STUDIES ON DEVELOPMENT OF FUNCTIONALIZED BIOPOLYMERIC NANOCOMPOSITE BASED EDIBLE NANOCOATING ON FOOD PRODUCTS AND SUSTAINABLE SECONDARY PACKAGING

Thesis submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

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Dedication

This thesis is proudly dedicated to

my sweet and loving parents

for their endless love, support and encouragement of day and night which make me able to get this honor.

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CERTIFICATE

This is to certify that the research work in the thesis entitled "**Studies on Development of Functionalized Biopolymeric Nanocomposite Based Edible Nanocoating on Food Products and Sustainable Secondary Packaging**", is carried out by me at the Department of Chemical Engineering, Indian Institute of Technology Guwahati, as partial fulfillment for the award of Doctor of Philosophy under the supervision of Dr. Vimal Katiyar, Professor at Department of Chemical Engineering and Dean (Research and Development), Indian Institute of Technology Guwahati, Assam, India. The research outcomes documented in this thesis are accomplished by me and has not been submitted to any other Institute or University for the award of any degree or diploma.

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CERTIFICATE

This is to certify that the thesis entitled **"Studies on Development of Functionalized Biopolymeric Nanocomposite Based Edible Nanocoating on Food Products and Sustainable Secondary Packaging",** being submitted by **Ms. Tabli Ghosh (Roll No: 166107020),** a student of Department of Chemical Engineering, Indian Institute of Technology Guwahati as partial fulfillment for the award of Ph.D. under my guidance and supervision. The research work documented in this thesis has not been submitted to any other University or Institute for the award of any degree or diploma.

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Blessings of Almighty are always solicited

Abstract

In the current century, the rising environmental issues caused by increased carbon footmarks and waste generation from conventional packaging systems have gained concern in developing environment benign polymers based edible and non-edible food packaging systems availed from renewable resources and their modified forms, which can transport the former produce to end-users in a safe condition. Additionally, the use of renewable biomaterials for the fabrication of edible coated food products (a kind of edible food packaging) has become indispensable to reduce the food waste generations. In this context, the present thesis work directs the use of bionanostructures based on modified polysaccharide (iron functionalized cellulose nanofiber, and sodium tripolyphosphate crosslinked nanochitosan) and protein (silk nanodisc) to tailor the inherent properties of biopolymers to be used as edible coating materials on perishable fruit products (a type of primary packaging) and their sustainable secondary packaging (a secondary packaging for edible coated food products) for safe delivery. The cellulose nanofiber (CNF), being fabricated from cellulose, is investigated for developing edible coating on food products. The fabrication of iron functionalized CNF (mgCNF) following single step co-precipitation technique is a way to adsorb iron particles on CNF molecule to be used as a reinforcement in developing chitosan (CS) biocomposites. Additionally, the formulation of curcumin (Cur) (a potential anticancerous agent) loaded mgCNF dispersed CS as edible nanocoating on cut pineapple delivers a tailored-made functionalized nanocomposite for anti-cancerous and iron-fortified ready to eat pineapple fruit products. The anti-cancerous property of edible-nanocoating materials are analysed, where the loading of Cur with CS based biocomposites shows anti-cancer activity by disrupting the cell membrane and further indicates the death of cancer cells with cell viability of ~17% and ~98% for CS/mgCNF (1.5 wt.%) with and without Cur, respectively. Further, the

nanocoatings are stable at heat sterilization temperature providing a new approach to treat the edible coated fruit products at high temperatures for obtaining enhanced shelf life. The optical properties and appearance of Cur loaded films provide red and yellow colorant effect with uniform transparency, which can be well known from a*, and b* values, and further increase consumer acceptance. It is noteworthy to mention that the developed edible nanocoating materials help to improve the storage conditions of cut pineapple. Therefore, the study delivers a technique to supplement the iron functionalized food products with anti-cancerous activity and provide improved performance to reduce food waste and, delivery of active compounds. Additionally, the formulated edible nanocoating materials are applied to coat whole kiwifruits and the keeping quality of kiwifruits is analysed during the storage period (10 days and 10 °C). The influence of edible nanocoating on firmness, weight loss analysis, color parameters, and physicochemical properties has been studied. Interestingly, at 10 days of storage, there is noticed a sharp decrease in the firmness of uncoated kiwifruit (~5 N), whereas, the edible coated kiwifruits using Cur-CS-mgCNF (1.5 wt%) has maintained the firmness above 15 N till 10 days of storage. Thus, a well-developed new edible coating system based on Cur loaded functionalized CNF/CS nanocomposite has been fabricated for perishable food products. However, as discussed, the system has been targeted for iron fortified, anticancerous materials for improving the shelf life of food products. Thus, a simple edible coating system based on nanochitosan (developed by ionic gelation method) modified starch and guar gum biocomposites has been fabricated, which is suited for improving the product life.

Nanochitosan (NCS) is a kind of polysaccharide based nanostructures used as a component in developing edible coating material. The biopolymeric composites based on starch (ST) and guar gum (GG) based edible coatings are generally used to maintain the food properties during storage

life. However, the increased hydrophilicity and reduced transparency of ST-GG biocomposites have limited its use, thus requires strategic modifications to overcome the existing shortcomings. In this regards, the inclusion of NCS (a food based nanomodifier) in ST-GG biocomposites can modify surface properties, thermal properties, optical properties, antimicrobial properties, mechanical properties, and others. The development of edible nanocoating on cut apple using NCS modified ST-GG biocomposites has significantly improved the food properties during storage life. The application of NCS aided ST-GG biocomposites has been done as an edible coating on fresh cut apple and the materials are beneficial for maintaining weight loss at time lapse, color attributes, reduced microbial growth, pH change and other properties. In this context, a simple and new edible nanocoating system has been developed compared to existing system for perishable food products.

Besides, a protein nanostructure material has been used further to strategical conversion of waste to edible coating material. The nature-based silk fibroin is a protein material and its nanoform are significant in developing packaging system, specifically as edible coatings. The specified protein nanostructures based edible CS nanocomposite coating for fresh produces acts as a candidate with superior thermal, hydrophobic, optical, mechanical and food properties. Thus, the formulation of silk nanodisc (SND) dispersed CS based edible nanocoating on fresh banana fruit provide improved storage life. The source waste muga cocoon is a type of agro-based waste (sericulture), which can be utilized in food packaging through chemically modifications is considered as an interesting fact. In this context, waste muga cocoons through chemically modification routes including degumming process to obtain silk fibroin and acid hydrolysis of obtained silk fibroin (SF) fabricates silk nanodisc (SND). This is an interesting utility for strategical conversion of waste into wealth and further aids sustainability to the environment by reducing the food based waste using edible packaging. Further, the obtained disc shaped SND has amplified crystallinity

(~95.0%) with high heat stability (5 wt% loss for SND is obtained at ~193 °C which is found to upgrade drastically as compared to SF (~68 °C)) delivering a new area to be used in heat unstable food items. Due to various synergistic properties of silk nanostructures in terms of thermal properties, surface properties, optical properties, mechanical, and physicochemical properties of CS based biocomposites, the silk nanostructures has gained interest to be used in developing edible food packaging. The thermal property of SND (1.5 wt%) dispersed CS has been found to be improved by 9 °C (~275 °C) as compared to CS (~266 °C). The surface wettability of SND dispersed biocomposite enhanced by ~10° suggesting improved hydrophobicity. Thus, the use of noteworthy material obtained from agro-based waste (waste muga cocoons) to be utilized as edible food packaging for fresh produce *via* functionalizing the waste muga cocoons for increased food value and storage quality of fresh produces. The improved tensile strength for CS/SND biocomposite is beneficial to protect perishable fruit products against mechanical damages. To recognize the edible coating materials as safe, the component analysis has been done using energy dispersive X-ray (EDX) spectroscopy. It is noteworthy to mention that SND dispersed CS edible coatings provide an improved texture of banana fruits compared to uncoated banana fruits at 25 °C. Thus, the current thesis work addresses the use of functionalized biopolymeric nanocomposite as an edible coating (primary packaging) to improve the storage life of perishable fruit products.

In line with this, the development of secondary packaging can provide a complete set of packaging for the delivery of food products. The development of industrially viable secondary packaging for edible coated food products such as fabrication of blown films of poly (lactic acid) (PLA) based biocomposites. The study manifests an in-depth understanding on the effect of modified biofillers on the bulk properties of PLA blown films from the aspects of thermomechanical, crystallinity,

thermal stability, color, migration, surface wettability and others to be used as secondary packaging materials. Further, the study provides a new area of the application utilizing biodegradable blown films for food packaging application of edible coated food. Based on this, the current doctoral work focuses to develop functionalized biopolymeric nanocomposite based edible nanocoated food products and their sustainable secondary packaging materials for the safe delivery of food products.

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Nomenclature

Abbreviations

ALT	Alanineaminotransaminase
ANOVA	Analysis of variance
AST	Aspartateaminotransaminase
ATCC	American type culture collection
ATR	Attenuated total reflectance
BC	Bacterial cellulose
BCNC	Bacterial cellulose nanocrystals
BCNF	Bacterial cellulose nanofibers
BNC	Bacterial nanocellulose
BUR	Blow up ratio
CEO	Cinnamon essential oil
CFU	Colony forming unit
CMC	Carboxymethyl cellulose
CNC	Cellulose nanocrystal
CNF	Cellulose nanofibers
CNP	Cellulose nanoparticles
CNW	Cellulose nanowhisker
CS	Chitosan
CSNP	Chitosan nanoparticle
Cur	Curcumin
CW	Cellulose whisker
DCP	Dicumyl peroxide
DMA	Dynamic Mechanical Analyzer
DMCS	Dicumyl peroxide linked modified chitosan
DMEM	Dulbecco's modified eagle's medium
DMGA	Dicumyl peroxide linked modified gum Arabic
DMSO	Dimethyl sulfoxide
DSC	Differential Scanning Calorimetry
EDX	Energy Dispersive X-Ray Spectroscopy
ELZCD	Erlong Zuoci decoction

EOs	Essential oils
FBS	Fetal bovine serum
FCMs	Food contact materials
FDA	Food and Drug Administration
FESEM	Field Emission Scanning Electron Microscope
FETEM	Field Emission Transmission Electron Microscope
FTIR	Fourier Transform Infrared Spectroscopy
GA	Gum Arabic
GG	Guar gum
GPC	Gel permeation chromatography
GRAS	Generally Recognized As Safe
HPMC	Hydroxypropyl methylcellulose
ICP-MS	Inductively coupled plasma mass spectrometry
LA	Lactic acid
LBL	Layer-by-layer
LC-MCC	Lipid coated microcrystalline cellulose
MAP	Modified atmospheric packaging
MCC	Microcrystalline cellulose
MCS	Oligomer grafted CS
MD	Machine direction
MGA	Functionalized gum Arabic
mgCNF	Magnetic cellulose nanofibers
MTCC	Microbial type culture collection
MTT	3-(4,5-dimethylthyazol-2-yl)-2,5-diphenyltetrazolim bromide
NCF	Nanocellulose fibers
NCS	Nanochitosan
NFC	Nanofibrillated cellulose
NPLA	Neat poly lactic acid
NPs	Nanoparticles
OLLA	Oligomer lactic acid
PBS	Polybutylene succinate
PBSA	Polybutylene succinate-co-adipate
PCA	Plate count agar

PCL	Polycaprolactone
PDI	Polydispersity index
PE	Polyethylene
PES	Poly (ethylene succinate)
PET	Polyethylene terephthalate
PGA	Polyglycolic acid
PHA	Poly hydroxy alkanoates
PLA	Poly lactic acid
PP	Polypropylene
PPAd	Polypropylene adipate
PS	Polystyrene
REO	Rosemary essential oil
RH	Relative humidity
ROP	Ring opening polymerization
SA	Sodium alginate
SF	Silk fiborin
SND	Silk nanodisc
ST	Starch
STPP	Sodium tripolyphosphate
TA	Titratable acidity
TGA	Thermogravimetric Analysis
TiO_2	Titanium NPs
TS	Tensile strength
TSS	Total soluble solids
TVB-N	Total volatile basic nitrogen
UTM	Universal Testing Machine
UTS	Ultimate tensile strength
UV	Ultra-violet
VSM	Vibrating sample magnetometer
WG	Wheat gluten
WMC	Waste muga cocoons
WPI	Whey protein isolate
WPNF	Whey protein isolate nanofibrils

WS	Water solubility
WVP	Water vapour permeability
WVTR	Water vapour transmission rate
XPS	X-ray Photoelectron Spectroscopy
XRD	X-ray diffraction
ZEO	*Zataria multiflora* essential oil

Notations

% E	Elongation at break
$\%X_c$	% Crystallinity values
RR_{o_2}	Respiration rates in terms of O_2
RR_{Co_2}	Respiration rates in terms of CO_2
V_f	Free headspace volume of the chamber
ΔC_{CO_2}	Difference in initial and final gaseous concentrations for CO_2
ΔC_{O_2}	Difference in initial and final gaseous concentrations for O_2
Δt	Storage period between two consecutive observations
A	Area of the tested sample through which permeation occurs
a* values	Red to green coloration effect
As	Arsenic
b* values	Blue to yellow coloration effect
C. I.	Crystallinity index
E'_g	Storage modulus at glass transition state
E'_r	Storage modulus at rubbery state
L values	Brightness to darkness
LA-*g*-CS	Functionalized CS
LA-*g*-GA	Functionalized GA
Mn	Manganese
M_n	Number average molecular weight
M_w	Weight average molecular weight
p	Pendulum factor
Pb	Lead
PDI	Polydispersity index

s	Main scale reading in the direction tested
S	Saturation vapour pressure of water
$S_{amorphous}$	Area under amorphous peaks
$S_{crystalline}$	Area under crystalline peaks
T_{10}	Sample temperatures at 10% mass reduction
T_{50}	Sample temperatures at 50% mass reduction
T_{cc}	Crystallization temperature
Tfact	Tearing Factor
T_g	Glass transition temperature
Tind	Tearing Index
T_m	Melting temperature
T_{max}	Maximum degradation temperatures
T_{offset}	Offset thermal degradation temperature
T_5	Sample temperatures at 5% mass reduction
T_{onset}	Onset degradation temperature
T_p	Peak temperature
Tres	Tearing resistance
W	Weight of sample taken for the analysis
W_{650}	Weight residue at 650 °C
W_{max}	Weight residue at maximum degradation temperature
Y	Thickness of the tested films
Zn	Zinc
ΔE	Total color difference
ΔH_{cc}	Latent heat of crystallization
ΔH_m	Latent heat of melting

CHAPTER 1

भारतीय प्रौद्योगिकी संस्थान गुवाहाटी
Indian Institute of Technology Guwahati

Introduction and Literature Review

The edible coating, being a kind of eco-friendly packaging, is designed to be eaten and biodegradable in nature similar to the food materials. This packaging technique delivers a synergistic approach for enhanced product life as a preservation practice and further aids in sustainability to the society for reduced food packaging waste. The chapter puts a focus on addressing the status of edible food packaging in terms of edible films and coatings in the global market and its trend in research and development in regards to other available packaging materials. The classes of biopolymers and others to be used as a component of edible coating and films include polysaccharides, proteins, lipids and waxes, plasticizers, and additives. The edible coating based food packaging counts on good sustainable packaging as it helps in reducing food waste and also provides sustainability to the world by reducing plastic based waste. However, the present research work mainly focuses on developing tailored-made nanotechnology based edible coatings on perishable food products. It is noteworthy to mention that the inclusion of bionanostructured materials in edible coating can provide multifunctional edible packaging materials contributing tunable packaging property, food texture, and to keep food products fresh for a longer duration. Moreover, the transportation and exportation of edible coated food can be made easily available using various secondary packaging. Thus, the importance and the need of using green biocomposite based secondary packaging for safe delivery of coated food products has been made.

The work in this chapter are published as:

1. Katiyar, V. and **Ghosh, T.,** (2020) Book entitled "*Nanotechnology in Edible Food Packaging: Food Preservation Practices for a Sustainable Future*" in the series Materials Horizons: From Nature to Nanomaterials by Springer Nature, ISBN 978-981-336-169-0.
2. **Ghosh, T.,** & Katiyar, V. (2019). Chitosan-Based Edible Coating: A Customise Practice for Food Protection. In *Advances in Sustainable Polymers* (pp. 167-182). Springer, Singapore.
3. **Ghosh, T.,** Borkotoky, S. S., & Katiyar, V. (2019). Green Composites Based on Aliphatic and Aromatic Polyester: Opportunities and Application. In *Advances in Sustainable Polymers* (pp. 249-275). Springer, Singapore.

1.1 Introduction

Edible food packaging (edible films and edible coatings) is defined as a customized, and sustainable packaging practice that can be consumed with the food product or can be removed before eating the edible packaged food, where the packaging materials have the characteristics features of biodegradability similar to the food materials. With the current trend towards making a sustainable world, food processing industries are continuously developing the sustainable packaging materials as a replacement to available conventional packaging materials. The petrochemical plastics generate environmental pollution such as production of toxic components, increase in plastic based waste, etc. (Ghosh, Borkotoky, & Katiyar 2019; Katiyar, Gupta, & Ghosh 2019**)**. In the United States, about one third of municipal wastes are generated from packaging and containers (Aldred Cheek, & Wansink 2017). In this regards, several available biomaterials are continuously utilized for the upbringing of sustainable packaging due to their versatile nature such as non-toxicity, biocompatibility, biodegradability, renewability, easily available, degrade faster, no greenhouse gas emission, reduce carbon footprint, etc. (Ghosh, Borkotoky, & Katiyar 2019; Katiyar, Gupta, & Ghosh 2019; Chiellini, 2008; Mondal et al., 2019). Additionally, the food packaging is an essential component for delivering the food products to target consumers in a safe condition. The benefits of using plastic packaging include flexible nature, versatility, lightweight, economic value, etc. Adversely, the drawbacks of using plastic as packaging materials include degradation of the environment, durability, generates harmful agents to the environment, etc. Thus, the plastic based packaging materials are focused to be tailored for their various characteristics attributes, such as development of sustainable packaging materials in terms of edible or non-edible packaging, which has an ability to reduce the generation of harmful agents to the environment.

