

Biopolymer based edible coating for enhancing the shelf life of horticulture products

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ARTICLE INFO

Keywords:

Edible coatings
Silk fibroin
Nanofibers
Dip-coating

ABSTRACT

As per the report of the United Nations, half of the fruits and vegetables loses annually. Industries are trying to reduce the postharvest loss by using coatings. Wax coating is the most preferred way to preserve fruits and veggies. Sometimes wax is mixed with some chemical compounds that are known to be carcinogenic. Recently many edible films have been developed using natural polymers to enhance the shelf life of food. The edible films act as a barrier between the food and the external environment to prevent the direct interaction of food with atmospheric gases and microbes, which reduce the rate of respiration, keeping the food fresh for an extended period. But, the cost of edible biofilms is high and restricted at the industrial level; the local fruits and vegetable vendors are not able to buy such costly biofilms. We have developed the solution for dip-coating and nanofiber coating using a blend of silk fibroin, PVA, honey and curcumin, which is a cost-effective method for fruits and vegetable vendors. The material used for coating is FDA approved. The techniques utilized for synthesizing the biofilm are electrospinning and dip-coating. Coating found to increase the shelf-life of fruits and vegetables.

1. Introduction

Food preservation is one of the most challenging tasks, especially horticulture crops (FAO, 2015); the whole world has recognized the problem and targets to cut half per capita global food waste at the retail and consumer levels by 2030. Food losses occur in many ways, along with production or via supply chains, including post-harvest losses (Blakeney, 2019; Verghese, Lews, Lockrey, & Williams, 2013). Crop production varies seasonally due to various factors, viz. in floods or droughts conditions, the production drops down, and demand is more. As a result, prices rise, and poor people are deprived of nutritious food. In a particular season, if the production is enormous and demand is less in the market, then the farmers are forced to destroy the crop production in the field itself instead of preserving the same because of the unavailability of cold storage across the nations and cost-effective methods of safeguarding the horticulture crops (Marsh & Bugusu, 2007). To balance the market demand and prices in off season, there is a need to preserve the crop in the cold storage by adopting some preservation method instead of directly putting the horticulture crops in cold storage; before this, some treatment should be done to maintain the nutritious value, texture and quality of horticulture crops. By doing this will

increase the supply in the more demanding season to balance the demand annually and maintain the prices in the market. According to the Food and agriculture organization of the United States, more than 1300 million Food are squandered annually. These waste horticulture crops (fruits and vegetables) contribute about 45–55% globally, which is very high compared to all other crops. The contribution of other varieties of crops in food waste and loss annually is 45% which includes cereals (30%), oil (20%), meat, dairy, and fish (35%). The amount of this total food loss and waste calculated was around US\$670 billion in developed and US\$300 billion in developing nations. The quantity of food lost or wasted annually is comparable to more than half of the world's annual cereals crop (2300 million tonnes in 2009–2010) (Rhim & Ng, 2007; Milinčić et al., 2019). There are many traditional food preservation techniques are being employed to preserve the food; some of the methods are given below with their drawbacks (i) Canning: very much time consuming, glass jars can break, spoilage may occur if the lid is not tightly applied (ii) Freezing: vitamins B and C are lost during the freezing process. (iii) Drying: taste changes and Food becomes hard. (iv) Vacuum: Very expensive and out of the reach of local vendors that cannot afford this technique. (v) Waxing: specially done for fruits and vegetables, but some carcinogenic chemicals are mixed with the wax to

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<https://doi.org/10.1016/j.fochms.2022.100085>

Received 31 October 2021; Received in revised form 2 January 2022; Accepted 4 February 2022

Available online 7 February 2022

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apply the thin and uniform coating (Kumar, 2019), which is hazardous for health, e.g. In the majority of the wax coatings, a compound named morpholine and its derivatives are mixed with wax to make sure that the wax coatings are applied thinly and evenly to the surface of the fruit. But this morpholine compound, in the presence of nitrate (contained in the diet), can be chemically nitrosated to form N-nitro-so-morpholine (NMOR), which is a potent carcinogenic compound and causes the risk of damage to kidneys, liver, and also causes allergies (Kumar & Kapur, 2016). However, limited preserving methods are available to preserve horticulture products shelf life. The highly familiar method is thin-film edible coating. These coatings are categorized into three types considering the nature of their elements such as hydrocolloids or natural gums (comprising proteins, polysaccharides or alginates), lipids (composed of fatty acids, acylglycerols or waxes) and their blends. These edible coating materials have a distinct barrier, physicochemical and mechanical characteristics against the atmospheric gases. Edible coatings can be prepared using polysaccharides, proteins, lipids or from the composite of these compounds. These edible coatings act as a barrier against the various atmospheric gases, humidity or water vapors, oxygen, carbon dioxide, microbes and help in decreasing the respiration and oxidation reaction rates in food. However, none of the three constituents can provide the needed protection by themselves and so these are usually used in a combination to achieve best results. Consequently, these days, most of the coatings are made up of more than one material, along with the supplement of low molecular weight compounds that works as a plasticizer and certain active agent to provide additional property in edible film (Şümmü & Baymdirh, 1995). Active agents are added to edible coatings to improve the coated product's antimicrobial, antioxidant, flavor, color, or nutritional properties. Such coatings act as a carrier of beneficial compounds that have a vital effect on the coated item for e.g., plasticizers are generally low molecular-weight compounds that imparts strength and elasticity to coatings and improve coating penetrability to water vapors and gases. Common plasticizers comprise polyols such as glycerol, mannitol, propylene glycol, honey, sucrose, sucrose and fatty acid esters that can be used as plasticizers. Similarly, other active agents, e.g., curcumin, rosemary oil, piperine, ascorbic acid, chitosan, etc., act as antioxidants and antimicrobial agents. The evolution of nanotechnology in the food and agriculture sector has shown tremendous opportunities to make food preservation and packaging possible without causing any side effects on health and the environment (Singh et al., 2017). Different types of edible biopolymers (Pradhan et al., 2015) and semi-synthetic polymers (Pavoni, Perinelli, Bonacucina, Cespi, & Palmieri, 2020) are available for food packagings (Zambrano-Zaragoza et al., 2018), such as chitosan, starch, alginate, pectin, xanthan, gellan, psyllium, carrageenan, basil seed, arabic gums, corn, cellulose and its derivatives for e.g. CMC (carboxymethylcellulose), and MC (methylcellulose), Pullulan, alginate, and guar gum (Salehi, 2020; Hashemi & Mousavi Khaneghah, 2017), silk fibroin, gelatin, poly (vinyl alcohol). Thin edible coating is applied on perishable fruits and vegetables using nanotechnology and different methods like dip coating, spray coating, and electrospun nanofibers coating (Ghorani & Tucker, 2015). Several edible thin film coating has been applied on the perishable fruits or vegetables by dip-coating methodologies using different biopolymer composites for e.g. (i) banana fruit coated by carrageenan composite (blend with carboxymethyl cellulose) have increased the shelf life by six days as compared to the control (Dwivany, Aprilyandi, Suendo, & Sukriandi, 2020); (ii) rice starch edible coating blended with sucrose esters have delayed banana ripening for 12 days in comparison with the untreated control when kept at 20 ± 2 °C; (iii) Gamma Irradiated Plasticized Poly(vinyl alcohol)/Carboxymethyl Cellulose/Tannin Composites were used as edible coating for enhancing the Shelf-Life of Banana Fruit from 9 to 19 days (Senna, Al-Shamrani, & Al-Arifi, 2014); (iv) arabic gum, soyabean gum, jojoba wax and glycerol were used independently as an edible coating for apple fruit and their effectiveness were studied during cold-storage, a significant delayed in the weight loss (3.11% to 4.66%) during the

