Post-harvest dip of enhanced freshness formulation to extend the shelf life of banana (*Musa acuminata* cv. Grand Naine) in India

Kanmani Venkatachalam[1], Iyamperumal Muthuvel[1], Srivignesh Sundaresan[2], Kizhaeral S. Subramanian[2]*, Janavi Gnanaguru Janaki[2], J. Alan Sullivan[3], Gopinathan Paliyath[3] and Jayasankar Subramanian[3]

[1]The Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam, Tamil Nadu, India
[2]Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India
[3]Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada
**Corresponding author email: kssubra2001@rediffmail.com*

A laboratory study was undertaken to determine the effects of a nano-emulsion carrying hexanal, an enhanced freshness formulation (EFF), as a post-harvest dip technology to minimize the post-harvest losses and to extend the shelf life of bananas. The banana fruits were harvested at three maturities (95%, 85%, and 75%), dipped or not dipped in the EFF, and studied under both ambient and reduced temperature storage conditions. During the experiments, the fruit's physical, physiological, and biochemical parameters were periodically evaluated. The treated fruit had lower physiological loss of weight and higher firmness throughout the study period, regardless of maturity level at the start. Treated fruit had higher total soluble solids and total sugars, and less acidity indicating improved fruit quality during storage, in addition to an extended shelf life. High resolution imaging using scanning electron microscopy showed that EFF-treated fruit exhibited well maintained structural lenticels on the fruit skin and deposition of starch granules in the fruit pulp, regardless of maturity level at the start. Overall, the results clearly indicated that the EFF-treated banana fruit were delayed in the ripening process and had an extended shelf life of up to six days in ambient conditions and nine days in cold storage conditions. Post-harvest dipping using hexanal formulation is a potential technology that could be adopted in pack houses for domestic and export markets.

Keywords: Hexanal, shelf life, banana, storage, dip technology, enhanced freshness formulation, EFF, post-harvest technology

India is the largest producer of bananas in the world (30 million tonnes) produced from an area of 850,000 hectares (National Horticulture Board 2016). Among the many cultivars commercially grown, cv. Grand Naine is gaining popularity and may soon be the most preferred, due to its tolerance to biotic stresses, extended shelf life, uniform colour development during ripening, and export-quality bunches. In India, the magnitude of post-harvest losses for fruit is estimated at 30% to 35%, creating a huge economic drain on the country—in the order of 13,560 crores Indian rupees annually (Murthy et al. 2009). Such massive post-harvest losses in perishables are due to poor harvesting, handling, storage, transportation, and marketing practices. Although physiological and biochemical activities involved in fruit ripening are irreversible, many technologies have been developed targeting the pre-ripening stage to reduce post-harvest losses and extend the shelf life of fruit.

Bananas being a climacteric fruit, have a distinct ripening pattern with increased respiration and ethylene biosynthesis rates during ripening (Lelièvre et al. 1997), which limit their shelf life. Furthermore, biochemical and physiological changes during ripening take place within a short time. As the activities of these enzymes are mainly ethylene dependent (Lohani et al. 2004), it is necessary to understand the ripening process in banana to develop successful post-harvest technologies (Yanez et al. 2004). The

softening of banana is caused by enzyme activities in cell walls, which involves polygalacturonase (PG), pectin methyl esterase (PME), pectatelyase (PL), and cellulases. The commercial application of 1-methylcyclopropene (1-MCP), a potent ethylene inhibitor (i.e. receptor blocker), has been successfully employed in climacteric fruit such as apple, plum, banana, strawberry, and pear for extending shelf life (Blankenship 2001; Zhu et al. 2015). European countries, particularly the United Kingdom, prohibit the direct use of 1-MCP in fruit preservation.

