The effects of hexanal incorporated composite material (HICM) made of banana fibre and polymers on extending the storage life of mango fruit (*Mangifera indica* L. var TEJC) in Sri Lanka

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Maintaining the quality of perishable commodities during storage and transportation remains a challenge to the fruit and vegetable industry in many countries. In order to minimize post-harvest loss, a hexanal incorporated composite material (HICM) was created using hexanal, banana fibre, polymeric materials, and biopolymers. Efficacy of the HICM was tested on mango variety TEJC. Trials were conducted over two consecutive fruit seasons in 2016 and 2017. Six fruits were packed in corrugated cardboard cartons and eight cartons per treatment (with and without HICM) were stored at 13.5°C ± 2 °C and 92% relative humidity. Quality observations for each treatment were recorded at 7-day intervals for 28 days. Higher retention of fruit firmness and marketability were observed in fruits packed with the HICM with 50% of the fruit marketable after 21 days storage at 13.5 °C ± 2 °C (p <.05). Control fruits were not marketable at 21 days. Qualitative headspace gas chromatography molecular spectroscopy (GCMS) analyses were executed to determine the stability of released hexanal and the fate of hexanal when absorbed into fruit. Hexyl esters and hexanoate esters were observed in the headspace of HICM-treated fruits. Results from this study indicate that HICM treatment could be used along with low temperature storage to promote the marketability of TEJC mangoes.

Keywords: Mango storage, hexanal, polymer composite, banana fibre, fruit firmness, post-harvest loss, Sri Lanka

Mango (*Mangifera indica* L.*)* is among the five most widely grown fruits in the world. It is a popular fruit in many tropical countries with increasing availability and growing markets in developed nations. However, meeting the quality requirements of these new, lucrative markets poses many challenges. Post-harvest losses of mangoes continue to be high in most producing countries, particularly during storage and transportation.

Ripening plays an important role in the development and expression of mango characteristics such as colour and flavour. In mangoes and other climacteric fruits, ripening is initiated by the release of required (i.e. threshold) levels of ethylene, the hormone that triggers the natural ripening process (Burg and Burg 1962). Ripening results in fruit softening caused by cell wall and plasma membrane deterioration. Soft, ripe fruits are more susceptible to diseases and physical injury when passing through the supply chain.

Mature mango fruits are harvested just before initiation of natural ripening and at the firm-to-touch stage in order to minimize loss during storage and transportation. Fruit firmness is maintained at this stage of maturity when fruits are held at suitably low temperatures. Ethylene-inhibiting compounds, such as 1-methylcyclopropene (1-MCP), aminoethoxyvinylglycine (AVG), and abscissic acid, are also known to inhibit

ethylene synthesis and can be used to delay ripening of fruits (Zarah et al. 2013; Yuan and Li 2008). The compound 1-MCP has been registered for commercial use in several countries for climacteric and some non-climacteric commodities. However, it is a competitive gaseous ethylene inhibitor that binds irreversibly to ethylene receptors present in plant tissue (Blankenship and Dole 2003). It has been used to regulate tissue response to ethylene by blocking ethylene receptors in different fruits by varying application parameters such as concentration, time, and temperature (De Ell et al. 2001; Gong et al. 2002).

Phospholipase D activity is an autocatalytic reaction that takes place during the process of fruit ripening and senescence, and results in the destabilization of plant tissue (Tiwari and Paliyath 2011). Membrane degradation can be prevented by inhibiting the action of phospholipase D (PLD), present in the membranes of plant tissue, and the key enzyme involved in the process (Cheema et al. 2014). Hexanal has been used as a PLD inhibitor and both pre- and post-harvest applications have been shown to inhibit ripening in some commodities (Cheema et al. 2014; Tiwari and Paliyath 2011; Sharma et al. 2010).

Hexanal has also been observed to show anti-fungal properties. For instance, exposure of apple slices to hexanal vapour (4.1 µL/L for 48 hours) was reported to inhibit growth of pathogenic fungi by 50% (Song et al. 1996).

