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RESEARCH ARTICLE

Slow Release of Hexanal By Biodegradable Electrospun Nanofibres for Increasing Shelf-Life of Harvested Mango Fruits

Badal Kumar Biswal[1] and Kizhaeral S. Subramanian*[2]

[1]Department of Nanoscience and Technology, Tamil Nadu Agricultural University, Coimbatore - 6410 03.
*[2]Director of Research, Tamil Nadu Agricultural University, Coimbatore - 641 003.

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ABSTRACT

Phospholipase D (PLD) is a crucial enzyme indeterioration of membrane phospholipid by a cascade of metabolic reaction which can be effectively inhibited by hexanal, a C6 aldehyde (volatile) naturally produced asplant defense mechanism. In this study, hexanal is encapsulated by PVA nanofibre intercalated with nanocellulose using electrospinning. In ourstudy, nanocellulose was prepared using biodegradable wastes from banana pseudostem of Grand naine and Poovan cultivars through acid hydrolysis method to increase the loading capacity of matrix. anocellulose extracted from Grand naine and Poovan varieties were found to be 15-25 nm respectively.Further, the prepared matrices were loaded with hexanal in both passive and active method. The effect of hexanal loaded nanofibre on mango fruits in extending shelf life was studied. The actively loaded matrix showed slower and uniform release of hexanal inside the packaging box, while passively loaded matrix initially exhibited burst release pattern, followed by a decrease in the hexanal release pattern. Fruit ripeness parameters such as physiological loss in weight (PLW), vitamin C content, total soluble solids (TSS) and change in fruit colour were positively correlated with the hexanal release pattern till 11 days of storage period at room temperature.These findings give an opportunity to explore electrospinning and encapsulation of various volatile compound for slow release application in post-harvest studies of perishables.

Keywords: *Electrospinning, Hexanal, Nano-fibrillated Cellulose (NFC), Encapsulation, slow release, post-harvest losses, mango.*

INTRODUCTION

India is the largest producer of mango (*Mangifera indica*) contributing 77% of global production (Rekha priyadharshini, 2015) and Andhra Pradesh (25.39%) (Singh et al., 2018) leads the mango production among Indian states. But in various stages such as farm level, transporting, marketing, storage, retail, processing unit and at consumer level mango fruits incurred about 35% of loss (Sab *et al*., 2017). Harvested mango fruit subjected to many desirable physiological changes such as senescence, ripening, loss of water etc. which influences its colour, flavour, texture and nutritive values (Esguerra and Rolle, 2018). These changes make a mango fruit attractive and edible but also susceptible to deterioration. Though these processes are programmed and cannot be shut down completely, but it can be regulated by using various technologies.

Abscisic acid (ABA) regulates the fruit maturation and senescence by enhancing respiration and ethylene production in fruits (Yokotani *et al*., 2009) which causes softening and ripening (Zhang *et al*., 2009). Phospholipase D initiates membrane deterioration which causes ripening and senescence (Paliyath *et al*., 2012; Paliyath *et al*., 2008). PLD catalyses the breakdown of phospholipids to a higher amount of phosphatidic acid (PA) which causes rapid membrane deterioration (Bargmann and Munnik, 2006) by generating various reactive oxygen species (Peng and Mao, 2011). Further, PLD activity have been reported to be enhanced in the presence of optimum concentration of ABA and ethylene (Fan *et al.,* 1997).Hence, post-harvest deterioration of fruits (mango) can be delayed by inhibiting PLD activity or ethylene biosynthesis. Several chemical inhibitors such as 1-methylcyclopropene (1-MCP) (Fan *et al.,* 2000; Jiang *et al.,* 1999), nitric oxide (Zhu *et al.,* 2006), polyamines (Valero *et al.,* 2002), lysophosphatidyl-ethanolamine (Ryu *et al.,* 1997), chitosan (Hong *et al.,* 2012), hexanal (Sharma *et al.,* 2010) etc. have

*Corresponding author's e-mail: kssubra2001@rediffmail.com

been used to inhibit ethylene biosynthesis, PLD activity as well as increasing antioxidant activity.