In order to improve the shelf life of fruit, various types of dip treatments have been adopted in several countries for different fruit. Several chemicals, such as combined solutions of calcium chloride, ascorbic acid, and cysteine (Bico et al. 2009), natural lysophospholipid along with soy lecithin (Ahmed and Palta 2016), salicylic acid (Srivastava and Dwivedi 2000), phenylurea [CPPU] and gibberellins [GA3] (Huang et al. 2014), 1-MCP (Blankenship 2001), nitrous oxide (N_2O) (Palomer et al. 2005), potassium permanganate ($KMnO_4$) (Hassan 2000), and oxalic acid (Huang et al. 2013), were found effective in minimizing the losses of fruit during storage and transport, and in extending the shelf life of fruit. In all cases, the chemicals inhibit ethylene production thus enabling the extension of shelf life of fruit. Despite the number of chemicals and technologies available for fruit preservation, the adoption of these technologies is very low due to practical difficulties, non-availability, and prohibitive costs. Non-invasive storage technologies, such as modified atmospheric storage, have been used to enhance the post-harvest shelf life of banana (Noomhorn and Poety 1993; Yahia 2009). The respiration rate of fruit is affected by the development stage and the respiration patterns (Nicolai et al. 2009), and the optimal storage temperature for banana appears to be 13°C to 14°C, with a relative humidity of 85% to 90% (Kader 2005). Although multiple options are available to extend the shelf life of fruit to some extent, various constraints make it necessary to introduce new formulations that are eco-friendly and economically feasible.

Hexanal, a naturally occurring, six-carbon aldehyde formed from linoleic acid via the lipoxygenase pathway in plants (Hildebrand 1989), is highly volatile and has antifungal properties against *Alternaria alternate*, *Botrytis cinerea*, and *Penicillium expansum* (Hamilton-Kemp et al. 1992; Song et al. 1998). Hexanal extends the shelf life of fruit when it is externally applied as a pre-harvest spray, post-harvest dip, or vapour. It is generally recognized as safe (GRAS), has been observed to be a strong inhibitor of phospholipase D, and so technologies for its application to enhance shelf life and the quality of fruit, vegetables, and flowers are currently under development (Paliyath et al. 1999, 2003; Paliyath and Murr 2007). Hexanal formulations applied as pre-harvest treatments, post-harvest dips, and vapour treatments were found to be effective in enhancing the shelf life of many fruit, such as apple, pear, peach, grape, sweet cherry, strawberry, mango (Paliyath et al. 1999; Paliyath and Murr 2007; Anusuya et al. 2006) and tomato (Utto et al. 2008). In addition to the antimicrobial activity of hexanal, its aroma volatiles increase the sensory attributes of ripe fruit (Archbold et al. 2000). It was also found to stimulate aroma production in Jonagold and Golden Delicious apple slices (Song et al. 1998). Hexanal formulations also prevented browning reactions for 16 days at 15°C when added under modified atmospheric conditions (Lanciotti et al. 1999). Despite hexanal formulations having been extensively studied in temperate fruit and vegetables, they have been rarely studied in tropical fruit. It is hypothesized that dipping of banana fruit in a hexanal formulation facilitates the inhibition of phospholipase D enzyme in the skin of the fruit and slowing down ethylene production; these two physiological processes enable the extension

of the shelf life of the fruit. Banana fruit is harvested at three distinct maturities to target domestic and export markets. This study focuses on the evaluation of the effectiveness of post-harvest dipping of fruit in an enhanced freshness formulation (EFF), a hexanal based formulation, at different levels of fruit maturity and under two distinct post-harvest storage conditions (ambient and reduced temperature storage) to extend shelf life.

Materials and methods

Fruit samples and treatments

The banana fruits were obtained from the Farm Fresh Banana, at Chinnamanur, Theni, Tamil Nadu, India. The fruits were harvested at three different stages of maturity (75, 85, and 95%) based on the number of days from shooting, using the nylon rope harvest method. However, only fruits harvested at 85% maturity are being discussed in this manuscript as this is the most common stage for commercial harvest for most practical purposes. The studies were undertaken from 2015 to 2016 at the Department of Fruit Crops, Horticultural College and Research Institute, Periyakulam and the Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore, India. The banana fruit hands were cleaned carefully by washing with potable water and maximum efforts were made to select uniformly-sized fruit that were free from injuries and diseases. Fruits were treated with EFF for 5 minutes and then air-dried and washed once in clean water. Fruits dipped in water for 5 minutes and then air-dried served as control. Treated and untreated samples of fruits were stored under: (a) ambient (Temperature 28°C ± 2°C, RH 60 ± 10%) and (b) cold (14°C ± 2°C, RH 90 ± 5%). For each treatment, 100 kg of banana fruit were used. Fruits were sampled on predetermined dates between 10 a.m. and 11 a.m., frozen in liquid nitrogen, and stored at −80°C until analysis.

