Journal of Siberian Federal University. Biology 2021 14(4): 475–497
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DOI 10.17516/1997-1389-0366
УДК 577.114.4:664

Aloe vera Incorporated Chitosan/Nanocellulose Hybrid Nanocomposites as Potential Edible Coating Material under Humid Conditions

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Received 13.06.2021, received in revised form 23.07.2021, accepted 23.08.2021

Abstract. Innovative post-harvest technologies are in demand to meet the requirements of farmers and agricultural industries to ensure global food security and to avoid food wastage. Edible coatings that can prevent food spoilage and/or enhance shelf life have taken on increasing importance. This work involves the development of edible coatings based on easily available bio resources, chitosan and nanocellulose, and utilizing their unique properties as an effective coating material. *Aloe vera*, known for its antioxidant and antimicrobial properties, has been proposed as an active ingredient that can be incorporated into the biodegradable film. Varying volumes of *Aloe vera* (0.25 ml, 0.35 ml, 0.5 ml, and 2.5 ml) were added to fabricate nanocomposite films by solvent casting. Transparent films were obtained, and their morphology was analysed using scanning electron microscope (SEM). The incorporation

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of *Aloe vera* was confirmed in various spectroscopic studies, which clearly show reduction in light transmittance for the nanocomposite films containing *Aloe vera*. The contact angle study showed an increase in hydrophobicity initially. Maximum tensile strength was obtained with 0.25 ml of *Aloe vera*. The potential use of nanocomposite solution as edible films was demonstrated in green chillies, which showed lower weight loss after 3 days when compared with uncoated chillies. In the first phase of this study, chitosan/nanocellulose nanocomposites enriched with *Aloe vera* have been proposed as a potential edible food coating material.

Keywords: edible coating, chitosan, nanocellulose, bio nanocomposite, *Aloe vera*.

Acknowledgements. Authors acknowledge support from School of Energy Materials and IIUCNN (Mahatma Gandhi University), Jaivam project (Mahatma Gandhi University) and School of Environmental Sciences (Mahatma Gandhi University).

Citation: Sali A. K., Ravi A. P., Shamsudeen S. P., Mathew S. S., Joseph B., Tharayil A., Vijayamma R., Maria H. J., Spatenka P., Kalarikkal N., Thomas S. *Aloe vera* incorporated chitosan/nanocellulose hybrid nanocomposites as potential edible coating material under humid conditions. J. Sib. Fed. Univ. Biol., 2021, 14(4), 475–497. DOI: 10.17516/1997-1389-0366

Гибридные нанокомпозиты хитозан/наноцеллюлоза с включением *Aloe vera* как потенциальный материал для изготовления съедобных покрытий, применяемых в условиях повышенной влажности

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Аннотация. Инновационные технологии переработки и хранения сельскохозяйственной продукции широко востребованы в сельскохозяйственной отрасли и нацелены на обеспечение глобальной продовольственной безопасности и снижение потерь продуктов питания. Все большую значимость приобретает разработка съедобных пищевых покрытий, которые могут предотвратить порчу пищевых продуктов и / или продлить срок их хранения. Это исследование посвящено созданию эффективного материала для изготовления съедобных покрытий на основе легкодоступных биоресурсов с уникальными свойствами – хитозана и наноцеллюлозы. *Aloe vera*, известный своими антиоксидантными и антимикробными свойствами, предложен в качестве активного ингредиента, который может быть включен в биоразлагаемую пленку. Различные объемы экстракта *Aloe vera* (0,25 мл, 0,35 мл, 0,5 мл и 2,5 мл) добавляли при изготовлении нанокомпозитных пленок методом литья из раствора. Морфология полученных прозрачных пленок изучена с помощью сканирующей электронной микроскопии. Включение *Aloe vera* в композит подтверждено различными спектроскопическими исследованиями, которые показали снижение светопропускания пленок нанокомпозитов, содержащих *Aloe vera*. Исследования краевого угла выявили увеличение гидрофобности композита. Максимальное значение прочности на разрыв получено при включении в состав композита 0,25 мл *Aloe vera*. Возможность использования полученного нанокомпозита в качестве съедобной пленки оценена в эксперименте с зеленым перцем чили. Покрытые пленкой образцы перца показали более низкую потерю веса через 3 дня по сравнению с образцами без покрытия. Исследование продемонстрировало потенциал разработанного нанокомпозита хитозан/наноцеллюлоза, обогащенного добавками экстракта *Aloe vera*, в качестве материала для изготовления съедобных пищевых покрытий.

Ключевые слова: съедобное покрытие, хитозан, наноцеллюлоза, бионанокомпозит, *Aloe vera*.

Благодарности. Авторы выражают благодарность за поддержку Институту энергетических материалов и IIUCNN (Университет Махатмы Ганди), проекту Java (Университет Махатмы Ганди) и Институту наук об окружающей среде (Университет Махатмы Ганди).

Цитирование: Сали, А. К. Гибридные нанокомпозиты хитозан/наноцеллюлоза с включением *Aloe vera* как потенциальный материал для изготовления съедобных покрытий, применяемых в условиях повышенной влажности / А. К. Сали, А. П. Рави, Ш. П. Шамсудин, С. С. Мэтью, Б. Джозеф, А. Тараил, Р. Виджаямма, Х. Дж. Мария, П. Спатенка, Н. Калариккал, С. Томас // Журн. Сиб. федер. ун-та. Биология, 2021. 14(4). С. 475–497. DOI: 10.17516/1997-1389-0366

Introduction

Consumer demands for high quality food are increasing day by day. Different kinds of synthetic materials or preservatives are used to enhance the food quality, which in turn improves the shelf life of the fresh food products by incorporating substances that have strong antimicrobial and antibacterial properties (Arvanitoyannis, 1999). Food products are vulnerable to the attack of microbial and bacterial microorganisms that will reduce the nutritional value of food commodities. There are many synthetic materials available in the market like fungicides or insecticides that protect the food commodities from harmful microorganisms. As a major constituent of food coatings, chemicals are potential sources of food adulteration. Chemicals usually enter into the food supply chain in the form of antioxidants, stabilizers, preservatives, and so on. It should be emphasized that such chemicals can be malicious in the long run. Nowadays most of the research is aimed at developing bio based antibacterial coatings that can be consumed without any side effects (Geueke et al., 2018; Aloui, Khwaldia, 2016). Significance of edible coatings increased due to their environment friendly nature and their potential use in the food industry. Edible films or coatings based on natural polymers or biopolymers cause no environmental issues, as they are biocompatible and obtained from agricultural and animal products like proteins, gums, lipids, etc. They improve the quality of food by limiting the migration of moisture, lipids, flavours /aromas, and colours between food components, carrying active ingredients (e. g., antioxidants, antimicrobials, flavour), and improving the mechanical integrity or handling characteristics (Khwaldia et al., 2004; Joshy et al., 2020b). Bioactive compounds such as essential oils (EOs) are added into such coatings to improve shelf life, prevent the growth of microorganisms, and preserve nutritional value of food. EOs have been found to exhibit excellent antimicrobial and antifungal properties, which makes them a natural alternative to fight against foodborne pathogens and normal food decay caused by bacterial and mould growth. The immobilization of the active compounds in polymer can result in their high concentrations on the surface of food in order to achieve a longer storage time (Ju et al., 2019; Vu et al., 2011). The prepared film coating should be non-toxic and possess high barrier properties to moisture, antibacterial properties, and high transparency. Bio polymers such as chitosan are easily available and are well known for their antimicrobial, biodegradable, biocompatible, and cost-effective properties (Joseph et al., 2020b; Ramadan et al., 2020; Dutta et al., 2019). Chitosan based films were produced by blending with biopolymers such as polysaccharides or proteins, by adopting solution-casting, layer-by-layer, extrusion, and other techniques (Kumar et al., 2020; Mohammadi et al., 2018; Fitch-Vargas et al., 2016; Ribeiro et al., 2020).