The use of edible films and coating started in the 12^{th} and 13^{th} centuries (Erkmen, & Barazi, 2018) and now is one of the dominant food packaging materials throughout the world. This

kind of packaging can be obtained in different types and are developed from several substances. The type of edible packaging is generally selected based on the targeted food products. Further, the use of different edible materials should have the status of Generally Recognized As Safe (GRAS) according to Food and Drug Administration (FDA). The most familiar use of edible packaging for consumers is found as the ice cream cone, where waffles or sugar based cones are utilized to carry ice-cream. The innovations and development of edible ice cream containers (Parr, 1931), and combine edible cones and ice cream (Saladino, & Samson, 1986) have put a remarkable impact in reducing the waste due to edible nature of the ice cream containers. Further, a fruit like casing, known as WikiCell, has been developed by a company *viz.* WikiFoods, which surrounds the foods and further, the casing can be broken similar to the skin of the foods (Katiyar & Ghosh, 2021). The WikiCell is a kind of film like membrane having characteristics attributes of biodegradability, thin, soft, and held to carry a small portion of food products, which plays a role in replacing the plastic materials. The other available edible food packaging include which are not available in the market are sugar casings, seaweed packaging, beeswax container, etc. The development of an edible coffee cup using a hard cookie lined with a chocolate layer provide heat resistant property. Further, cupcake wrappers and candy wrappers are also developed from starch (ST) (from potato fibres) and rice paper, respectively. The food based industries such as grains, sugar, beverages, edible oils, fruits and vegetable processing industry, dairy industry, poultry processing industry, meat processing industry, fisheries, etc. are focused to utilize edible food packaging for reduced waste. Interestingly, the edible films and coatings as edible food packaging have a great deal of interest in both research and development section and industrial section because of the greenery and sustainable approaches. In this regards, the biobased polymers (as shown in **Figure 1.1**) are thoroughly researched for the development of edible coatings and films materials including cellulose, ST, chitosan (CS), gum Arabic (GA), guar gum (GG), carrageenan, pectin, proteins (Sources:

casein, whey, soy, zein, wheat gluten (WG), cottonseed, collagen, egg white, wool keratin, collagen, etc.), lipids and waxes (fatty acids, acylglycerols, carnauba wax, beeswax, candelilla wax, rice bran wax, mineral oils, vegetable oils, paraffin wax, etc.), resins (wood rosin, shellac), etc. (Arnon et al., 2014; Park et al., 1993; Rodríguez et al., 2006; Oriani et al., 2014; Wang, & Gao 2013; Hosseini et al., 2013; Ali et al., 2010; Xu et al., 2019; Karbowiak et al., 2006; Tavassoli-Kafrani, Shekarchizadeh, & Masoudpour-Behabadi 2016; Moalemiyan, Ramaswamy, & Maftoonazad 2012; Espitia et al., 2014; McHUGH, Aujard, & Krochta 1994; Khwaldia, et al., 2004; Krochta 2002; Gontard et al., 1994; Baldwin et al., 1997; Rhim, & Shellhammer 2005; Lenart, & Piotrowski, 2001**)**. Ideally, the edible food packaging provides a remarkable opportunity in the innovations of food packaging due to it's biodegradable nature. Several available biopolymers include ST, cellulose, protein, CS, and lipids are naturally derived polymers. Further, these biopolymers are mainly derived from renewable resources, which are processed chemically and are surface treated for advance properties.

However, the global market size of edible packaging is done based on (1) Available sources such as plants and animals; Between 2019 and 2025, among the two available sources, the plants based sources are dominating the current market trends of edible packaging; (2) Available input materials such as polysaccharides, proteins, lipids, waxes, plasticizers, and additives (**Figure 1.1**); (3) End use of developed materials such as pharmaceuticals, food packaging, beverages, edible cups, cutlery items, fresh foods, baby foods, etc.; (4) Targeted packaging application such as antimicrobial packaging, edible coatings, and edible films; (5) Regions such as North America, Asia Pacific, Europe, South America, and others.

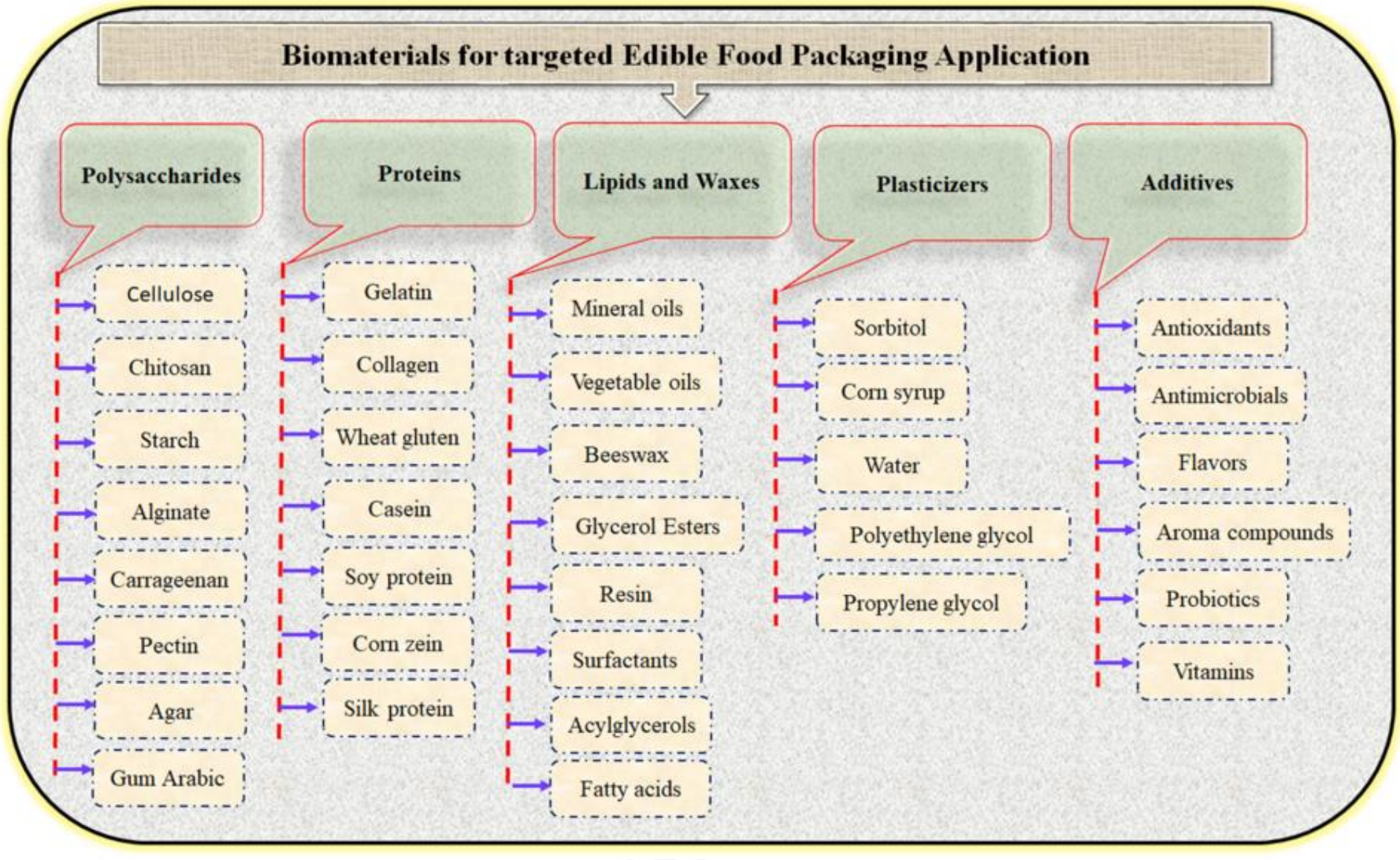


Figure 1.1 *Targeted biomaterials for edible food packaging in terms of edible coatings and films.*

Moreover, there are many benefits of using edible food packaging in regards to other available packaging materials such as edible packaging is eatable, edible packaging have a biodegrading nature within a very short period of time, no waste cycling is required, provide health beneficial agents to human health, etc. Additionally, this kind of packaging is used as single served food products. The limitation of edible packaging includes water solubility (WS) of edible packaging, which may degrade the quality in humid climates, further, edible packaging needs another packaging material as secondary packaging for the transportation of the products due to hygiene concern. The edible food packaging should be compatible with consumers, where some of the edible packaging may create allergies due to variations in components.

As mentioned, the foremost advantages of edible packaging over synthetic/biodegradable packaging are that it can be ingested along with the packaged food products. A food packaging is termed as "edible food packaging" only if it possesses potential qualities similar to the food components and also acts as an integral part of food that is designed to be consumed along with food. The primary roles of edible coatings and films include a selective barrier to gas exchange, moisture transfer or oxidation processes by delaying food deterioration, and retaining food property in order to enhance the product life (**Figure 1.2**). In other words, the major functions of food packaging involve preserving and protecting the food products from the surrounding environment, and further preventing surface contamination (minimizing exposure to spoilage components; i.e. moisture, microorganism, solutes, vapors, oils and off flavors). The use of food packaging also helps to protect desirable components (flavor volatiles) of food products by protecting them from chemical, physical, and biological activities, which is a promising outcome in fresh food preservations. The prime functions of edible coating/films are represented in **Figure 1.2**. This specific packaging also provides structural protection to limit mechanical damage during storage, transportation, and handling; and also protect the food

against oxidation and other chemical reactions. In addition, they are also utilized to transfer bioactive components such as antimicrobial, antioxidants, colors, flavors, and nutraceutical agents (**Figure 1.2**). Additionally, the utilization of available emerging bio-based nanostructured materials is considered as remarkable agents for improved food properties and shelf life, where the nanostructured materials further act as a delivery agent for nutraceuticals. Interestingly, the bio-based nanostructured materials can be functionalized and modified according to the targeted food products for prolonged shelf life.

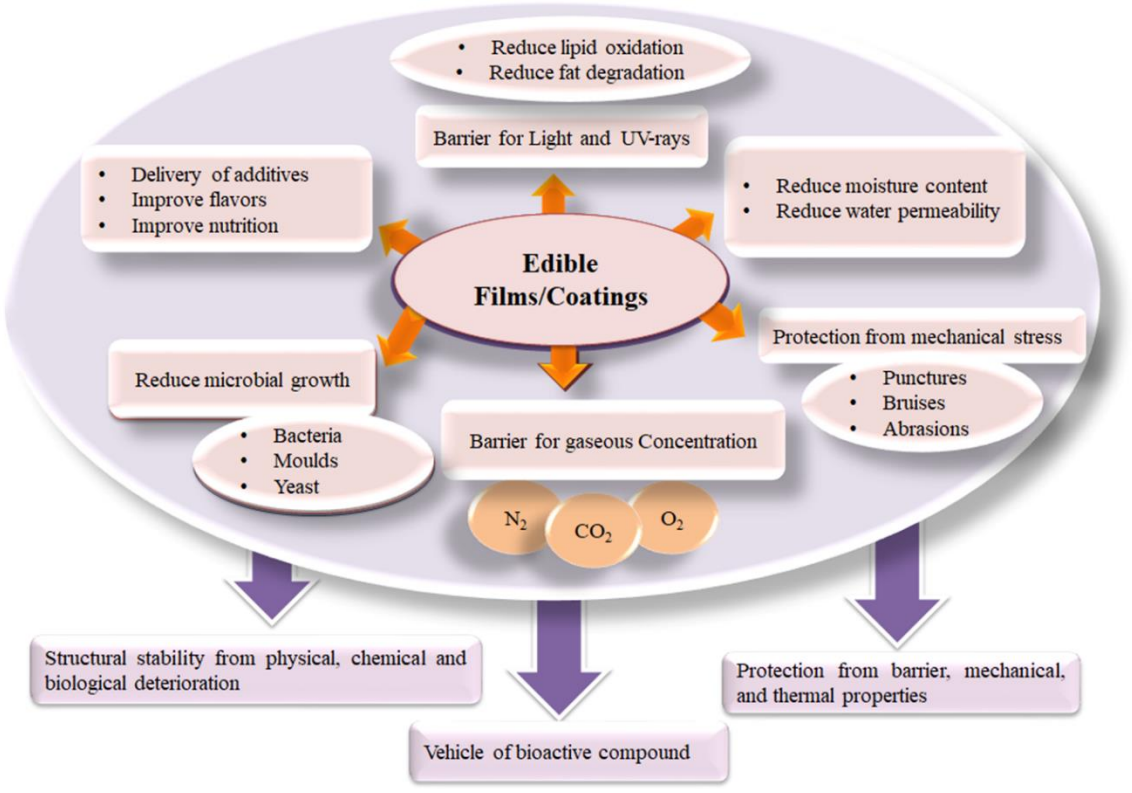


Figure 1.2 *Functions and trends of edible films and coatings in preserving food products.*

The edible coating is commonly used as a food preservation approach, where various edible materials are used to provide a thin layer of selected materials on targeted food products. In the 12th century, the edible coating was noted to be developed in China for the first time to

coat citrus food products (oranges and lemons) using waxes to prevent water loss. In England, the lard or fats are used to develop a coating material on meat products during the 16th century, which was known as larding. During the 1930s, the citrus food products are coated with hot-melt paraffin waxes. In later days, the fresh fruits and veggies are coated with the aid of carnauba wax and oil in water emulsions. In the past days, the various beneficial properties of edible coated food products were not well known by the consumers, which resulted in their unacceptability. However, the edible coated food products gained consumers' acceptance after they became familiar with the beneficial properties of edible coated food products. The edible coating can be obtained by utilizing various kinds of approaches such as dip coating, spray coating, foaming, brushing, wrapping, dripping, fluidized bed coating, and panning, which help to create a barrier between the food product and the external environment. The inclusion of edible coating also helps to improve the appearance of food products with the aid of edible materials on the surface of food products. The edible food packaging in terms of coatings is used to give a semipermeable barrier against harmful environmental agents such as light, temperature, gaseous agents, microbial agents, etc. Further, this kind of postharvest preservation technique is extensively utilized for reduced respiration rate, maintaining the weight of fruit products, total soluble solids, appearance, and others. The respiration of fruit and vegetables is also affected by storage temperature, time and gaseous conditions, etc. (Ghosh, & Dash 2018; Ghosh, & Dash 2020). The edible coating includes the widespread use of biopolymers including polysaccharides, protein, lipids and waxes, bioactive compounds, and others. The materials that are used for edible coating should be safe for human consumption, nutritionally rich, and are accepted by consumers. GA is another kind of polysaccharide used in the food industry having several beneficial attributes such as antimicrobial, stabilizers, adhesiveness properties, etc. (Borkotoky et al., 2019**)**.

The edible coating on food products is obtained by various processes, where, dip coating of fruit products is one of the widely used processes. The dip coating is obtained by dipping the food product in selected coating solutions, and drying the coated food products. In some cases, multiple layers of coating are also applied to obtain more effective food properties. The application of edible coating using dripping (applying the coating materials on the food products) is very cost effective, where the uniform coating can be obtained. Foaming based edible coating is obtained by using a foaming agent, and is added to coating solution, where compressed air is further blown into the applicator tank. Spray coating involves spraying of coating solution on the food products, this technique is used when a thin layer of coating is needed on food products. Besides biopolymeric materials, the components from fruits and vegetables are also used for the development of edible food packaging such as purees, pomaces, juices, active components, etc. (Otoni et al., 2017). The use of edible coating on food products has several aspects such as providing sweet flavor, enhanced texture of cereal products, reduced moisture loss of dried fruit (using mineral oil), improved appearance in fruits and chocolate candy, reduce mold growth in cheese, smoked fish, reduce fat uptake in fried products, and is used as a carrier for active compounds (Baldwin, Hagenmaier, & Bai, 2011). The critical concerns of edible coatings include chemical safety, eatable food products, cost, barrier property, shelf life, food quality, nutritive value, environment, etc.

Edible films are another class of edible food packaging which have been an attractive packaging material in the current trend of packaging market with the immediate effect in commercialization of developed materials. The term “edible films” has two considerations, where the first term “edible” defines the designing of eatable materials and considered safe, non-toxic and the second term “films” define the film forming properties of the materials similar to packaging materials (Erkmen, & Barazi, 2018). Edible films are a thin layer of edible materials including polysaccharides, proteins, lipids and being used as pouches, films, wraps

and others on food products or between food components as sandwich materials. During the 15th century, the development of edible films using soymilk, known as Yuba film was done in Japan, which is the first free standing edible film. The Yuba is developed by using denatured soy protein and the film is used for ground meat, vegetables, and as a component in the soups (Umaraw, & Verma, 2017). The yuba film has gained popularity in China and Japan having high nutritional and digestible properties. Further, the edible films are developed using casting (Du et al., 2008), extrusion and compression molding (Krishna, Nindo, & Min, 2012), compression molding (Ortega-Toro et al., 2014), and others which can further be used on or between food products. The proper application of edible food packaging depends on various characteristics such as selected food properties, selected materials, effect of materials, cost, etc. The properties of edible films are improved by adding various food additives such as natural food colouring agents, spices, antimicrobial agents, plasticizers, antioxidants, and others, which help in improving film properties such as optical properties, microbial properties, roughness, etc. The focused characteristics features of edible films include biodegradability, enhanced shelf life of food products, improved sensory properties, active functions of films, physical properties, optical properties, etc. The properties of food products are dependent on several factors such as film compositions, thickness of the film, transparency, etc. The versatile applications of edible films include active packaging, oral-disintegrating films, edible oven bags, fruit and vegetable leathers, food wrappings, etc. The edible food packaging in terms of edible films and coatings provide various benefits for improved life of food products by reduced water leakage from food products, reduced gas diffusion, reduced solutes movement, improved appearance of food products, reduced microbial growth, reduced oil and fats movement, and others. The most crucial characteristics features of edible packaged products are microbiological test, sensory properties, nutritional properties, wettability, mechanical properties, optical properties, etc. Additionally, the edible food packaging is also involved in

the encapsulation of active compounds, aroma compounds, antioxidants, pigments, and others (Debeaufort, Quezada-Gallo, & Voilley 1998). The development of edible food packaging in terms of edible films and coatings are displayed in **Figure 1.3**.

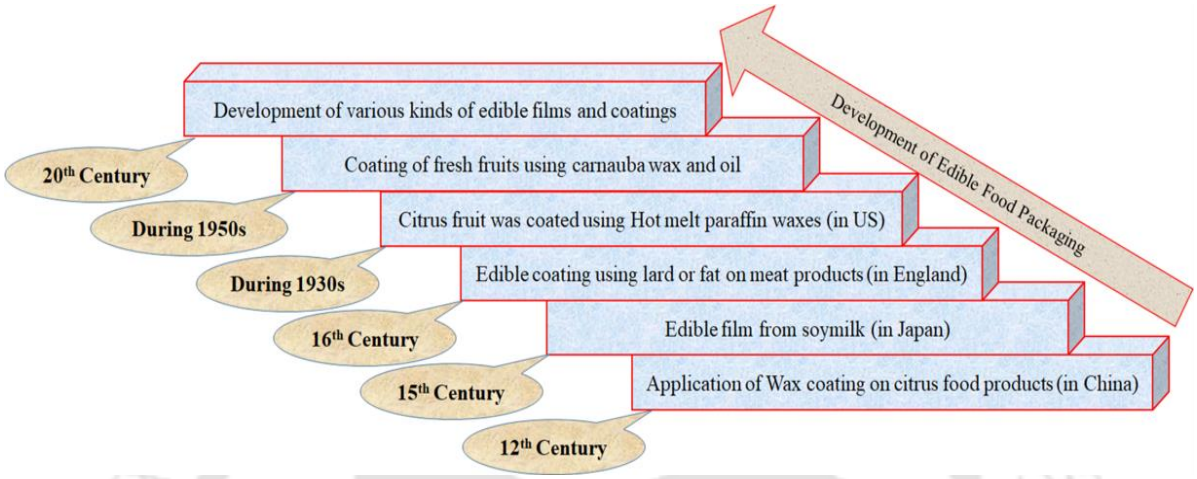


Figure 1.3 *Development of edible food packaging in terms of edible films and coatings.*

Additionally, several traits of edible food packaging have been displayed in **Figure 1.4**. The addition of health beneficial agents such as antioxidant agents, antimicrobial agents, to food products by means of edible food packaging is considered as another effective way for an enhanced product life of perishable food products. The edible food packaging acts as a safeguard against various kinds of injuries such as mechanical, thermal, chemical, physical, microbiological, and others. The single layer edible coating cannot provide an effective barrier against the environment. Thus, multiple layers of edible coating can serve the purpose of improved shelf life of food products efficiently. From very early days, cellulose based biopolymers are widely utilized for food packaging applications such as paper, polymer composites, edible packaging, etc. The biopolymer extraction and its usability are dependent on several factors such as temperature, relative humidity (RH), microbial spoilage, etc. In

recent past years, the use of biopolymers has attained remarkable attention in food packaging technology for its wide applicability. Based on this, the use of biopolymers as edible food packaging materials are gaining significant attention.