Shelf life

The time from the day of harvest, taken by fruit to reach the optimal, edible ripe stage was counted and reported in days.

Physiological and biochemical parameters

The physiological and biochemical traits were measured in 5 fruit sampled at three-day intervals, from each of treated and untreated banana fruit kept under ambient and reduced temperature storage conditions.

Physiological loss in weight (PLW)

The PLW was calculated by subtracting final weight from initial weight of the fruit and then expressed as per cent weight loss with reference to the initial weight as recommended by the Association of Office Analytical Chemists (2001) using the following formula: PLW (%) = Initial weight (g) − Final weight (g) / Initial weight (g) × 100. Ten fruits in each treatment were used for PLW estimation.

Respiration rate

One-kilogram of fruit of each batch, (EFF treated and control, ambient-stored and cold-stored) was placed in airtight plastic containers of 5L capacity with rubber septa placed in the top of the container lids. Changes in the O_2 and CO_2 levels inside the plastic containers were measured using Headspace Gas Analyzer (CheckMate 3 Dansensor) and measurements were made at 2-day intervals with three replicates for each treatment.

Firmness

Fruit firmness was measured using a Texture Analyzer (TA-HDi, Stable Micro Systems, UK) fitted with a 4 mm cylindrical probe (P/4) by the method proposed by Camps et al. (2005). Textural values were obtained from a three-point slope (A to D measurements) for each banana and the average values were determined and recorded.

Fruit quality

Quality parameters such as titratable acidity and total sugars were determined using standard operational protocols in the same set of fruit that were used for the fruit firmness analysis. The titratable acidity was estimated by titration of the juice against 0.1 N KOH using phenolphthalein as an indicator and expressed as citric acid (Srivastava and Kumar 1993). The total sugars were determined colorimetrically by an Optima UV-VIS spectrophotometer (Model SP-3000) using anthrone reagent (Hedge and Hofreiter 1962). A standard graph was prepared using known concentrations of glucose solutions. The sample values were plotted on the standard graph for total sugars and total sugars were expressed as percentages. The ascorbic acid (vitamin C) content was determined using the 2,6-dichlorophenol-indophenol titration method described in Association of Offical Analytical Chemists (2001). L-ascorbic acid was used to prepare a standard solution (1 mg/mL). The ascorbic acid concentration was calculated by comparing it with the standard and expressed as mg/100 g fresh weight.

Scanning Electron Microscope (SEM) Analysis

Fresh samples were collected from treated and untreated banana fruit after 12 days and 18 days under reduced temperature storage conditions. The fruit pulp and peel surface were cut into 2-3 mm long segments, processed as required for scanning electron microscopy and observed with a scanning electron microscope (SEM-FEI-Quanta 250, Netherlands).

Statistical analysis

A completely randomized factorial design was employed to understand the main effects of treatments on different maturities (75%, 85% and 95%) under ambient and reduced temperature storage conditions and their interactions for the different parameters examined in fruit samples in the laboratory. Mean comparisons were made after computing ANOVA and Least Significant Difference (LSD) at the $P < 0.05$ level. All the statistical analyses were performed utilizing the statistical analysis software AGRES.

Results

Only data from the 85% level of maturity are reported in the tables. There was no significant treatment by maturity interaction for any trait measured. The 85% level of maturity represents the stage of ripening at which most bananas would be harvested for the local markets. Any notable results for the 95% and 75% maturities are noted in the text.