Nanocellulose has gained increasing interest for a wide range of applications in different fields of engineering due to its renewability, anisotropic nature, excellent mechanical properties, good biocompatibility, tailorable surface chemistry, and interesting optical properties (Iwamoto et al., 2009; Joseph et al., 2019); moreover, nanocellulose based edible coatings have been found to have anticancer properties (Joshy et al., 2020a). The most beneficial attributes of nanocellulose are the green nature of the particles, their physical and chemical properties, and the diversity of applications that can be derived from this material (Sultana et al., 2020; Joseph et al., 2020a). It is a natural nanoscale product, and it also possesses characteristics like special morphology and geometrical dimensions, crystallinity, high specific surface area, rheological properties, liquid crystalline behaviour, alignment and orientation, mechanical reinforcement, barrier properties, surface chemical reactivity, biocompatibility, biodegradability, lack of toxicity, etc. (Lin, Dufresne, 2014). Nanocellulose can be extracted from natural sources and has antimicrobial properties. Paper coatings with cellulose nanofibrils have been shown to have resistance to air permeance and enhanced tensile properties (Azeredo et al., 2017). Films made of cellulose nanofibrils (CNF) and cellulose nanocrystals (CNC) have high mechanical strength and outstanding oxygen barrier properties (Cherpinski et al., 2018). Poor water barrier properties of biopolymers like nanocellulose can be controlled by combining with edible oils or essential oils (Joshy et al., 2020b).

Aloe vera is well known for its medicinal and therapeutic properties (Valverde et al., 2005). The predominant medical uses of the orally ingested gel juice are against ulcerous, gastrointestinal, kidney, and cardiovascular problems and also to reduce the cholesterol and triglyceride levels in blood. Due to its other properties like anti-inflammatory and antibiotic activities, it is also used against some diseases (diabetes, cancer, allergy, AIDS) (Eshun, He, 2004). *Aloe vera* extract is tasteless, odourless, and colourless, and, therefore, it can be used in edible coating as an additive. *Aloe vera* contains many constituents, which results in anti-microbial properties. Due to the presence of carbohydrates, saccharides, etc., *Aloe vera* can form a barrier on the fruit surface, which will considerably reduce the respiration rate, decay rate, and water loss (Misir et al., 2014). *Aloe vera* based coating was used for enhancement of storage life and quality maintenance of papaya fruits due to its antifungal activity (Marpudi et al., 2011). Inner gel extract of *Aloe vera* was shown to be effective against Gram positive and Gram negative bacteria (Habeeb et al., 2007; Sánchez et al., 2020b). A study by Hazrati et al. (2017) showed that *Aloe vera* gel was an effective coating material for peach fruits during cold storage period. The coating was effective against weight loss and colour change (Hazrati et al., 2017). Strawberries coated with banana starch-chitosan and *Aloe vera* gel showed decreased decay rates under commercial refrigerated conditions and shelf life was found to increase up to 15 days (Pinzon et al., 2020).

Films based on chitosan and nanocellulose crystals have shown superior antimicrobial characteristics (Amirabad et al., 2018; Naseri et al., 2015). It is also reported that reinforcing nanocellulose with other long-chain polymers, such as chitosan, improves the quality of the packaging material as well as ensures long storage life (Sundaram et al., 2016). Chitosan/polycaprolactone based bilayer films containing grape seed extract and nanocellulose were used to enhance the quality of chicken breast fillets. The modified films showed antioxidant activity and higher antimicrobial effect and further restricted the growth of mesophilic aerobic and coliform bacteria in fresh chicken breast fillets (Sogut, Seydim, 2019). The effect of chitosan based edible coating on post harvested fruit quality of strawberry fruits showed that chitosan treatment could inhibit oxidative enzyme activity in strawberries during storage, and decay did not begin in chitosan treated fruit 9 days longer than in untreated fruit (Wang, Gao, 2013). A recent study on chitosan/*Aloe vera* combination for extending shelf life of blueberries demonstrated that addition of chitosan and *Aloe vera* enhanced the shelf life of the fruits and showed the potential to fight against fungi (Vieira et al., 2016). To the best of our knowledge, rather few studies reported the effectiveness of chitosan based nanocellulose films as edible coatings incorporating *Aloe vera* as an additive.

The right combination of new bio-materials and preparation methods is significant for improving the effectiveness of coating material to preserve fruit and vegetables. This study details the preparation of chitosan/nanocellulose based films containing *Aloe vera* by solvent casting method. The study was focused in this initial phase on investigating the physicochemical characteristics of the coating material along with its effectiveness as an edible coating material for vegetables stored in humid conditions.

Materials

Chitosan (Sigma Aldrich), nanocellulose suspension (extracted from 1000 g of pineapple leaf fibres and processed using an autoclave), glycerol (Sigma Aldrich), *Aloe vera* oil (AL KAMIL Factory for Natural Products), glacial acetic acid (Emplura 99–100 %).

Preparation of chitosan based nanocellulose film

Synthesis of nanocellulose suspension

Cellulose is obtained from raw material taken for alkali treatment and bleaching, as this will break the intermolecular and intramolecular hydrogen bonding between the hydroxyl group of cellulose and hemicellulose and can increase the hydrophilicity of fibres (Abraham et al., 2011).

Nanocellulose was extracted from pineapple leaves using the following procedures.

(a) Alkaline treatment of pineapple fibre: About 1000 g pineapple leaf fibres were collected and processed using an autoclave set at 100 °C for 1 hour (Mahardika et al., 2018; Cherian et al., 2011). After that, steam explosion was done and then the alkali treated fibres were exposed to water until it reached neutral pH. The alkali treatment process was repeated five more times and then the treated fibres were completely neutralized by washing with water and were subjected to bleaching process (Cherian et al., 2010).

(b) Bleaching process: For bleaching process, 1:1 mixture of the two solutions was used. The solution A (1000 ml) consisted of sodium hydroxide, acetic acid, and water (26.5 g, 73.3 ml, and 900 ml, respectively) and solution B (1000 ml) consisted of 1:3 mixture of sodium hypochlorite and water (250 ml and 750 ml, respectively). The bleaching was carried out in an autoclave at a pressure of 1.5 kg/cm^2 at 100 °C for 1 hour. Fibres were subjected to washing after the steam explosion and the process was continued until the fibres became clear white (the process was repeated four times). Finally, the bleached fibres were thoroughly washed using distilled water until the odour of the bleaching agent had been removed completely. The bleached fibres were then subjected to oxalic acid treatment (Cherian et al., 2011; Asrofi et al., 2017).

(c) Oxalic acid treatment: Acid hydrolysis treatment was found to increase the crystallinity and reduce the diameter of the fibres. 11 % oxalic acid was used, and the bleached fibres were treated with oxalic acid in an autoclave (1.5 kg/cm^2, 100 °C). The process was repeated six times, and the treated fibres were washed with distilled water. After neutralization, the fibres were subjected to homogenization (Asrofi et al., 2017; Fahma et al., 2011).

(d) Mechanical treatment: The extracted fibres were suspended in distilled water and stirred using homogenizer (10000 rpm for 5 hours), which resulted in 3.33 wt.% nanocellulose suspension formation. The obtained nanocellulose was analysed using FTIR, SEM, and TEM (Chandra et al., 2016; Santos et al., 2013).