There are several advantages of edible packagings, such as:

- Reduce plastic based waste and solid waste
- Reduce carbon footprint, and global warming
- Increase the nutritional value of food products
- Edible packaging can act as a carrier for antimicrobial, antioxidant, or other active agents
- Edible packaging can capture various active agents as encapsulation
- Improve aesthetic property of food products

The limitations of edible packaging include:

- Increased cost
- Secondary packaging is required to carry coated foods such as blown film materials
- Secondary packaging materials are costly
- Environmental sensitive packaging materials

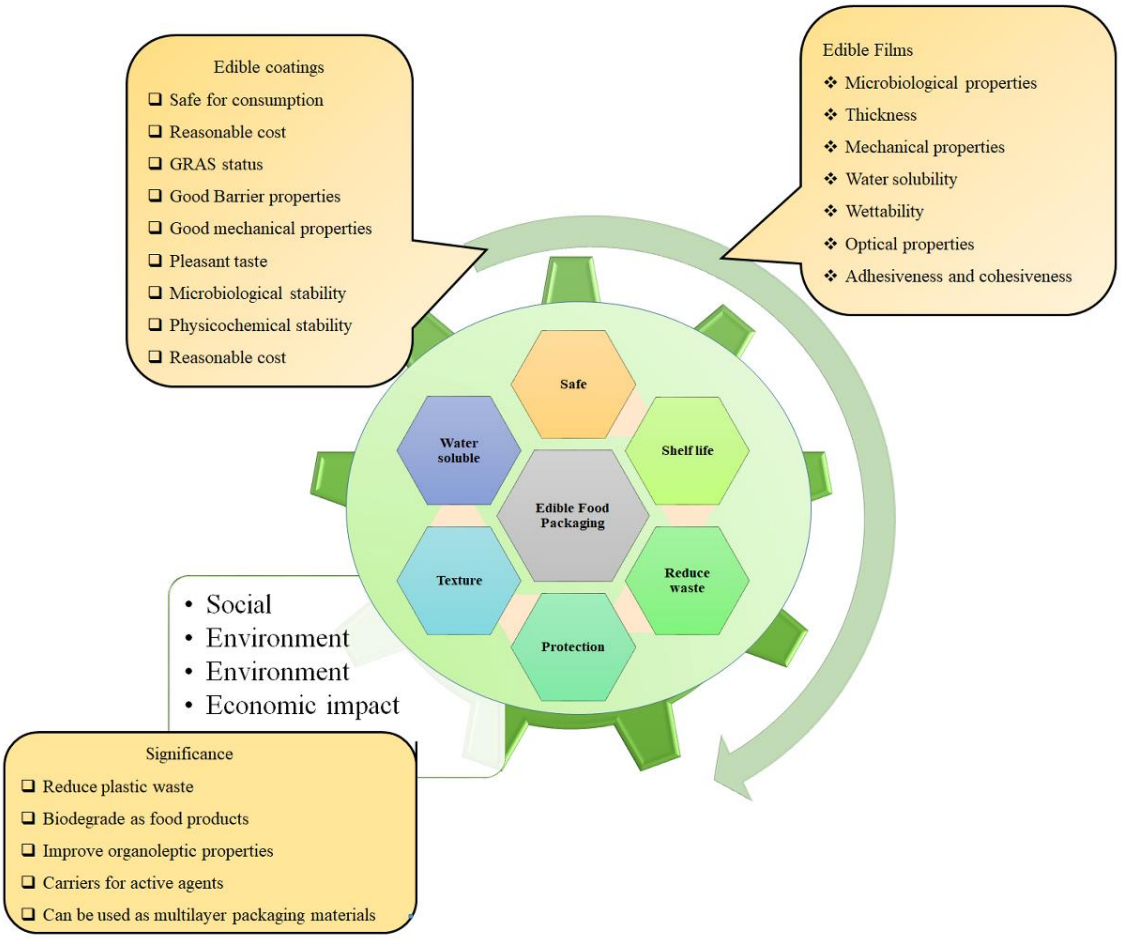


Figure 1.4 *Significance of edible food packaging in terms of edible films and coatings.*

Additionally, the transportation of coated food products can be obtained using secondary packaging approach. The food packaging is classified into three categories such as primary packaging, secondary packaging, and tertiary packaging. The primary packaging materials remain in contact with food products. The secondary packaging materials are used to transport the primary packaged food products such as carton boxes, paper boxes, etc. On the otherhand, the tertiary packaging materials are used to carry the secondary packaged food products. The secondary and tertiary food packaging materials can be recycled and reused if used properly. The edible food packaging materials are generally considered as primary food packaging materials, which is a potential candidate in preserving, transporting, and marketing food products. The addition of various plasticizers and additives can be added to different

biopolymers to obtain the improved physical properties, surface functionality of the biopolymers. The intermolecular forces within biopolymers include electrostatic, hydrophobic, covalent bonds, ionic interaction, and others. Thus, the present work focuses on developing edible coating on food products and their secondary packaging.

1.1.1 Nanotechnology based Edible Coating: Primary Food Packaging

The nanotechnology in food processing has a great deal of interest to provide potential benefits such as producing functional food products, extended shelf life of food products, intelligent packaging using nanosensor, etc. as shown in **Figure 1.5.** However, the current trend of the food packaging market is widely utilizing the sustainable nanostructured materials for delivering tailored-made properties of eco-friendly food packaging. The advantages of sustainable nanostructured materials for edible coating (a type of edible food packaging) have been represented in **Figure 1.5**. The sustainable nanomaterials are extensively used in edible coating for nano-food safety, nano-functionality, nano-preservation, etc. (**Figure 1.5).** As mentioned earlier, the sustainable nanomaterials are used to improve the functionality of food products. The sustainable nanomaterials are considered as a remarkable source to be used as safety materials, where sustainable nanomaterials are used against microbes, allergens, and to reduce metal content. The nanocellulose such as cellulose nanocrystals (CNC), cellulose nanofibers (CNF), cellulose nanoparticles (CNP) and others are extensively used to improve the mechanical property of neat biopolymers for edible coating. The CS nanoparticles (CSNPs) provide an effective antibacterial activity against gram-positive (*Staphylococcus aureus*, *Bacillus subtilis, Enterococcus faecalis*) and gram-negative bacteria (*Escherichia coli, Salmonella typhi*). Thus, the antimicrobial activity of CSNPs provides considerable attention from the past few years, where CS and its various derivatives are used to treat many postharvest diseases as antifungal coating materials. However, the antibacterial activity of sustainable nanomaterials is observed due to the repeating units of primary amine groups and the property

is further influenced by various factors such as molecular weight of CS (low molecular weight/ medium molecular weight/high molecular weight), degree of deacetylation, temperature, pH, sources of CS, etc. In this regards, the use of antimicrobial/antibacterial agents for the fabrication of polymeric components, solid lipid NPs, and liposomes help in improving product life. The addressed nanoparticles (NPs) are used as an additive in improving the qualities of food products in terms of texture, crispiness, flavor, color, etc. CS is used as supplements with other biopolymers for improved antibacterial activity. The cellulose, and CS based NPs are extensively used to provide nano-functionality to food products. The cellulose based nanomaterials have attained considerable attention for their tunable rheology, physical and chemical property, other structure-property relationship, etc. Cellulose nanostructured materials have a remarkable mechanical property, and biocompatibility, availability, low density, which increase the use of specific nanomaterials in edible packaging application. The NPs are performed as nanoencapsulation, and preservative materials, where nanoencapsulation helps to increase the bioavailability of active compounds, and protect bioactive compounds and further, offer sustained release of the active compounds. The wide application area of the specified NPs includes the area of edible nanocoatings, active packaging, intelligent packaging, and food supplements to improve the product life and acceptance. As food supplements, NPs are added to food products as additives, anticaking agents, systems for delivery of bioactive compounds, flavor and nutrient delivery, etc. The NPs are used as nanoemulsion for the delivery of bioactive materials, nutraceuticals or nutraceutical rich food and can be consumed with nanoemulsion materials. In this regards, the bioavailability of developed materials is tested for their bioaccessibility, absorption, and transformation in biochemical properties across mucus layers and epithelium cells.

In the present research work, the focused polysaccharide based bionanostructured materials for developing edible coating is cellulose nanostructured materials, CS based

nanostructured materials. Additionally, the various forms of nanocellulose are available as nanocrystals, nanofibers, and NPs which can be availed by modifying the routes of synthesis. The various forms are comparable in terms of obtaining the tunable properties of packaging materials for both edible and non-edible packaging forms. The characteristics traits of nanocellulose include rheological behavior, high mechanical property, lightweight, barrier properties, nutritional properties, etc. The cellulose based nanostructured materials are utilized in food packaging, developing ST based foods, food stabilizers, delivery systems, etc. CS nanostructured materials have some advantageous properties in comparison to commercial CS including high surface area, small particle size, high reactivity, and improved antimicrobial property. In this regards, the fabrication of CSNP is commonly obtained using ionotropic gelation method, which is a simple method and the desired particle size can also be optimized. Additionally, silk nanodisc (SND) is an emerging protein nanostructured materials. SND is a kind of protein based nanostructured materials having noteworthy properties and can be used in developing edible coating on food products. Additionally, the mentioned nanomaterials having noteworthy properties in terms of surface chemistry, non-toxicity, biodegradability, biocompability, and reinforcement effect can be used to modify the inherent properties of available biopolymers for edible coating application.

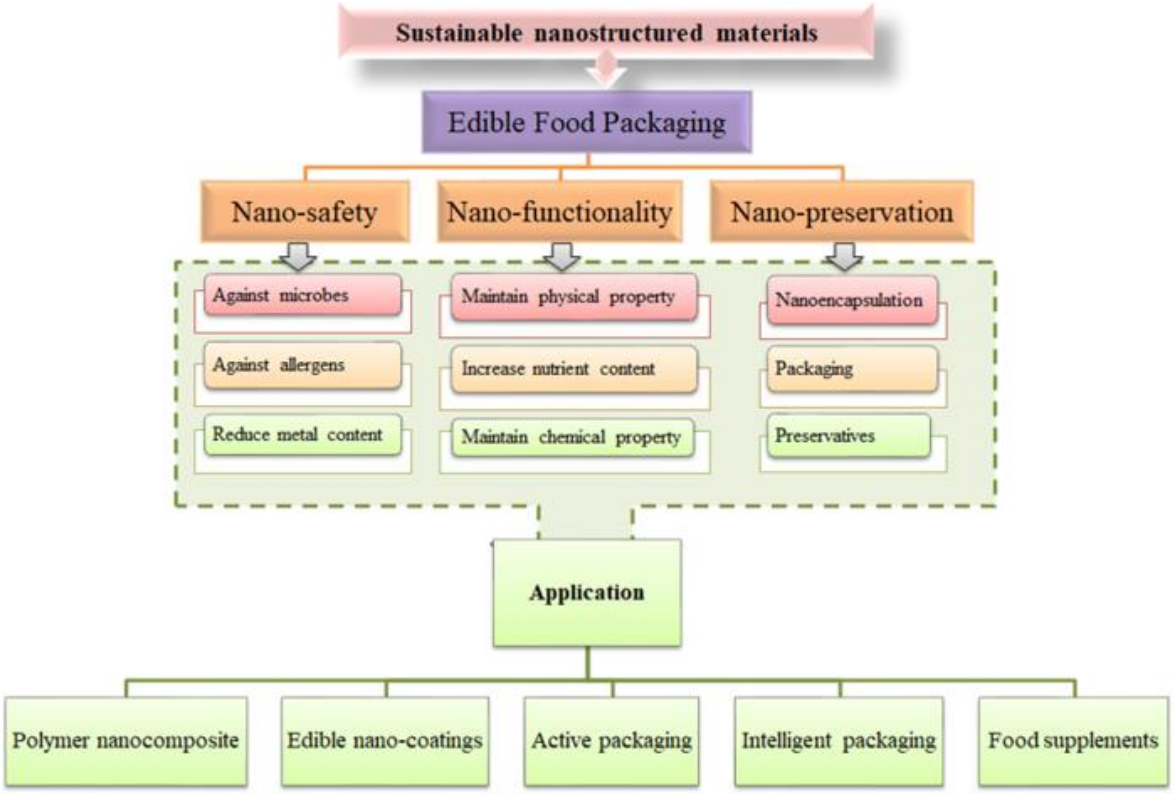


Figure 1.5 *Sustainable nanostructured materials in edible food packaging.*

1.1.2 Nanotechnology based Blown Films: Secondary Packaging

The blown film based plastic processing technique is a kind of extrusion process, where film products are obtained for packing films, bags for clothing and textile, covering films for items, etc. The main parts of a blown film include extruder (Screw, barrel), die head, winder, tower, cooling system, hopper, etc. as shown in **Figure 1.6**. In this process, the raw polymer materials are generally obtained in the form of granules or pellets, where the raw polymer materials are mixed with biofillers or other materials, which are further dried before the extrusion. The dried polymer pellets are extruded *via* heating, where a viscous liquid is obtained between the rotating screws and barrels of the extruder. The extruder is linked with a blown machine, where the liquid fed of polymer is passed and air is blown, which helps in obtaining blown films. The stability of bubble formation is dependent on various factors such as the type of polymer used, the property of extruder, etc. The blow-up ratio and thickness of

films are affected by various factors such as the speed of rotational screw, speed of pull up nip rollers, the air pressure inside the inflated plastic, etc. However, the bubble of plastic may be unstable forming defective films such as bubble tear, bubble breathing, draw resonance, helical instability, freeze line height instability, heavy-bubble instability, bubble flutter, and others. From very early days, different conventional plastic materials are used to develop blown films. Recently, the various biodegradable polymers are used to develop blown films, however, the biofillers are utilized to improve the property of blown film materials. In this regard, plasticizers, biofillers (ST, CS, cellulose, and others), compatibilizer, cross-linking agents, etc. are also used to modify biodegradable polymers. There are several advantages of blown film extrusion process, which include (1) flat as well as tube-shaped films can also be obtained, (2) the film thickness and width can be controlled by controlling air volume, and (3) different plastic materials can be mixed for the blown film development, etc.

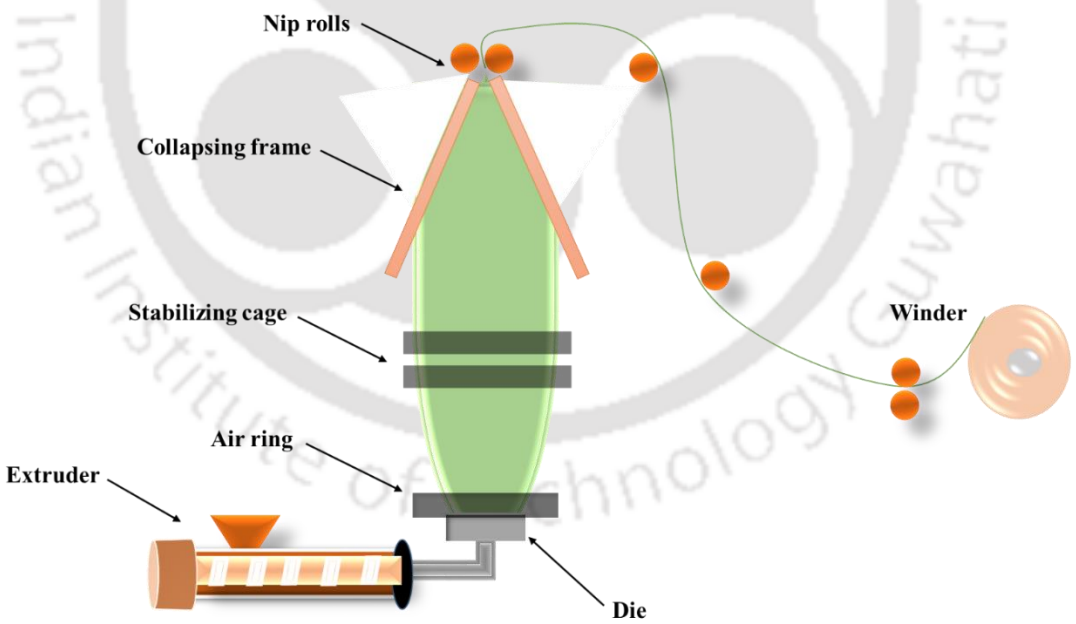


Figure 1.6 *Processing steps in Blown Films as secondary packaging materials.*

1.2 Scope and Structure of the Thesis

In the above sections, an overview of the research and development and global market analysis on edible food packaging specifically in terms of edible coatings has been made briefly. Additionally, the importance of the nanotechnology in edible coatings and films has been discussed. However, the use of curcumin doped iron functionalized cellulose nanofibers/magnetic cellulose nanofibers (mgCNF) dispersed CS based edible nanocoating on fruit products having iron fortified and health beneficial properties with an improved shelf life of cut pineapple and whole kiwifruits are considered as a novel approach (**Objective 1**). Additionally, the use of nanochitosan (NCS) as a nanofiller material in ST-GG biocomposite can overcome the existing shortcomings including surface property, thermal stability, wettability, and others to be used in developing edible coating on food products (**Objective 2**). The SND/CS based edible coating has been developed to offer an improved shelf life of perishable fruits (**Objective 3**). In the present work, the remarkable bionanostructures based on mgCNF, NCS, and SND are the focused nanofillers for developing functionalized biopolymeric nanocomposite based edible nanocoating on fruit products. Additionally, the development of secondary packaging materials using biocomposite of PLA based blown films for coated food products have been targeted (**Objective 4**).

Therefore, the PhD Research work aims at utilizing remarkable bionanostructured materials with their modified form to tackle the existing shortcomings of biopolymers based edible coating. The curcumin doped mgCNF/ CS based edible coating is a superior candidate for delivering iron fortified ready to eat food products with enhanced food properties including mechanical, thermal, food texture and optical properties. In this study, mgCNF is used for improved properties of available chitosan materials for edible nanocoating applications. Further, the existing shortcomings of ST/GG biocomposites to be used as an edible coating including hydrophilic nature, reduced water barrier properties, and transparency can be tailored

using several strategies. In this study, NCS has been used as a noteworthy agent to tailor the existing properties for targeted edible coatings on cut apple products. Thus, the two remarkable polysaccharide based modified bionanostructures can be used for developing superior edible coating. Additionally, another protein based nanostructures such as SND has been utilized to develop edible coating on food products. The edible coating generally acts as a primary food packaging, where the inclusion of secondary packaging is helpful in the delivery of coated food products to end users. Besides, development of green biocomposite based secondary packaging has been done for the packaging of coated food products. In this context, the outcome and findings of the research work based on "*Studies on Development of Functionalized Biopolymeric Nanocomposite Based Edible Nanocoating on Food Products and Sustainable Secondary Packaging*" are represented in the thesis as four chapters (Chapter 3 to chapter 6). The structure and content of the thesis organization have been briefly discussed in the below sections.

Chapter 2 describes the detailed experimental protocols, testing materials and methodologies used for developing mentioned edible nanocoating and adopted strategies to fabricate the bionanocomposite materials. The chapter discusses in detail fabrication protocols of bionanostructured materials, biocomposite formulation and process for developing dipped coated fruit products (mgCNF, NCS, SND). Additionally, the analytical instruments, processing conditions and methods to develop edible coat on food products have also been described. Further, the chapter also details the development of functionalized CS, functionalized GA to be used in developing sustainable secondary packaging based blown films.