storage was observed on compared to the control which shows 5.82% weight loss during the 60 days time period (El-Anany, Hassan, & Rehab Ali, 2019). Some other methodologies for apple post-harvest preservation apart from the coating are ultra-low oxygen storage systems (ULO 1 2.0 kPa CO₂ and 1.0 kPa O₂) and (ULO 2 2.5 kPa CO₂ and 1.5 kPa O₂) and 1-methyl cyclopropane (1-MCP) treatment. The least occurrence of microbial action was observed on apples stored under ultra-low oxygen conditions during six months of storage, while 1-Methylcyclopropene has put an encouraging result on apple epidermis firmness (Radenkova & Juhnvevica-Radenkova, 2018). Many other novel technologies for example, microwave treatment, vacuum, infrared or gamma irradiation, high pressure, pulse electric, etc. have been tried on fruits like apple and banana to eliminate the browning problem of fruits chemical treatment such as sulphitation or citric acid application performed. During transportation, banana was treated with chemicals such as nitrous oxide, salicylic acid and 1-methylcyclopropane for delaying the aging process (Mohapatra, Mishra, Singh, & Jayas, 2011). All the methodologies and materials discussed, above used chemical preservatives, requires cold storage along with the dip-coating, sophisticated techniques like ultra-low oxygen storage system to enhance the shelf-life of fruits and vegetables, which are quite difficult to perform and very costly for local fruit vendors or farmers. So, there is a need to synthesise cost-effective coating material and utilize coating methodologies like dip-coating for farmers and local vendors and electrospinning for food industries.

In this paper, we have performed both dip-coating (for local farmers) as well as electrospun nanofibers coating (for industrial use) on perishable fruits and vegetables. We used the silk fibroin protein as the base biomaterial because of its biocompatibility, non-toxic, higher stability and good mechanical strength (Wei, Kim, & Kim, 2011), PVA as a supporting polymer for electrospun coating and curcumin and honey were used as the active agent (Sabliov, Chen, & Yada, 2015), here honey acts as a natural moisturizer (Katouzian, Faridi Esfanjani, Jafari, & Akhavan, 2017) while curcumin as an antioxidant and antibacterial agent (Teow, Liew, Ali, Khoo, & Peh, 2016; Tamjidi, Shahedi, Varshosaz, & Nasirpour, 2013). The thin coating of electrospun nanofibers was directly applied by putting the fruits and vegetables over the collector plate (Dumitriu, Mitchell, Davis, & Vasile, 2017), and dip-coating was applied by dipping the fruits and vegetables in silk fibroin solution (Marelli, Brenckle, Kaplan, & Omenetto, 2016), carrying active agents and results were analysed. All the material used for edible coating is the Food and Drug Administration (FDA) approved.

2. Materials and methods

2.1. Materials

B. mori silk cocoons were procured from silk board Dehradun, Uttarakhand, India. Curcumin (MW 368.125 g/mol) (cat no. C1386) (Sigma-Aldrich), dialysis membrane (MWCO-12 kDa) (HiMedia, cat. no. LA401), lithium bromide (HiMedia, cat. no. GRM 3535), sodium carbonate (HiMedia, cat. no. GRM851), sodium bicarbonate (HiMedia, cat. no. MB045), dimethyl sulfoxide acid (DMSO) (SRL, cat. no.43404), ethanol (CSS, cat. no. 1170), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, cat. no. 1898-66-4), EDTA (Ethylenediaminetetraacetic Acid) (SRL, cat. no. 054448), Polyvinyl alcohol (HiMedia, CAS No. 9002-89-5) ultrapure water (18MΩ cm) (Millipore) was purchased and used as received. Natural Honey was collected directly from the field of the mustard plant in a village of Uttar Pradesh. *E. coli* (DH5α) and *S. aureus* (MTCC 737) was obtained from IMTECH, India. The bacterial culture medium, Luria Bertani (LB) and Nutrient Broth (NB), used for bacterial assays, were procured from Merck (Germany) and Himedia (India), respectively. The 3T3 mouse embryonic fibroblasts cell lines were received from the National Centre for cell science (Pune, India), and the [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] (MTT \geq 97.5%), was received from Sigma-Aldrich (Bangalore, India) Dulbecco's Modified Eagle Medium (DMEM) (LOT0000481561),

Dulbecco's Phosphate Buffered Saline (LOT SLBZ6118). All the reagents were of analytical grade and used without further modification.