Hexanal is reported to be highly volatile when applied or stored under ambient temperature. Hexanal is a potent inhibitor of phospholipase D which is highly responsible for senescence and rapid ripening and deterioration of climacteric fruits. To address this problem, hexanal has been used through various approaches such as vapour exposure (Ashwini *et al.,* 2018; Thavong *et al.,* 2010; Utto *et al.,* 2008), enhanced freshness formulation (EFF) (Gill *et al.,* 2015; Sharma *et al.,* 2010), β-cyclodextrin inclusion complex(Almenar *et al.,* 2007) etc. to increase shelf-life of different fruits. Due to volatile nature of hexanal, it is crucial to devise a method in which it is released gradually for a prolonged period of time. Recently, by using nanotechnology tools and techniques volatiles like hexanal can be trapped for longer period of time and released in controlled manner. Nanotechnology has been used to solve various day-to-day problems with precision in the field of health, engineering and agriculture by implementing different techniques (Subramanian and Tarafdar, 2011). Electrospinning (Formhals, 1934) is a technique, which is used to synthesize nanosized polymeric fibre by applying high voltage. In electrospinning, the polymer solution is subjected to high potential difference, which reduces surface tension of liquid resulting in formation of nanofibres accelerated towards collector of opposite polarity (Bhardwaj and Kundu, 2010; Liang *et al.,* 2007). Electrospinning process helps in controlled delivery of active compounds, volatiles, growth factors and cells within polymeric or biomaterials carrier (Liebmann and Klimov, 2011; Yu *et al.,* 2009). In the present study, hexanal was loaded in the electrospun fibres consisting of nano-fibrillated cellulose (NFC) derived from pseudostems of Grand naine and Poovan blended with PVA to extend the shelf-life of mango. Their effectiveness was accessed through laboratory scale shelf-life evaluation tests.

MATERIAL AND METHODS

Materials

All the experiments were conducted in Department of Nano Science and Technology, Tamil Nadu Agricultural University (TNAU), Coimbatore. Chemical such as hexanal, ethanol, glacial acetic acid, nitric acid, sulphuric acid, toluene, acetone, anthrone reagent, decahydronapthalene, sodium sulphite, tween 60, oxalic acid, ascorbic acid, 2,6- dichlorophenol indophenol and poly-vinyl alcohol (Cold) of AR grade were purchased from Sisco Research Laboratories. All glasswares were purchased from Borosil pvt. ltd. Electrospinning instrument (ESPIN NANO), used for production of nanofibres was purchased from IIT, Chennai. Double distilled water was used whenever necessary. Fresh unripe and matured mangoes var. Bangalora was used for the shelf-life study to understand the effect of hexanal.

Extraction and quantification of cellulose from banana pseudostem

Banana pseudostems of two cultivars (Grand naineand Poovan) were harvested from TNAU orchard and fibres were extracted by RASPADOR banana scrapper located at Eco-green unit, Madampatti, Coimbatore. The banana sheaths were combed using the machine to get the fibre in which the moisture and muscilages got removed. Moisture content in the extracted fibers were calculated by gravimetric method.

The extraction of cellulose from banana pseudostem was done in 2 stages i.e. dewaxing (Brinchi *et al.,* 2013) and delignification (Brendel *et al.,* 2000). Dewaxing was done by boiling the fibres in a mixture of toluene: ethanol (2:1) for 6 hours. The residual fibres were washed with ethanol for 30 minutes and dried in hot air oven till constant weight is attained. The dewaxed fibres were treated with 69% nitric acid: 80% acetic acid (1:10) at 130ºC for 30 minutes to get the delignified cellulose fibres. The unprocessed reddish-orange delignified cellulose was washed with 99% ethanol and doubled distilled water in alternate sequence until white coloured cellulose fibres were obtained. The white coloured delignified holocellulose was washed with analytical grade acetone twice and then it was suspended in acetone for further use in future (Scheme 1). The amount of holocellulose produced was recorded for each 100mg of banana fibre by gravimetric method.

Quantification of 3 major components (cellulose, hemicellulose and lignin) of banana pseudostem fibers were carried out to estimate yield recovery. Total cellulose, hemicellulose and lignin were estimated by UpDegroff method (Updegraff, 1969), detergent solution method (Soest and Mcqueen, 1973) and Tappi protocol (Tappi, 2006), respectively.

Synthesis of nano-fibrillated cellulose (NFC)

Dried holocellulose fibers (100 mg) were immersed in acetone (50 ml) and probe sonicated (Spinco analytica) for 30 minutes at 40% amplitude. The NFC (1%) was uniformly mixed with the PVA (10%) solution to get PVA-NFC (1%).

Production of nanofibers and hexanal loading by electrospinning

Electrospinning of nanofibres were carried out on a conducting aluminium sheet (0.5 gauge) with 25 KV voltage, tip to collector distance of 20 cm, drum rotation (600 rpm/min.), syringe translation (0.8

m/min) and flow rate (0.2 ml/h) at 25°. Hexanal was loaded both actively and passively to produce hexanal loaded nanofibers.