Hexanal occurs naturally in plants and is categorized as a generally recognized safe (GRAS) compound (Gunasekaran et al. 2015). It is recommended for use as a food grade additive as it is naturally produced through fatty acid degradation (Tiwari and Paliyath 2011; Sharma et al. 2010). Linoleic acid and linolenic acid are the biological precursors of hexanal and several enzymatic reactions are responsible for the generation of hexanal in biological systems (Gunasekaran et al. 2015).

Our study was initiated for the purpose of developing technology to facilitate the export of premium quality, Sri Lankan TEJC mangoes to distant markets. The retention of fruit firmness over extended storage periods plays an important role in preventing post-harvest loss. The technology developed is thus focused on maintaining firmness and quality of this perishable commodity during storage and transportation via the slow release of hexanal into the individual cartons carrying fruits.

Banana pseudo-stem fibre extracted from common banana varieties was utilized as the matrix for the HICM. Milani et al. (2016) have described banana fibres in detail. In light of their research and together with the fibres' porous structure, high cellulose content, and the possibility of its use as a substrate for absorption and the slow release of absorbed materials, banana fibre was determined to be a suitable ingredient for the composite material developed in our study and its effectiveness was tested against a control.

Materials and methods

Materials

Pseudo-stems of common banana varieties cultivated in Sri Lanka were used for fibre extraction (Milani et al. 2016). The mango variety TEJC harvested from Ellawala Horticultural Farm at Galkiriyagama, Dambulla, Sri Lanka, was used to test the efficacy of HICM. Hexanal (> 97% purity, Food Grade) and kappa-carrageenan were purchased from Sigma-Aldrich Inc., USA. Polyvinylpyrrolidone (M.W. approximately 44,000; product code P4405) was purchased from Superchem Products Ltd, Needham Market, Suffolk, England. Tapioca starch and polythene sheets were purchased from local, food-grade ingredient suppliers.

Methods

HICM preparation

Banana pseudo-stems were collected from local plantations. Dried sheaths and debris were cleaned manually. Pseudo-stem sheaths were separated and fibre was extracted using a mechanical fibre extractor. Separated fibre was sun dried for 24 to 48 days, depending upon weather conditions. The extracted fibre was then cut into pieces of less than 2 mm in length using a mechanical cutter.

The HICM was prepared as single units. Each unit of HICM consisted of banana fibre (11.0%), polymeric material such as polyvinylpyrrolidone (3.0%), a biopolymer mixture including tapioca starch (15.5%) and kappa-carrageenan (5.0%). The remaining percentage of material was comprised of low-density polyethylene film (50.5%). Each HICM unit was prepared by mixing hexanal (15.0% of final weight) with biopolymers and coating this mixture onto the banana fibre with polyvinylpyrrolidone, followed by storage in a closed glass bottle for 24 hours. The mixture was then transferred to a polyethylene sleeve and sealed. Thereafter, each sealed sleeve was wrapped in aluminum foil and subjected to a hot press for one minute at 130°C.

Headspace analysis

Headspace studies were carried out to investigate the hexanal release pattern of the HICM and to investigate the role played by hexanal in extending the storage life of mango. The slow release pattern of hexanal from the HICM was observed via two repeated trials using the headspace gas chromatographic technique. Observations were recorded over 31 days.

Freshly prepared composite material (2 g) was transferred into a 610 mL airtight bottle. Headspace gas samples (0.5 mL) for the analyses were drawn manually from the bottle via the septum on the lid, at daily intervals. The peak area was determined by gas chromatography (Agilent 6890 series system) using a Supelcowax 10 (fused silica) capillary column (30 m x 0.25 mm x 0.2 µm). The peak position for hexanal was observed at 5.68 min. The analyzing temperature was 60°C. The concentration was calculated using the constructed calibration curve. Cumulative release of hexanal was determined using the following equation:

$$X_n = 0.5\ (X/610)$$

Where, X is the derived concentration of headspace hexanal in bottle at the n^{th} time and X_n is the extracted concentration of hexanal at the n^{th} time interval. The cumulative extracted concentration was determined and plotted against the time of analysis. The analysis was done at both room temperature (28°C ± 2°C) and at 13.5°C ± 2°C - the optimum temperature for extending storage life of TEJC mangoes. Chilling injury in TEJC mango is observed below 11ºC (authors unpublished data).