2.1.1. Preparation of regenerated silk fibroin (SF) solution

5 g of cocoons were cut into half and washed and dried for 3 h in a hot air oven. After that, dried cocoons were degummed by boiling in 0.02 M of sodium carbonate for 1 h in a 2L beaker on a heating mantle to allow the removal of sericin protein, followed by 3 times washing and drying degummed silk fibroin fibers for 8 h in hot air oven. These degummed silk fibroin fibres were then dissolved in 9.3 M LiBr in a 1:4 ratio (SF:LiBr) and kept at 60 °C for 4 h in a hot air oven to allow the complete dissolution of silk fibroin fibers. Now dialyze the dissolved silk fibroin solution for four days in a dialysis membrane having MWCO 12 kDa by changing the water every 6 h interval. After the dialysis is complete, remove the solution from the dialysis bag and centrifuge at 9000 rpm for 20 min twice. After that store, the tinted yellow solution at 4 °C (Rockwood et al., 2011).

2.1.2. To find the concentration of the silk fibroin obtained

Initially, weigh a dry Eppendorf (T1) then, add 1 mL of regenerated silk fibroin solution and weigh again (T2). Keep it to dry at 60 °C overnight in a hot air oven. Now weigh the dried part also (T3). Calculate the silk fibroin concentration as follows: $(T3-T1)/(T2-T1) * 100$

2.2. Synthesis of nanocurcumin from curcumin

150 mg of curcumin was dissolved in 10 mL of DMSO. After that water bath sonicator, having a power output supply of 300 W and frequency of 70 Hz, was used. Curcumin (15 mg mL^{-1}) dispersed in 10 mL of cell culture grade DMSO in Falcon tubes was exposed to ultrasonication in the water bath sonicator at 55 ± 5 °C for 30 min. After sonication, the solution was stirred at 400 rpm at room temperature for about 15 min. Falcon tubes with the nanocurcumin solution were then wrapped with aluminium foil and kept in a dark place (Gopal, Muthu, & Chun, 2015).

2.3. Preparation of SF composite solution for coating (dip and nanofibers based)

Take the regenerated silk fibroin (SF) aqueous solution that was stored at 4 °C after the dialysis. Prepare the 3% PVA aqueous solution by dissolving the PVA in milipore water, allow the PVA to dissolve completely by putting it on a magnetic stirrer for 6 h at room temperature at 600 rpm and then, add it to the regenerated silk fibroin solution in a 1:1 (SF: PVA) ratio in a vial. Put the vial on a magnetic stirrer for 15 min at 500 rpm at room temperature. After that, add nanocurcumin (250 µg/mL concentration) and 5% honey (w.r.t. SF-PVA volume) to the silk fibroin-PVA solution. Then, silk fibroin-PVA solution loaded with nanocurcumin and honey is put on a magnetic stirrer for 10 min at room temperature to allow the complete dissolution of honey and nanocurcumin. The SF-PVA composite solution loaded with nanocurcumin and honey is injected into the 5 mL syringe. After that, the syringe was fixed in the electrospinning machine and the model fruits or vegetables were directly placed on the collector plate of electrospun machine for coating the electrospun nanofibers on fruits or vegetables, after that the required parameters are set to synthesize the electrospun nanofibers such as, the flow rate was set to 0.5 mL/h, and the voltage was set to 20 kV after optimisation. The electrospun nanofibers start to deposit on the fruits and vegetables to coat them from all sides by changing their position after 40 min; the total electrospinning time is 2–3 h. In the same way, solution for dip-coating was prepared by using the above protocol used for nanofibers synthesis except without adding the PVA solution. Dip-coating was done on the model fruits and animal by simply dipping them thrice at an interval of 5 min in the solution for 30–40 s, then the coated food was hung to dry at room temperature.

2.4. Physicochemical characterization

2.4.1. FTIR measurements

The FTIR (Cary 630 FTIR spectrometer) spectrum of silk fibroin composite, nanocurcumin, PVA, and honey were recorded by directly putting the sample between diamond crystal and knob of FTIR machine at a range of $4000\text{--}400 \text{ cm}^{-1}$

2.4.2. Field-Emission electron microscopy (FE-SEM) analysis

The morphology of the silk fibroin nanofibers (SFNFs) was analyzed by using Field-Emission Electron Microscope (FE-SEM) (Ultra-Plus Carl Zeiss). The samples were gold-sputtered using Denton gold sputter unit for 70 s and then, mounted in FE-SEM at 5.0 kV. The obtained images of SF composite nanofibers were then processed by using Image J software to calculate the mean diameter of the SF composite nanofibers.

2.4.3. Contact angle measurement

The contact angle was measured by using the instrument optical Tensiometer. Contact angle, θ , is a quantitative measure of the wetting of a solid by a liquid. It depicts the nature of the material, whether it is of hydrophobic or hydrophilic nature if the contact angle is less than 90° then liquid wets the surface and it is hydrophilic while, if the contact angle is more than 90° then it does not wet the surface and is of hydrophobic nature.

2.4.4. UV-Visible spectroscopic measurements

UV-Visible spectrophotometer (Lasany double-beam L1 2800) is used to measure the absorption peak of materials by performing scanning in the range of 200 nm – 800 nm. Samples were dissolved in their respective solvents (such as water, DMSO) in specific concentrations and absorbance was measured using Quartz cuvette (path length:10 nm)

2.4.5. Thermogravimetric analysis

Thermogravimetric analysis of electrospun nanofibers (both SF-PVA and SF-PVA-honey-nanocurcumin) was performed in a TG/DTA SII 6300 EXSTAR thermal analyzer by heating them to the temperature 800 °C at a heating rate of 5°/min with flowing air (200 mL/min) Thermo Gravimetric Analyzer (TGA) (TG/DTA SII 6300 EXSTAR) was used to check the moisturizing ability of the honey present in SF-PVA-curcumin composite nanofibers by comparing it with the SF-PVA nanofibers.