Chapter 3 deals with the fabrication of curcumin doped mgCNF dispersed CS based nanocomposite for focused edible coating application on perishable fruit products. The fabrication of mgCNF can be obtained through single step co-precipitation method which acts

as a remarkable candidate to modify the characteristics attributes of CS based biocomposites in terms of mechanical, optical, thermal and other properties. The fabricated iron added biocomposites have iron content within the permissible limit as per standard legislations. Additionally, the doping of curcumin in the developed CS biocomposites provides anticarcenogenic property. Thus, the enhanced packaging properties alongwith the medicinal properties make it a noteworthy candidate to be used as edible coatings for perishable food products with improved shelf life.

Chapter 4 describes the fabrication of NCS aided ST-GG based biocomposites for edible coatings on cut apple fruits. In this chapter, a detailed study has been made to investigate the structure-property-performance analysis of NCS (fabricated using ionic gelation method) added ST/GG based biocomposites as edible coatings. The fabricated NCS is analysed in terms of modifying the properties of ST-GG biocomposites in terms of packaging properties (Barrier, thermal, optical, color, surface properties, antimicrobial properties, and others). Additionally, the application of developed materials on the effectiveness of storage life of cut apple has been studied. In this study, a new approach to modify the properties of ST-GG biocomposites has been made.

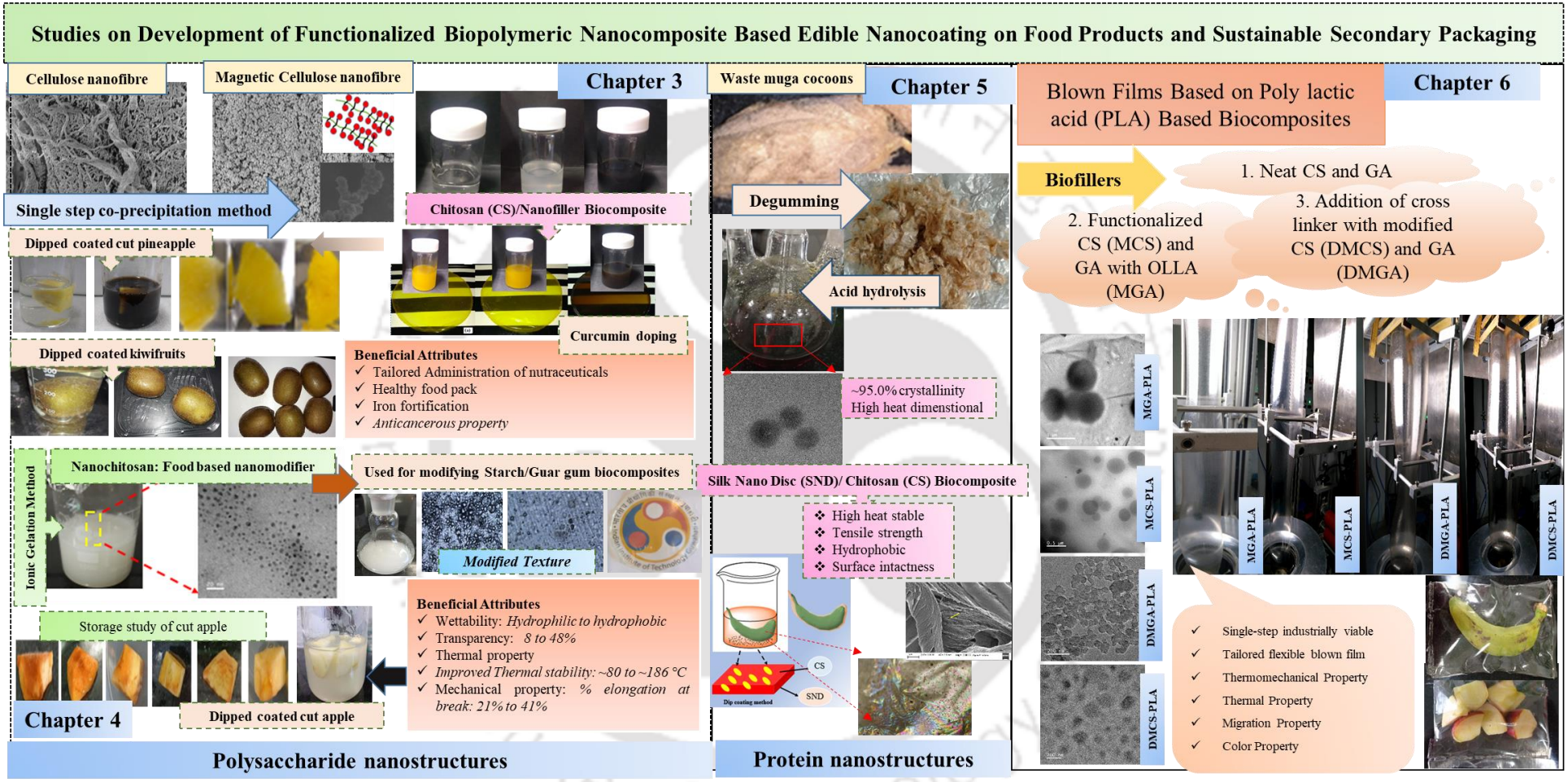
Chapter 5 deals with the fabrication of SND/CS nanocomposite based edible nanocoating on whole banana fruits. The fabricated edible coatings act as a noteworthy candidate due to the application of SND materials with modified packaging properties in terms of thermal property, surface wettability, mechanical property, color and other properties. Additionally, the use of SND is effective in modifying the inherent properties of CS to be used as an edible coating.

Chapter 6 demonstrates the development of a single-step industrially viable and flexible blown film based food packaging using biocomposites of Poly lactic acid (PLA)/functionalized biofillers and cross linking agents for targeted secondary packaging. The fabrication of

functionalized biofillers (CS and GA) has been obtained through grafting oligomers of lactic acid with specified biofillers separately, to make it compatible with NPLA (neat PLA). The used neat biofiller, functionalized filler (oligomer grafted CS and oligomer grafted GA), and cross linking agent help in obtaining the tailored properties of NPLA based blown films with various level of compatibility, and modified properties for the targeted application. It is noteworthy to mention that the biocomposite of PLA can overcome the existing shortcomings of NPLA based blown films, providing a sustainable secondary packaging.

Chapter 7 provides a conclusion of the findings of the research work and highlights the fundamental issues alongwith the future scope of the current work aiming to convert small scale to large scale production. The effect of using mgCNF dispersed CS facilitated by curcumin in multifunctional packaging properties has been discussed and a comparison has been made. The interference of using NCS on the packaging properties of ST-GG biocomposites has been made with noteworthy properties for developing edible coating on food products has been concluded. Additionally, the utilization of SND/CS based biocomposites for targeted edible coating has been concluded to convert small scale to large scale conversion. Further, the importance of using secondary packaging for transporting coated food products has been discussed with some suggestions for future work perspective.

Graphical abstract of the PhD Thesis



1.3 Literature Review

1.3.1 Biomaterials for Edible Coating

The focused biopolymers for developing edible coating include polysaccharide, protein, and lipid materials, their derivative, nanostructured forms, and others. Additionally, the other focused components for fabricating edible coating including the biopolymers are (i) Puree, juices (Sources: Fruits and vegetables); (ii) Inorganic materials; (iii) Filler materials (Natural fibres and nanomaterials); (iv) Plasticizers (sugars and alcohols); (v) Functional additives (Antioxidants, nutraceuticals, antimicrobial, probiotics, and others); and (vi) Other additives (Browning inhibitors and cross linking agents). Considerably, the components of edible packaging have the advantageous features of non-toxicity, biodegradability, biocompatibility, and reduce solid-waste management problems. In this regard, **Figure 1.7** represents the characteristics features of edible coating in terms of (i) Packaging property: Barrier, mechanical, thermal, optical and others; (ii) Sustainability: Renewable and biodegradable materials; (iii) Deliver improved product life of food products due to reducing the weight loss phenomena of fruits, maintained firmness of food products, microbiological properties and others; (iv) Active functions such as antibacterial, antifungal, and antioxidant property; (v) Sensory property: Improved texture, color, flavour, and other taste attributes of coated food products; (vi) Health beneficial property: Food fortifications with active agents and others.

In this context, the several biopolymers, nanostructured forms, and derivatives for targeted edible coating have been discussed in the subsequent sections. The fabrication methods, modification strategies, and edible/non-edible food packaging application of the biomaterials have been discussed. Further, the synthesis strategies of edible coating on food products have been detailed.

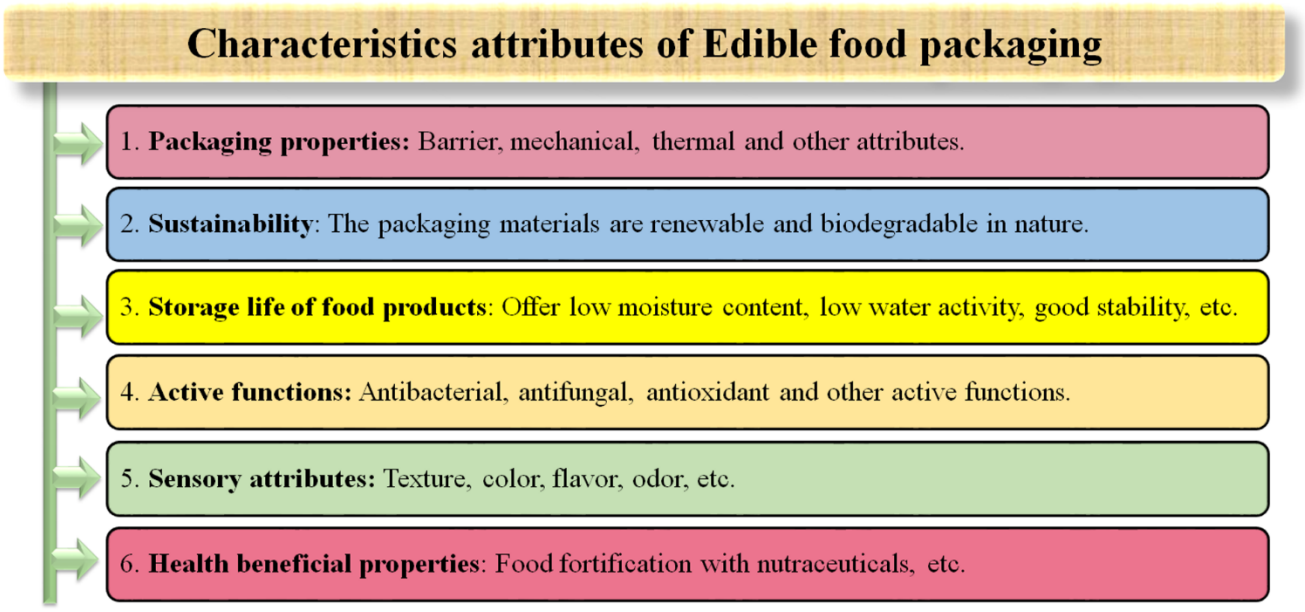


Figure 1.7 *Characteristics attributes of edible food packaging.*

Polysaccharides are a class of carbohydrates consisting of chains of monosaccharide units linked together by glycosidic bonds. The other classes of carbohydrates are termed monosaccharides and oligosaccharides consisting of single and 2 to 10 monosaccharide units, respectively. The polysaccharides are classified based on various factors such as repeating units, sources, origin, etc. The polysaccharides based on origin are categorized as (i) Animal based origins: CS/chitin, hyaluronic acid, and chondroitin sulfate (obtained from marine wastes such as cartilage, fish eyeballs, crustacean shells); (ii) Microbial origin: Xanthan gum, dextran; (iii) Marine origin: Agar, alginic acid, carrageenan; (iv) Plant origin: ST, cellulose, GA, gum karaya, and GG. Additionally, the polysaccharides are also classified into main three types such as storage polysaccharides (ST, glycogen, and inulin), structural polysaccharides (cellulose, chitin, pectin), and mucosubstances (mucopolysaccharides). The classes of polysaccharides based on structure include linear and branched polysaccharides. The linear polysaccharides include cellulose, amylose, algin, pectin and the branched polysaccharides include amylopectin, GA, galactomannans, xanthan, and xylan. The several kinds of polysaccharides are considered

as potential candidates to be used in edible food packaging (edible films and coatings) as bio-composites and blends. The presence of hydroxyl groups and other hydrophilic moieties in the chemical structure of polysaccharides serves a significant role in the film formation. However, the low water resistance property, mechanical property, and cost-effectiveness are the main drawbacks of polysaccharide based materials to be used in food packaging. The structure of polysaccharides can be tuned to improve their physicochemical properties by salt-addition, solvent-change, pH-change, and chemical modification of hydroxyl groups. Besides, there are several strategies applied to modify the properties of polysaccharides to act as a better reinforcing agent to develop composite materials. Cellulose is recognized as extensively available polysaccharide molecules being used as resources for polymeric molecules. Cellulose consists of β(1→4)glucose units, which is generally indigestible by human beings as they lack symbiotic bacteria in the intestinal tract required for producing necessary enzymes in digesting cellulose molecules. Cellulose can be extracted and is available in various forms. Pretreatment of biomasses yields cellulose, and under controlled acid hydrolysis of cellulose yields nanocellulose, where acid hydrolysis helps to break the glycosidic bonds and removes the amorphous region of the cellulosic materials keeping crystalline region. Except for its property of biodegradability, non-toxicity, biocompatibility, nanocellulose acts as a potential nanofiller for most of the biodegradable polymers. Further, CS is a unique cationic polysaccharide with several functional properties including non-toxicity, biocompatibility, antioxidant, antimicrobial, and other health beneficial property, film forming property, lipid lowering activity, gelling property, etc. The characteristics attributes of CS, related derivatives, and CS based nanostructured materials have attained remarkable importance for several existing noteworthy functional properties. The biological properties of CS are bioadhesive in nature, adsorption enhancers, cytocompatible, antimicrobial active, antioxidant active, macrophage activation, and others, which depend on the degree of acetylation and molecular weight of CS.

The physicochemical properties of CS include solubility, crystallinity, viscosity, and others, which vary from source to source. Further, it is worth mentioning that the remarkable biological, antimicrobial, and physicochemical properties of NCS provide multifaceted applications specifically in sustainable food packaging. ST is another kind of polysaccharide consisting of amylose and amylopectin, where the properties of ST are mainly dependent on the composition of both the units. The pure form of ST is insoluble in cold water and it is tasteless and odorless in nature. ST is cheap, easily available, biocompatible, non-toxic, and biodegradable in nature, which makes it a focused polymer for edible packaging application. Besides, ST is a widely used biopolymer in various sectors including drug delivery systems, edible coatings, edible films, biodegradable films, food functionalization, etc. ST is usually modified for improving its inherent properties to be used in edible packaging application, wherein, breadfruit derived ST hydrolysates possesses better properties than commercial ST hydrolysate with same ranges of dextrose equivalent from other sources. Similarly, the composites of sago ST, CS and silver NPs can be used for edible packaging materials.

Additionally, protein based materials such as silk nanocrystals, soy protein, whey protein isolates, soy protein isolates, keratin, milk protein, corn protein, egg protein, etc. can be used for the preparation of edible coatings for enhanced shelf life. Additionally, the inclusion of pretreatment can improve the quality of food products such as edible coating and blanching as a pretreatment technique is a promising method for drying pumpkins (Molina Filho, Frascareli, & Mauro, 2016). The formation of acrylamide (a procarcinogen) in banana chips can be reduced with the aid of pre-frying treatments such as blanching and pectin based coating (Suyatma, et al., 2015). The application of coating materials based on ST and pectin for osmotic dehydrated and convective dried food products can effectively influence the drying attributes, where the drying characteristics also depend on the type of coating materials (Lenart & Piotrowski, 2001). A report suggests that the use of edible coatings (whey protein isolate and

pullulan) on freeze dried Chinese chesnut can effectively improve the quality and shelf life of chesnut (Gounga et al., 2008**)**. The growth of *Listeria monocytogenes* (a pathogen which causes foodborne infections) in roasted turkey (at chiller storage) can be effectively controlled with combined processing techniques such as edible antimicrobial coatings (pectin) with frozen storage (Jiang, Neetoo, & Chen, 2011). In this regards, the application of freezing with edible coating can further minimize the risks of listeriosis (an infection) caused by *Listeria monocytogenes*. A report further suggests that the application of pectin and green tea powder as edible coatings can effectively improve the quality of irradiated pork patty (Irradiation at 0 and 3 kGy using cobalt-60 gamma rays) (Kang et al., 2007). The edible coated pork patties have a reduced count of total aerobic bacteria in comparison to uncoated pork patties. Further, in 2003, Vachon et al., has studied the effectiveness of edible coating (based on caseinate) and gamma-irradiation treatment on the keeping quality of fresh strawberry fruit products (Vachon et al., 2003). The application of both the treatment helps in delaying the mold growth and the irradiated caseinate is more effective in comparison to the unirradiated caseinate for obtaining improved storage life of strawberry. The combined application of gamma irradiation, ascorbic acid, and protein based edible coating can help to improve the keeping quality of ground beef in terms of biochemical and microbial attributes (Ouattara et al., 2002). Additionally, the edible films based on milk protein such as calcium caseinate and whey proteins cross linked using radiative and thermal treatments can also provide tunable mechanical and structural properties (Vachon et al., 2000**)**. The storage quality of fresh cut pears can be maintained with the aid of pure oxygen pre-treatment, CS and rosemary extract based edible coating (Xiao et al., 2010**)**. The combined effect of the treatments has an ability in inhibiting polyphenol oxidase activity, and further, increase the beneficial properties of fresh cut pears and reduce the browning effect, etc. There are various other pretreatments using additives (ascorbic acid, citric acid, sodium benzoate, and others), controlled atmosphere storage and CS coating can improve the quality

of fresh cut Jackfruit bulbs slices by retaining phenolic content, sensory attributes, phenolic content, etc. (Saxena et al., 2013). A report suggests that the characteristics quality attributes of strawberries can be monitored during freezing when whey protein based coating is used as a pretreatment method (Soazo et al., 2015). Thus, the inclusion of food preservation techniques and edible food packaging has a beneficial property of improved product life. The available food preservation techniques are used for improved shelf life of food products, however, the inclusion of some preservation techniques may alter the taste and nutritional quality of specific food products due to different processing conditions and use of different agents. In this regards, edible food packaging in terms of edible films and coatings helps in maintaining the nutritional quality of food products. Further, the edible coating and films enhance the nutritional quality of food products by delivering active agents from available natural agents. The use of edible coating and films are different, where, edible coating is developed on food products by developing a thin layer of edible material on food products and edible film is used as a layer of edible material between food products or onto food surface.

1.3.2 Global Overview of Edible Food Packaging in Research and Development

In this section, the past and existing trends in edible food packaging, which are obtained by using continuous matrices of polymers, NPs, and active agents, etc. have been discussed. Edible food packaging is one of the potential candidates utilized as a food preservation technique for the improved shelf life of food products. The global consumerization of edible food packaging as edible coating and films are increasing due to several advantageous features such as reduced plastic waste, use of naturally available materials, renewability, biocompatibility of materials, increase food value, ready to eat food products, etc. The improved food quality generally depends on several factors such as type of coating materials, processing condition, concentration of materials, storage condition, type of food products, etc. The existing biomaterials or biopolymers are used individually or in a combined form for

maintaining the food property during storage. Based on the discussion, the present section will provide a general global status of edible food packaging.