Headspace gas chromatography/mass spectrometry (GCMS) analysis was carried out for the qualitative detection of the headspace constituents of HICM, the respective converted products of the hexanal throughout the storage time, and the aromatic volatiles created by the HICM-treated fruits. The gas samples drawn from the headspace were injected into a Thermo Scientific Trace 1300 series gas chromatograph coupled with an ISQ QD single quadruple mass spectrometer. A Thermo Scientific fused silica capillary column (Supelcowax: 30 m x 0.25 mm x 0.2 µm) was used for the analysis. Mass spectrometry was carried out with an ion capture detector operating in electronic impact mode with impact energy of 70 eV, a scan interval of 0.50 fragments, and fragments detected in the range of 50 Da to 450 Da. The headspace aromatic compounds were identified through the mass spectrum with spectra from the equipment database (NIST11). Analyses were done at room temperature (28°C ± 2°C).

In-vivo fruit testing

The aluminum foil cover enclosing the HICM was opened immediately before use and pasted inside the lid of individual, corrugated cardboard cartons after fruits were packed, and just prior to taping the cartons for storage. Six randomly selected mango fruits harvested at the mature green stage were placed in each carton (approximately 3.5 kg of fruit per carton) prior to low temperature storage at 13.5°C ± 2°C and 90% relative humidity. The trial consisted of TEJC mango fruits packed in cartons with the HICM, and control fruits packed in cartons without HICM. There were eight replicate cartons per treatment.

Trials were repeated over two consecutive seasons to test the reproducibility of results. Two randomly selected cartons containing 12 TEJC mangoes per treatment were analysed at 7-day intervals over a period of 28 days. Fruit firmness, flesh firmness, total soluble solid content (°Brix), acidity (expressed as % citric acid), pH, and overall cosmetic appearance for marketability were recorded.

Fruit and flesh firmness of mangoes were measured using a Guss Fruit Texture Analyser (GS-25) by compression to a depth of 5 mm and expressed in kilograms. Total soluble solid content was determined in juice obtained from mango fruits at room temperature using a hand-held prism refractometer (Kruss, 0-30, Germany). To determine the acidity, mango flesh (10 g) was blended with distilled water (40 mL) using a domestic blender (Summit, India), and the extract was filtered and titrated against 0.1 N NaOH using phenolphthalein as an indicator. Acidity was calculated as the equivalent fresh weight of mango fruit and expressed as per cent citric acid. The pH of extract was measured at room temperature by a pH metre (Hatch, Loveland, USA). Marketability of TEJC mango fruits was determined by visual examination of fruits in accordance with an index (developed by the Postharvest Technology Laboratory, ITI, Sri Lanka) from 1 to 10 where 1 is "marketable" and 10 is "not marketable." Percentage marketability was expressed as the number of marketable fruits as a percentage of total fruits for each treatment.

Statistical analysis

The experiment was conducted as a Completely Randomized Design (CRD) with eight replicate boxes per treatment and the trial was repeated in two consecutive fruit seasons. The experimental means for physico-chemical parameters were analysed via Student's t-test at a $p<0.05$ significance level using SPSS statistical software (version 13).

Results

The hexanal release pattern of HICM is presented in Figure 1. Hexanal release was observed to take place at a rapid rate during the first 200 hours and slow down thereafter as seen in Figure 1. The pattern of release of hexanal vapour was the same at both 13.5°C ± 2°C and at an ambient temperature of 28.0°C ± 2°C. Cumulative release of hexanal vapour did not reach a plateau for up to 744 hours).