Preparation of solution for edible coating film

For preparing nanocellulose/chitosan solution (Sample 1, presented as NC/CH), varied compositions of chitosan were weighed (0.5 g, 1.0 g, 1.5 g, 2.0 g) and kept in a beaker. Weighed chitosan was dissolved in 3 % acetic acid solution and kept overnight to get maximum solubility. Nanocellulose suspension containing 3.2 g solids in 100 ml water was added to the chitosan solution at a 1:1 ratio. Then, around 30 ml of water was added to the mix along with 2 drops of glycerine. Water and glycerol acted as plasticizer. The mixture was then stirred for 1 hour using a magnetic stirrer. After that, the mixture was homogenized for 10 minutes using a probe homogenizer at 10,000 rpm. The homogenized mix was sonicated in a probe sonicator for 10 minutes. After sonication, solution was cast. Sample 2 (presented as NC/CH/V) was prepared at a 1:0.25 ratio of chitosan and nanocellulose. The procedure described above was used to prepare Sample 2 except that different volumes of *Aloe vera* extract (0.25 ml, 0.35 ml, 0.5 ml, and 2.5 ml) were also added to the solution as an additive before homogenization.

Characterization techniques

Scanning electron microscopy (SEM)

The morphology of the nanocellulose/chitosan composite surface was examined at 15 kV under high vacuum conditions. A small part of the sample was cut and kept on the aluminium stage covered by carbon tapes, and then the samples were coated with an ultrathin gold layer (Au) using an ion sputtering machine.

Fourier transform infrared spectrometry (FTIR)

FTIR spectra were collected using a Nicolet 6700 Fourier Transform infrared spectrometer and measured over a 4000–650 cm^{-1} range. Both sets of samples (Samples 1 and 2) were examined. For each sample, 200 scans were averaged with a spectral resolution of 4 cm^{-1}.

Contact angle measurement

The hydrophilicity of edible coating films before and after adding *Aloe vera* was measured using digital contact angle analyser equipped with a CCD camera (Pheonix-KGV-5000) at room temperature. The films were placed on the stage of the machine. The contact angle was measured after carefully adding a water droplet (Milling-Q-Water) on the surface of the scaffold by using the software-controlled system. A series of images of the solution droplet was taken and the contact angle was measured accordingly. The measurements were taken at 3 different portions of the sample.

Tensile properties

Tensile measurements were done on a universal testing machine (Tinius Oslen H50K) by setting a crosshead speed of 50 mm/min and gauge length of 50 mm. Measurements were performed at ASTMD882 standard by using rectangular specimens. Average values were obtained from at least three successful determinations. The testing was conducted at 23±2 °C and 50±5 % relative humidity.

UV-visible spectroscopy

UV–Vis spectroscopic studies of the samples were carried out using a Shimadzu UV-2600 UV–Vis spectrophotometer. For the study, 1 ml solution of each sample was taken in a sample vial.

Weight loss study

The nanocomposite solution was applied on green chillies purchased at a local market with the objective of prolonging their shelf life during storage. The assessment was based on weight loss. The weight loss was measured for three days with a three decimal precision weighing balance. The value was expressed as a relative percentage and calculated as weight loss (%) = (Wi – Wt)/Wi *100 (where Wi is the initial weight and Wt is the weight measured during storage).

Results and discussion

Solvent casting method was used for fabrication of films (Saldanha, Kyu, 1987; Suh et al., 2002; Kuila, Nandi, 2004). It included solubilizing polymer in a suitable solvent followed by casting the solution in a Petri dish and then drying (Rhim et al., 2006). Suitable filler materials like plasticizers, cross linking agents, etc. were added prior to casting. Furthermore, solution filtration, sonication, or centrifugation for removing insoluble particles and air bubbles were also performed. Solution casting method is mainly used at laboratory scale to prepare films, and further research is needed in order to analyse its feasibility on commercial scale. The protocol for preparation of Sample 2 is given in Fig. 1.

The films were dried at 40 °C for about 24 hours. The films were transparent and exhibited uniform appearance and plastic-like properties (Fig. 2, 3). All films were easily peeled off from Teflon covered Petri dish. Increase in the plasticiser amount made the films stickier. Transparency of the prepared film will depend on the amount of nanocellulose, as an increase in nanocellulose content decreases the transparency of the film (Xu et al., 2019; Cazón et al., 2018). It can be safely assumed that chitosan is the material that is responsible for better film forming property.

SEM images of chitosan-nanocellulose composite films with 0.5 g, 1 g, 1.5 g, and 2 g chitosan, respectively, are shown in Fig. 4 (a-d). The nanocellulose fibres are clearly visible and

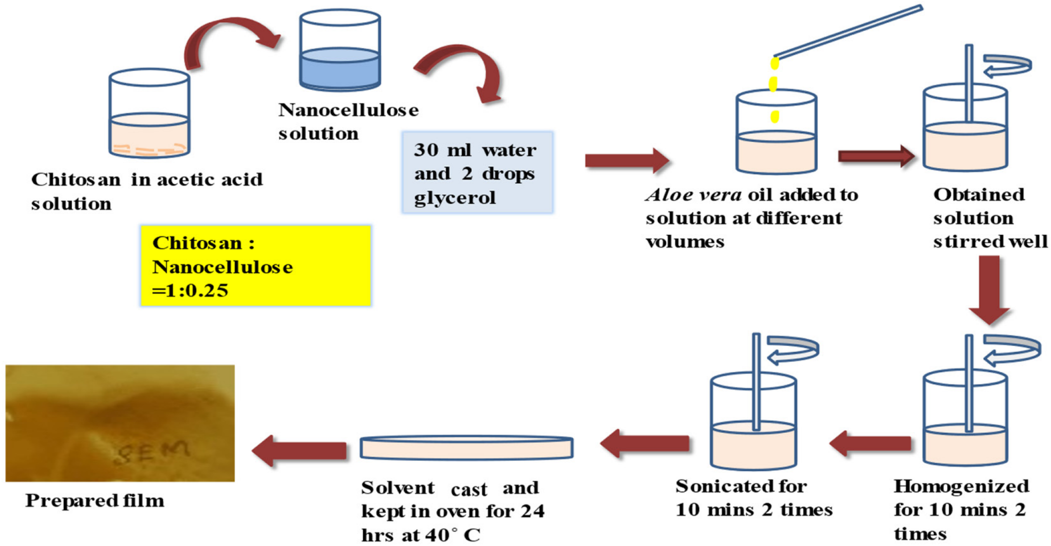


Fig. 1. Flowchart for the preparation of edible coating films of sample NC/CH/V (sample 2)

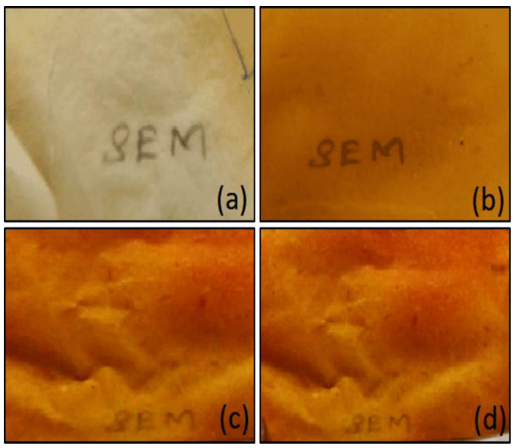


Fig. 2. Transparency of (a) NC/0.5 CH, (b) NC/1CH, (c) NC/1.5 CH, and (d) NC/2CH films