2.5. Bacterial culture and antibacterial studies

The freshly cultured bacterial cells were used to perform the antibacterial test. Curcumin incorporated silk fibroin composite solution were evaluated for their antibacterial studies against Gram-positive *S. aureus* and Gram-negative *E. coli* bacteria by measuring optical density using UV-vis double beam spectrophotometer (Lasany, LI-2800) on a certain wavelength of 600 nm. To evaluate the bactericidal activity Gram-negative (*E. coli* DH5 α) and Gram-positive bacteria (*S. aureus*) were cultured in L.B. (Luria-Bertani) and N.B. (Nutrient Broth) media, respectively by adding different volumes of curcumin ($V_1 = 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 \text{ }\mu\text{L}$) from the stock solution of curcumin ($C_1 = 15 \text{ mg/mL}$ in DMSO) in test tubes, after that the bacterial cells were incubated at 37 °C in shaker at 300 rpm for 8 h. The growth of the bacteria was firstly determined by turbidness of the medium, after 8 h of incubation, take the O.D. (optical density) at 600 nm (Meyers, Furtmann, & Jose, 2018) and calculate the minimum inhibitory concentration (MIC) using $C_1V_1 = C_2V_2$, where C_2 is the unknown concentration (MIC), and V_2 (6 mL) is the volume of sample in a test tube. Here, the antibacterial experiment was replicated thrice ($n = 3$).

2.6. Antioxidant activity measurement using DPPH assay

The antioxidant potential of the nanocurcumin and honey present in composite NFs were determined by 2, 2-diphenyl-1-picrylhydrazyl

(DPPH) free radical assay. Basically, 0.5 mL of 50 μM methanolic DPPH solutions, different concentrations of nanocurcumin, honey and their composites were added to make up a final volume of 0.6 mL. The samples and appropriate controls were incubated for 20 min in an unlighted place at 37 °C. After that, 100 μL of each sample was added to 96 well plates. The difference in absorbance was taken at 517 nm by a multiplate reader or UV-vis spectrophotometer. The free radical scavenging activity (%) of nanocurcumin and its composites were calculated by the following formula (Blois, 1958):

Scavenging activity (%) = $(AD_{517} - AS_{517}) / AD_{517} \times 100$. {A.D. and AS are the absorbance of DPPH solution (without-antioxidants) sample solution (with antioxidants)}, respectively.

2.7. Electrospun nanofibers and dip coating study by time-lapse photography

Electrospun nanofibers coated and dip-coated model fruits, vegetables and animals (zebrafish) are taken in a triplicate ($n = 3$) to study and compare the morphological changes in the coated and uncoated (control) fruits, vegetable and animal food, the time-lapse photography for banana (both riped and unriped), apple and zebrafish as a model was done at a regular interval of time depending on the nature of food type and its shelf-life for e.g. in banana photography was done in every 24 h. The external appearance, color change, stiffness, smell and texture were studied in detail by time-lapse photography using 64 megapixels camera.

2.7.1. Weight loss

The weight of coated and uncoated fruits and vegetables were recorded by using a standard weighing scale to know the effectiveness of coating in shielding the food against external factors like gases, light, temperature, moisture.

2.8. Cell culture

3T3 cells (mouse embryonic fibroblasts cell lines, NCCS Pune) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin and kept at 37 °C in a humidified incubator supplied with 5% CO_2 . The media was changed every other day, and cells were subcultured after 48 h and harvested from subconfluent cultures (70–80%) using 0.25% trypsin-EDTA.

2.8.1. MTT assay

The cytotoxicity of the curcumin in silk fibroin composite nanofibers towards 3T3 cells was assessed by MTT assay. The cells were seeded onto the surface of the nanofibers (curcumin free and curcumin loaded composite nanofibers) matrices at a seeding density of 10,000 cells/well and cultured for 24 h in 96-well plates. Afterwards, the growth media was removed from each of the wells and 10 μL of MTT solution was added to the respected well and incubated at 37 °C for 4–5 h, resulted in the formation of dark-blue formazan crystals, which were dissolved in DMSO by incubating them for 15 min at 37 °C and the absorbance was taken at 570 nm using Biorad plate reader. The MTT assay was performed in triplicate, and the cell-viability percentage was calculated using the following equation (Sauraj et al., 2018)

$$\% \text{ Cell viability} = [(A_{570})_{\text{treated}} / (A_{570})_{\text{control}}] \times 100$$

2.9. Statistical analysis

The entire experimental records were completed in triplicate, and data were expressed as mean \pm standard deviation, wherever relevant. Data were statistically managed using one way analysis of variance (ANOVA) to assess significant differences among various groups, followed by student t-test using GraphPad Prism 6.0, and Origin Pro 8.0 software.

3. Results and discussions

In the current study, silk fibroin-based dip coating and electrospun nanofibers have been used as edible coating for the preservation of horticulture crops. Further, it has been loaded with antimicrobial and antioxidant agents curcumin and honey as a natural moisturizer. The antibacterial study was done by taking O.D. at 600 nm by UV-visible spectroscopy. The presence of curcumin and honey in silk fibroin solution and electrospun nanofibers was confirmed by FTIR analysis. FE-SEM revealed the actual morphology and average size of the SFNFs. Antioxidant properties of curcumin were determined by DPPH assay. Moisturizing properties of honey in SF composite nanofibers were studied by T.G. analysis. The wettability of the nanofibers was confirmed by measuring the water contact angle. Time-lapse photography of horticulture crops (fruits and vegetables) was done to study the external appearance, stiffness, taste and texture changes with respect to time.

3.1. Characterization of surface morphology

The surface morphology of SFNFs was examined by field emission scanning electron microscopy. FE-SEM analysis revealed that the nanofibers were in a random mesh-like arrangement having beads like pattern, which ensured the enhanced hydrophobic characteristics. The average size (calculated from 60 fibers taken from 2 images) of nanocurcumin loaded SFNFs was calculated using Image J software as was found to be 91.797 ± 43.51 nm with the highest value reported 212.459 nm, and the minimum value reported was 29.098 nm. The inset image of the histogram represents the nanofibers diameter range against the number of counts, as shown in Fig. 1A and B.

3.2. Silk fibroin concentration

The silk fibroin concentration calculated after dialysis was found to be 5.95 % approximately, calculated from the formula given in section 2.3. with values $T_1 = 1.11$ g, $T_2 = 2.10$ g, $T_3 = 1.169$.