1. Active Loading

The formulation for active loading hexanal was prepared in 2 concentrations such as 5% and 10%. Initially, 20% and 40% of hexanal solution was prepared in tween 60. Then 1 ml of hexanal solution were added to a mixture of 3 ml of 10% PVA/PVA-NFC (1%) and 1 ml of 0.01% gum arabic to prepare final electrospinning solution of 5% and 10%, respectively.

2. Passive Loading

Hexanal solution for passive loading was prepared for both 5% (0.5 ml hexanal, 4 ml tween 60 and 5.5 ml distilled water) and 10% (1 ml hexanal, 4 ml tween 60 and 5 ml distilled water). Few drops of this solution were added to the 10% PVA/PVA-NFC (1%) electrospun nanofibres to obtained the passively loaded matrix.

Characterization

The degree of physical changes in active loaded electrospun fibers were visualized through Quanta 250, FEI scanning electron microscope (SEM). NFC from both Grand naine and Poovanwere characterized in FEI TECHNAI SPRIT transmission electron microscope (TEM). EDAX and XRD of both NFCs were observed to assess the elemental composition and crystalline nature, respectively. Functional groups were identified by Bruker-Tensor 27 FT-IR spectroscopy. The hexanal release kinetics was evaluated by TRACE GC ULTRA GC-MS. In this experiment, 1 cm x 4 cm of electrospun matrix was cut and placed in a 20cc vial. The experiment was done in triplicates and sampled for 2.5 h at 30 minutes interval.

Shelf-life evaluation

A 4 cm x 10 cm of electrospun film was positioned on the internal top side of mango packaging box. This experiment is conducted in ambient temperature and pressure. Each treatment consists of 3 replication and each box containing 5 nos. of mangoes. The study was conducted for 2 weeks by examining physiological weight loss (PLW) (Ding *et al.,* 1998; Yadav *et al.,* 2013), Total soluble solids (TSS) content (Islam *et al.,* 2013), study of fruit colour by HUNTERLA Bcolorimeter (Srinivasa *et al.,* 2004) and estimation of vitamin C content(Patrick *et al.,* 2016). The readings were observed at 3 days interval upto 11 days.

RESULTS AND DISCUSSION

Quantification and characterization of NFC

Nanofibrillated cellulose (NFC) has beenwas selected for the active packaging material for sustained release of hexanal. The moisture content of the extracted fibres from Grand naine and Poovan was found to be 23.1% and 21.3%, respectively. Chemical analysis of banana pseudostem fibres of Grand naine and Poovan variety resulted in 33.6% and 24.3% of cellulose (Figure. 1a) which is in contrast to earlier reports clarifying that banana

Scheme 1. A schematic representation of preparation of nano-fibrillated cellulose (NFC) from banana pseudostem fibres by the process of 1. Delignification, 2. ultra-sonification

pseudostem fibre contain more than 50% cellulosic fibre (Jayaprabha *et al.,* 2011; Preethi and Murthy, 2013). Holocellulose extracted from banana pseudostem of Grand naine and Poovan were found to be 27.82% and 28.4%, respectively (Figure. 1b). This might be due to lower percentage of lignin and ash content found in Poovan variety (Gopinathan *et al.,* 2017; Khan *et al.,* 2013; Preethi and Murthy, 2013). Further, NFCs synthesized from both varieties were characterized in TEM (Fig. 2a, 2b) and the diameter ranged from 15–25 nm and possess net like fibre structure (Gopinathan *et al.,* 2017; Sofla *et al.,* 2016). EDAX analysis revealed that Poovan NFC contain more carbon than Grand naine NFC