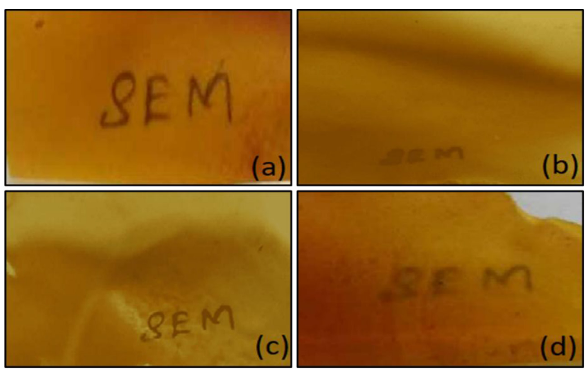


Fig. 3. Transparency of (a) NC/CH/0.25V, (b) NC/CH/0.35V, (c) NC/CH/0.5V, and (d) NC/CH/2.5V films

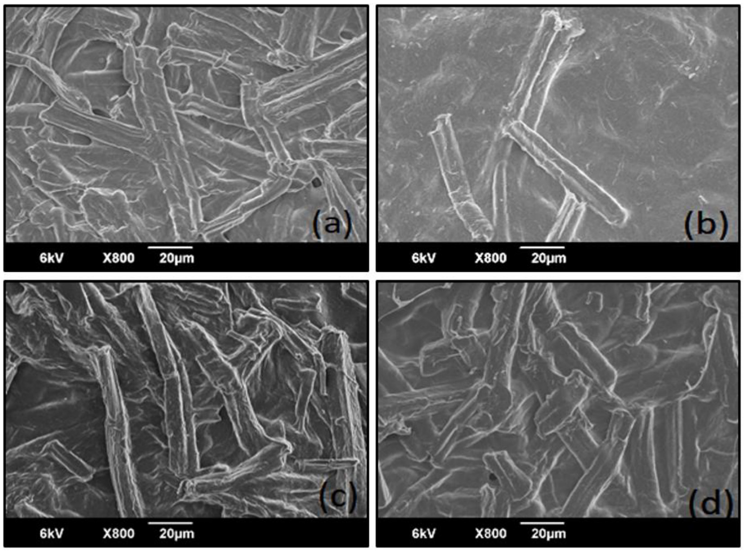


Fig. 4. SEM images of films of sample NC/CH with (a) 0.5 g, (b) 1 g, (c) 1.5 g, and (d) 2 g chitosan, respectively

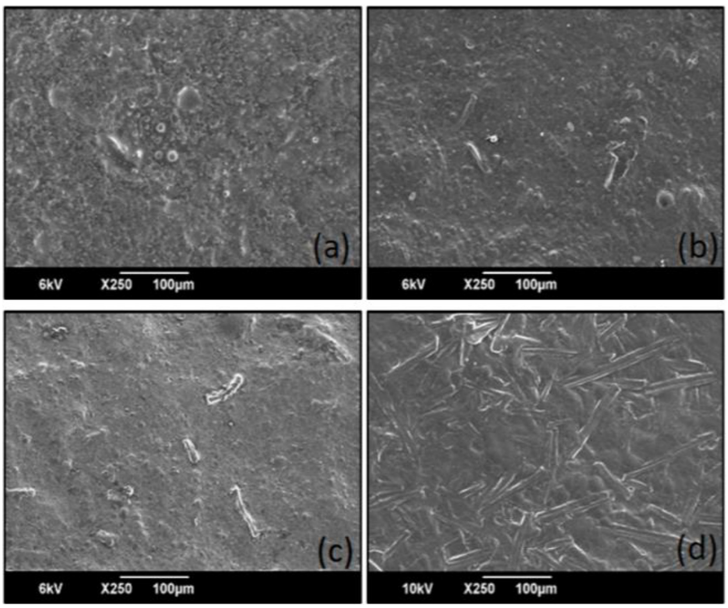


Fig. 5. SEM images of films of sample NC/CH/V with (a) 0.25 ml, (b) 0.35 ml, (c) 0.5 ml, and (d) 2.5 ml *Aloe vera*

are distributed throughout the chitosan matrix, and the fibre diameter varies from 3.96 μm to 7.26 μm. These images clearly show that the amount of nanocellulose fibre as filler is very high in these compositions. Fillers are dispersed in the matrix as multilayers and these contribute to the high strength of the film and its stiffness. It can be seen that the length of the fibres varies in the nanocellulose suspension. Mechanical properties of the film completely depend on the amount of filler (Corsello et al., 2017; Khan et al., 2012; Rubentheren et al., 2015). Fig. 5 shows SEM images of films with varied compositions of *Aloe vera*. The incorporation of *Aloe vera* made the film surface smooth. A coating-like appearance is also seen on the substrate.

The FTIR spectra were recorded to characterise the specific chemical groups present in each one of the materials and to analyse the effect of chitosan addition on nanocellulose films. The spectra of pure nanocellulose and chitosan were used as the reference. Fig. 6A shows the FTIR spectra of chitosan nanocellulose composite films with 0.5 g, 1 g, 1.5 g, and 2 g chitosan. The nanocellulose spectra contain typical bands for cellulose. The region between 3600 and 3200 cm^{-1} is assigned to OH stretching vibrations with the maximum at 3343 cm^{-1}, which is related to OH vibration due to hydrogen intramolecular bonding (Salari et al., 2018). Absorption peak in this range shows an increasing trend with the molecular weight of chitosan. This increase is due to the increase in hydrogen bonding between chitosan and nanocellulose. The 3000–2800 cm^{-1} region is related to CH and C-H_2 stretching vibrations. This peak also shows an increase in intensity with increased molecular weight of chitosan (Balti et al., 2017). The 1500–1250 cm^{-1} region is related to CH deformation and OH out-of-plane bending vibrations. The 1180–800 cm^{-1} region has the same pattern for the nanocellulose and chitosan samples due to their similar structure. This region is also sensitive to the C-O and C-H stretching vibrations. Moreover, there was a drastic increase in the intensity of the absorption bands at 1054 and 1032 cm^{-1} with increasing molecular weight of chitosan (Khan et al., 2012). This is due to the increased interaction of nanocellulose with chitosan. However, other changes, which occurred due to the increased molecular weight of chitosan, were minor at 1538 and 1340 cm^{-1}, and their intensity increased with increasing molecular weight of chitosan. The main characteristic feature is the presence of bands at 1610 cm^{-1}, which is related to stretching of carbonyl group, while bands at 1740 cm^{-1} are related to ester-stretching (Salari et al., 2018).

Fig. 6B shows the FTIR spectra of chitosan-nanocellulose composite films with different amounts of *Aloe vera*. For comparative study, spectra of neat chitosan (1 g) and chitosan-nanocellulose (1 g chitosan) without *Aloe vera* are also included. Except chitosan-nanocellulose film without *Aloe vera*, all other films show a band at 3000–3600 cm^{-1}, which is attributed to the hydroxyl groups of the adsorbed water (Bajer et al., 2020). This band is broad in the case of plain chitosan film. In composites with