Similar results were obtained in both seasons—January 2016 and July 2017—with respect to variation in fruit firmness, total soluble solid content, pH and acidity (% citric acid) in the HICM treated and the untreated controls during storage at 13.5°C ± 2°C, over 7, 14, and 21 days. The means of the above fruit quality parameters recorded during the second season (July 2016) are summarized and presented in Table 1.

The percentage marketable fruits in the untreated controls and those in the HICM treatment are presented in Figure 2. Both HICM treated and the untreated controls remained in good condition at 13.5°C ±2°C during the first two weeks. However at 21 day, due to the emergence of disease symptoms associated with the anthracnose and stem end rot pathogens, only 50% of HICM treated fruits were of marketable quality whereas all control fruits were not in marketable condition.

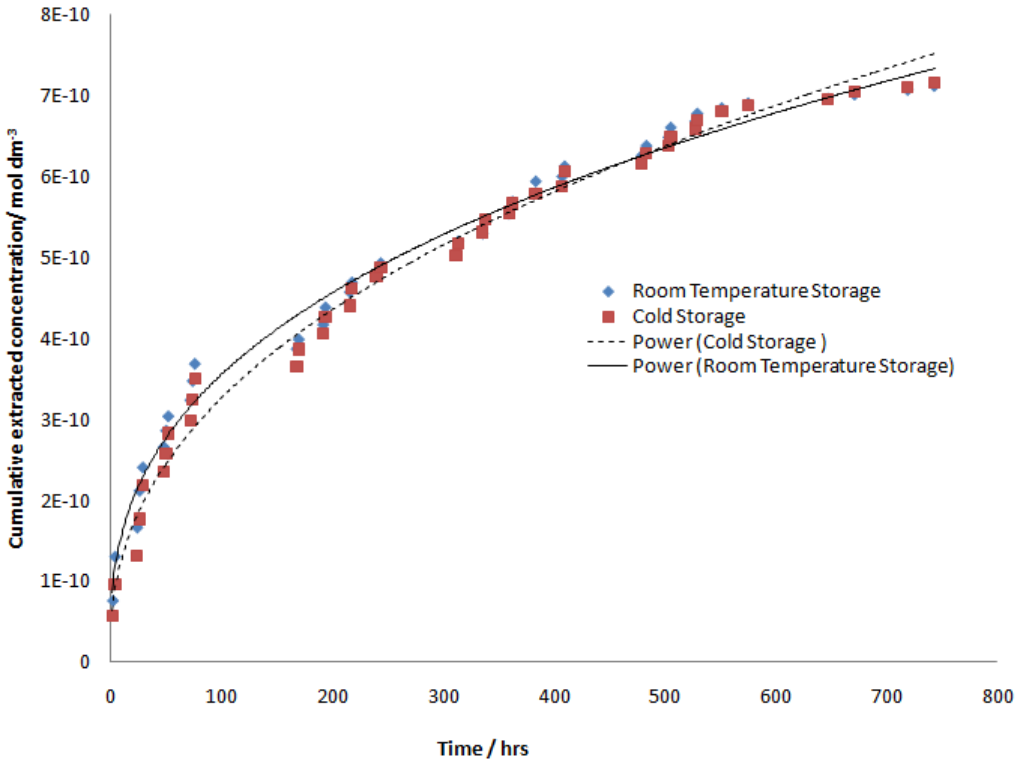

Figure 1: Cumulative release pattern over time of hexanal from hexanal incorporated composite (HICM) Material during storage at room temperature (28°C ± 2°C) and at cold temperature (13°C ± 2°C).