The materials in edible packaging should be eatable both in the initial and the final packaging forms (Cerqueira, Teixeira, & Vicente, 2016**)**. The first patent on edible coating was available in the year 1933 in the United States, where wax coating was mainly applied to citrus fruit products (Cerqueira, Teixeira, & Vicente, 2016**)**. In later days, many inventions have been done on edible coatings such as edible film coated dried fruits (Miranda, & Marco, 2005), coating of dehydrated foods (Cole, 1969), meat (Allingham, 1949), frozen confection (Sabin, 1940), frozen fish (Sortwell, 1980**)**, etc. Edible coatings provide a lot of beneficial properties in retaining food factors such as reduction of fat uptake in the deep fat fried food products (meat, potatoes) based on hydrocolloids as edible coating with better nutritional quality (Kurek, Ščetar, & Galić, 2017**)**. In this regards, the edible oil barrier properties in edible films and coatings are very essential to obtain nutritional fried products. The patents on edible films available are gelatin films (Yuzhu, Zheng, & Yimin, 2019) and edible food casings from carboxymethyl cellulose (CMC) (Verrall, & Brown, 2017), casein based edible films (Bonnaillie & Tomasula 2018), etc. The patents on edible films for their versatile use are also available such as transmucosal delivery of terpenes (Sanders, 2019). Further, the tunable film forming properties of edible food packaging are attained *via* heating, enzymatic modification, salt addition, drying, cross-linkers, food additives, etc. In this way, the innovations in research and development are providing a new trend in the field of food packaging with many beneficial traits.

1.3.3 State of Global Market and New Trends in Edible Food Packaging

The global consumerization and market value of the edible food packaging market are increasing day by day. In this regards, according to the available report on Global Opportunity Analysis and Industry Forecast, 2017-2023, the edible food packaging market was marked at

$697 million in 2016, which is forecasted to reach $1097 million by 2023. The popularization of edible food packaging market relates to the increased in hygiene concern, reduced conventional packaging waste, etc. The plastic based waste is a critical concern to the society which further increases the carbon footprint, global warming, which acts as a facilitator for market growth of edible food packaging. On the otherhand, manufacturing regulations, high manufacturing cost, and safety concerns may lead to a decrease in market value of edible food packaging. The main marketing features of edible food packaging include a class of materials, targeted end users, and major market areas. The principal class of materials includes polysaccharide, proteins, lipids and waxes, biocomposites, blends, surfactants, active agents, etc. The targeted consumers of edible food packaging include food and beverages, pharmaceuticals, medicinal use, etc. The major market area of edible food packaging includes north America, Europe, Asia-pacific, etc. The global food packaging markets are WikiCell Designs Inc., MonoSol LLC, Tate & Lyle Plc, JRF Technology LLC, Safetraces, Inc., Bluwrap, Skipping Rocks Lab, Watson, Inc., and Devro plc., etc. The region-wise analysis of the global edible packaging market covers North America (US, Canada, Mexico), Europe (UK, Germany, France, Rest of Europe), Asia-Pacific (India, China, Japan, Rest of Asia-Pacific), and LAMEA (Latin America, Middle East, Africa) as represented in **Figure 1.8**. Various countries covered under each region are studied and analysed to identify the major trends demonstrated by these respective regions. Europe dominated the global edible packaging market in 2016, followed by North America.

The Global market for edible food packaging products is available with many products such as seaweed, edible cutlery, ooho edible water, loliware edible cups, casein, etc. The company for edible food packaging materials are Bakeys, India (sorghum, rice, wheat flours), Coolhaus, Los Angeles (potato wafer paper wrapping), Do Eat, Belgium (water and potato ST), Ecovative, New York (mycelium packaging and Founder: Gavin Mcintyre), Eco Six Pack Ring

(E6PR), Mexican (compostable matter and by-product waste), Evoware, Indonesia (seaweed), Loliware (seaweed, organic sweeteners, fruit and vegetable coloring), Monosol, Indiana (edible pods for instant beverages), NVYRO, United Kingdom (Cassava plants), Poppits, Florida (food grade edible films), Scoby, Poland (edible, recyclable package), TIPA, Israeli (biomaterials and technology), etc. In this regards, the available edible food packaging in the worldwide has been represented in **Table 1.1 (**Katiyar & Ghosh, 2021**).** Further, the commercial edible coatings and films available in market are BioEnvelop®, Chris-Kraft Polymer Inc. COGIN ®, ENAK ®, Freshseel™, Fry Shield™, GREENSOL®, Nature Seal™, Nutrasave™, Opta Glaze™, Seal gum, Spray gum™, Semperfresh™, SHELLAC(E904), Z-Coat™ etc. (Erkmen & Barazi 2018). The market trend in edible food packaging is growing day by day due to the increased socio-scientific demand in the current situation.

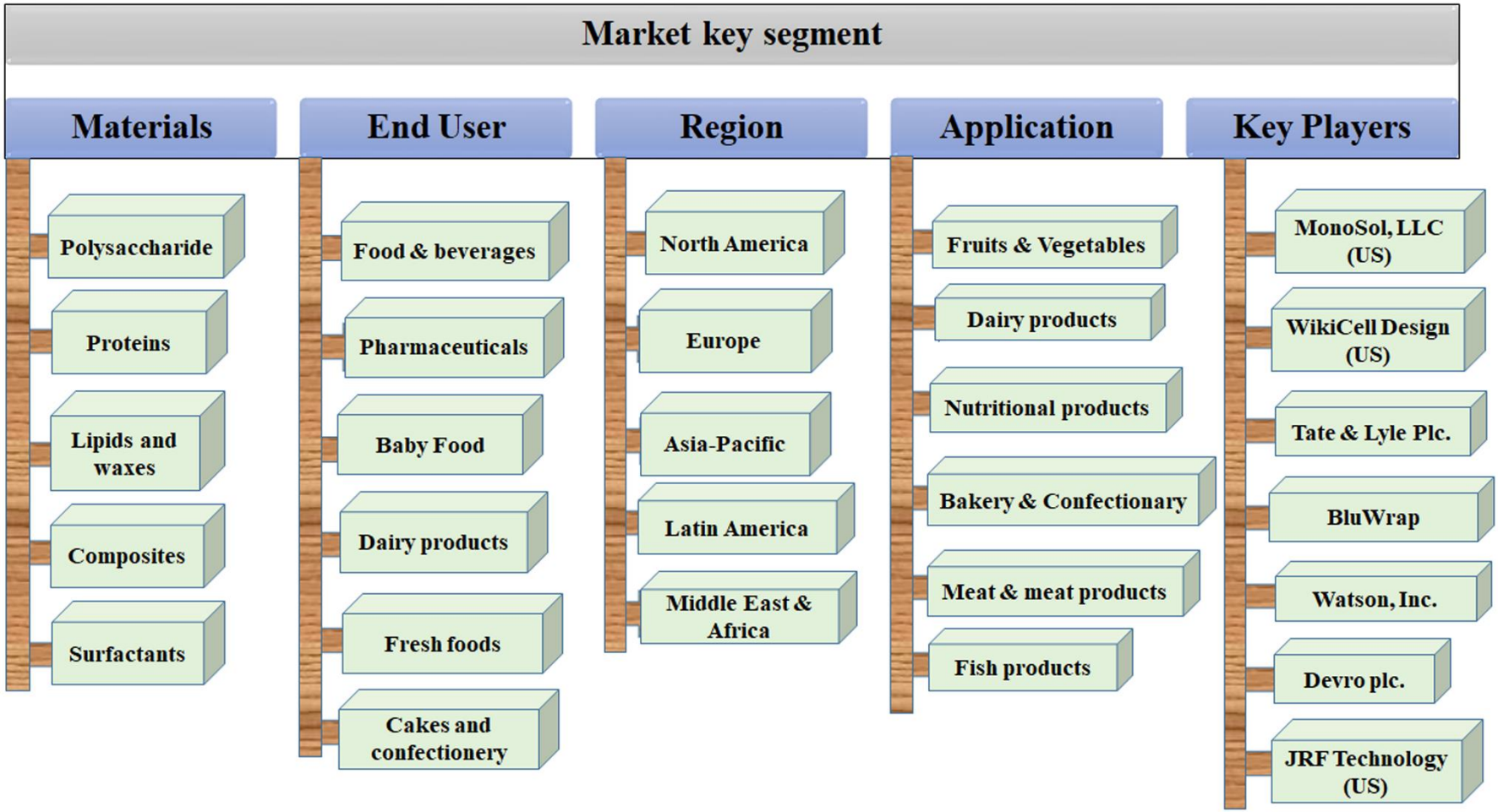


Figure 1.8 *Market key segments of edible food packaging.*

Table 1.1 *Global Market of Edible Packaging.*

No.	Company	Components of Packaging	Product and properties
1.	Bakeys, Hyderabad, Telangana, India	Different types of flour such as Sorghum, wheat, rice, millet Several flavors in spoon: cumin, mint-ginger, sugar, carrot-beetroot.	Edible Cutlery, Edible spoons, Edible forks, Edible chopsticks Trade Name: Bakeys Foods Private Limited
2.	Coolhaus, Los Angeles, California	Potato ST	Edible wrappers for ice cream sandwich
3.	Do Eat, Belgium	Water and Potato ST	Edible Tableware, Verrines, Sandwich rings, Cupcake Holders, Food bags
4.	Ecovative Design, New York	Mushroom materials from Fungal mycelium, Non-food agricultural materials	Mycelium packaging, MycoComposite, MycoFlex, Atlast Protective packaging, structural biocomposites, thermal insulation, etc.
5.	Eco Six Pack Ring (E6PR), Mexico	Compostable matter and by-product waste	Holder for beer cans
6.	Evoware, Indonesia	Burger wrapper, Instant noodle seasoning sachets, Coffee pouches	Seaweed
7.	MonoSol, Indiana, US	----	Food grade and water soluble films, Edible Pod for instant beverages, Wrapper and sachets, Soluble in hot and cold water, Water soluble films
8.	NVYRO, United Kingdom	Cassava ST Tapioca ST	Nvyro disposable food packaging products: Plates, Cups, Lunch boxes, Trays, Lunch plates, Ready to eat foods, Eatable plates Suitable food products: Liquid, cold, hot, dry, semi-liquid foods
9.	TIPA, Israel	Fully compostable plastic packaging, End life is like orange peel, Decomposting in 180 days,	Biomaterials and technology

		Can be used for dry, baked and frozen products	
10.	Scoby, Poland	Kombucha	Edible, recyclable package Properties: Fully edible and recyclable, Sachet, bag, and bowl, Zero waste production
11.	Loliware, New York	Alginate (Seaweed), agar (Red algae)	Flavored straws Properties: Behave like plastic for 24 h

In this regards, the edible food packaging has grown interest rapidly for reduced packaging waste and food waste. However, the inclusion of bionanomaterials in developing biopolymeric nanocomposite based edible coating can improve the shelf life of food products.

1.3.4 Biopolymeric Nanostructured Materials in Edible Nanocoating

The organic and inorganic nanomaterials are used in modifying the inherent properties of biopolymers to develop edible coating materials. In this context, the emerging bionanomaterials such as cellulose, CS and silk nanostructured materials are used as edible coating for different food products. Moreover, it is noteworthy to mention that the nanomaterials can be utilized to develop functionalized biopolymeric nanocomposites for improved/maintained shelf life of food products with value addition. In this regards, the fabrication methodologies, modification strategies and their application in developing edible coating have been discussed in the subsequent section.

1.3.5 Cellulose Nanostructured Materials in Functionalized Biopolymeric Nanocomposite based Edible Nanocoating: Fabrication, and Modification Strategy

The cellulose based nanostructured materials obtained from several renewable resources (plant fibers, wood, microbes, etc.) are used for edible food packaging based emerging techniques. The nanocelluloses having characteristics attributes such as renewability, biocompatibility,

biodegradability, tunable surface chemistry, nano-dimensions, low cost, and others make it a remarkable candidate to be used in developing edible food packaging. In this regard, the present section provides an insight into the use of several cellulose nano-form fabrication methods to obtain tailored-made properties for targeted edible food packaging. It is noteworthy to mention that the addition of nanocellulose for developing biocomposite based edible food packaging can help to improve the packaging properties such as barrier property, mechanical property, thermal property, optical property, and other physicochemical properties providing an ability in delivering tunable packaging attributes. The approaches utilized to modify the surface of nanocellulose for obtaining the better dispersion in the fabrication of polymer composites with required properties are briefly discussed. Additionally, cellulose nanomaterials are commonly applied in the development of both edible and non-edible food packaging materials for their remarkable properties which can further be modified by varying the forms of nanomaterials such as nanocrystals, nanofibers, nanowhiskers, etc. The section also details various case studies of developing biocomposites using nanocelluloses for design and development of edible packaging for perishable food products.

The development of cellulose based nanostructured materials has a great interest in the current trend of edible food packaging. The cellulose derivatives and its nanostructured materials are utilized in various food and beverage industries for obtaining tailored-made properties of food products. As shown in **Figure 1.9,** the targeted food based industries for using cellulose based products include bakery industry, meat industry, dairy industry, cereal industry, veterinary foods, food packaging industry, and others due to its tunable physicochemical properties. In food industries, the cellulose and its derivatives are used in various formulations such as emulsifiers, bulking agents, anti-caking agents, fat substitutes, texture enhancers, etc. Additionally, cellulose

and various derivatives play a remarkable role in improving the food quality in terms of texture, colour, and others with an enhanced shelf life of food products. The use of cellulose in food products and packaging (edible and non-edible) are largely applied due to increased food waste for careless handling, mechanical damages, and increase of plastic based waste as packaging materials. Cellulose and its nano-forms are available extensively and have the characteristics attributes of biodegradability, biocompatibility, surface chemistry, improved packaging property, and non-toxicity, which make them an ideal material to be used as a replacement for available conventional materials. Cellulose has a great market value for reducing the overall carbon footprint in the packaging industry and further provide improved food value. Interestingly, cellulose derivatives and nanoforms are also used in edible packaging to aid improved properties to other materials such as CS, ST, agar, etc. Further, the use of nanocellulosic materials in edible and non-edible sectors has been increased for having tailored barrier, mechanical, thermal, and color properties which further improve the shelf life of food products. As shown in **Figure 1.9,** the properties of nanocellulosic materials can be modified *via* chemical, physical, mechanical methods, and developing biocomposites of nanocellulose**.**

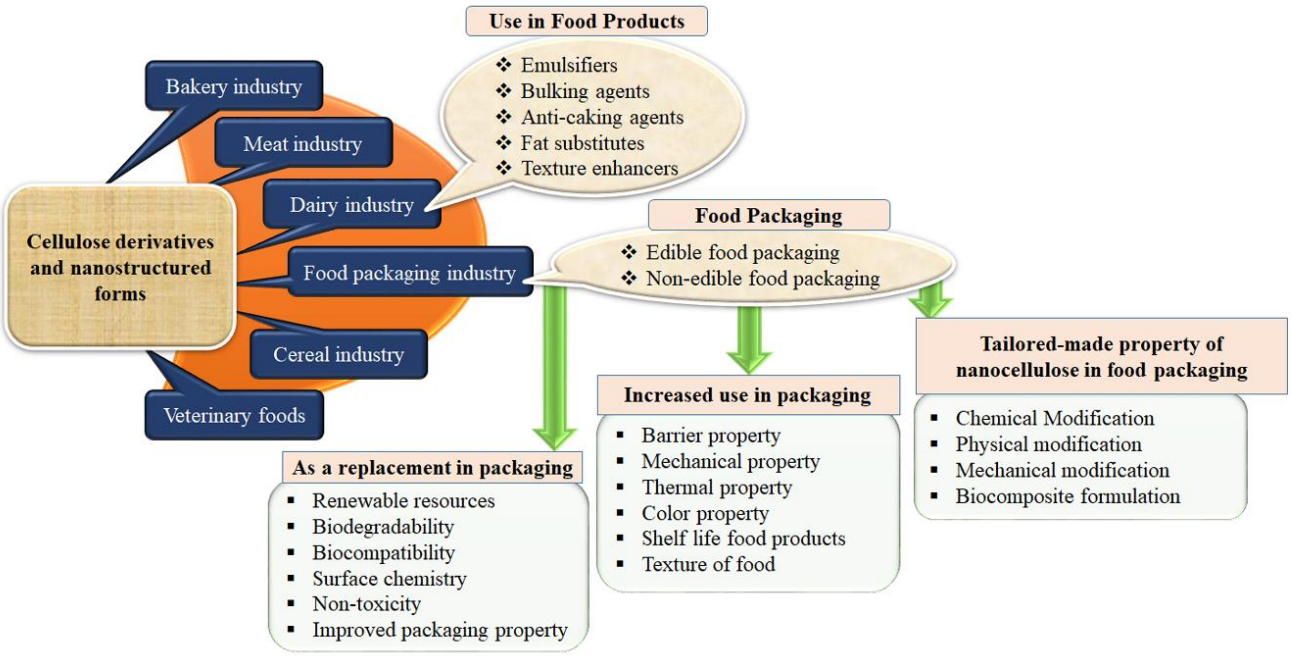


Figure 1.9 *Prospect of cellulose derivatives and nanostructured form in food sector.*

The cellulose delivering various health beneficial properties and further having a capability to improve the packaging properties and shelf life of food products are used in the development of edible food packaging. The use of cellulose and its derivatives in edible food packaging includes CMC/CS bilayer based edible coating on citrus fruit (Arnon et al., 2014), methylcellulose/ sodium alginate (SA) for peaches (Maftoonazad, Ramaswamy, & Marcotte, 2008), cellulose derivatives for 'Berangan' banana (Malmiri et al., 2011), CS/CMC/moringa leaf extract based edible coating for avocado fruit (Tesfay, & Magwaza, 2017**)**, CMC/zataria multiflora essential oil/grape seed extract based edible coating for rainbow trout meat (Raeisi, et al., 2014), CMC/calcium/ascorbic acid based edible coatings for fresh cut apples (Saba, & Sogvar, 2016), etc. The application of nanocellulose in edible food packaging includes carrageenan/ cellulose nanowhisker (CNW) as edible films (Sánchez-García, Hilliou, & Lagarón, 2010), alginate-acerola puree/ cellulose whisker (CW) as edible films (Azeredo et al., 2012), fish myofibrillar protein/ bacterial cellulose nanofibers

(BCNF) as edible films (Shabanpour et al., 2018), agar/ nano-bacterial cellulose (nano-BC) as edible films (Wang et al, 2018), etc.

The well dispersion of nanocellulose in various matrix materials helps to improve the characteristics attributes of packaging materials such as barrier, thermal, mechanical, wettability, and other properties to be acted as ideal packaging materials for improved shelf life of food products. Since the past decades, nanocellulose based polymer composites are considered emerging materials to be used in various forms of food packaging. The high mechanical strength, water resistance property, stability, and barrier properties make it a superior candidate for edible food packaging. In this regards, nanocellulose based materials are widely utilized as a potential alternative for available petrochemical based packaging.

Tailored Nanocellulose Based Materials for Edible Nanocoating

The biodegradable, non-toxic, high surface area with high crystalline nanocellulose including NFC, CNC, and bacterial nanocellulose (BNC) with their unique barrier layer properties such as enhanced oxygen and water vapor properties are used for food packaging. The percolated structure of CNF, and its long, flexible fiber structure with good mechanical properties provide proper dispersion in the matrix. Various investigations have been put forward to utilize nanocellulose derived biopolymers as edible films in food packaging. An article was reported on edible films from pectin, a natural polymer reinforced with crystalline nanocellulose (2, 5 and 7 w/w %) developed using solution casting evaporation method (Chaichi et al., 2017). Considering the most important functional properties in case of edible films and coatings are their barrier properties for water vapour and oxygen, carbon dioxide gases, migration of compound, appearance such as color and gloss, physical and mechanical protection for proper handling of food products. The high surface area feature of nanoscale cellulose demonstrates its application as reinforcements for

polymer matrix giving a new research area. However, its poor dispersion is a critical concern, which affects the mechanical properties of the composite materials leading to a challenging task. Thereby modification of the nanofiller is needed for good dispersion in the polymer materials *via* physical and chemical methods (Ng, et al., 2015; Dufresne, 2008).