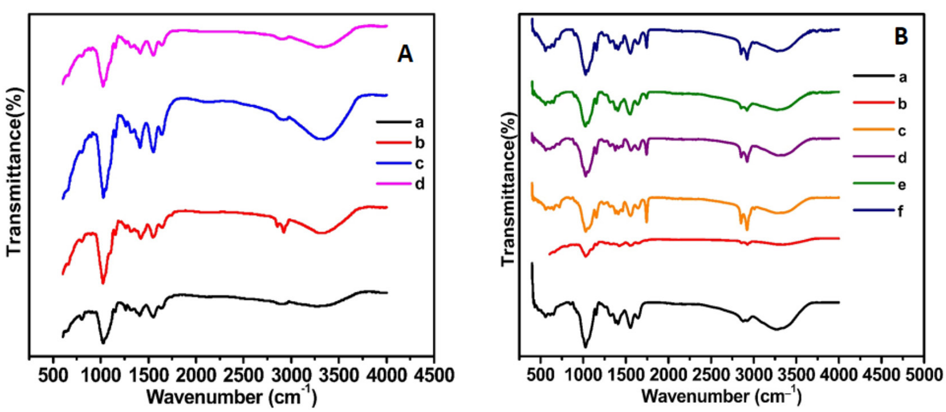
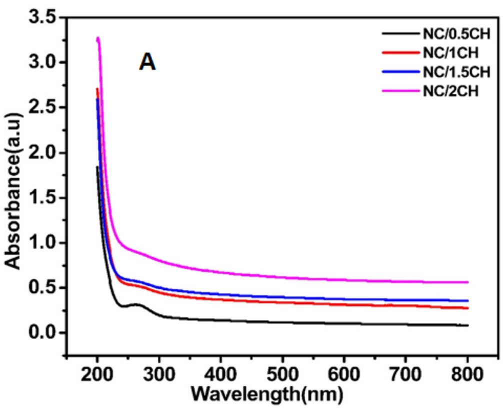


Fig. 6. (A) FTIR spectra of films of sample NC/CH with (a) 0.5 g, (b) 1 g, (c) 1.5 g, and (d) 2 g chitosan, respectively and (B) FTIR spectra of samples with (a) 1 g chitosan only, (b) sample NC/CH with 1 g chitosan and then spectra of samples NC/CH/V with (c) 0.25 ml, (d) 0.35 ml, (e) 0.5 ml, and (f) 2.5 ml *Aloe vera*, respectively

Aloe vera, these bands become narrower. A clear assignment of characteristic modes in this spectral range is not trivial because the hydroxyl stretching vibration bands overlap with the vibrations of N–H stretching of chitosan. This band can also be assigned to –OH stretching groups of *Aloe vera* constituents (e. g. uronic acid, mannonse or galactruonic acid) or phenolic groups in traquinones such as aloinand emodin present in *Aloe vera* (Torres-Giner et al., 2017). Such a wide range may also indicate the formation of intermolecular hydrogen bonds caused by dipole – dipole attraction forces. It can be assumed that in the studied systems, this type of bonds may be formed between the same (i. e., chitosan-chitosan) or various (*Aloe vera*-chitosan) molecule interactions.

Spectral region between 900 and 1200 cm^{-1} has an intense absorption band. This band is predominantly visible in the case of composites with *Aloe vera* and in the plain chitosan sample. These are present due to the C–O–C bond (in anhydrase glucose ring) stretching vibration of polysaccharide in *Aloe vera* gel but also in chitosan. This moiety, called «saccharide band», is sensitive to some conformational changes and is directly related to the crystal and amorphous phase in samples. It has been reported that a small band at ~ 1200 cm^{-1} is assigned to C–O stretching vibration and intramolecular hydrogen bonding of OH groups in chitosan (Arias et al., 2018; Anicuta et al., 2010; Pawlak, Mucha, 2003). The band at 1200 cm^{-1} in plain chitosan sample is shifted to higher frequencies (1210 cm^{-1}) in the spectra of the sample with 0.25 ml, 0.35 ml, 0.5 ml, and 2.5 ml *Aloe vera* addition. It is probably due to some interactions between chitosan and glycerol with *Aloe vera* macromolecules through H-bonding, which facilitates the mutual intercalation of substrates. This characteristic band is also sensitive to water content, and it shifts into higher frequencies when water content increases (Bajer et al., 2020, El Fawal et al., 2019).

UV–Vis adsorption spectra were recorded to determine the optical properties of chitosan-nanocellulose films as shown in Fig. 7A. Film opacity is one of the important factors that determine the quality of nanocomposite films. Transparency/light transmittance of composite films was measured between 200 and 800 nm, using a UV–Vis recording spectrophotometer. The films obviously showed transmittance values in the range of both visible light (400–800 nm) and ultraviolet light (200–400 nm) (Salari et al., 2018). As we increased the molecular weight of chitosan, the films showed an increase in absorbance and therefore a decrease in transmittance (Fig. 7A) (Valencia-Chamorro et al., 2011; Kaya et al., 2018).



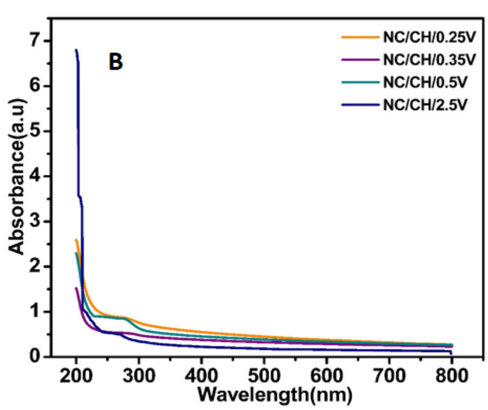


Fig. 7. UV–Visible spectra of (A) samples NC/CH and (B) samples NC/CH/V

Fig. 7B shows the UV–Vis absorption spectra of chitosan-nanocellulose films with different quantities of *Aloe vera* extract. An adsorption band between 250 and 350 nm is shown for the films containing *Aloe vera*. However, no such band was seen in the given range for chitosan-nanocellulose films without *Aloe vera*. The absorbance of nanocomposites near UV region of 250 nm to 350 nm increases with an increase in *Aloe vera* content (Fig. 7B). This absorbance is notably higher in the case of 0.5 ml *Aloe vera* incorporated film. In this region, the transmittance decreases significantly. This reduction in the light transmittance value in the UV region compared to the chitosan-nanocellulose films without *Aloe vera* confirms the UV absorption potential of *Aloe vera* incorporated in the matrix. This will certainly help to protect the food items from UV degradation (Nieto-Suaza et al., 2019; Sánchez et al., 2020a).

Contact angle measurements give the information about hydrophobicity or hydrophilicity of materials. This property is very important for their application as edible coating materials, especially in food industry, where the use of limited water permeability is crucial to maintaining the durability of food products (Noorbakhsh-Soltani et al., 2018). In this work, wetting characteristics of chitosan based nanocellulose composite films with different compositions were studied. Wetting characteristics such as work of adhesion, total surface free energy, and spreading coefficient were studied in detail.

Fig. 8 shows the contact angle images of 0.5 g, 1 g, 1.5 g, and 2 g chitosan films. All the samples show contact angle less than 90 degrees, indicating its hydrophilic nature. The contact angles increase with an increase in chitosan concentration up to 1.5 wt.% and after that, they are reduced. This may be due to better interaction between nanocellulose and chitosan through hydrogen bonding. However, at a higher concentration of nanocellulose, the presence of OH groups in nanocellulose makes it more hydrophilic. A detailed analysis of the

