sugars such as glucose, sucrose, and fructose [36]. Siriboon et al. [26] reported that increased breakdown of starch to soluble sugars contributed to the increase in TSS in banana fruit. According to [37] and [9] revealed that inhibition of Phospholipase-D (PLD) enzyme retains the quality and soluble solid content during the storage period in tomato. The present results were concomitant with the experiment of [28] that hexanal vapour treatments were effective to delay the breakdown of starch content in fruits and which is one of the important ripening indicators for tropical fruits. Similar

results have also been observed in mango [10], banana cv. Grand Naine (AAA) [34], guava [11] and oriental sweet melon [38].

3.2.2 Sugar content

Hexanal treated fruits not gives significant (P = 0.05) results in case of all sugars (Total, reducing and non- reducing sugars) but, it shows a significant difference among the cultivars. At the end of the storage period, the sugar content of all treated fruits was much lower than the control fruits (Fig.3a, 3b). The main biochemical change in fruit pulp during ripening is the conversion of starch to sugars. In the present study, it was noted that the starch content of banana fruits tended to decrease under the storage. Fruits treated with hexanal displayed significantly higher starch content than the control. This indicates that hexanal application delays the decrease of starch content in banana.

In contrast, it was observed that the sugar content of the fruits was increased with the ripening process irrespective of the cultivars. The observed increment in the total amount of sugar could be due to the conversion of starch to sugar as ripening progress which is in agreement with [39]. In the present study, among the compared cultivars, Grand Naine (AAA) and Rasthali (AAB) fruits exhibited the highest total sugars throughout the storage period. However, hexanal treatment significantly delayed the increment in the sugar content in the fruits. In general, 2% EFF dip has significantly delayed the accumulation of total sugars, reducing sugars, non-reducing sugars in all tested cultivars except Ney Poovan. Lima et al.[40] have found an appreciable increase in the activity of amylase, reducing and non-reducing sugars contents and decrease in the starch content during ripening in banana.

Jincy et al.[14] reported that the hexanal treated fruit had lower TSS content because of decreased reducing, non-reducing and total sugars contents. The soluble sugar content increases with the ripening process, the decreased sugar content in hexanal treated fruits indicates delayed ripening compared with control fruit. Similar results have also been observed in mango [10], banana cv. Grand Naine (AAA) [34], guava [11]. Our data had shown that higher total solids in treated fruits indicating the enhanced quality as a hexanal fruit dip, on other hand, [8] have shown no hexanal effect on the total soluble solids in cherries.

3.2.3 pH of the fruit pulp

The pH of the pulp of ripened banana decreases due to increased accumulation of malic acid, citric acid and oxalic acid in the vacuole of pulp cells, which will be further used in the tricarboxylic acid (TCA) cycle. Generally, the banana fruits are harvested at the mature green stage, were the pulp pH will be higher, however during ripening the pH levels tend to decrease [41]. In our experiment, it was observed that the pH of pulp decreased over the period of ripening in control fruits, whereas hexanal formulation treated fruits retained higher pH even at the final stage of storage irrespective of cultivars (Fig. 4b).

3.3 Effect of hexanal formulation on ethylene, respiration rate and Phospholipase D (PLD) activity,

The lowest ethylene evolution rate recorded at the treatment of 2% EFF in Grand Naine (AAA), Poovan (AAB) and Rasthali (AAB) and at 1% in Ney Poovan (AB). This could have contributed to the enhanced shelf life of the four cultivars in the present study (Fig. 5a). Our data shows that hexanal suppress ethylene production throughout the storage period. [7] reported that ethylene production was inhibited in hexanal and 1-MCP-treated tomato fruit within a day of treatment and delays the ripening by regulating the PLD gene expression and ethylene signaling regulation through downstream components. Binding of ethylene to its receptors accelerates the process of fruit ripening and senescence. It has been proposed that after binding to its receptors, ethylene releases calcium from storage compartments, which initiates phospholipid degradation by binding of Phospholipase D (PLD) to the plasma membrane.

The respiration rate of banana is directly influenced by the increasing levels of CO_2 during the storage. At low levels of oxygen (1–3%), ethylene production is reduced because oxygen is a co-substrate for 1-aminocyclopropane- 1-carboxylic acid oxidase (ACO) [42]. From the present study, it could be inferred that the control fruits of all cultivars exhibited maximum respiration rate by producing a higher amount of CO_2 than the hexanal treated fruits (Fig 5b). Among the cultivars, Grand Naine (AAA) and Ney Poovan (AB) showed higher levels of CO_2 than Rasthali (AAB) and Poovan (AAB) under ambient storage conditions. These results are in accordance with those reported by [43] and [44]

who found that the CO_2 production in the fruits depends upon the gas level in tissue, endogenous ethylene level, exposure time, storage temperature and cultivar of the fruits. [10] reported that the application of hexanal as postharvest dip treatment in mango cv. Neelum decreases the respiration rate.