3.3. FTIR studies of SFNFs

To confirm the entrapment of nano-curcumin and honey in the silk fibroin nanofibers, FTIR analysis was performed. Silk fibroin regenerated solution revealed peaks at 3420 cm^{-1} refers to O-H stretching vibration due to absorbed water molecules. Peaks between 1650 and 1635 cm^{-1} correspond to amide I (C = O) stretching vibration, 1540 – 1520 cm^{-1} corresponds to amide II (N-H bending and C-H stretching), and 1270 – 1230 cm^{-1} corresponds to amide III (C-N stretching and C = O bending) of silk fibroin (Ha, Tonelli, & Hudson, 2005; Wang & Zhang, 2013). The PVA film showed absorption bands between 3550 and 3200 cm^{-1} corresponds to stretching of O-H bond, vibrational band corresponds to 2840 – 3000 cm^{-1} indicates to stretching C-H from an alkyl group, 1750 – 1735 cm^{-1} corresponds to (C = O) vibration, 1150 – 1085 cm^{-1} corresponds to (-C-O-C) (Mansur, Sadahira, Souza, & Mansur, 2008). The honey showed absorption bands at 3280 – 3271 cm^{-1} corresponds to O-H bond stretching, C-H stretching occurs at 2935 – 2931 cm^{-1} bandwidth, O-H bending in H_2O occurs at a maximum range of 1643 – 1642 cm^{-1} , C-H and C-O stretching and O-H, C-H, O-H bending in organic acids and carbohydrates corresponds to 1416 – 1252 cm^{-1} . C-O, C-C stretching in organic acids and carbohydrates occurs at a bandwidth of 1031 – 1020 cm^{-1} (Mail, Ab Rahim, Amanah, Khawory, Shahudin, & Seeni, 2019). Curcumin showed the characteristic phenolic vibration at 3504 cm^{-1} , C-C stretching occurs at 1610 – 1560 cm^{-1} , C = O corresponds to 1640 cm^{-1} , C = C corresponds to 1520 cm^{-1} , C-O stretching occurs at 1250 cm^{-1} , 3547 cm^{-1} corresponds to O-H group (Gopal et al., 2015). The same absorption bands were found in the SF based nanofibrous blend with low absorption intensity, as shown in Fig. 1C.

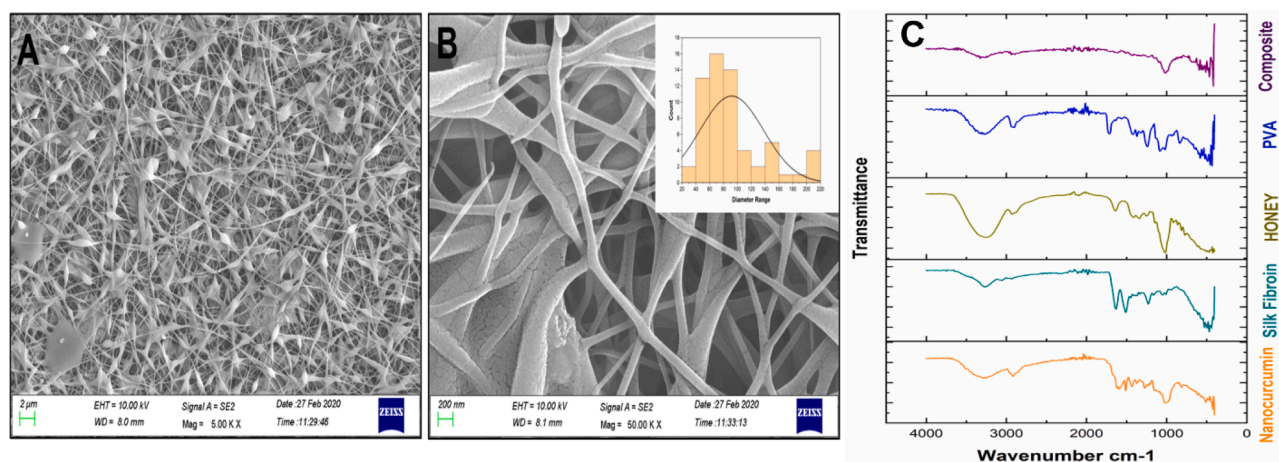


Fig. 1. (A) Field emission scanning electron microscopy (FE-SEM) images of Silk fibroin composite nanofibers (SF-honey-nanocurcumin-PVA) (A) 2 μm scale bar (B) 200 nm scale bar and the average size of NFs (91.797 ± 43.51 nm) was calculated by using Image J software (C) FTIR spectra of nanocurcumin, silk fibroin, honey, PVA (powder form) and composite of silk fibroin-nanocurcumin-honey-PVA.

3.4. Thermogravimetric analysis

The chemical decomposition behavior of the nanofibers as a function of time was investigated using thermogravimetric analysis shown in Fig. S1, which represents the comparative thermogram of SF-PVA nanofibers and SF-honey-nanocurcumin-PVA composite nanofibers. The Silk fibroin (SF) blend with PVA-honey-nanocurcumin nanofibers (NFs) undergoes more weight loss compared to SF blend with PVA NFs between the temperature ranges of 70–85 $^{\circ}\text{C}$, further again the weight loss observed is more in the SF blend with PVA-honey-nanocurcumin nanofibers (around 20%) in comparison to the SF-PVA nanofibers (12–15%) in the temperature range of 250–320 $^{\circ}\text{C}$. Such difference in weight loss is due to the presence of water content in honey released from the glucose, fructose and other sugar molecules while heating at a very high temperature which confirms that honey acts as a natural moisturizer that prevents the peel of fruits and vegetables from dehydration and makes the skin of the fruit stiff.

3.5. Contact angle measurement

The water contact angle was done to verify the hydrophobicity or hydrophilicity of SF composite NFs. The water contact angle depicts the nature of the SFNFs; the contact angle results are shown in Table S1; from these results, the NFs were found to be moderately hydrophilic due to the presence of PVA and honey in the SFNFs composite, as shown in Figure S2.