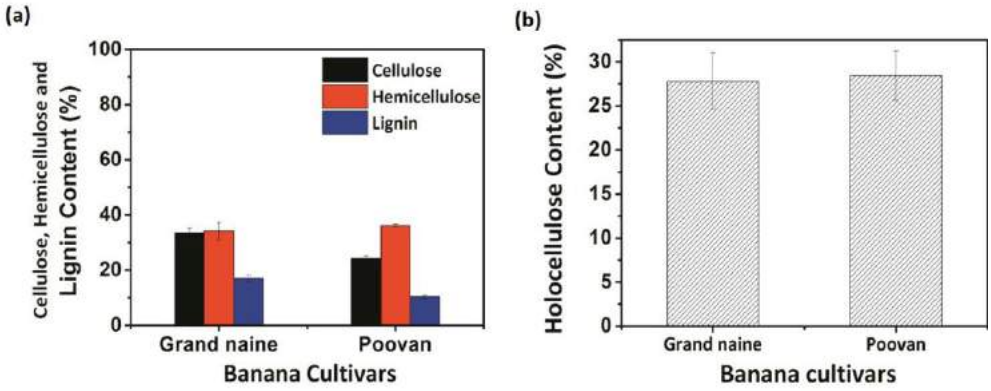

Figure 1. Holocellulose content extracted by delignification process compared with total cellulose, hemicellulose and lignin content from Grand naine and Poovan fibres.

(inset in Figure. 2a, and 2b) (Sofla *et al*., 2016). FT-IR spectra were compared for functional group analysis between both NFCs (Figure. 2d). FT-IR spectra of both NFCs can be divided into 2 regions. A broad band was observed in the range of 3600–3100 cm^{-1} which corresponds to O-H stretching vibrations (Fengel, 1993). The band is was narrower in case of Grand naine NFC and also the peak value (3428.14cm^{-1}) is was at lower wave length than Poovan NFC (3434.43 cm^{-1}) which is attributed to reduced intra- and intermolecular hydrogen bonds. Confirmation of other cellulose peaks attributed to cellulose such as 2911.56 cm^{-1} corresponding to H-C-H (alkyl) vibrations, 1639.04 cm^{-1} corresponding

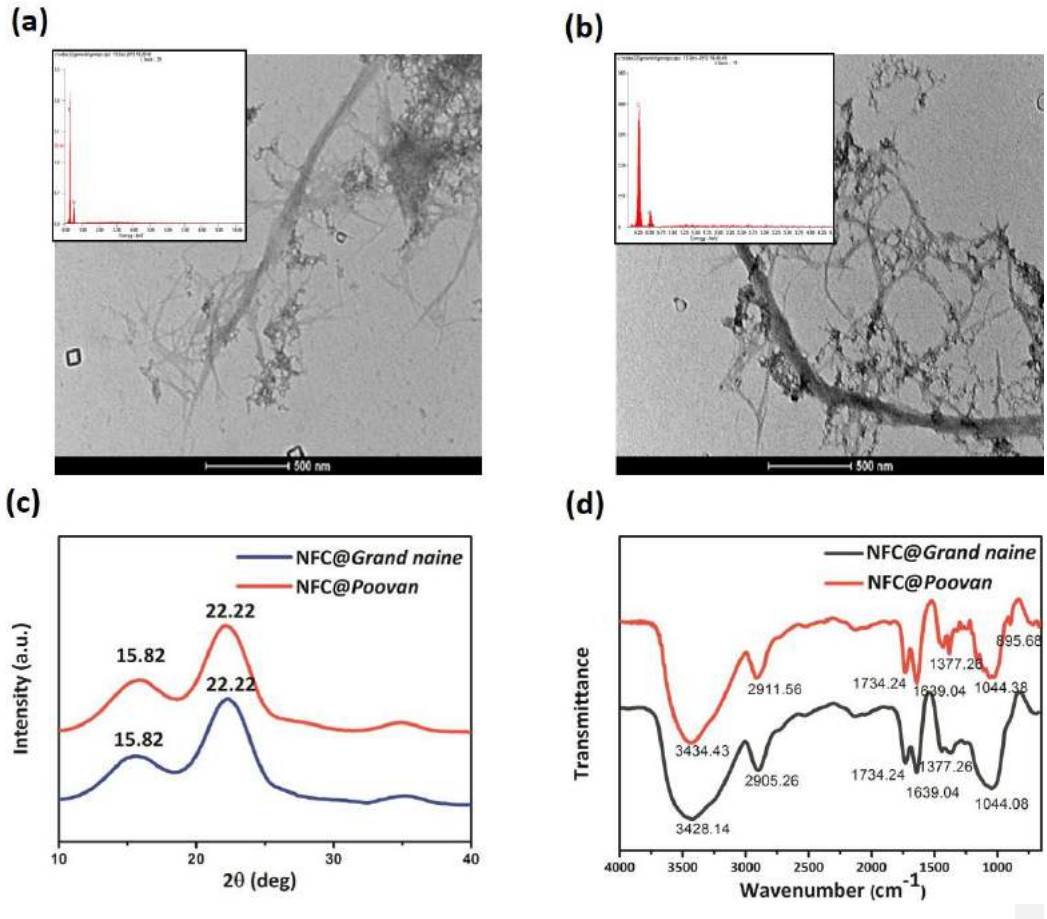

Figure 2. Characterization of nano-fibrillated cellulose (NFC) derived from Grand naine and Poovan (a) Transmission electron microscope image of the NFC derived from Grand naine banana variety with its EDAX (inset). (b) Transmission electron microscope image of the NFC derived from Poovan banana variety with its EDAX (inset). (c) XRD spectral comparison between Grand naine and Poovan NFCs. (d) FT-IR (ATR mode) spectra of Grand naine and Poovan NFCs.