Hexanal is naturally produced in plants during lipid peroxidation mediated by lipoxygenase and hydroperoxide lyases [13]. The enzyme PLD is gradually stimulated during the fruit ripening process in an autocatalytic manner which can result in membrane degradation and destabilization [13]. Hexanal is a potent inhibitor of PLD activity [45]. El Kayal et al. [18] noticed that in raspberry, hexanal formulations delay the ripening process by reducing PLD transcript levels and altering the calcium-binding proteins. A significant difference (P = 0.05) in ethylene evolution rate, respiration rate, and Phospholipase D enzyme activity were observed in the control and treated fruits of all cultivars. The EFF-2% treated fruits of AAB genome cultivars show reduced ethylene and respiration rate at the end of the storage period whereas, in AB genome types low concentration of (EFF-1%) hexanal formulation inhibits PLD activity by which it reduced the respiration and ethylene evolution (Fig. 6a).

3.4 Effect of Hexanal Formulation on Shelf Life

Enhanced shelf life due to both spray and vapour form of hexanal application has been reported in several fruit crops like apple, banana, grape, peach, pear, strawberry, nectarine, etc [13,8,14,12,34]. Our results revealed that the application of hexanal formulation as postharvest dip treatment in different banana cultivars significantly enhances shelf life. The shelf life of the fruit was directly proportional to the hexanal formulation concentration. In Grand Naine (AAA), Poovan (AAB) and Rasthali (AAB), 2% EFF treated fruits recorded higher shelf life than the other treatments, whereas in Ney Poovan (AB), 1% EFF treated fruits recorded the best result in ambient storage condition (Fig. 6b). This change in response behavioral could be due to the genomic composition (ploidy level) since the Ney Poovan is diploid in nature, but all other cultivars are triploid in nature.

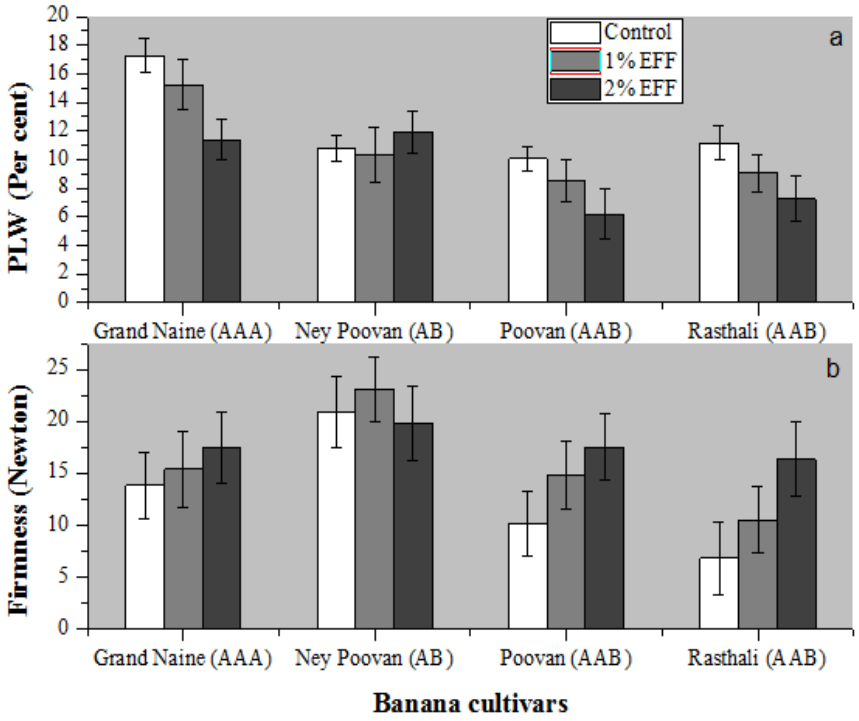

Fig. 1. Effect of hexanal formulation on physiological loss in weight (1a) and fruit firmness (1b) of different banana cultivars. Each value is the mean ± standard error (n = 12)