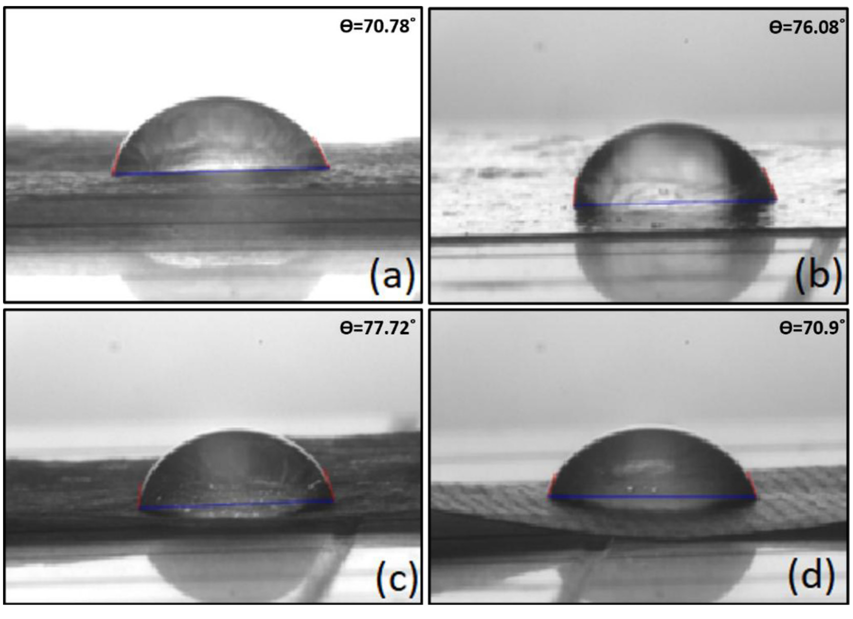


Fig. 8. Contact angle images of films of sample NC/CH with (a) 0.5 g, (b) 1 g, (c) 1.5 g, and (d) 2 g chitosan, respectively

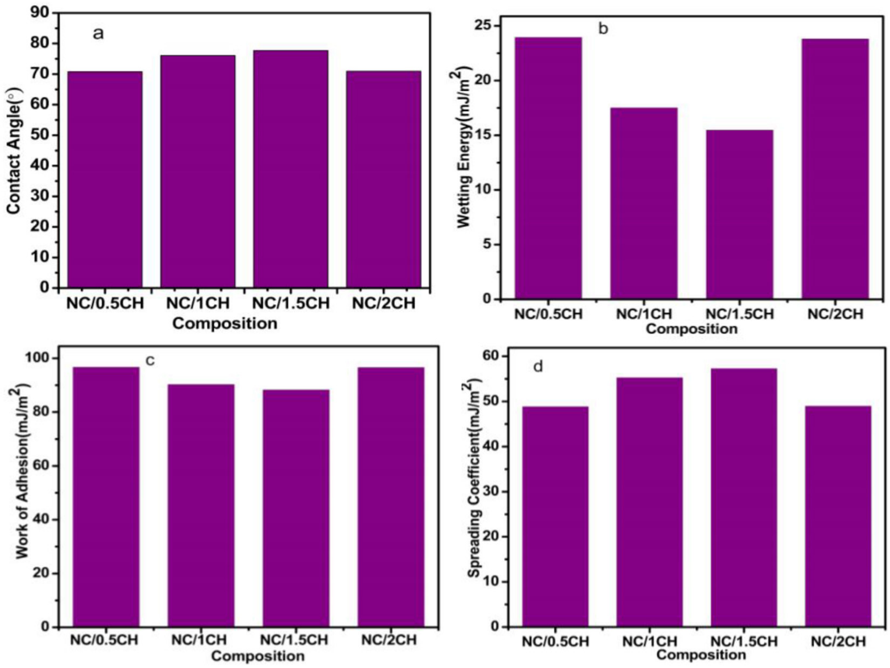


Fig. 9. Graph shows (a) contact angle v/s composition, (b) wetting energy v/s composition, (c) work of adhesion v/s composition, and (d) spreading coefficient v/s composition of sample NC/CH

films was done by plotting other parameters like wetting energy, work of adhesion, and spreading coefficient (Fig. 9). It is evident that the maximum contact angle is observed in the case of 1.5 g chitosan composite and, thus, this composition will have the highest hydrophobicity and lowest surface energy.

According to the theory of wetting process, if the solid–vapour interfacial energy is low, the tendency of liquid for spreading will be less and, thus, the system will be more hydrophobic. The solid surface is rich in hydrocarbon molecules. The forces that hold hydrocarbons together are much weaker than the force that acts between water molecules. A higher value for wetting energy means the liquid will spread over the surface. So, the film with the lowest wetting energy is less hydrophilic in nature. This is a favourable characteristic for edible coating application. The lowest wetting energy is shown by 1.5 wt.% of chitosan, indicating more hydrophobic nature as explained.

The work of adhesion (WA) is the work required to separate the composite surface and the liquid droplet. It is evident that film with 1.5 g of chitosan will have the lowest value for work of adhesion. Generally, the work of adhesion is found to decrease with an increase in the complexity of the composite and with a decrease in interfacial bonding. The polar and non-polar interactions across interface are a measure of adhesion between the test liquid and composite surface. Work of adhesion can be correlated to the nanocellulose-chitosan interaction. The effective dispersion of nanocellulose into the chitosan matrix decreases the work of adhesion, and, thus, an increment in the hydrophobic nature of the composite can be seen. Spreading coefficient gives the idea about the wetting and spreading of a liquid on the surface of a solid. A positive value of spreading coefficient indicates spontaneous wetting and spreading of the liquid on the surface of a solid, and a negative value indicates poor wetting. It also depends on the surface tension of the test liquid: a liquid with high surface tension would not spread much. Spreading coefficient (Sc) values indicate that a liquid will spontaneously wet and spread on the solid surface if the value is positive, whereas a negative value of spreading coefficient implies the lack of spontaneous wetting. This means that there is a finite contact angle (i. e., u>0). The polar–polar interactions across the interface are a measure of wetting. Spreading coefficient increases with the amount of chitosan and, thus, hydrophobicity of nanocomposites will be enhanced.

Fig. 10 presents the contact angle images of chitosan-nanocellulose composite film with 0.25 ml, 0.35 ml, 0.5 ml, and 2.5 ml *Aloe vera*, respectively. The contact angle shows an initial increase, and above 0.5 ml of *Aloe vera*, it is reduced. The quantified data of contact angle, wetting energy, work of adhesion, and spreading coefficient for Sample NC/CH/V are given in Fig. 11. The data suggest that film containing 0.5 ml *Aloe vera* has favourable properties for edible film such as high contact angle, low wetting energy, low work of adhesion, and high spreading coefficient, which results in hydrophobic nature. It is evident from the analysis that addition of *Aloe vera* oil enhanced hydrophobic nature of films.

Fig. 12 shows the stress-strain graph of chitosan-nanocellulose films with 0.5 g, 1 g, 1.5 g, and 2 g chitosan. From the graph, it is clear that film containing 1 g chitosan has the maximum strength, which indicates that this particular composition has good dispersion of nanocellulose.

Successful coating should provide adequate mechanical strength to fruit and should be free from minor defects. This attribute can be accomplished by having a flexible coating that is resistant to breakage and abrasion. Tensile strength and elongation at break are parameters that affect this property. Factors that are critical to mechanical properties include polymer structure, type of plasticizer, concentration of plasticizer, molecular weight of coating forming materials, types of solvent, and thickness of coating. The tensile strength of chitosan-based bio composite increased with molecular weight of chitosan (Fig. 13). The increase in the TS

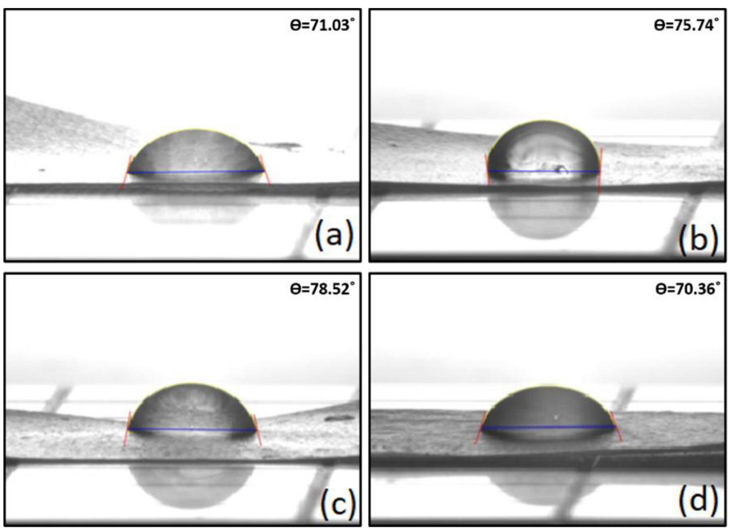


Fig. 10. Contact angle images of films of sample NC/CH/V with (a) 0.25 ml, (b) 0.35 ml, (c) 0.5 ml, and (d) 2.5 ml *Aloe vera*, respectively