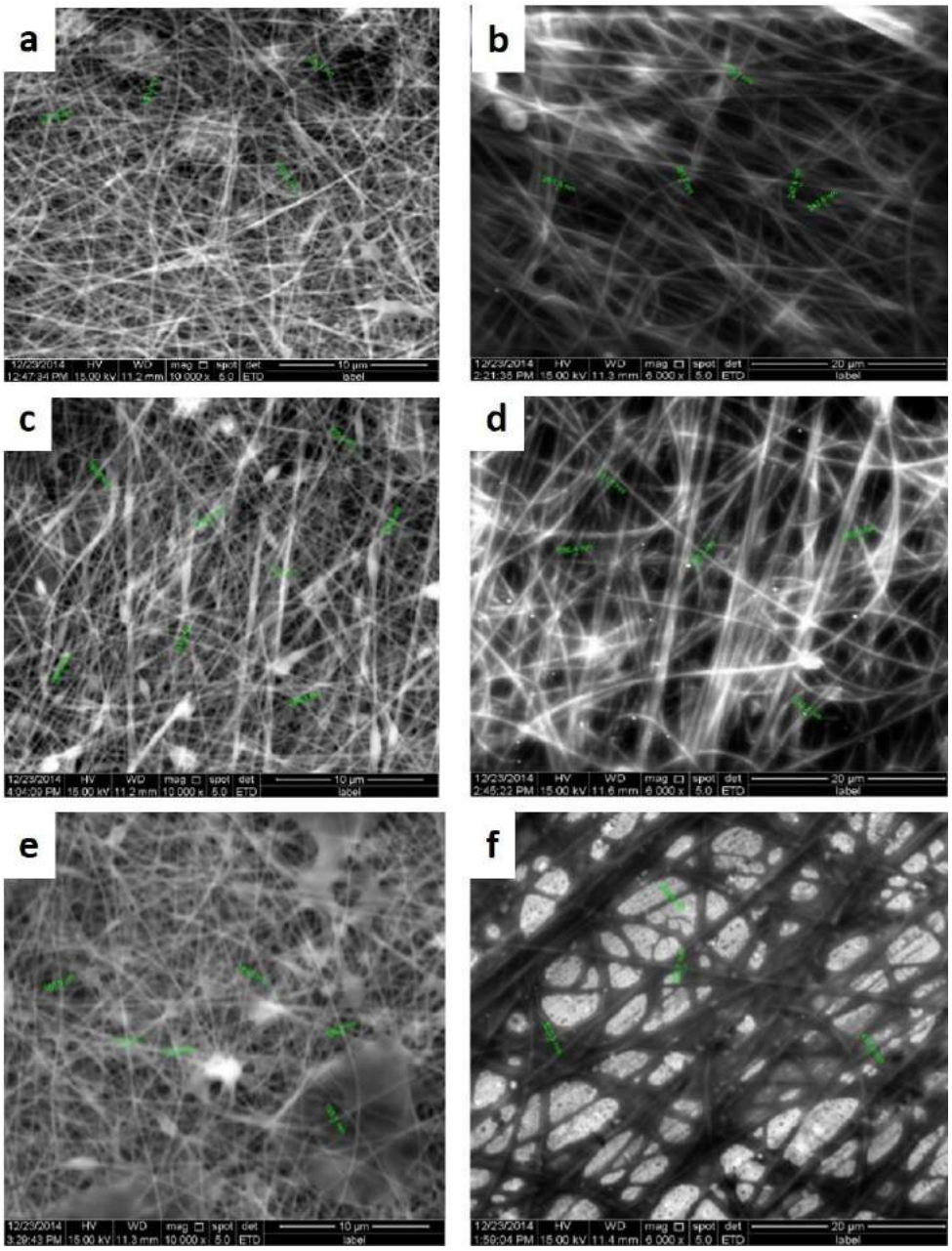

Figure 3. SEM image of electrospun fibres showing the morphological changes before and after loading hexanal (a) PVA electrospun fibres at 10000x magnification, (b) PVA electrospun fibres loaded with hexanal at 6000x magnification, (c) PVA-NFC@Grand naine electrospun fibres at 10000x magnification, (d) PVA-NFC@Grand naine electrospun fibres loaded with hexanal at 6000x magnification, (e) PVA-NFC@ Poovanelectrospun fibres at 10000x magnification and (f) PVA-NFC@Poovan electrospun fibres loaded with hexanal at 6000x magnification.