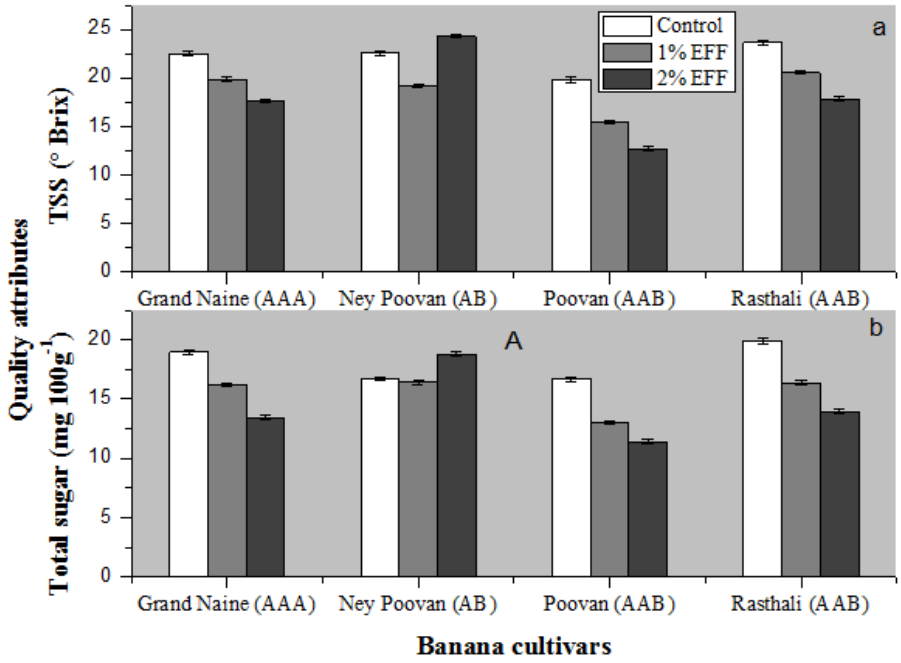

Fig. 2. Effect of hexanal formulation on total soluble solids (^{O} Brix) (2a) and total sugars (2b) in different banana cultivars. Each value is the mean ± standard error (n = 12)

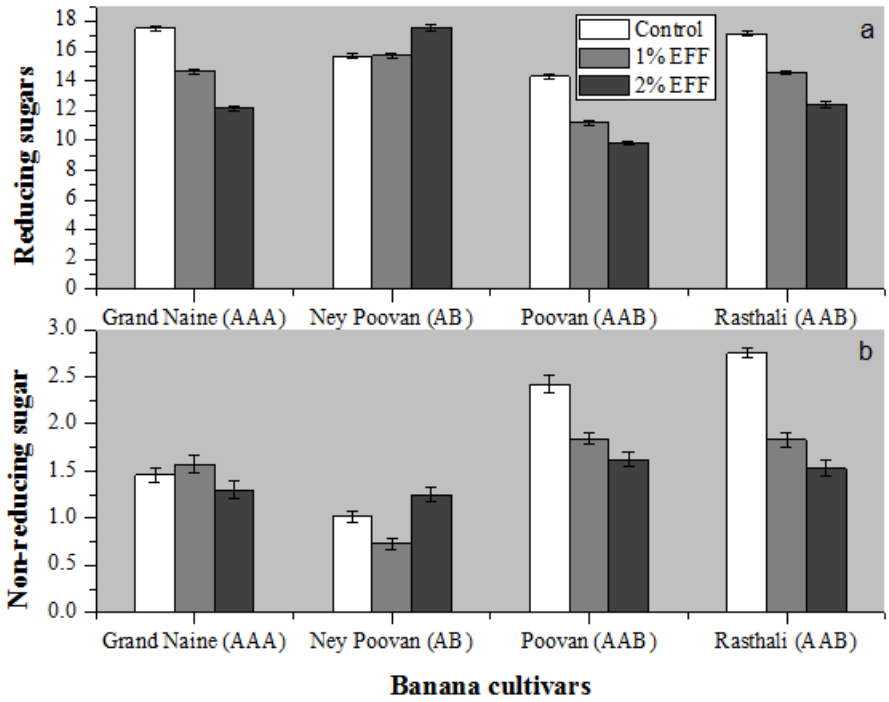

Fig. 3. Effect of hexanal formulation on reducing sugars (3a) and non-reducing sugars (3b) in different banana cultivars. Each value is the mean ± standard error (n = 12)