3.6. Antibacterial test

The antibacterial activity of nanocurcumin was carried out with gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria, and nanocurcumin was found to be effective in inhibiting the growth of both gram-positive and gram-negative bacteria. The growth was measured by taking the O.D. at 600 nm, as shown in Figure S3. The minimum inhibitory concentration (MIC) calculated for gram-negative bacteria was 200 $\mu\text{g}/\text{mL}$ and for gram-positive bacteria was 225 $\mu\text{g}/\text{mL}$. The mechanism behind the inhibition of bacterial growth is already well-researched and is found that curcumin particles attached at the cell wall of bacterial cell, breakdown the peptidoglycan layer and breached inside the cell, thus triggering disruption of the structure of cell organelles and causing the cell lysis (Bhawana, Basniwal, Buttar, Jain, & Jain, 2011).

3.7. DPPH assay

Antioxidant activity is the ability of a material to neutralize and scavenge free radicals. DPPH assay is used to determine the antioxidant activity of a compound. DPPH is a nitrogen-free radical containing deep purple colour solution which turns yellow as interacts with antioxidants. Different concentration of nanocurcumin was added to 50 μL methanolic DPPH solution, and the decrease in absorbance was determined within minutes of incubation at 517 nm. The free radical scavenging depends on a dose-dependent manner, as shown in Fig. 2A. With the increase in the concentration of nanocurcumin, there was an increase in % scavenging activity from 15% to 55%. From the curve, the EC_{50} value calculated was approximately equal to 17.64 $\mu\text{g}/\text{mL}$, as shown in Fig. 2B. It is defined as the volume of antioxidants needed to reduce the concentration of DPPH assay by 50%. With an increasing concentration of nanocurcumin the color intensity of DPPH solution changes from deep purple to colorless and then to yellow.

3.8. Time-lapse photography

3.8.1. Banana coated with silk fibroin nanofibers

Banana is selected as a model fruit because of its miserable shelf life of 4–5 days. Banana coated with edible SFNFs showed increased shelf-life by additional four days, and its texture, stiffness and quality were maintained while the uncoated banana on the 6th days lost its stiffness and emitted a foul smell, as shown in Fig. 3A. In another coating experiment, two bananas were taken; one was taken as control (uncoated), and another banana was coated with nanofibers; and after six days, banana without peel (uncoated on the left side and coated on the right side) was examined, and it was reported that uncoated banana started decaying from the bottom while the coated banana was fresh and stiff, as shown in Fig. 3B.

3.8.1.1. Green banana (model vegetable) coated with silk fibroin nanofibers.

The unripe green Banana used as a vegetable, and after coating of nanofibers, the ripening process was delayed by two weeks, and after ripening, it can be used as a fruit, while the uncoated banana ripens after two weeks, but it cannot be consumed because side by side it starts decaying at the same time. It may be noticed that fungal growth occurs on the uncoated banana, which was one of the main reasons for its early decaying and coated banana did not show any fungal growth due to the nanocurcumin, which functions as an antimicrobial agent present in SFNFs, as shown in Figure S4.

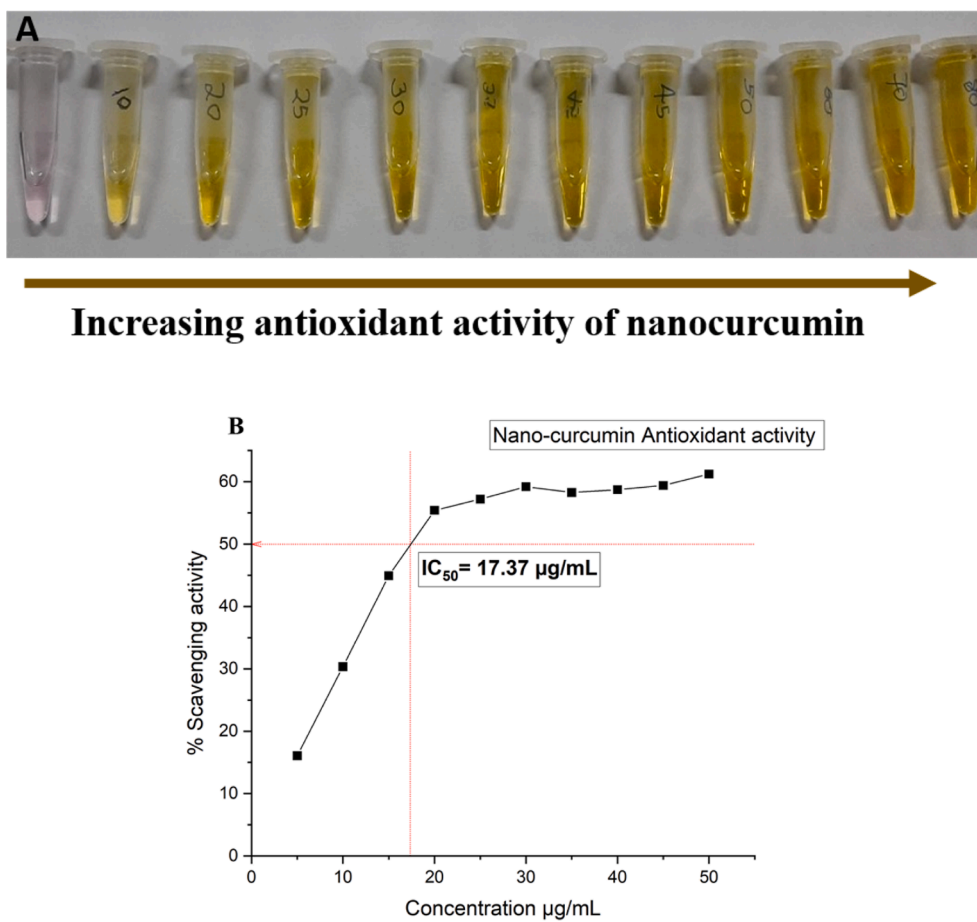


Fig. 2. (A) DPPH assay using nanocurcumin for antioxidant properties and (B) calculation of IC₅₀ (scavenging activity).