Shelf life (days)

The shelf life of banana fruit dipped in EFF was 33 days under ambient conditions while the controls stayed fresh for 27 days only (Fig. 1). Under reduced temperature storage conditions, dipped fruit stayed fresh for 42 days, while control fruits were fresh for 36 days only. (Fig. 1). The results indicated that the shelf life was extended by 6 days (Fig. 2). However, the marketability of the treated fruits kept in reduced temperature storage improved by 18 days (15 days in control and 33 days in reduced temperature storage; Fig.2).

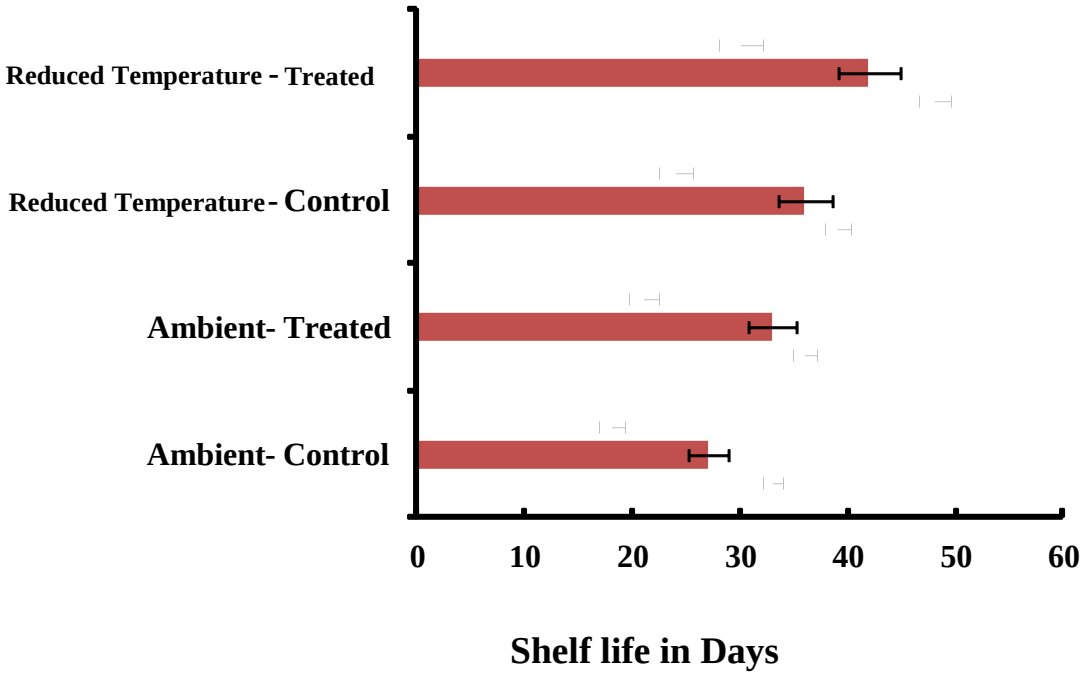

Figure 1: Shelf -life of bananas in EFF treated and control bananas at 85% maturity under ambient and reduced temperature storage conditions with ± SD.