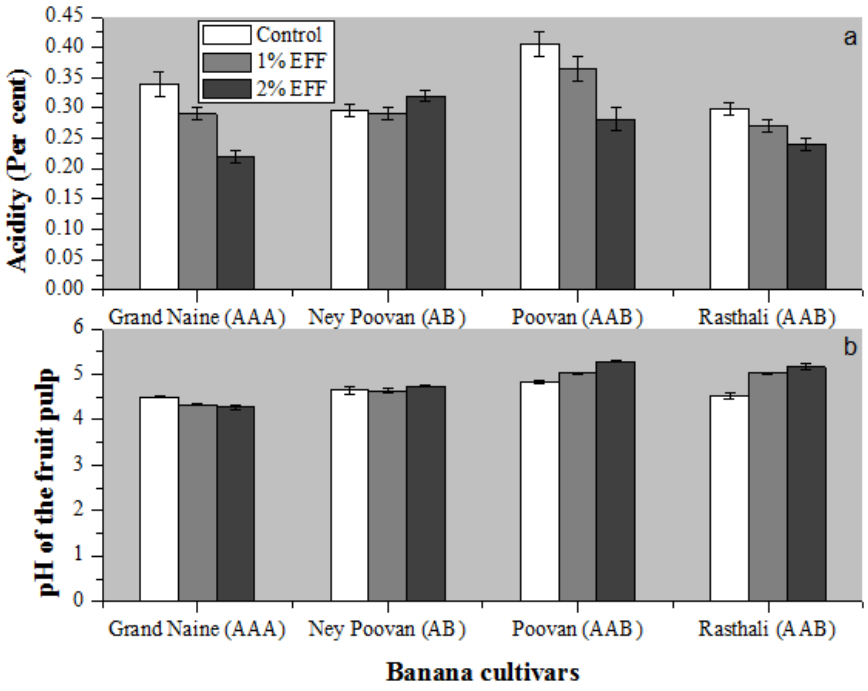

Fig. 4. Effect of hexanal formulation on fruit pulp acidity (4a) and pH (4b) of different banana cultivars. Each value is the mean ± standard error (n = 12)

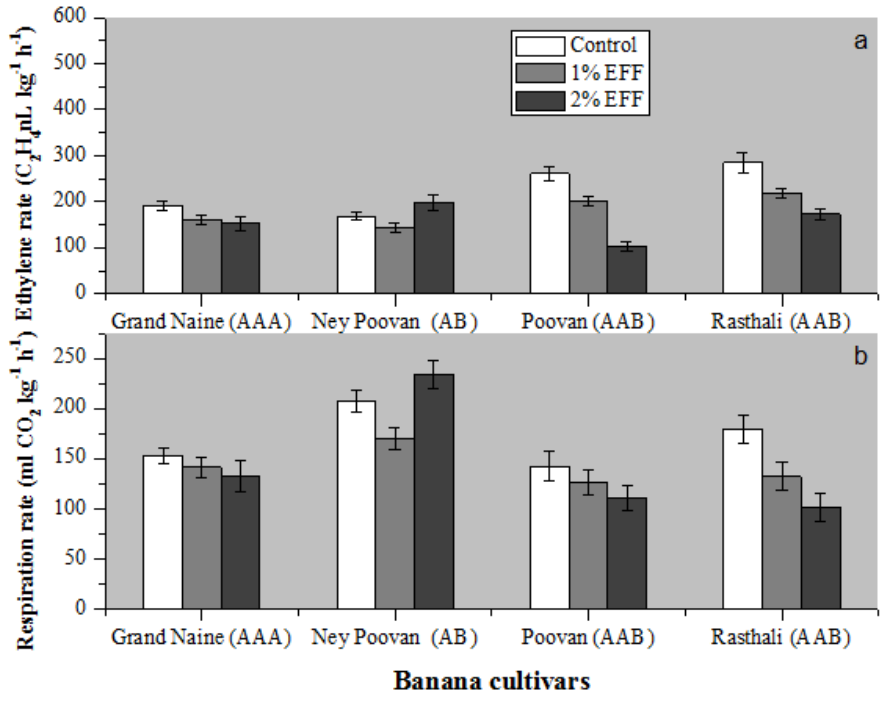

Fig. 5. Effect of hexanal formulation on ethylene evolution rate (5a) and respiration rate (5b) of different banana cultivars. Each value is the mean ± standard error (n = 12)