Table 1: Fruit quality parameters of TEJC mangoes in relation to hexanal incorporated composite (HICM) and control treatments after cold storage at 13.5°C ± 2°C

Parameter	Storage period (days)	Control	HICM
Fruit firmness (kg)	7	5.48 ± 0.61	6.62 ± 0.05
	14	3.00 ± 0.16	3.80 ± 0.11
	21	-	1.62 ± 0.32
Total soluble solids content (°Brix)	7	15.75 ± 0.25	15.50 ± 0.29
	14	16.75 ± 0.25	16.75 ± 1.31
	21	-	15.50 ± 0.64
pH	7	4.07 ± 0.05	3.93 ± 0.02
	14	4.88 ± 0.06	4.82 ± 0.06
	21	-	5.27 ± 0.07
Acidity (% citric acid)	7	0.81 ± 0.02	0.83 ± 0.03
	14	0.36 ± 0.02	0.37 ± 0.01
	21	-	0.22 ± 0.02

Note: Control fruits were not in marketable condition at day 21 and thereafter. Data presented as mean ± standard deviation. Statistical significance (*) defined as $p < .05$ was determined by t-tests

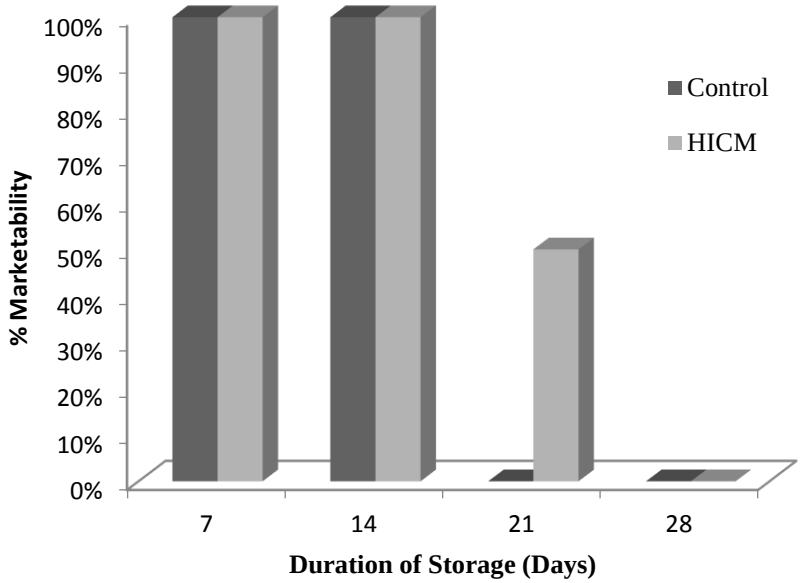

Figure 2: Percentage difference in marketability of TEJC mangoes in relation to treatments (HICM and control) over 28 days of cold storage (13°C ± 2°C) with significant difference observed after 21 days (p=0.05).

Discussion

TEJC mango fruits were developed as an export-oriented mango variety in Sri Lanka. The variety is in high demand due to its inherent good taste, cosmetic appearance, and high flesh content. Shelf life extension of the said variety provides the opportunity to ship them by sea to lucrative overseas markets with economic benefit to Sri Lanka. Therefore, the HICM was developed to achieve the slow release of hexanal vapour into the fruit pack to extend the storage shelf life of TEJC mango fruits.

The HICM released hexanal at a rapid rate during the first 200 hours but was observed to slow down thereafter (Figure 1). The releasing pattern of hexanal vapour was the same at 13.5°C ± 2°C and 28.0°C ± 2°C. The in vivo HICM treatment trials with mango fruits were conducted over 28 days. The cumulative release of hexanal vapour in the closed environment in vitro studies did not reach a plateau up to 744 hours (day 31), indicating the possibility of using the active composite material (HICM) during storage for a period of 31 days. This would make it particularly useful for the export of mango.