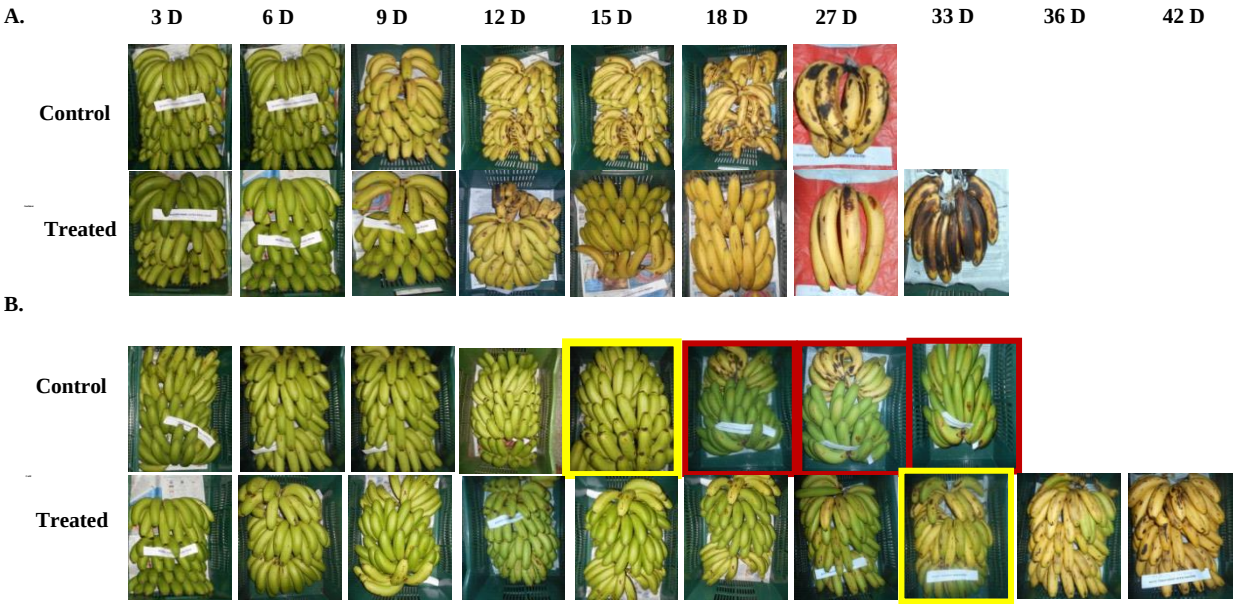

Figure 2: Shelf-life extension of banana fruits at 85 % maturity in treated and control bananas under ambient (A) and reduced temperature storage (B) conditions. Yellow box shows the latest date of marketability and red box in control denotes- unmarketable as they did not ripen normally.

Physiological loss in weight

The PLW values increased progressively with the advancement of storage, regardless of maturity, treatments, or storage conditions. The PLW values registered in EFF-dipped fruit were significantly ($P \leq 0.05$) lower than controls in all the days of observation (Table 1).

Table 1: Effect of enhanced freshness formulation on physiological loss in weight (PLW) % and fruit firmness of banana fruits

Physiological Loss of Weight					**Firmness**			
Days	**28°C**		**4°C**		**28°C**		**4°C**	
	C	**T**	**C**	**T**	**C**	**T**	**C**	**T**
3	2.09	1.67	1.30	1.00	19.78	20.25	20.01	21.36
6	3.23	2.24	2.65	2.44	19.32	19.98	20.89	20.89
9	4.21	3.42	3.51	3.22	18.75	19.15	19.56	19.56
12	5.12	4.25	4.56	4.24	17.89	18.98	18.65	19.38
15	6.83	5.78	5.64	5.32	15.38	16.85	16.89	17.65
18	8.23	6.32	5.87	6.23	14.12	15.56	16.25	17.42
21	9.12	7.34	6.98	7.54	13.05	14.25	15.54	16.52
24	10.31	8.34	7.52	9.33		13.12	14.45	15.89
27	10.67	9.34	8.12	10.30		12.54	13.78	14.36
30		10.34	8.56	10.98			12.85	13.56
33		10.89	9.12	11.23			11.24	12.85
36			10.25	12.45			10.65	11.35
39				13.56			9.58	10.25
42				15.25				9.28
LSD*	**0.101**		**0.132**		**0.191**		**0.165**	

LSD* Least Significant Difference (P<0.05) used to compare means within a treatment (temperature)
C-Control; T-Treatment- dipped in EFF solution for 5 minutes

Firmness

The firmness of fruit decreased with the progression of storage duration. On the 18th day the treated fruit had higher firmness (15.56; 17.42 N/mm) than the control (14.12; 16.25 N/mm) under ambient or reduced temperature storage conditions, respectively (Table 1). In reduced temperature storage temperatures, the firmness of fruit was retained for a longer period of time (48 days). In general, the treated fruit had significantly ($P \leq .05$) higher firmness than the control, regardless of storage conditions.

Total sugars

The total sugar content of banana fruit increased with the advancement of ripening in both control and treated fruit, as expected. However, the sugar content was significantly lower in the treated fruit compared to control at all days of observation (Table 2). This indicates that the ripening progressed normally due to EFF treatment, but at a reduced pace.