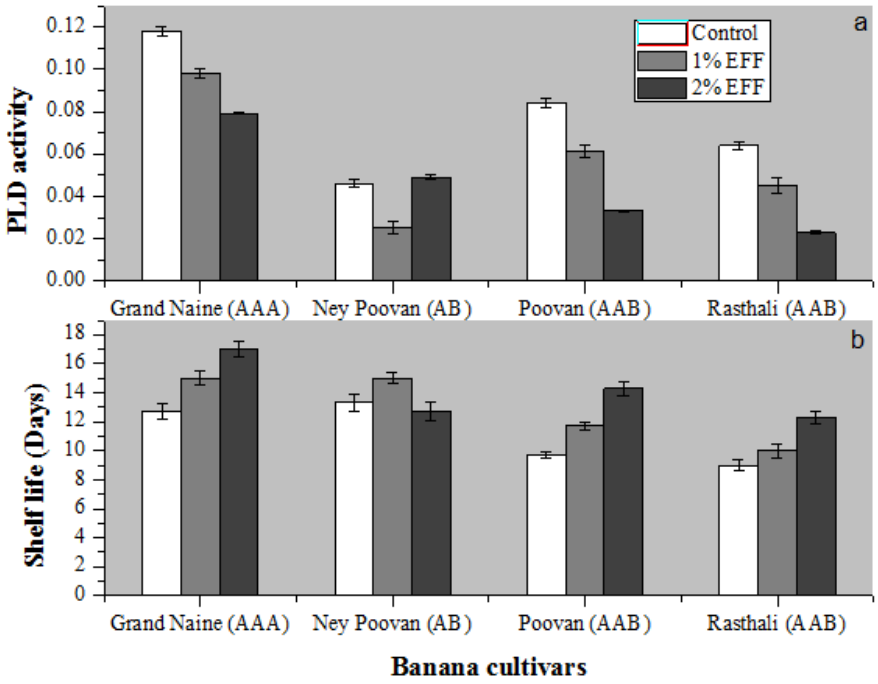

Fig. 6. Effect of hexanal formulation on phospholipase D enzyme activity (6a) and shelf-life (6b) of different banana cultivars. Each value is the mean ± standard error (n = 12)

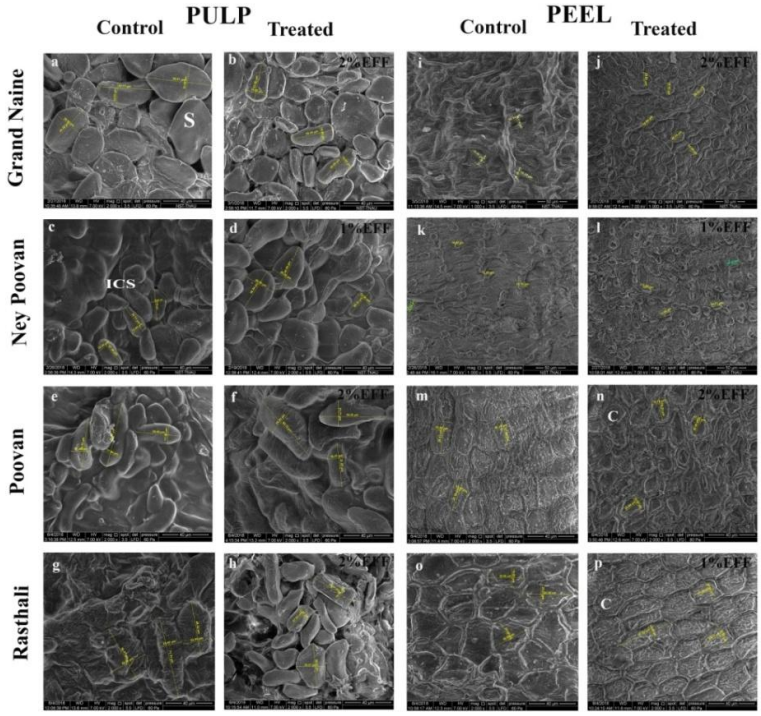

Fig. 7. Representative scanning electron microscopy images of ultrastructural changes in peel (a to h) and pulp (i to p) of different banana cultivars. (s – starch granules, ICS – intercellular space, C- cell)

3.5 Effect of Hexanal Formulation on Ultrastructural Changes

In the present study, the differences in the structural integrity of tissue layers could be easily detected in fruits treated with hexanal formulations that of untreated control in all the cultivars by Scanning electron microscopy (Fig. 7). In hexanal treated fruits, prominence of starch granules could be observed in between the parenchyma cells in the pulp which is indicative of delay in the ripening process as against the absence of such starch granules in control fruits.

4. CONCLUSION

Hexanal formulation treated fruits exhibited reduced physiological loss in weight and increased firmness along with quality attributes such as sugars and ascorbic acid throughout the storage period. In the line of foregoing, Grand Naine (AAB) dipped in 2% hexanal formulations for 5 minutes could extend the shelf-life up to 17 days in ambient storage condition and 1% hexanal formulations could increase shelf life up to 15days in Ney Poovan (AB), Poovan (AAB) with the added advantages of improved fruit quality.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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