Chemical Modification. The chemical modification of nanocellulose can be obtained *via* utilizing the available hydroxyl groups on nanocellulose. The chemical agents such as compatibilizing agent, coupling agent, acetylating agent, polymer grafting agent, co-polymerization, non-covalent surface modification, sulphonation, esterification, etherification, silation etc. are used to improve the properties of nanocellulose for food packaging application. The chemical modifications further help to improve the interfacial compatibility with the various polymer matrix by changing and controlling the molecular interactions. However, in case of surface compatibilization of nanocelluloses, one reactive group should be present. On the otherhand, for the co-polymerization aided modifications, at least two functional groups are required for improved interactions. In this case, the functional group in cellulose can react with polymer matrix *via* grafting, radical reactions, and organometallics for improved properties (Habibi, 2014; Sharma, et al., 2019**)**.

Physical Modification. The physical treatment of cellulose is a challenging method to alter the cellulose polymorphs, thereby, improving the matrix mechanical bonding and other properties (Islam, Alam, & Zoccola, 2013**)**. The various physical methods such as cold plasma (electric discharge), dielectric-barrier discharge (creation of ions, free radicals and other species generated by high energy electrons), ultra-sonification (include no use of organic solvent), and irradiation by gamma rays are used to enhance the CNC's adhesion properties, and further, mechanochemical

treatments are applied through shearing and compressing forces, etc. (Besbes, Alila, &Boufi, 2011; Kalia et al., 2011; Sonia, Priya Dasan, & Alex, 2013 ; Zhang, et al., 2011).

Development of Nanocellulose based Composites. The alternative of petroleum based plastics such as the active biobased food packaging has received great attention for improved hydrophobicity, mechanical properties, and antimicrobial activity of individual biopolymers. Li et al. have reported that developing CW with CS matrix displays an increase in tensile strength (TS) upto 120 MPa by incorporating 20% CNW along with excellent thermal stability and water resistance (Li, Zhou, & Zhang, 2009). A work has been reported on the preparation of bionanocomposites using casting/evaporation method based on WG, CNC and Titanium NPs (TiO_2), which provide improved breaking length of 56 % and burst index of 53% by incorporating 7.5% CNC and 0.6% TiO_2 for 3 coating layers over kraft paper. The bionanocomposite also showed excellent antimicrobial activities almost 100 % against yeast *S. cerevisiae*, gram- negative bacteria *E. coli*, and gram-positive bacteria *S. aureus (*Wakil et al., 2005**)**. Jung et al. has investigated developing coating using Fe^{3+}-anthocyanin complexation with CNF/SA using layer-by-layer (LBL) coating method on blueberries and found that leakage of anthocyanin pigments can be eliminated (Jung et al., 2015). Another study on acerola puree and alginate reinforcing with CW provides improved water vapour barrier property in the form of nanocomposite edible film (Azeredo et al., 2012).

Case Studies on Nanocellulose Based Edible Nanocoating

In this section, a brief discussion has been made about the use of various nanoforms of cellulose such as CNC, CNF, BNC, CNW, which are utilized with other biopolymers as edible food packaging materials. However, the characteristics attributes of fabricating composite based

edible packaging can be modified using plasticizers, flavouring agents, and others as shown in **Table 1.2.** Different types of cellulose based materials are utilized as primary, secondary packaging, flexible packaging, and developing containers, etc. However, in this section, the use of nanocellulose as a component of edible packaging has been made.

Storage Study of Edible Coated Food Products. Nanocellulose has been widely utilized for the fabrication of composite materials to be used as edible coatings for enhanced shelf life of food products as given below:

- Storage study of fresh strawberry has been done using edible coating based on CNC reinforced gelatin plasticized with glycerol (storage condition: under refrigeration for 8 days) (Fakhouri et al., 2014)
- Storage study of strawberries has been done using apple pectin, CNCs, essential oils of lemongrass (Storage condition: 8 days and 10 ± 1 °C) (Da Silva, et al., 2019)
- Storage study of pears has been done using CNC reinforced CS based edible coating (Storage study: 3 weeks and ambient storage: 20 ± 2 °C and 30 ±2 % RH) (Deng et al., 2017)
- Storage study of grape fruits has been done using CNF, carrageenan, glycerol, and Hydroxypropyl Methylcellulose (HPMC) (Storage Condition: refrigerated storage for 41 days) (Silva-Vera et al., 2018)
- Storage study of strawberry has been done using CS and cellulose nanofibril based composites (Storage condition: Cold storage for 21 days) (Resende et al., 2018)

Packaging Property of Nanocellulose based Edible Films and Coatings. The nanocomposite based on CNF reinforced mango puree provides tunable packaging properties for developing edible films. The incorporation of CNF provides improved mechanical properties in terms of TS

and Young's modulus for forming a fibrillary network within mango puree. The development of edible films based on microcrystalline cellulose (MCC) and lipid coated MCC NPs (LC-MCC) incorporated HPMC has a promising attribute in the field of food packaging for being flexible and transparent materials. The TS of MCC and LC-MCC incorporated HPMC films provide increased TS up to 53 and 48%, respectively in comparison to neat HPMC films (Bilbao-Sáinz et al., 2010). In this regards, several types of nanocellulosic materials are used to improve the appearance and quality of various food products during storage time. Further, CW from cotton fibres is used as a filler material in developing composites of alginate-acerola puree with plasticizer which can be used as edible food packaging with a tailored-made property of overall tensile property and water vapor barrier property (Azeredo et al.,2012). The fabrication of pectin/ crystalline nanocellulose based biocomposite edible films deliver an improved film property in terms of mechanical property, and barrier property (Chaichi, et al., 2017). Further, this kind of pectin based packaging materials can be considered as fully biodegradable and renewable packaging for food products. The properties of CS based edible films can be tailored-made with the aid of nanofiller nanocellulose and glycerol as a plasticizer. With the aid of central composite design considering two variables CNF concentration (0 to 20 g/100 g) and glycerol (0 to 30 g/100 g) are used for optimizing the development of CS based edible films, where with 15 g/100 g CNF concentration and 18 g/100 g glycerol concentration are considered as optimum condition (Azeredo et al., 2010). The CNF dispersed CS films have antimicrobial property, with an efficacy in improving barrier property (Yu, et al., 2017). Further, Pereda and others have developed sodium caseinate/ nanocellulose based edible films, where the TS and tensile modulus has been found to be improved with increased nanocellulose (Pereda et al., 2011). The application of CNF, TiO_2, rosemary essential oil (REO) and whey protein isolate (WPI) based edible nanocomposite films helps in improving

the sensory quality, organoleptic properties, and shelf life of lamb meat (Sani, Ehsani, & Hashemi, 2017). The synthesis of biocomposite films has a great interest in delivering the controlled release of antimicrobials. The fabrication of WPI/CNF composite films containing TiO_2 and REO are very efficient in preserving quality of meat for maintaining food properties, where the films with TiO_2 and REO have an ability to inhibit pathogenic bacteria in meat, and is considered economic. The nanocellulose and CS based edible films in packaging ground meat has an ability to decrease the lactic acid bacteria, which helps in improving the shelf life of ground meat (Dehnad et al., 2014). Further, the storage study of grape fruits with edible coating application using CNFs, HPMC, and Ƙ-carrageenan has been reported, where application of spray system is utilized for coating fruits (Silva-Vera et al., 2018).

Table 1.2 *Application of cellulose nanostructured materials in edible food packaging.*

No.	Filler Type	Matrix Type	Targeted Food product and obtained property	Reference
1.	CNF	Mango puree	Edible films Property: Improve mechanical property Improve barrier property	Azeredo et al., 2009
2.	MCC NPs	HPMC	Edible Films Property: Better moisture barrier properties, reduced water barrier properties.	Bilbao-Sáinz et al., 2010
3.	CNC	Gelatin	Edible coating on fresh strawberry Property: Antimicrobial effect Reduce weight loss Prolong shelf life Retention of ascorbic acid	Fakhouri et al., 2014
4.	BCNF	Fish Myofibrillar protein	Edible films Property: Improved thermal property Reduce WVP	Azeredo et al., 2012

			Reduced swelling index	
5.	CW	Alginate-acerola puree Plasticizers: Corn syrup	Edible films Property: Improved TS Improved Young's modulus Improved water vapor barrier	Shabanpour et al., 2018
6.	Crystalline nanocellulose	Pectin	Edible films Property: Improved TS Improved elongation at break Improved water barrier properties	Chaichi et al., 2017
7.	CNF	Gelatin Glycerol (A plasticizer)	Edible films Property: Tunable mechanical property Tunable optical property Tunable barrier property	Andrade-Pizarro, Skurtys, Osorio-Lira 2015
8.	CNF Titanium dioxide REO	WPI	Edible films Food Products: Lamb meat Property: TiO_2 and REO improve the organoleptic property of lamb meat Increase shelf life of lamb meat Inhibit the growth of spoilage and pathogenic bacteria in meat	Sani, Ehsani, & Hashem, 2017
9.	Nanocellulose	CS Glycerol (Plasticizers)	Edible films Property: Good dispersion Tunable properties in terms of TS, elongation at break, Young's modulus, barrier properties, thermal properties	Azeredo et al., 2010
10.	CNF	CS	Edible films and active packaging Property: Antimicrobial property Barrier property	Yu, et al., 2017
11.	NCF	Sodium caseinate Glycerol as plasticizer	Edible films Property: Less transparency for composite films More hydrophilic than neat sodium caseinate films	Pereda et al., 2011

			Improve mechanical property with the aid of nano-filler material	

(Note: CNF: Cellulose nanofibers; CS: Chitosan; BCNC: Bacterial cellulose nanocrystals; BC: Bacterial cellulose; BCNF: Bacterial cellulose nanofibers; MCC: Microcrystalline cellulose; TS: Tensile strength; HPMC: Hydroxypropyl Methylcellulose; CNC: Cellulose nanocrystal; REO: Rosemary essential oils; WPI: Whey protein isolate; CW: Cellulose whiskers; NPs: Nanoparticles; NCF: Nanocellulose fibers; WS: Water solubility)

1.3.6 Chitosan Nanostructured Materials in Functionalized Biopolymeric Nanocomposite based Edible Nanocoating: Fabrication, and Modification Strategy

The inclusion of CS nanostructured materials as a constituent of edible food packaging is a potential candidate to be used as an alternative against existing conventional packaging materials for attaining enhanced product life of perishable food products. The several properties such as excellent physicochemical properties, bioactivity, antimicrobial, antifungal, health benefits, and packaging properties are better obtained by the use of CS nanomaterials as a biocomposite or as an individual material. Further, CS nanostructured materials are generally availed in the forms of nanocrystals, nanofibers, and NPs, which can be fabricated using several processes, where optimization of the processing conditions deliver targeted morphology (**Figure 1.10**). The several processes for fabricating CSNPs include ionotropic gelation, microemulsion, emulsification solvent diffusion, polyelectrolyte complexes, reverse micellar, solvent evaporation, co-precipitation, complex coacervation, and other methods. The section discusses the fabrication methods and property analysis of CS based nanostructured materials for delivering the tailored packaging properties to act as a potential material in developing edible films and coatings.

Interestingly, CSNPs aided composites help to improve the shelf life of food products while maintaining the several quality parameters throughout the storage life.

Prospective of Chitosan Nanostructured Materials in Edible Food Packaging

CS is a renewable, and non-allergic material which is used in developing edible coating. CS nanostructured materials are also utilized as drug delivery materials. The CSNP with insulin has an ability to enhance the pharmacological bioavailability. CSNPs has some remarkable attributes such as non-toxicity, small size, high surface reactivity and provides additional benefits to be used in edible food packaging. As shown in **Figure 1.10**, the NCS is obtained in the form of CSNP, nanofiber, nanocrystals, and the noteworthy several characteristics attribute increase its use in edible food packaging.

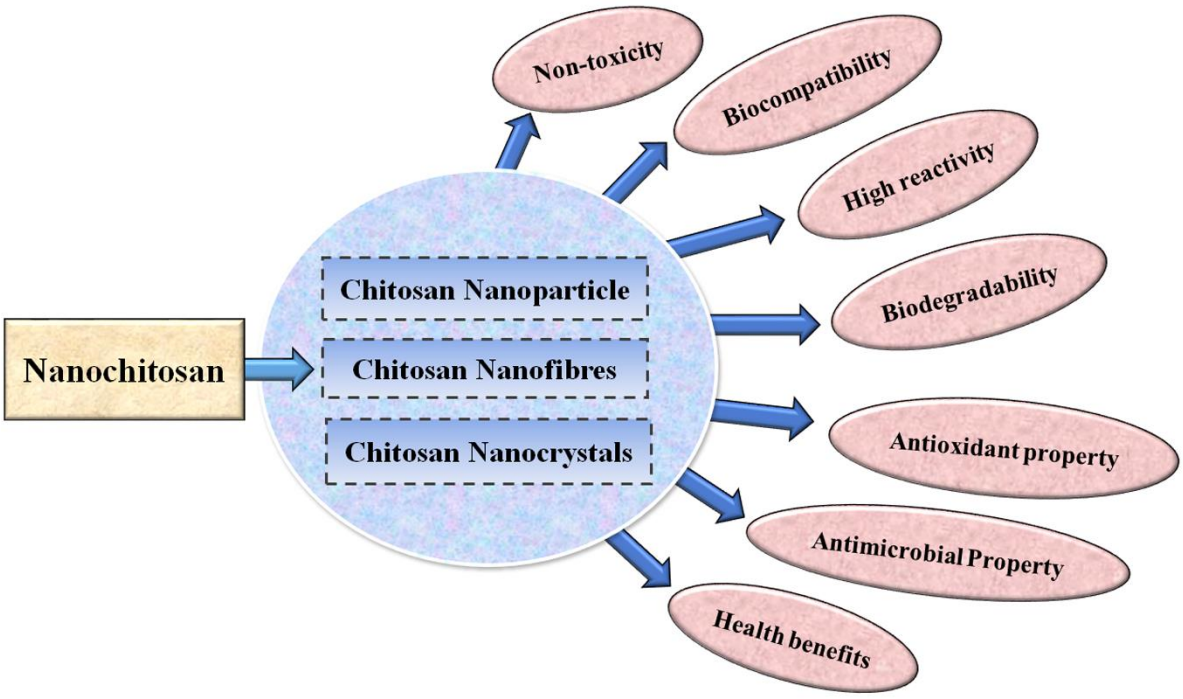


Figure 1.10 *Prospective of nanochitosan in edible food packaging.*

Biocompatibility and Non-Toxicity. The non-toxicity and biocompatible nature of NCS make it a potential alternative to use as food components and to develop edible films or coatings for an improved shelf life of food products. The toxicity determination of CS (source: fungi and shrimp) and NCS (fabrication process: Ionic gelation) are evaluated using brine shrimp bioassay, and rat bioassay (Darwesh et al., 2018). The toxicity evaluation using brine shrimp bioassay shows that there are no toxic components at several concentrations such as 5000, 10000, and 15000 ppm. The blood parameters such as biochemical property of blood, liver, kidney functions, histopathology changes in liver, stomach and kidney tissues can be determined for toxicity evaluation. The normal functioning of the liver when feed with CS and NCS are observed by alanineaminotransaminase (ALT) and aspartateaminotransaminase (AST) values. Further, the levels of creatinine, urea, and uric acid in the blood can provide the normal kidney functioning. The intake of 100 and 200 mg kg^{-1}bw rat CS and NCS has no effect on liver and kidney functions. Histopathology of liver, kidney and stomach also provides no obvious changes in the tissues and inflammation, fibrosis has not found for the same. The biochemical property of blood and oxidative stress is also not affected by the intake of specified CS. Additionally, the CSNPs (50 µg. mL^{-1}) extracted utilizing the fungal enzyme *Trichoderma harzianum* are biocompatible in nature which provides no cytotoxicity effect as evident by cell viability and acridine orange/ethidium bromide staining assays (Saravanakumar et al., 2018). The CS releases amino sugars in human body by the action of enzyme lysozyme which makes it a biocompatible biomaterial (Leonida, & Kumar, 2016). However, the biodegradability rate of CS depends on degree of deacetylation, where the biodegradable nature of NCS is a critical parameter for acute and long term toxicity. Thus, CS nanostructured materials due to their non-toxic, biocompatible, and biodegradable nature make it an attractive biopolymer for microencapsulation, and edible food packaging.

Antimicrobial Property. CS and it's nanostructured forms have an antibacterial property which provides a great interest to be used as edible coating and film materials for several food products. However, CS provides antibacterial activity in the acidic medium due to its insoluble nature at pH above 6.5. Additionally, the antimicrobial property of CS depends on the type of sources, degree of deacetylation, physicochemical property, molecular weights, selected solvent, etc. The CS has a better antibacterial property against gram-negative bacteria than gram-positive bacteria. The CSNP is considered as a remarkable antimicrobial agent against several bacteria, viruses, and fungi, which makes it a promising candidate against some of the available bactericides and chemical fungicides (Divya, Smitha, & Jisha, 2018**)**. In this regards, the CSNPs (low molecular weight and high molecular weight) have an inhibitory action against *Bacillus cereus, Escherichia coli, Listeria innocua, Staphylococcus aureus, Salmonella typhimurium,* and *Yersinia enterocolitica* ***(***Madureira et al., 2015**)**. The encapsulated CSNP with polyphenols also provides the antimicrobial activity. NCS has better antimicrobial activity than CS for preserving silver carp fillets (Ramezani, Zarei, & Raminnejad, 2015). The antimicrobial property of NCS is better than CS due to the larger surface area and better interaction with microbes. Additionally, NCS has a better ability to inhibit total volatile basic nitrogen (TVB-N) than CS. Another research reports the antimicrobial activity of CS (sources: crustaceans and fungi) and NCS against gram-positive bacteria, gram negative bacteria, yeast, and fungi (Darwesh et al., 2018).

Antioxidant Property. Additionally, the CSNPs extracted utilizing the fungal enzyme *Trichoderma harzianum* provide antioxidant activity in a dose-dependent manner (Saravanakumar et al., 2018). The CSNPs with small size ranges and low molecular weight provide antioxidant activity and have scavenging activity against free radicals (Divya, Smitha, & Jisha, 2018**)**. The antioxidant property of CS can be evaluated by radical scavenging activity against superoxide

anion and hydroxyl radical. The lower molecular weight CS oligomers have better antioxidant activity than higher molecular weight, where the 50% inhibition concentrations for determining superoxide anion scavenging are 5.54 mg/mL, 8.11 mg/mL, and 12.15 mg/mL, for CS oligomers with molecular weights 2300, 3270, and 6120 Da, respectively. Additionally, for superoxide anion, the maximum inhibiting efficacy of CS oligomers with molecular weights 2300, 3270, 6120, and 15250 Da are 89, 75, 74 and 41 %, respectively. For hydroxyl radicals, the maximum inhibiting efficacy of CS oligomers with molecular weights 2300, 3270, 6120, and 15250 Da are 71, 65, 51 and 7 %, respectively (Sun et al., 2007). The antioxidant activity of high molecular weight CS based films provides a way to use it as a natural antioxidant material for food application, where high molecular weight CS can be evaluated for radical scavengers against 1,1-diphenyl-2-picrylhydrazyl radicals, hydroxyl radical, superoxide radicals (Wan et al., 2013). However, the antioxidant property of hetero-chitooligosaccharide depends on degree of deacetylation, and molecular weight (Je, Park, & Kim, 2004).