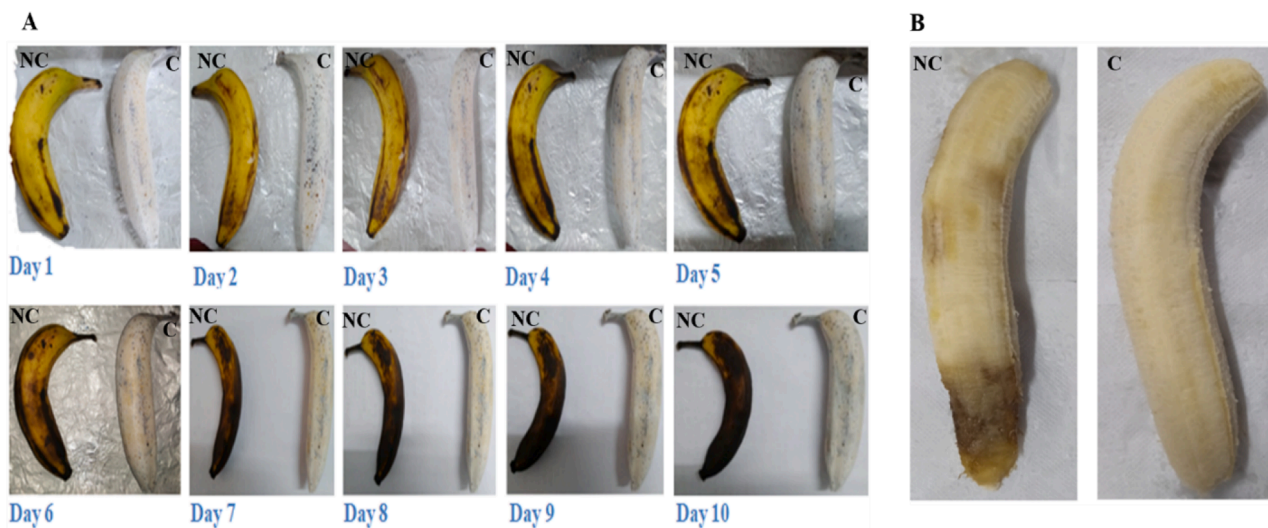


Fig. 3. (A) Time-lapse photography of silk fibroin composite nanofiber coated banana (150 min total electrospinning time by changing the position of banana to allow the proper coating from all sides) and uncoated banana. (B) Banana without peel on 6th day; Note: NC- non-coated, C- coated.

3.8.1.2. Apple coated with silk fibroin nanofibers. The apple shelf-life is tremendously increased after coating about a month with maintained texture, quality and smoothness and remain stiff for more than a month while uncoated apple starts rotten after one week and emits a foul smell, lost its stiffness on 18th day its sap starts leaking and burst out 20th day. In contrast, the coated apple survives for more than 3 weeks with maintained texture, stiffness and taste, as shown in Fig. 4A.

3.8.1.3. Dip coating. The silk fibroin composite aqueous solution containing nanocurcumin and honey was used for dip-coating directly. From Fig. 4B, the uncoated apple slice turns brown and size decreases due to loss of water content, while in the dip-coated slice, morphology, texture, and color were in better condition than the uncoated slice after three days.

Another experiment was performed to test the stability of fish with

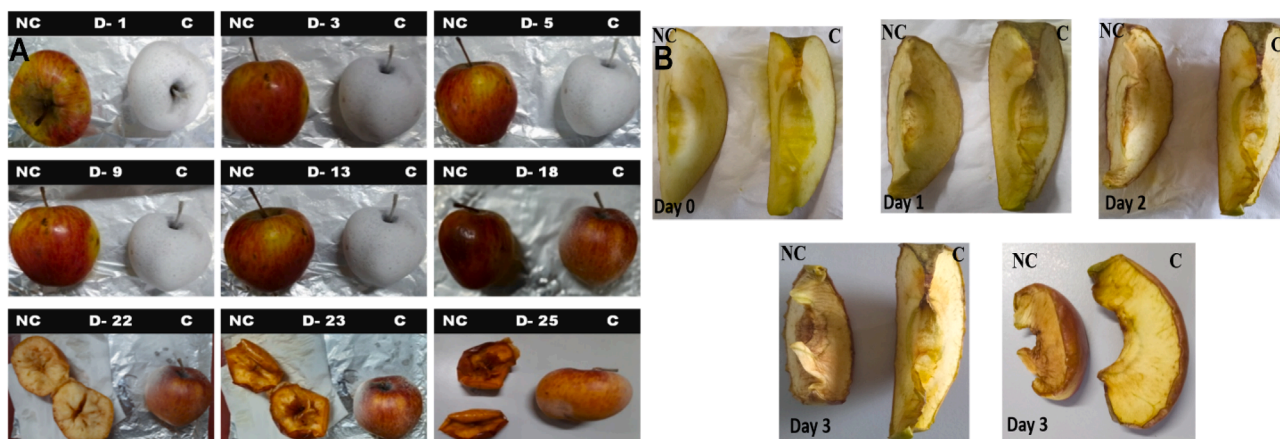


Fig. 4. (A) Morphological study of coated and uncoated apple using time-lapse photography. On the 18th-day apple, was dipped in water for 30 sec to check the withstand ability of silk fibroin composite nanofibers. After drying, the coating becomes transparent, but the nanofiber was remained tightly attached to the peel of apple (120-minute electrospinning time; NC-non-coated, D- day and C-coated). (B) Apple slice was used for dip coating and dipped in silk fibroin-honey-nanocurcumin solution for 30 s, and time-lapse photography was done to study the morphological change at a different angle (on day 3) shows that non-coated slice shrinks more compared to dip-coated slice; NC-non-coated, C-coated.

and without dip-coating here we used mature zebrafish as a model to test their shelf-life by continuously monitoring them for 48 h in an interval of 6 h, and it was found that coated fish retains the morphology and fluids in it while uncoated fish dried entirely and lost its structural morphology and become like a dry leaf, as shown in Fig. 5.