Table 2: Effect of enhanced freshness formulation on total sugars (per cent) of banana fruits

	Total Sugars (per cent)				**Ascorbic acid (mg/100g)**				**Respiration rate (mg $kg^{-1}h^{1}$)**			
Days	**28°C**		**4°C**		**28°C**		**4°C**		**28°C**		**4°C**	
	C	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**
3	10.32	9.78	10.06	9.32	9.00	8.65	8.23	8.02	22.54	23.12	23.98	24.78
6	10.63	10.31	11.32	9.78	9.45	8.99	8.67	8.34	21.12	22.45	22.78	23.67
9	11.21	10.65	11.86	10.12	9.67	9.13	8.98	8.89	20.13	21.23	21.34	22.45
12	11.98	11.23	12.05	10.65	10.00	9.78	9.23	9.00	19.34	20.12	21.12	21.09
15	12.03	11.78	12.35	10.86	11.01	10.23	9.45	9.10	17.23	18.67	20.09	20.89
18	12.65	12.14	12.98	11.32	12.23	11.56	10.48	10.12	16.34	17.22	19.23	19.34
21	14.98	12.87	13.42	11.76	13.24	12.24	11.67	10.78	14.23	16.23	19.01	18.23
24		16.07	13.54	13.31		13.09	12.23	11.09		15.78	18.78	17.54
27		18.54	16.01	16.98		14.56	13.89	11.89		14.98	17.56	16.56
30			18.65	18.47			14.24	12.67			16.21	15.34
33			19.47	20.65			14.89	13.80			15.23	14.09
36			20.78	21.01			15.35	14.23			14.08	13.67
39			21.31	21.54			15.98	15.32			13.56	12.45
42				21.98				16.12				11.67
LSD*	**0.156**		**0.168**		**0.135**		**0.130**		**0.200**		**0.183**	

LSD* Least Significant Difference ($P<0.05$) used to compare means within a treatment (temperature)
C-Control; T-Treatment- dipped in EFF solution for 5 minutes

Ascorbic acid

The ascorbic acid content increased during storage as expected. However, the ascorbic acid content in the EFF treated fruit was always significantly lower than control on any given day of measurement indicating the slowness of ripening due to EFF treatment (Table 2).

Respiration rate

The respiration rate of banana fruit declined gradually during storage, regardless of environmental conditions or treatments.

Although the respiration rate was higher in the treated fruit than control, the increase was not significant (Table 2).

Electron microscopy

The SEM images exhibited well-maintained structural lenticels on the fruit peel (Fig. 5) and the pulp cells had starch granules in the treated fruit, regardless of maturity (Fig. 3). Fruit skin was distorted and the pulp cells had fewer starch granules in the control fruit.