Other Properties. Besides, the above mentioned properties, the CSNPs have many health beneficial properties such as the increased immune response of living system, acts as a weight reducing agents, lowers the high density lipoprotein, provide antioxidant property, decrease the lipid peroxidation, decrease oxidative stress, reduce pain, bio-adhesive property, antitumor property, antiparasitic effects, etc. CS has an ability to bind with fat more effectively in comparison to other glycans, which helps in lowering the high density lipoproteins. Additionally, NCS based films are used as a therapeutic agent to treat cutaneous leishmaniasis and provide increased wound contraction rate, re-epithelialization rate, and formation of scar tissue (Bahrami et al., 2015). The nanosystem based on CS-sodium tripolyphosphate (STPP) also acts as a potential candidate for the delivery of active agents for several food grade properties.

Fabrication Routes of Chitosan Nanostructured Materials

In 1994, Ohya and coworkers first described CSNPs preparation using emulsifying and cross-linking process. Besides, the various methods for developing CSNPs include ionotropic gelation, emulsion cross-linking, emulsion-droplet coalescence, precipitation, reverse micellar method, sieving method, spray drying method, and others. The CSNPs formation can be obtained using chemical or physical cross linking process. The acid hydrolysis is another approach for obtaining chitin nanocrystals and nanofibers with controlled dimensions. The CSNPs are also used extensively in the agricultural sector as pesticide delivery for crop production, fertilizer delivery, micronutrient delivery, nanosensors, etc. (Kashyap, Xiang, & Heiden 2015). Additionally, NCS is a potential candidate in food sectors and is used in edible films, coatings, encapsulation, carrier materials, antimicrobial active films, dietary supplements, and others.

Ionotropic Gelation. The ionotropic gelation is a simple and mild process, which includes no use of chemical cross-linking and also reduces the possible toxicity of chemicals. CS (polycation) having an amino group possess positive charges, whereas STPP (polyanion) have negative charges, so the component can be self-assembled through the ionic gelation method. In this method, the CSNP extraction is developed through interaction between the oppositely charged molecules as represented in **Figure 1.11**. The ionic interactions between the biomacromolecules can be monitored by charge densities of CS and STPP. The STPP has the ability to form gels through ionic interactions. The CSNPs ionically cross-linked with STPP has the properties of haemocompatibility, non-toxicity, mucoadhesivity, and antimicrobial activity which also provide the benefits of incorporating macromolecules, hydrophilicity, etc. (Bugnicourt, & Ladavière, 2016**)**. For the fabrication of CSNPs *via* ionotropic gelation, CS is first dissolved in acetic acid solution, and before or after the addition of polyanion, stabilizing agent such as poloxamer can be

added to the CS solution. In the next step, STPP as a polyanion is added to the above solution under mechanical stirring at room temperature, and formation of CSNPs held. In this process, the processing parameter such as weight ratio of CS and STPP is a crucial parameter to control and obtain higher yield to fabricate CSNPs. In this process, the dimension of fabricated CSNPs can be modified *via* tailoring the ratio of CS and STPP. The several factors which affect the fabrication of CSNPs are concentration, pH, ratio of components, mixing method, etc. The particle size of CSNPs are 85 nm (CS concentration: 2.14 mg mL^{-1}, STPP concentration: 0.3 mg mL^{-1}), 110 nm (CS concentration: 2.14 mg mL^{-1}, STPP concentration: 0.6 mg mL^{-1}) and 221 nm (CS: 3.15 mg $mL^{-1}$and STPP concentration; 0.6 mg mL^{-1}) (de Moura et al., 2009). The STPP is used in food product development and has the characteristics attributes for acting as a carrier material for the delivery of bioactive components. The biomacromolecules are used as a preservative and food additives for meats, poultry, seafood, etc., where STPP is used to keep the freshness of the products by maintaining the natural color and improving the texture. The mentioned properties are attained by improving the water holding properties of the products. Further, it is used as an emulsifier in food products. Additionally, the particle size, polydispersity index and other properties of CSNPs can be modified by controlling the molecular weight, concentration, mass ratio, and pH of CS solution (Antoniou et al., 2015).

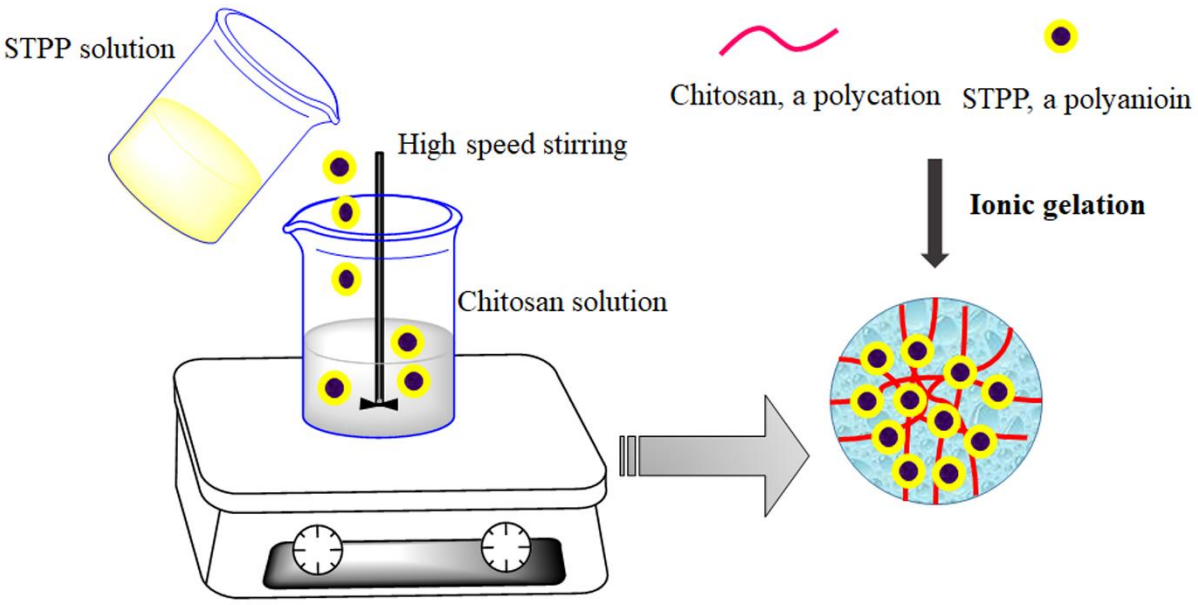


Figure 1.11 *Fabrication of chitosan nanoparticles through ionotropic gelation.*

Packaging Properties of Nanochitosan based Edible Food Packaging

CS nanostructured materials are extensively used to improve the packaging property of several polysaccharides such as HPMC and protein based films in terms of barrier property, mechanical property, thermal property, color property, and others. Furthermore, the CSNPs having the significant properties of antimicrobial, antioxidant, antifungal, and cytotoxic activities are used to wrap several food products. The fabrication of CS based edible films with tailored packaging properties in terms of functionality and performance can help to improve the existing shortcomings of individuals. The utilization of CS nanostructured based materials for edible packaging of food products in terms of edible films and coating can help to avail with fresh, seasonal food products, in several regions. The CS nanostructured materials in combination with other polymeric materials as polymer biocomposites are used to provide the improved shelf life of several categories of food products such as poultry products, seafood, agricultural products, etc. The emulsion droplet

coalescence method of developing CSNP involves both emulsions cross linking and precipitation methods. Further, the several NCS based edible films are represented in **Table 1.3**.

In this regards, the edible films based on HPMC and CS/STPP NP provide improved water vapour permeability (WVP), mechanical properties (tensile properties) and thermal properties (de Moura et al., 2009). The use of CSNPs in developing HPMC based biocomposite films has a tendency to occupy the empty spaces of the pores of HPMC matrix, which helps to improve the packaging properties. The CSNPs with a particle size of 85 nm, 110 nm and 221 nm provide elastic modulus of ~1264, ~1190, and ~1204 MPa, respectively. Further, the elongations of HPMC/CSNP based edible films are ~11.1, ~5.2, and ~5.7 % for particle sizes of 85 nm, 110 nm and 221 nm, respectively. The CSNPs loaded HPMC films improve the thermal stability of the films and the thermal degradation temperature changed from 232 to 271 °C due to NP loading. Additionally, the CSNPs incorporated tara gum based edible films improve the mechanical property and physicochemical property (Antoniou et al., 2015**)**. The use of CSNPs help to reduce the film hydrophilicity and WS which is a desirable property for food packaging application. As discussed earlier, the free volume of HPMC edible films can be reduced when CSNPs are used as a nanofiller materials due to the compact structure and further, create an obstruction in the path of water diffusion decreasing the moisture content of the films. The TS of CSNP (10% w/w) incorporated tara gum increased to 58 MPa from 23 MPa (neat tara gum films). The incorporation of CSNP in HPMC films helps to increase the TS by 35.73 MPa and decrease the elongation by 7.21%. Additionally, the WS and WVP are reduced by 74.3%, and 22.7% for CSNP reinforced HPMC films, respectively. The development of CSNP and fish gelatin based edible film provides increased superficial hydrophobicity, thermal stability, and crystallinity (due to nucleating effect of nanofiller) in comparison to neat fish gelatin films (Hosseini et al., 2016a). Additionally, the

addition of *Origanum vulgare L.* essential oils (EOs) develop more flexible films with increased crystallinity and further reduce the WVP (Hosseini et al., 2016b).

The edible films based on biocomposites of zein film with CSNP, and cinnamon EO (CEO) provide significant tunable packaging properties in terms of mechanical property, physical attributes, structural and antimicrobial attributes (Vahedikia, et al., 2019)**.** The zein based biocomposite films containing CSNPs (2% w/w) and CEOs (4 % w/w) provide increased TS and decreased elongation at break compared to neat zein films. The WVP of neat zein film and zein biocomposite films (with CSNPs and CEOs) are ~ 6.4 and ~ 2.0 g $Pa^{-1}h^{-1}m^{-1}$, respectively. Additionally, the color parameters of neat zein film and zein biocomposite films are ~ 36.30 and ~ 27.05 in terms of L value; ~6.28 and ~14.22 in terms of a* values; ~13.96 and ~19.00 in terms of b* values, respectively. The edible films based on guava puree, HPMC, and CSNP deliver a sustainable packaging material with improved thermal, and mechanical property, whereas, the WVP and WS also reduce due to added CSNPs (Lorevice et al., 2012). The edible films based on banana puree extracted from over-ripe peeled bananas, glycerol, pectin and CSNPs reduce the water vapour permeation by 21% (Martelli et al., 2013). The active edible films based on NCS and pectin can improve the shelf life of food products which inhibit the growth of *Aspergillus niger*, *Escherichia coli*, *Saccharomyces cerevisiae*, and others (Ngo et al., 2020).

Table 1.3 *Packaging properties of chitosan nanopartilces based edible films.*

No	Film Composition	Type of Packaging Materials	Packaging Property	Reference
1.	CSNP and HPMC	Polysaccharide based edible or biodegradable films	Improve mechanical property, barrier property, water solubility, thermal stability, and others	de Moura et al., 2009
2.	CSNP and tara gum	Polysaccharide based edible films	Improved tensile strength, thermal stability, antimicrobial effects and others	Antoniou et al., 2015
3.	CSNP, zein film, and CEO	Protein based active Edible films	Provide improved tensile strength, decreased elongation, decreased water vapour permeability, tailored solubility, and others.	Vahedikia et al., 2019
4.	CSNPs and Fish gelatin	Edible films	Increased superficial hydrophobicity, thermal stability, and crystallinity	Hosseini et al., 2016a
5.	CSNP, HPMC, guava puree	Edible film	Increased mechanical and thermal property Decreased WVP and water solubility	Lorevice et al., 2012
6.	Fish gelatin, CSNP, *Origanum vulgare* L. EO	Edible films	Increased crystallinity, flexible films, decreased water vapor permeability Antibacterial film	Hosseini et al., 2016b
7.	CSNPs, banana puree films, glycerol	Edible films	CSNPs improves the mechanical property And reduces the mechanical property	Martelli et al., 2013
8.	NCS and pectin	Active edible films	Increased tensile strength, reduced water solubility, hydrophobic films	Ngo et al., 2020

(**Note:** CSNPs: Chitosan nanoparticles; HPMC: Hydroxypropyl methylcellulose; CEO: Cinnamon essential oil; EOs: Essential oils; NCS: Nanochitosan; WVP: Water vapour permeability)

Application of Chitosan Nanostructured Materials in Edible Food Packaging

The use of CS nanostructured materials in developing edible food packaging has attained a great interest due to several remarkable properties including antimicrobial, antibacterial, and cytocompatible nature. The CS nanomaterial based edible coating also helps to decrease the weight loss of fruits and vegetables, while, delaying the ripening of perishable fruit products. In this regards, several research and developments are continuously undergoing for its wide consumer acceptance and commercialization purpose. Additionally, CS nanostructures as edible nanomaterials added coatings provides significant advantages in order to maintain quality, safety, and enhanced shelf life of highly perishable food products. The edible coating based technology has been considered as one of the widely used food preservation techniques, which minimizes the postharvest loss of fresh produces.

1.3.7 Silk Fibroin based Nanostructured Materials in Functionalized Biopolymeric Nanocomposite based Edible Nanocoating: Fabrication, and Modification Strategy

The potential sources of silk fibres are Muga (*Antheraea assamensis*), Mulberry (*Bombyx mori*), Eri (*Philosamia Cynthia ricini*), Tasar (*Antheraea mylitta*) and others. The silk fibres of silkworms consist of two components such as sericin (20 to 30%) and fibroin (70 to 80%). The sericin is present as a glue-like layer and fiborin is present as two singular filaments in cocoons. The sericin is hydrophilic in nature, soluble in warm water, and can be removed from silk fibre using the degumming process (boiling in sodium carbonate or autoclaving). The silk sericin has many beneficial properties including antibacterial, oxidation resistance, and moisture regulating quality. The extraction of fibroins is done by removing sericin from the cocoons. The silk fibroin (SF) has many beneficial properties to be used in food packaging applications. However, SF is a potential candidate to be used in developing edible coating on food products. SF is a kind of

structural protein obtained from *Bombyx mori* silkworm cocoons, having noteworthy properties including non-toxicity, biocompatibility, edibility and others. The properties of SF can be monitored by formulating blends and biocomposites of SF with other polymers. SF is a kind of insoluble protein in silk, which can be extracted from cocoons using acid hydrolysis method. The bilayer edible coating based on SF and poly(vinyl alcohol) for fresh-cut produces provides improved food properties (Ruggeri, et al., 2020). SF is considered as an effective water based coating to obtain enhanced freshness of perishable food (Marelli et al., 2016). The reduced respiration rate and water evaporation property of fruit can be obtained using SF based edible coating. In this context, SF can be considered as a promising material for edible coating.

The nanosystem assisted protein based edible food packaging include the use of protein based nanostructured materials such as whey protein isolate nanofibrils (WPNF), zein NPs, SF, gelatin NPs, egg albumin NPs, casein-silver conjugated NP, and others. However, the inclusion of plasticizers, and compatibilizers with protein based nanostructured materials can further improve the edible films and coating properties. The noteworthy attributes of protein NPs include non-antigenic property, biodegradability, surface chemistry, surface modifications, and further, controlled dimensions of protein NPs can also be obtained (Jeevahan & Chandrasekaran, 2019). Additionally, protein based biocomposites are also used to develop edible coating materials and incorporation of nanofiller materials can improve the protein coating properties in terms of barrier property, mechanical property, thermal property, and others.

1.3.8 Synthesis Strategies to Develop Coated Food Products

The several methods used for developing edible coated food products include dipping, spraying, fluidized-bed coating, dripping, foaming, panning, brushing, etc. In wet processes of developing edible coating, liquid forms of coat materials are used to coat regular and irregular

products *via* dipping, spraying, electrospraying, and others followed by drying. Additionally, the selection of a particular coating method depends on several attributes such as surface property, selected coating materials (rheology and other property), adhesion property, etc. However, the stability between targeted food products and coating materials is essential to develop a good coat on food products. The layer by layer coating is another approach to improve the shelf life of food products, where the coating materials can be applied simultaneously or sequentially on food products. Further, the selection of processes also depends on large scale coatings development and required time, where a discussion related to available techniques for developing edible coating has been made in the below sections.

Dip Coating Method

The dip coating method is an extensively used lab scale approach for developing edible coating on food products, which provides several beneficial approaches in comparison to other available techniques such as simple, cost effective, and develop uniform coverage on both regular and irregular food products. The several processing steps for developing edible coating using dipping are represented in **Figure 1.12**, which involves dipping of the selected food products in the developed edible coating solution. However, to develop a dipping based edible coating on fruit and vegetables, viscous solutions are preferred **(**Valdés et al., 2015**)**. The dipped food products are further allowed to drain the excess materials from the food products and then dried *via* open air or hot air dried technique to solidify the coating on food products followed by storage in specific conditions. This kind of coating process is commonly used to develop a uniform coating on food products. The coating thickness on food products is a critical parameter to consider in edible coated food products, where the coating thickness on food products *via* dip coating treatment depends on various factors such as dipping time, product temperature, temperature of prepared coating

materials, etc. (Soares, Fernandes, & Vicente, 2016). However, the controlling of processing conditions and continuous production of dipped coated food products are generally the shortcomings in developing dipped coated food products. Moreover, the dipping method involves the direct contact of the selected food products and coating solution, which may lead to microbial contaminations in the coating solutions. In dip coating, the fruit products are washed using distilled water, disinfecting agents to avoid contamination before dipping into the coating solution. Further, the layer by layer coating can also be developed using dip coating method, where the selected food products are dipped into different coating solutions followed by draining the excess solution after successive dipping (Sipahi et al., 2013).