to adsorbed water (hydroxyl), 1734.24 cm^{-1} corresponds to the presence of hemicellulose (C=O) and 1038.48 cm^{-1} corresponding to C-OH vibrations have been similar in both NFCs (Fan *et al.,* 2012; Oh *et al.,* 2005; Poletto *et al.,* 2014; Yang *et al.,* 2007). The absence of 1745 cm^{-1} peak value confirms that processed cellulose did not undergo acetylation during delignification process (Moran *et al.,* 2008). The XRD pattern of NFCs showed amorphous spectra with broadened peak at 2θ = 15.82° and 22.22° with miller indices 101 and 200 (Park *et al.,* 2010; Zuluaga *et al.,* 2009), respectively but no major difference was observed between both NFCs (Figure. 2c) while FT-IR spectra of Poovan showed a low intensity peak value at 895.68 cm^{-1} which is assigned to β-(1→4)-glycosidic linkage (Ciolacu *et al.,* 2011). The crystalinity of cellulose can be estimated by the difference between ratio of absorbance/transmittance at 1377.2 cm^{-1}, 2900 cm^{-1} and 1430 cm^{-1}, 895 cm^{-1} which confirms Grand naine is more crystalline than Poovan NFC (Ciolacu *et al.,* 2011).

Characterization of electrospun nanofibers

Using electrospinning technique, PVA and PVA-NFC nanofibres were loaded with hexanal. The SEM image of electrospun PVA nanofibre without addition of NFCs observed formation of beadsmight be due to irregular flow rate of polymer solution (Figure 3a). The average diameter of individual fibreiswas 98.63 ±6.48 nm. When loaded with hexanal solution (Figure 3b), nanofibresobserved increase in diameter (237.7 ±32.17 nm) because of interaction between hydrophilic polymer (PVA), hydrophobic volatile active ingredient (hexanal) and non-ionic sur

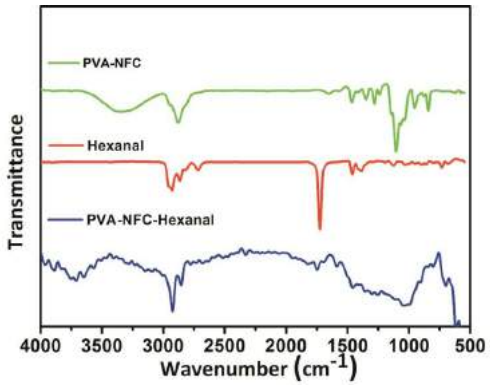

Figure 4. FT-IR characterization and comparison study of PVA-NFC e-spin fibre,

hexanal and hexanal loaded PVA-NFC e-spin fibre factant (Tween 60) under electrospinning condition (Dvores *et al.,* 2012). Rapid evaporation of the solvent increases the PVA concentration which induces depletion-flocculation of hexanal micro-droplets which leads to aggregation (Arecchi *et al.,* 2010). Hence, an increase in fibre diameter has been observed in which PVA encapsulated the micro-droplets ofhexanal. Furthermore, PVA impregnated with NFC showed increased fibre diameter for PVA-NFC@Grandnaine (184.76 ±37.12 nm) and PVA-NFC@Poovan (151.82 ±31.43 nm) as compared to only PVA electrospun fibres (Figure 3c and 3e). From Figure. 3d and 3f, demonstrates that PVA-NFC@Grandnaine-hexanal fibres were cylindrical in structure with beads formation at regular distance. In contrast, PVA-NFC@ Poovan-hexanal electrospun fibres were branched, beaded, multi-fibrillated and ribbon shaped. There was also a significant difference in fibre diameter between both matrices. This might be due to higher hexanal loading in PVA-NFC@Poovan-hexanal fibres due to more amorphous nature of Poovan NFC which is in agreement with the FT-IR spectral data of NFCs.