Hexanal is a reactive aliphatic aldehyde and has high oxidizing potential on exposure to air. Our qualitative headspace GCMS study of HICM indicated that hexanal is oxidized into hexanoic acid and 2-butyl, 2-octenal—a crotonated product of hexanal—at day 7 at room temperature (28°C ± 2°C). According to our qualitative headspace GCMS study on aroma volatiles, β-ocimene, α-pinene, ethyl butanoate, and caryophyllene were observed as prominent aroma compounds for the TEJC variety. Hexyl esters and hexanoate esters, such as hexyl acetates, hexyl butanoate, and hexyl hexanoate, were also found in our qualitative GCMS headspace analysis of HICM-treated fruits. These hexyl esters and hexanoic esters were released into the headspace on absorption of hexanal by the

fruit, indicating the occurrence of both oxidative and reductive metabolic pathways. Aldehyde dehydrogenase is the enzyme responsible for oxidation of hexanal in living tissues. The condensation of hexanal with pyruvate in the presence of the pyruvate dehydrogenase enzyme reduces hexanal to hexanol and other by-products (Jaar et al. 1999). Hexanoic esters and hexyl esters are reported as natural aroma compounds in Sri Lankan mango varieties such as *Jaffna* (MacLeod and Pieris 1984).

Variation in fruit quality parameters in relation to treatments and the storage duration (7, 14, and 21 days) at 13.5°C ± 2°C is summarized in Table 1. Reduction of fruit firmness and fruit softening are natural phenomena associated with ripening and are the result of degradation of the cell wall architecture (Tiwari and Paliyath 2011). Determination of the extent of softening of stored fruits through firmness methodologies is practiced as a predictive measure of post-harvest condition. Firm fruits are less susceptible to bruising. Reduction of losses due to damage during post-harvest operations and retention of fruit firmness through Phospholipase D inhibition have been reported previously (Sharma et al. 2010; Valero et al. 2007). Decrease in firmness is a common phenomenon observed during storage of fruits and is due to weakness of cells below the outer skin of fruits (Sirisomboon and Lapchareonsuk 2012). The current study revealed that controlled release of hexanal vapour results in a significantly higher ($p=0.05$) fruit flesh firmness at day 7 (Table 1). Firmness has an impact on fruit quality as excessive degradation of polymeric components present in the cell wall results in loss of fruit quality (Sharma et al. 2010). Retention of fruit firmness helps to reduce post-harvest loss as well as reduce the incidence of disease due to fungal invasion through the weakened fruit skin. After absorption, hexanal is not accumulated in fruit tissue, but is further converted to hexanol which is metabolized during the respiratory cycle (Cheema et al. 2014). Unlike the ripening inhibitor, 1-methylcyclopropene (1-MCP), which down-regulates several genes involved in cell wall degradation and does not have significant effect on maintaining cell wall rigidity and firmness, hexanal treatment preserves the firmness of fruits without the heavy involvement of down-regulating genes related to cell wall degradation (Tiwari and Paliyath 2011).

Our study revealed that pH was low at day 7 of low temperature storage in fruits exposed to the HICM treatment compared to controls. Hexanal vapour released by the composite material had no effect on the total soluble solids content and per cent acidity of the fruits, and enabled the normal development of these characters during the ripening process. Our study indicates that HICM treatment has the potential to maintain the marketability of mangoes for more than 21 days, while 100% of the control fruits deteriorated at 13.5°C ± 2°C with symptoms of senescence and disease after 21 days of storage.

Conclusion

While all control TEJC mangos in this study were not of marketable quality after prolonged storage, 50% of the HICM treated fruits remained marketable even after 21 days when stored at 13.5 °C ± 2 °C. Incorporation of an effective slow release volatile antifungal agent into the current HICM formulation is necessary to reduce incidence of disease and extending the storage period of these fruits at commercially acceptable levels of loss for 21 to 28 days. Sri Lanka's TEJC mangoes have a rich, exotic flavour and an attractive appearance and are well suited for both domestic and lucrative, distant export markets. The slow release of hexanal by the HICM at room and cold storage temperatures as observed in this study indicates the potential

for further adaptation of this product for commercial use in the shipment of mangoes to distant destinations. Further, the utilization of banana pseudo-stem fibre as an ingredient provides the opportunity for exploiting a hitherto underutilized agricultural waste product, which would otherwise act as a breeding ground for pathogens and pests in banana orchards, and contribute to the emission of greenhouse gases when it is burned.

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