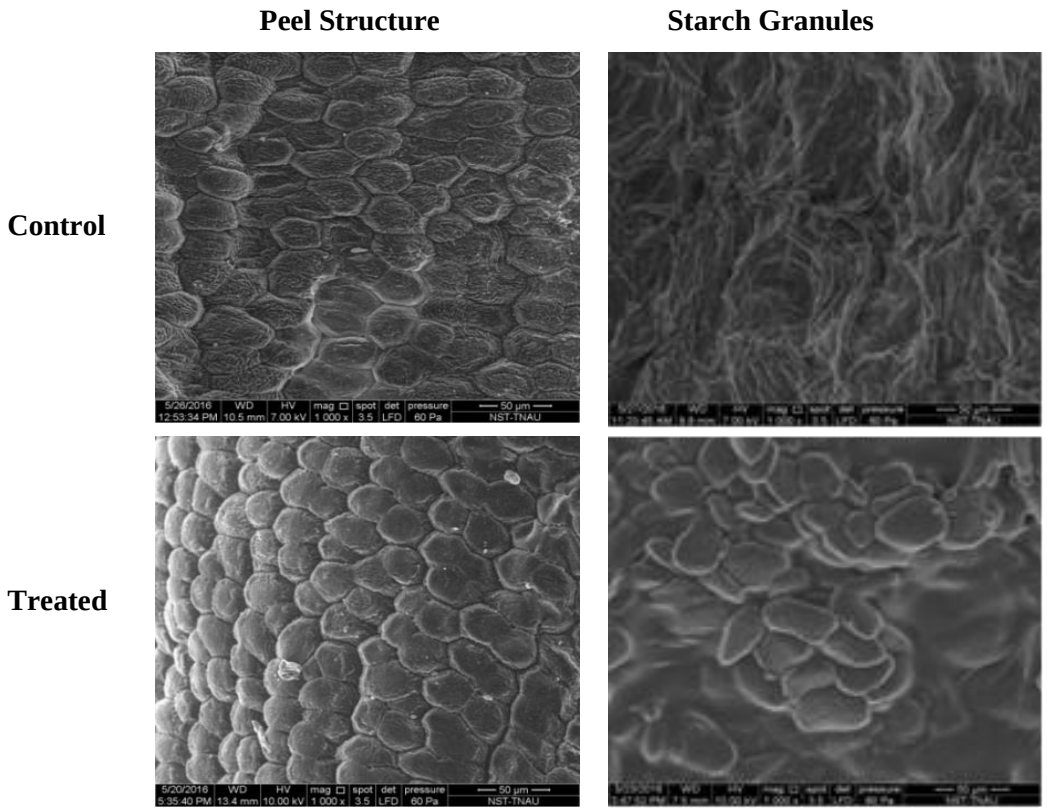

Figure 3: Peel structure and starch granule deposition of banana fruit in control and EFF-treated fruits on 18th day as visualized with scanning electron microscopy at 1000 X magnification. The lenticels in the peel are intact and plump in treated against a dehydrated and flat appearance in the control. Similarly the starch granules are intact in the fruit indicating that it has not fully ripened while they have completely broken down in the control.

Discussion

Hexanal is a plant-derived compound that has been used to inhibit the phospholipase D enzyme in the skin of the fruit and it has been associated with the extension of shelf life of fruit in temperate (Paliyath and Subramanian 2008; Sharma et al. 2010) and tropical climates (Anusuya et al. 2016; Jincy et al. 2017). The banana fruit dipped in EFF maintained significantly lower PLW values throughout the experiments, regardless of maturity at harvest or storage conditions. This may be attributed to the thickening of the cell wall as a consequence of the inhibition of the lipoxygenase enzyme. Our data, in combination with that in the literature, provides a body of evidence that supports the hypothesis that hexanal formulations facilitate skin thickening, which contributes to the reduced PLW. Our EFF had a similar effect in all the maturities of harvest indicating that the dipping technology could be useful for both domestic and export markets.

It is well established that climacteric fruit, such as banana, continue to respire even after being harvested and this leads to fruit spoilage. Many post-harvest management strategies have been designed to minimize the respiration rate to extend the shelf life of fruit. Our results demonstrated that the EFF lowered the respiration rates, which corresponded closely with shelf life extension during storage, regardless of maturities. Kader (2005) reported that increasing the CO_2 concentration by 7-8% and lowering the oxygen to 1% in controlled atmospheric storage extended the shelf life of

banana fruit as a consequence of the reduced respiration rate. Furthermore, Paliyath and Subramanian (2008) extended the shelf life experimentally, of temperate fruit treated with hexanal formulation in combination with 1-MCP and have shown that hexanal has a unique advantage of slowing down the respiration naturally without ill effects. A potent ethylene blocker, 1-MCP has been used in various commercial applications to extend the shelf life of fruit, vegetables, and flowers (Blankenship and Dole 2003). The only constraint with 1-MCP application in certain fruit and vegetables is that it completely arrests the ripening process with no possibility of reversing the arrested reaction. Reversing the reaction, however, is necessary for certain fruit and vegetables after long distance transport and before reaching the market. Hexanal treatment offers several advantages over 1-MCP treatment as it does not impair colour nor flavour development but delays senescence in (at least) apples and tomato fruit (Kondo et al. 2005; Cliff et al. 2009). The EFF-dipped bananas maintained higher firmness compared to control fruit throughout our experiments. The fruit retained higher firmness due to the action of hexanal, which seems to have reduced the activities of enzymes promoting pectin and hemicellulose degradation. The delay in softening may also be due to the reduced biosynthesis of cell wall hydrolases in addition to ethylene inhibition. Most fruit soften during ripening and this is a major quality attribute that often dictates shelf life. Fruit softening could arise from one of the three mechanisms: loss of turgor, degradation of starch, or breakdown of the cell walls. Loss of turgor is largely a non-physiological process associated with the post-harvest dehydration of the fruit and it can become important during commercial storage. Degradation of starch probably results in a pronounced textural change, especially in those fruit like banana, where starch accounts for a high percentage of the fresh weight (Turner and Fortescue 2002). Our data clearly demonstrate that EFF-dipped fruit remain fresh for longer periods of time irrespective of maturity at harvest.