Functional groups and their properties of prepared nanofibre composite and loading of hexanal were studied by ATR mode (Figure 4). The characteristic peaks of PVA at 3353.53 cm^{-1} corresponds O-H stretching vibration, 2949.12 cm^{-1} is assigned to vibrations from C-H alkyl group, 1464.58 cm^{-1} for CH2 group and 1104.78 cm^{-1} as C-O-C group has been observed. NFC has been characterized by peak values of 2881.11, 1651.07, 1036.04 and 843.71 cm^{-1}. The hydroxyl band has shown more broadening as compared to both PVA and NFC FT-IR spectra. Hexanal loaded nanofiber showed prominent peaks at 2936.83, 2856.24 (Q-band) for aldehyde group and a sharp peak at 1725.65 attributed to C=O functional group confirms the hexanal loading in the PVA-NFC electrospun fibre (Adhikari *et al.,* 2003). Other peaks of PVA and NFC were also expressed at very low intensity in the FT-IR spectra of hexanal loaded electrospun fibre.

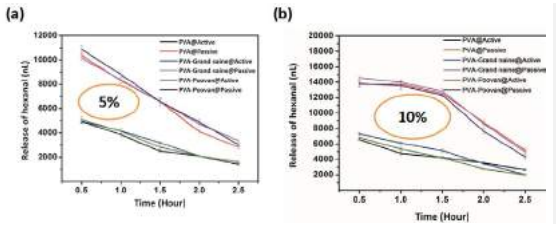

Figure 5. Efficiency of PVA, PVA-Grand naine NFC and PVA-Poovan NFC electrospun fibres to release hexanal by (a) 5% loading in both active and passive method, (b) 10% loading in both active and passive method

Hexanal Release Efficiency Using GC-MS

The release of the hexanal from the electrospun nanofibre was accessed by GC-MS. Although there were no significant differences found between overall releases of hexanal from fibres, 5% loaded matrices facilitate steadier release than 10% loaded matrices (Figure 5). It was observed that in passively loaded fibreundergo burst release for first one hour and then the release rate was decreased rapidly. PVA-NFC@Poovan and PVA have been found to be most persistent under active loading and passive loading, respectively. It might be due to amorphous nature of Poovan NFC (Figure 2c) which can retain more hexanal micro-droplets. In passive loading, due to the hydrophilic properties of PVA,the matrix can bind to the surfactant (tween 60) modified hexanal but NFC loaded PVA matrices did not resulted in major adsorption as NFCs were encapsulated by PVA which might act as a barrier to hexanal molecule.

Post-harvest shelf-life study of mango fruits

While in storage, fruits undergo many physico-chemical changes such as skin colour, metabolism, firmness, pH, TSS, acidity etc. In this study, Physiological loss in weight, peel colour changes, TSS, vitamin C content changes and the effects of controlled release of hexanal have been investigated for 2 weeks and discussed on mango fruits in the following sections.