3.8.1.4. Weight% loss. Weight loss % were calculated for uncoated and coated banana (Fig. 3A), apple (Fig. 4A) and apple slice (Fig. 4B). The coated fruit weight loss % was very low as compared to the uncoated fruits; the rate of decay of uncoated fruits is much higher in comparison to the coated ones. The rate of evaporation of water is high in uncoated fruits in contrast to coated fruits, as shown in Table 1. This further confirms that nanofiber coating is effectively limiting the rate of gaseous exchange and honey present in the coating, working as a moisturizer and preventing the water content from evaporating.

3.9. *In vitro* cytotoxicity assay

The *in vitro* cytotoxicity of curcumin loaded composite nanofibers was tested against 3T3 cells (mouse embryonic fibroblasts cells) by the MTT assay shown in Figure S5. The viability of 3T3 cell lines seeded on the nanofibers without curcumin was 94.43% while, cells treated with the nanofibers composites containing curcumin showed 77.10% viability. The slightly lower viability of the curcumin treated cell lines in

Table 1

Comparative study of weight loss in coated and uncoated fruits.

Fruit	Initial wt. gm	Final wt. in gm	Wt.% loss
Coated Banana (Fig. 3A)	69.15	60.14 (after 10 days)	13.02%
Uncoated Banana (Fig. 3A)	61.17	45.60 (after 10 days)	25.45%
Coated Apple (Fig. 4A)	93.82	78.15 (after 25 days)	16.70%
Uncoated (Fig. 4A)	95.71	29.46 (after 25 days)	69.21%
Uncoated apple slice (Fig. 4B)	24.33	7.99 (after 3 days)	67.15%
Coated apple slice (Fig. 4B)	25.2	17.49 (after 3 days)	30.59%

comparison to the control and the nanofibers that are devoid of curcumin was due to the antibacterial activity of curcumin, which exerts toxicity and concentration above 20 μ M or 7.35 μ g/mL are toxic to normal cell lines (Cianfruglia, Minnelli, Laudadio, Scirè, & Armeni, 2019). However, the daily permissible limit of daily curcumin intake for humans is 6–8 g/day, which is quite high in comparison to 250 μ g/mL. So, the dose added to the SF nanofibers composite is considered to be safe for humans.

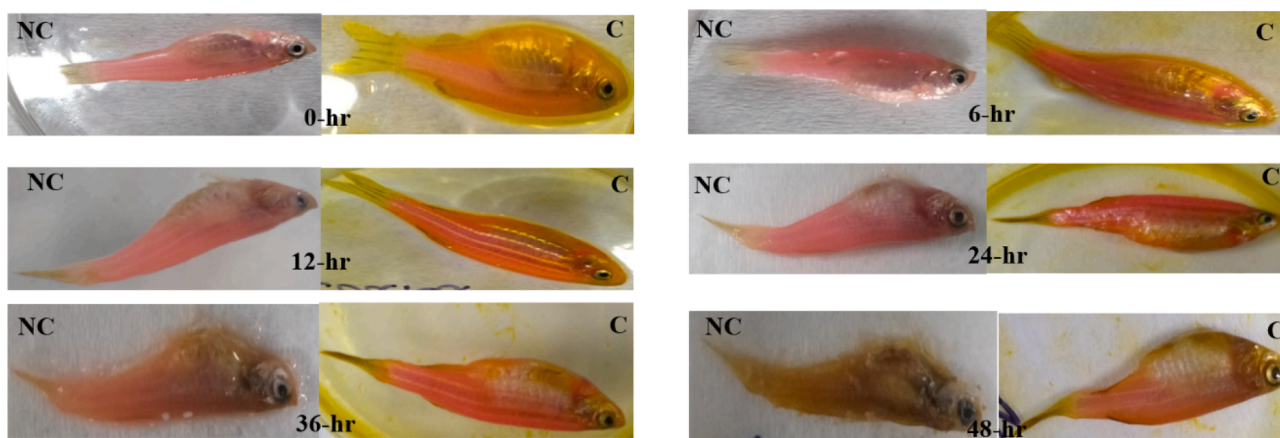


Fig. 5. Assessing the shelf-life of mature zebrafish (used as a model animal to study the dip-coating on non-veg food) using a blend of silk fibroin, honey and nanocurcumin solution; 30-sec dip coating; NC-non-coated, C-coated.

4. Conclusion

The aim of the study was to explore the capability of silk fibroin as an edible coating biomaterial loaded with the antioxidant and antimicrobial agent in electrospun nanofibers. Considering this work-done, various conclusion can be drawn. Silk fibroin is an attractive biomaterial that can be utilized for numerous biomedical applications and an edible coating material in the food nanotechnology field. A single step feasible, reproducible method has been described to incorporate antioxidant and antimicrobial agent (nano-curcumin) in SFNFs.

From the result discussed above, the coating on perishable fruits like banana (ripened and unripe), apple (including slices) is successfully able to increase the shelf-life and also maintains the weight, stiffness and fruit quality; shelf life is increased to almost double. The banana is one of the most perishable fruit, and within four days it starts decaying, but the banana coated with edible nanofibers is in an eatable condition for up to 8–9 days its texture and quality was also very good, while the uncoated banana is in eatable states for only initial 3–4 days. Same as for apple, the coated apple works for more than three weeks, and its weight loss is negligible compared to the uncoated apple, which loses its stiffness and burst out on the 22nd day while the coated apple is in perfect condition. Similarly, the dip-coating done on zebrafish, which was taken as a model, remained stable for 48 h. Such type of dip coating would be favourable for exporting meat or other non-veg foods that decays very fast during transportation. This food preservation method is cost-effective and can also be used as a simple dip-coating which does not need any special expertise, so; it is favourable for the local vendors. Most of the coating techniques are very costly and out of the reach of local vendors. We are utilizing the silk cocoons discarded by the industry, which proves very cost-effective to us, and the nano-coatings are edible and adds extra nutrition to them.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

DPS is thankful to the Ministry of Education, Government of India, for the fellowship. Sincere thanks to the Institute Instrumentation Centre and Centre for Nanotechnology, IIT Roorkee, for various instrumental and analytical facilities.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochms.2022.100085>.

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