Sugars, soluble portions of starch, organic acids, soluble pectin, and vitamin C are the components of total soluble solids (TSS) of banana fruit pulp. Reis et al. (2004) reported that a chemical dip, calcium chloride plus ascorbic acid, and modified atmosphere storage increased the TSS of banana pulp. In our experiment, total sugars in banana fruit increased with the progression of ripening under both ambient and reduced temperature storage conditions. In general, total sugar content in treated fruit was lower compared to controls, regardless of storage condition, suggesting the reduction in pace of ripening due to hexanal. Similarly, the cold-stored fruit had lower total sugar content compared to ambient-stored fruit. Our result is in agreement with that of Blankenship and Dole (2003) who also found that sugar level was dependent on the storage conditions. The lower sugars seen under reduced temperature storage conditions may be due to the inhibition of acid metabolism and dehydration, which reduced soluble sugar concentrations in fruit (Duan et al. 2008).

Ascorbic acid is one of the most important qualitative traits, especially with respect to human nutrition. The observed increase in ascorbic acid content might be due to a catalytic influence of hexanal on ascorbic acid biosynthesis from its precursor glucose 6-phosphate, or inhibition of its conversion to dehydroascorbic acid by the enzyme ascorbic acid oxidase or both. That ascorbic acid content increase is in agreement with the reports of Khan et al. (1976) in litchi. Pinaki et al. (1997) carried out an experiment with mature and fully developed banana fruit of uniform size that were dipped in GA3 at 150 ppm and they found that GA3 -treated fruit retained a higher titratable acidity and had lower ascorbic acid content during storage. Selvaraj (1993) conducted an experiment on mango fruit and showed that the acidity increased during maturity, which is closely associated with the

production of higher amounts of anti-oxidants. Our study showed an increase in ascorbic acid content in treated fruit.

Banana is a typical climacteric fruit in which the respiration rate reaches a peak during ripening (Albert 1926). The transformation from starch to sugar accelerates during the sudden increase of respiration (Clendennen and May 1997; Chen and Ramaswamy 2002). However, after the climacteric respiration phase, the respiration rate decreases (Cordenunsi and Lajolo, 1995; Waliszewski et al. 2003). Our results clearly show that starch granules are well preserved in the treated fruit compared to the controls, regardless of maturity indices; that peel structures with clear lenticels were well retained; and that respiration rates were lower in the treated fruit compared to the control fruit (data not shown). All of these results indicate that the ripening-associated processes were delayed by EFF in the treated fruit compared to the control fruit.

Conclusion

Overall, the results indicated that dipping of banana fruits in EFF extended their shelf life. The treated fruits maintained lower PLW and higher firmness throughout the study period, regardless of maturities at the start. In addition, treated fruit had lower total sugars, and higher firmness indicating less decrease in quality of fruit during storage compared to the control. The structural integrity of skin cells and fruit pulp seen in the high-resolution images of the treated fruit clearly showed a delayed ripening process. Based on these data we conclude that banana fruit dipped in 2% EFF for 5 minutes experience an extended shelf life of up to 6 days under ambient conditions and 9 days under reduced temperature storage conditions with the added advantage of improved fruit quality.

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