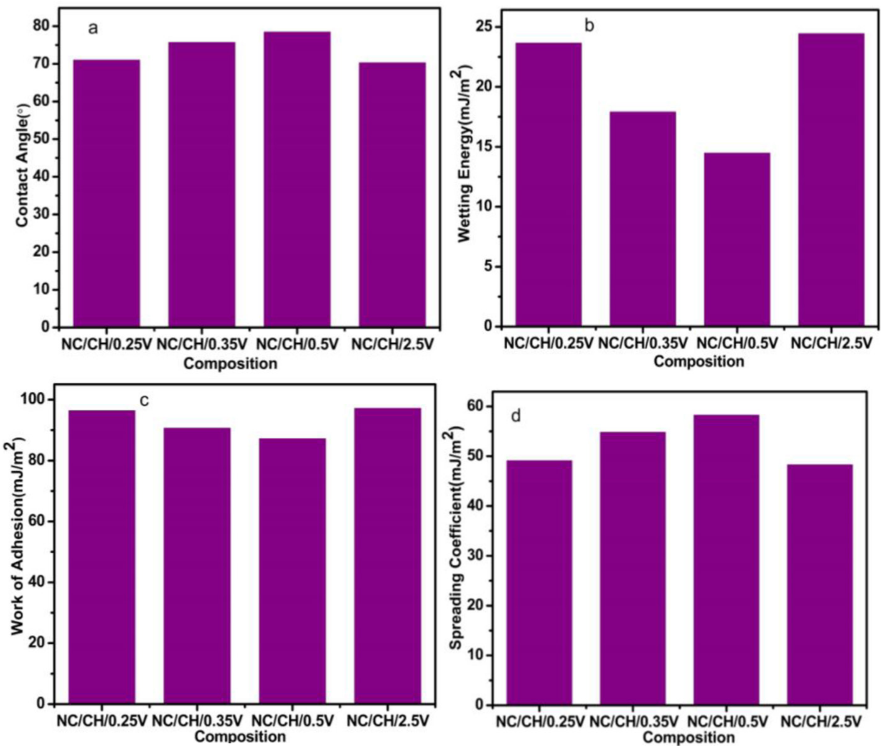


Fig. 11. Graph shows: (a) contact angle v/s composition, (b) wetting energy v/s composition, (c) work of adhesion v/s composition, and (d) spreading coefficient v/s composition for sample with *Aloe vera* (sample NC/CH/V)

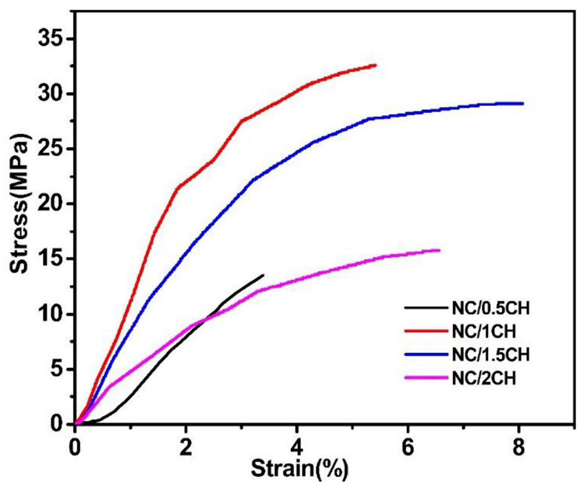


Fig. 12. Stress v/s Strain graph of films of sample NC/CH

values of the nanocellulose reinforced chitosan films can be attributed to two factors such as (1) the favourable nanofiber-chitosan interactions and (2) the reinforcing effect occurring through effective stress transfer at the nanofiber–chitosan interface (Valencia-Chamorro et al., 2011). The interaction between the anionic sulphate groups of nanocellulose and the cationic amine groups of

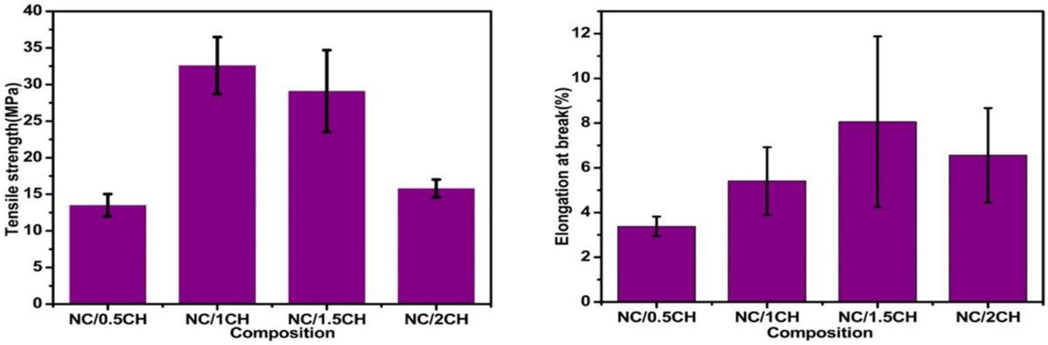


Fig. 13. Tensile strength vs composition, elongation at break vs composition of NC/CH

chitosan might favour a good interface between the matrix and the filler. This may lead to high tensile strength values of the nanocomposite films.

The elongation at break of chitosan-based bio composite increased with molecular weight of chitosan (Fig. 13). Elasticity of composite films is influenced by interaction of plasticizer with chitosan matrix. Thus, as molecular weight of chitosan increases, the interaction of chitosan with glycerol and water increases, which will result in increased elasticity or elongation at break with increasing molecular weight of chitosan.

Fig. 14 shows the stress-strain curve of composite film with 0.25 ml, 0.35 ml, 0.5 ml, and 2.5 ml *Aloe vera* and pure chitosan film (1-CH). The mechanical properties were enhanced by the addition of *Aloe vera* into the composite. Maximum ultimate stress is exhibited by the film with 0.25 ml *Aloe vera* (Fig. 14). The curve of this particular composition is ideal and film with this particular composition will have better strength for the application of edible coating.