1. Physiological loss in weight. The changes in the weight of individual mangoes were recorded

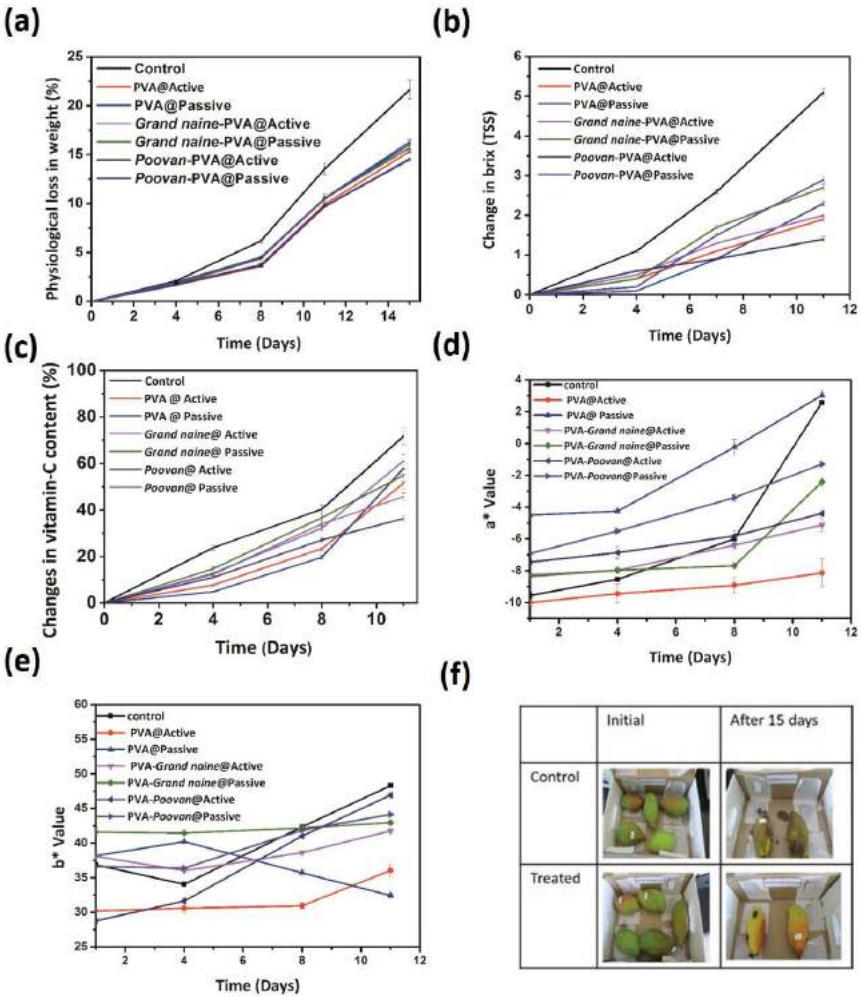

Figure 6. Assessment of mango shelf-life by (a) Physiological loss in weight, (b) TSS content and (c) Vitamin C content(d) a* co-ordinates (e) b* co-ordinates, (f) Visual colour changes of mangoes in both control and treated during 15 days of storage.

by electronic balance (max. limit- 220 g with error of 0.01 mg). The weight loss in both treatments (hexanal loaded and unloaded boxes) were similar in first week whereas, in second week the weight loss was significantly higher in unloaded (control) boxes in comparison with hexanal treated boxes of mangoes (Figure 6a). Among the treatments actively loaded fibres showed better results than passively loaded fibres, might be due to steady, prolonged and persistent release of hexanal from actively loaded electrospun nanofibres.PVA-Poovan@active is observed as the best treatment for its higher hexanal retention capacity.

2. TSS Content. TSS (Total soluble solids) content was observedincreasingtrend with time during storage. The increase in TSS content is directly related to the ripening or deterioration of fruits (mango). During 11 days of study it has been found that the TSS content began to rise significantly in control and was constantin actively loaded hexanal exposure specifically in PVA-Poovan@active (Figure 6b).

3. Vitamin C content. Vitamin C content was measured by DCPIP titration method. Green mango fruit is generally rich in vitamin-C which gives its characteristic sour taste. As ripening proceeds, the vitamin-C content decreases rapidly. The extent of change in vitamin-C content directly resembles the ripening rate. During 11 days of storage control mango showed 71.64±3.47% loss in vitamin C content whereas PVA-Poovan@active showed around 36.31±1.74% loss in vitamin C content. The results demonstratethat inhibition of deterioration of mango fruit mostly dependent upon the time of exposure rather than dose of hexanal (Figure 6c).

4. Changes in peel colour. The deterioration or morphological changes can be visualized and estimated through colour perception. Colour changes is one of the preferred indicators in fruit ripening study. The colour changes occurs generally due to decrease in peel chlorophyll content and increase in β-carotene content (Ketsa *et al.,* 1999). The peel colour changes can be estimated by L*, a* and b* co-ordinates where L* corresponds

to lightness, a* is attributed to red(+)/green(-) and b* is attributed to yellow(+)/blue(-). From the observed data, untreated mango fruit rapidly losses its colour as both a* and b* increases in positive direction (Figure 6d and 6e). The changes in a* value indicates the chlorophyll degradation and b* value correlates with β-carotene synthesis. It is observed that in most of the β-carotene synthesis was overshadowed by large proportion of chlorophyll. The degraded chlorophyll showed black colouration which isowing to lateral infection of pathogens. In treatments containing actively loaded electrospun fibres, the discolouration of mangoes was slower and steadier as compared to passively loaded electrospun fibre treatments (Figure 6f).

CONCLUSION

In the present study, electrospun matrix assisted hexanal delivery increased the shelf life of mango fruits. Hexanal is released slowly in the vapour form from the electrospun matrix and was found to be safer for handling and consumption. Introduction of nanocellulose in PVA matrix not only increased the loading capacity of hexanal, it also enhanced the slow release pattern. The electrospun hexanal-matrix treated mango fruits were able to remain fresh for 11 days of the study. However, enhancing the loading efficiency of hexanal and its slow release is still a challenge.

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