For further clarification plots of composition v/s modulus, elongation at break, and tensile strength graphs were analysed (Fig. 15). Tensile strength (TS, in MPa) indicates the maximum tensile stress that the film can sustain; elongation at break (E, in%) is the maximum change in the length of a test film before being broken; and elastic modulus (EM, in MPa) is a measure of the stiffness of the film as a function of *Aloe vera* incorporation. These mechanical

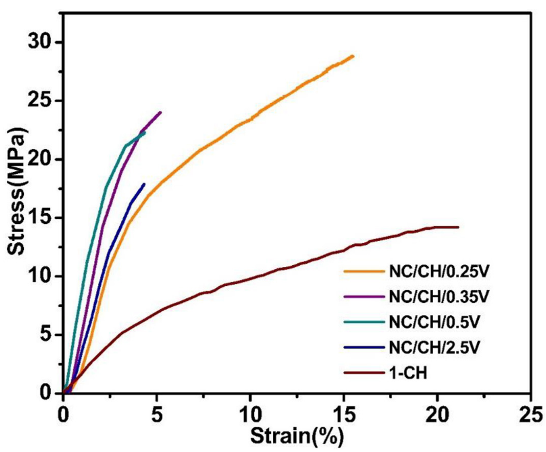


Fig. 14. Stress v/s Strain graph of films of sample NC/CH/V

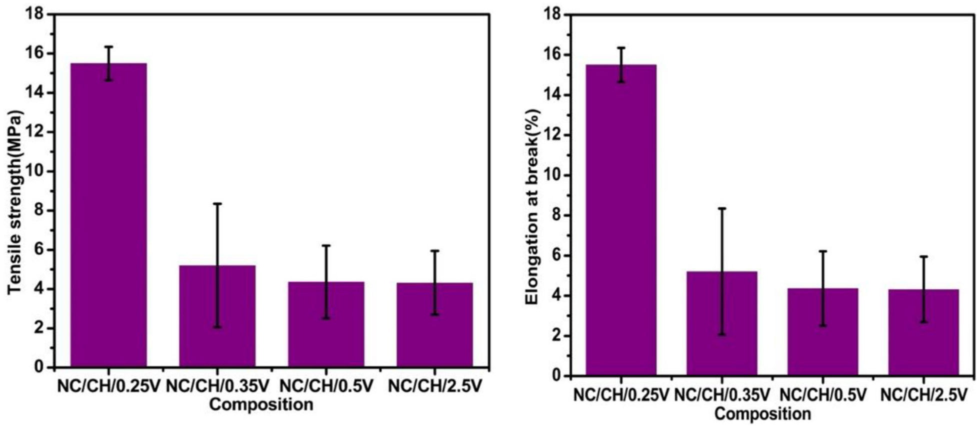


Fig. 15. The graphs of composition v/s tensile strength and elongation at break of composites with different compositions of *Aloe vera*

characteristics showed that by the addition of *Aloe vera* when compared to the plain composite film, a decrease in tensile strength was observed. However, it is quite clear from the graph (Fig. 14) that the modulus of the material is enhanced initially and tends to decrease with further addition. The tensile strength and elongation at break decrease with an increase in the amount of *Aloe vera* added. But data of plain composite films reveal that the tensile strength increases with the addition of *Aloe vera*. Results suggest that the optimum amount of *Aloe vera* needed to get an ideal film with maximum strength and elasticity is 0.25 ml.

The water content of fruits and vegetables is a major factor in maintaining quality of horticultural products. Low weight loss is important in maintaining the fruit quality over longer time. Weight loss is associated with respiration and transpiration of moisture. According to Lin et al. (2002), water loss in longan fruit was mainly from the pericarp rather than the aril (pulp). The water loss was also known to positively correlate with the pericarp browning of longan fruit (Lin et al., 2002; 2005). In this study, weight loss of nanocellulose (NC)/chitosan (CH)/*Aloe vera* coated samples and uncoated chillies was determined for a period of three days (Fig. 16).

NC/CH/0.25 V treated chillies were noted to have relatively lower weight loss throughout storage days, as compared to standard chillies without any treatment and chillies treated with NC/CH/0.35V. By Day 3, the weight loss of uncoated chillies was 43.7 % and that of NC/CH/0.25V coated chillies was 39.1 %. The weight loss of chillies coated with NC/CH/0.35 V was 40.3 %. Incorporation of nanocellulose and *Aloe vera* in the coating solutions shows a decrease in the weight loss percent. The barrier properties of coating film formed on the surface could be increased by the addition of nanocellulose, chitosan, and *Aloe vera* to coating solutions. Chitosan/nanocellulose/*Aloe vera* coated chillies show that the prepared film is effective, and it helps to enhance the storage life of chillies under humid condition.

Conclusions

An environmentally friendly bio nanocomposite edible coating material was successfully fabricated utilizing chitosan and nanocellulose with the addition of *Aloe vera* as an antimicrobial agent, via solvent casting

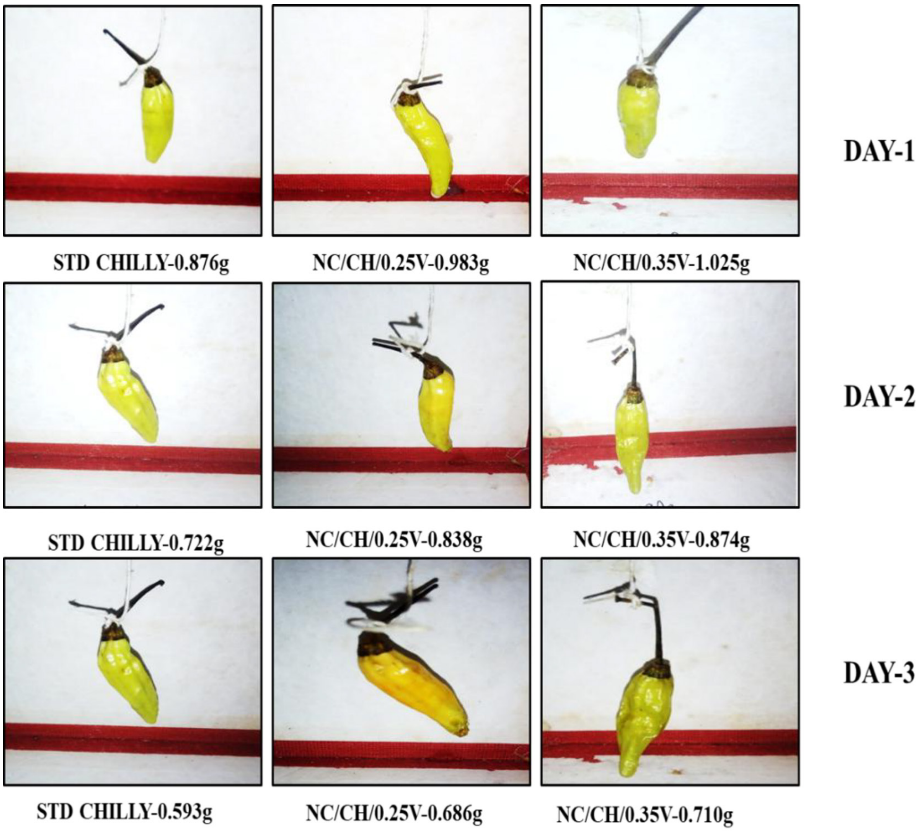


Fig. 16. Weight loss studies of uncoated and NC/CH/*Aloe vera* coated chillies for 3 days

technique. Due to the fibrous structure and fine dispersion of nanofibers over the chitosan matrix, there is an enhancement in properties of nanocomposites after reinforcement. But at higher filler loading, the properties decrease probably due to agglomeration of these nanofibers. Dispersion of nanocomposite is visible from the SEM images. Also, optical studies and UV visible studies show a nanoscale dispersion in the composite. Mechanical analysis guarantees the strength of the composite. Contact angle analysis demonstrates that the material is water repellent, which is a favourable property of edible coating materials. Transparency of the film is a great advantage, as we can provide a natural appearance to the fruits or vegetables to be coated. Coating application study using chilli gives a satisfying result, but further modifications are needed to prepare a better and promising material.

Chitosan based nanocellulose composite can be effectively used as an alternative to synthetic fruit coating. It can be used to increase the shelf life and freshness of fruit as well. Moreover, biodegradability is the main advantage of edible over polymer traditional synthetics, as they can be consumed with the products. Even if the films are not consumed, they are completely biodegradable and will not produce any detrimental effect on the environment.

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