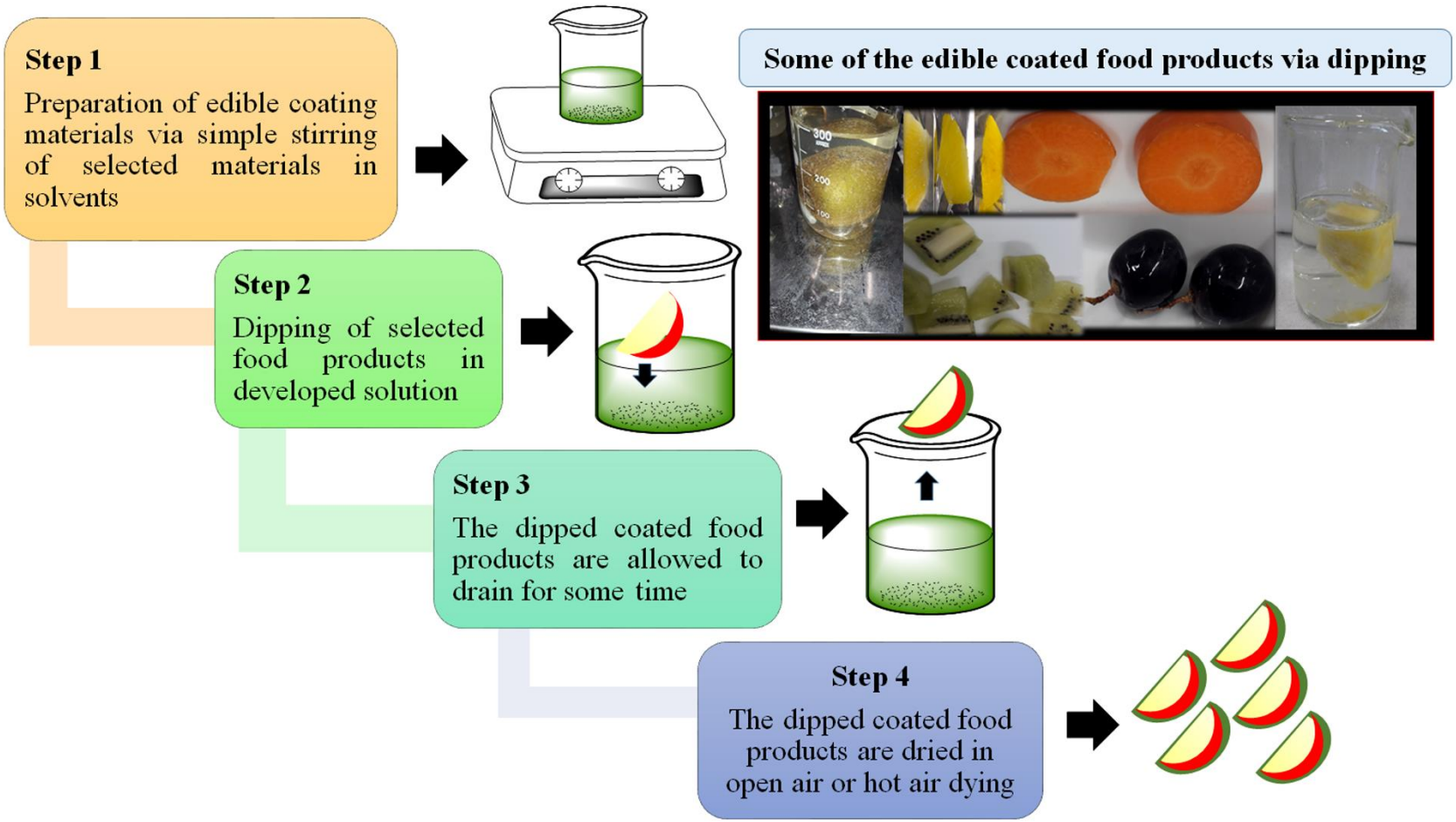


Figure 1.12 *Processing steps in developing dipped coated food products.*

The use of dip coating method has been applied for developing coating on several categories of food products such as fruits and vegetables, meat and meat products, fish and fish products, milk products, etc. (Soares, Fernandes, & Vicente, 2016; Sipahi et al., 2013; Cisneros-Zevallos, & Krochta, 2003; Bazargani-Gilani, Aliakbarlu, & Tajik, 2015; Petrou et al., 2012; Zhong, Cavender, & Zhao, 2014). In this regards, the development of edible coating on several fruit products using dip coating method has been represented in **Table 1.4**. The edible coating using dip coating can be given on whole fruits (apple, pear fruit, strawberry) (De León-Zapata et al., 2015; Dave, Rao, & Nandane, 2017; Del-Valle et al., 2005) and cut fruits such as apple slices (Rojas-Graü et al., 2007), cut pineapple (Ghosh, Teramoto, & Katiyar 2019), fresh-cut pears (Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2008), fresh cut melon (Roller, & Seedhar, 2002), fresh cut kiwi fruits (Roller, & Seedhar, 2002), and others. Further, edible coating using dip coating methods are also developed by dipping in different solutions such as edible coating of cut pears *via* dipping in polysaccharide solution (first dipping) and calcium chloride solution with antibrowning agents (second dipping) (Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2008)**.** The use of antibrowning agents can control the enzymatic browning and retard microbiological degradation of fruit products**.** The dipped coating of chicken breast meat can be obtained by first applying CS solution and then oregano essential oils under modified atmospheric packaging (MAP) conditions can retard lipid oxidation and significantly affected mesophilic count, lactic acid bacteria, yeast-moulds, etc. (Petrou, et al., 2012). The dip coating of meat samples using CS, glycerol, tween 80 and *Zataria multiflora* essential oil (ZEO) is reported to perform, where, development of edible coating on meat samples followed several steps such as (i) dipping in coating solution for 2 mins, (ii) draining of excess coating solution from the samples; (iii) dipping again for 2 min and (iv) drained for 5 h at 10 °C (Bazargani-Gilani, Aliakbarlu, & Tajik, 2015).

Table 1.4 *Development of edible coating on food products using dipping method.*

No.	Materials	Method of edible coating development	Targeted food product	References
1.	1.5% w/v CS solution	Dip coating •Dipped in prepared solution for 10, 20, 30, 40, 50, or 60 s •Draining of solution: 180 s	Frozen fish samples	Soares, Fernandes, &Vicente, 2016
2.	Formulation 1 (F1): CS, glycerol, acetic acid Formulation 2 (F2): Sodium alginate, glycerol Formulation 3 (F3): Soy protein isolate, glycerol	Dip coating Coating time for several formulations: F1: 20 Sec F2: 20 Sec F3: 15 Sec	Mozzarella cheese	Zhong, Cavender, & Zhao, 2014
3.	Polysaccharide based edible coating Sodium alginate and pectin	• Dipping time: 2 and 4 min • Removal of excess coating solution: 1 min	Sapota fruit	Menezes, & Athmaselvi 2016
4.	Pomegranate juice CS Acetic acid ZEO	Dip coating •Dipped in the coating solution for 2 min and draning • Dipping for second time (2 min) and drained for 5 h at 10 °C	Chicken breast meat	Bazargani-Gilani, Aliakbarlu, & Tajik, 2015
5.	CS Oregano oil under MAP	Dip coating • Dipped in CS solution for 1.5 min • Excess solution was drained immediately after dipping • Then oregano oil is applied to the samples.	Chicken breast meat	Petrou, et al., 2012
6.	Alginate, pectin, gellan, calcium chloride, antibrowning agents (N-acetylcysteine and glutathione)	• Dipping of cut pears in the polysaccharide solution for 2 min. • Draining of excess solution for 1 min. • Dipping in calcium chloride solution with	Cut pears	Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2008

		antibrowning agents for 2 min.		
7.	Soy protein isolate HPMC Olive oil Potassium sorbate	Dip coating • Washing of fruits in distilled water • Washing in sodium hypochlorite solution (disinfection) • Dipping of fruits in the solution for 2 min	Fresh pear fruits	Dave, Rao, & Nandane, 2017
8.	SF	Dip coating Dipping time is 10 s (for 1, 2 and 4 times) Drying conditions: 22 °C,38% RH, 4 h	Strawberry and banana	Marelli et al., 2016
9.	Sodium alginate Trans-cinnamaldehyde encapsulate powder (Antimicrobial agent) Calcium lactate Pectin	Layer-by-layer Dip coating method (Each dipping for 2 mins and allowed for draining 2 min) • Dipping in alginate and antimicrobial solution for 2 min • Dipping in calcium lactate solution • Dipping in pectin solution • Dipping in calcium lactate solution	Fresh cut watermelon	Sipahi et al., 2013

(Note: CS: Chitosan; ZEO: *Zataria multiflora* essential oil; MAP: Modified atmospheric packaging; HPMC: Hydroxypropyl methylcellulose; SF: Silk fibroin)

1.3.9 Sustainable Secondary Packaging for Coated Food Products

As mentioned, green polymers are used for their biodegradable, biocompatible, non-toxic nature and can be derived from naturally occurring materials and renewable resources, which create no pollution to the environment. The modified green packaging can be used as secondary packaging materials for coated food products. In this context, the properties of biodegradable

polymers such as aliphatic and aromatic polyesters for various applications need to be modified with the aid of various routes. The fabrication of green composites can be done using biodegradable polymers incorporating fillers, where the properties of biodegradable polymers can be tuned to a greater extent. Among available, Poly (lactic acid) (PLA) is a chemically derived polymer produced from carbohydrate-based feedstock (corn, sugar beet and others) following various routes of processing such as direct polycondensation reaction, solid state polymerization, enzymatic polymerization, and ring opening polymerization (ROP) having enormous use in biomedical, food packaging, textile, conductive materials, etc. Proper dispersion of filler materials in PLA aids in improving its gas barrier properties, mechanical properties, thermal properties, and biocompatibility, which provides an extensive claim in many work areas. Furthermore, use of some PLA based composites in food packaging sectors includes: (1) PLA/Zinc oxide composite with antimicrobial properties, enhanced mechanical, and barrier properties (Marra et al., 2016) and (2) PLA/Graphene (Sandwiched architectured) composite with enhanced gas barrier properties, and reduced water vapour permeability (WVP) by 87.6 % (Goh et al., 2016).

PLA/CH Based Green Composites

CH molecules being hydrophilic in nature are not easy to incorporate as filler materials in PLA films. So grafting of CH with hydrophobic lactic acid oligomer (OLLA) molecules overcomes the shortcomings of CH for acting as a filler material in PLA films, where grafting is defined as a technique to make a segmented copolymer. In OLLA-*g*-CH, grafting causes OLLA (branch chain) polymers to link with CH molecule (acts as the backbone of polymer unit) through amide-ester bonds. Moreover, OLLA-*g*-CH acts as a surfactant molecule bearing a hydrophilic head (CH) and a hydrophobic tail (OLLA) and is synthesized by *in-situ* condensation polymerization. OLLA-*g*-CH, an effective bionanofiller, reinforced PLA films enhance the properties of PLA film in terms of gas barrier properties, mechanical properties,

and thermal properties (Pal, & Katiyar 2016). The biocomposite offered uniform dispersion of filler materials with size range ~20-150 nm, which results in enhanced thermal, mechanical, and gas barrier properties. The analysis of thermal properties of formulated bionanocomposite films can be done in terms of glass transition temperature (Tg), melting temperature ™, and heat capacity (Cp), where increased amount of filler loading results in a decrease of Tg and Cp values up to 18 °C and 0.4 J/g.K, respectively. In addition, plasticizing effect of OLLA-*g*-CH has an ability to decrease Tg around more than 10 ℃ of prepared film. In comparison with NPLA films, the ultimate tensile strength reduces by ~18%. Moreover, with an increase in filler loading, Young's modulus of prepared composite decreases from 542±133 MPa to 298±31 MPa. CH being an antimicrobial and antibacterial agent is used as an application for the preparation of antibacterial textile.

PLA/ GA Based Green Composites

GA is a polysaccharide unit mainly consist of a highly branched galactose unit linked with β-1, 3-glycoside molecule, which is either neutral or slightly acidic in nature. Blending of PLA with GA is possible irrespective of their hydrophobic-hydrophilic properties with improved properties. The hydrophilic nature of GA makes its unsuitable for reinforcing the material in PLA composite film, so grafting of GA with LA converts hydrophilic GA to hydrophobic grafting molecules (LA-*g*-GA), which acts as great filler materials in PLA films and forms a good dispersion in the matrix material (PLA) (Borkotoky et al., 2019). LA-*g*-GA (functionalized GA) can be prepared by a polycondensation reaction, which involves grafting of LA (microwave synthesized) with GA molecules. LA-*g*-GA molecules enhanced the properties of PLA film by enhancing its gas barrier properties, mechanical properties, and thermal properties (Tripathi, & Katiyar, 2016). Incorporation of the specified filler materials (functionalised GA) decreases the thermal stability of PLA/LA-g-GA in comparison to neat PLA films. The observed T_g values for PLA, PLA/LA-g-GA (3 % wt loading), PLA/LA-g-GA

(5 % wt loading), and PLA/LA-g-GA (10 % wt loading), are ~ 65, 46, 42, and 41 °C, respectively, which is found to decrease with increase in filler. The mechanical properties of developed film are generally analysed based on its tensile strength, elongation at break, and Young's modulus. The composite films have reduced tensile strength by 14 % and enhanced elongation at break by 16 % for 3 % filler material. In this context, the modified PLA based packaging can be used to develop secondary packaging.

1.3.10 Storage Study of Food Products

The shelf life analysis of food products is evaluated based on various properties such as physicochemical properties, respiration rates of fruits and vegetables, microbiological study, color properties, texture properties, etc. The fruit products with a fresh smell and having a proper size, consistency, and shape are more acceptable in comparison to other non-uniform and foul-smelling fruit products (which occurs due to environment degrading factors). In this regards, the food products should maintain the consistency, food property, and microbiologically safe for the entire product life. Additionally, the packaging materials in terms of primary and secondary packaging materials are used to carry the fruit products, where the packaging materials or food contact materials (FCMs) should be safe for food products. Additionally, the FCMs such as food manufacturing equipment, food packaging materials, food preparation/dining wares, and others are tested with different food simulants including acetic acid 3% v/v, ethanol 10% v/v, ethanol 50% v/v, vegetable oil, distilled water, and others to ensure safe consumption. Interestingly, the level of migration from FCMs to foodstuffs generally depends on several factors such as storage temperature, storage time, type of food products, type of packaging, etc., where, the migration of several components from the printing inks, varnishes, adhesives, inner bags, foodstuffs, and others may occur. In this regards, the fruits and vegetables, fish products, meat and meat products, dairy products, and others are analysed for various components to ensure safe storage and quality analysis. The several physicochemical properties of food products include the analysis of

moisture content, total dry matter, total soluble solids (TSS), weight loss analysis, and other properties (ash content, protein content, dietary fiber content, fat content, pH, titratable acidity (TA), etc.). The pH of fruit products is determined using a pH meter. The pH of fruit products generally increases during storage due to ripening. Further, the TSS of fruit products is determined using a portable digital refractometer. The TSS content of fruit products increases during storage due to ripening. Additionally, the weight loss analysis of fruit products is measured by calculating the initial (weight at 0 days) and final (weights at sampling day) weights of the food products using a weighing balance. The texture of food products is measured using a firmness meter. In this way, the different edible coated foods with and without secondary packaging are commonly analysed to check the effectiveness.

CHAPTER 2

Materials and Methods

This chapter discusses the details of materials, chemical reagents and experimental procedures for undergoing the current thesis work. In this chapter, the development of selected functionalized biopolymeric nanocomposites has been detailed for edible nanocoating application. The development of iron incorporated cellulose nanofibers using single step co-precipitation method has been discussed to be used as a reinforcement in preparing chitosan (Source: crab shells) based functionalized nanocomposites. The preparation of nanochitosan from chitosan (medium molecular weight) and sodium tripolyphosphate using ionic gelation method has been discussed to be used as a reinforcement in starch and guar gum based functionalized nanocomposites. The fabrication of silk nanodisc from waste muga cocoons is done following degumming and acid hydrolysis method subsequently to be used as a reinforcement in chitosan (medium molecular weight) based functionalized nanocomposites. The processing of functionalized biopolymeric nanocomposites through solution casting process has been discussed. The detailed discussion on using the functionalized biopolymeric nanocomposites to develop edible coating (a kind of primary packaging) on fruit products using dipped coating method has been discussed. The details of shelf life study of the coated fruit products have been discussed. Additionally, the development of poly lactic acid based blown films using industrially viable technique to be used as a secondary packaging has been discussed. The experimental procedures, used equipment, and instrumentations for characterizing the biopolymeric nanocomposites as primary packaging and blown films as secondary packaging has been discussed in this chapter.

2.1 Materials

Cellulose nanofibers (CNF) as 2 wt% water dispersion is obtained from Sugino Machine, Ltd. The used CNF has the Trade name BiNFi-s cellulose (WMa- 10002) with a fiber diameter of 20-50 nm. $FeCl_2$ (extra pure), and $FeCL_3$ (extra pure) are procured from Nacalai Tesque Inc., Kyoto, Japan. Ammonia solution (28-30 %) is procured from Chameleon reagent, Osaka, Japan. Curcumin (a mixture of curcumin, demothoxycurcumin and bisdemothoxycurcumin; 98 % pure) is supplied by ACROS ORGANICS, Japan. The standard buffer solutions at pH 4.01±0.02 and 9.18±0.02 are supplied by Nacalai Tesque Inc., Kyoto, Japan. For objective 1, the used chitosan (Crab shells, small flakes) and acetic acid (99.7% purity) are supplied by Nacalai Tesque Inc., Kyoto, Japan and FUJIFILM Wako Pure Chemical Corporation, respectively. For objective 1, NaOH (97% pure) is procured from Nacalai Tescue Inc., Kyoto, Japan. For in vitro studies, Dulbecco's modified eagle's medium (DMEM), penicillin streptomycin, and fetal bovine serum (FBS) are obtained from Gibco™, USA. Tetrazolium dye MTT (3-(4,5-dimethylthyazol-2-yl)-2,5-diphenyltetrazolim bromide), trypan blue stain, and dimethyl sulfoxide (DMSO) are procured from Sigma Aldrich. Acridine orange stain is obtained from Thermo Fisher Scientific. Potato starch (pure) is supplied by Sisco Research Laboratories Pvt. Ltd., India. Additionally, for objectives 2 to 4, the utilized chitosan (medium molecular weight, deacetylated chitin, poly (D-glucosamine)) and acetic acid glacial are supplied by Sigma-Aldrich (India) and Finar limited, respectively. Guar (gum guar) is procured from Sigma-Aldrich (India). The glycerol is obtained from Himedia Company (Mumbai India). Additionally, the plate count agar is obtained from HiMedia laboratories. For the fabrication of silk nanodisc (SND), the utilized waste muga cocoons (WMC) as a source has been procured from Regional Muga Silk Station, Assam, India. Further, sulphuric acid (>99%), and sodium carbonate (>99% purity) are supplied by SISCO research laboratories (Analytical grade, SRL Chemicals, India). Cellulose acetate membrane (cut of membrane weight ~ 14 kDa) used for

dialysis of acid hydrolyzed SND is supplied by Sigma Aldrich. For the investigation relating to shelf life analysis, the cut pineapple and whole kiwifruits are obtained from a Supermarket of Gifu City, Japan. For the experiment, banana and apple fruits are collected from a local vendor at Indian Institute of Technology Guwahati, Assam, India. Poly lactic acid, PLA (4032D Grade; Granulated form, comprises of L-lactic acid/D-lactic acid in the proportion of 98.6:1.4) is acquired from Nature Works® LLC., USA. The used gum Arabic (GA), dicumyl peroxide (DCP) and L-lactic acid (20% assay) for blown film preparation are procured from Sigma-Aldrich (India). Acetone, sodium hydroxide, and ethyl alcohol are obtained from Sisco Research Laboratories Pvt. Ltd., India. For gel permeation chromatography (GPC) analyses, the reagents such as chloroform (Merck, India) is used. For contact angle analysis, Millipore® water, glycerol from Merck, India, Diiodomethane from Sigma Aldrich, India are utilized. All the chemicals are utilized for the required analyses the way it is received without any additional refinement.

2.2 Experimental Procedure

2.2.1 Fabrication of Iron Functionalized Cellulose Nanofibers Through Co-precipitation Method

The synthesis of iron incorporated CNF (mgCNFs) is carried out following the co-precipitation method, where iron particles get adsorbed onto CNFs molecules (**Figure 2.1**). For the synthesis, CNF (4 g) is dispersed in 50 ml of deionized water *via* sonicating for 2 min (amplitude 30 %, benchtop sonicator). The prepared CNF solution is stirred for 4 h under N_2 atmosphere, where $FeCl_2$ and $FeCL_3$ (molar ratio of 0.0125 M and 0.025 M) are successively mixed to the solution at 1000 rpm, 90 °C and the pH of the prepared solution is maintained using 0.1 N NaOH in alkaline side. The next step included the dropwise addition of 2.5 ml of ammonia solution to the above solution at 85 °C, and is continuously stirred for 4 h to obtain

mgCNF. In this way, mgCNF is fabricated, which is separated using a permanent magnet, and further washed using ethanol and water thrice and freeze dried (EYELA FDU-1200, Japan). The freeze dried materials are grinded using a mortar and pestle which are used for further analysis.

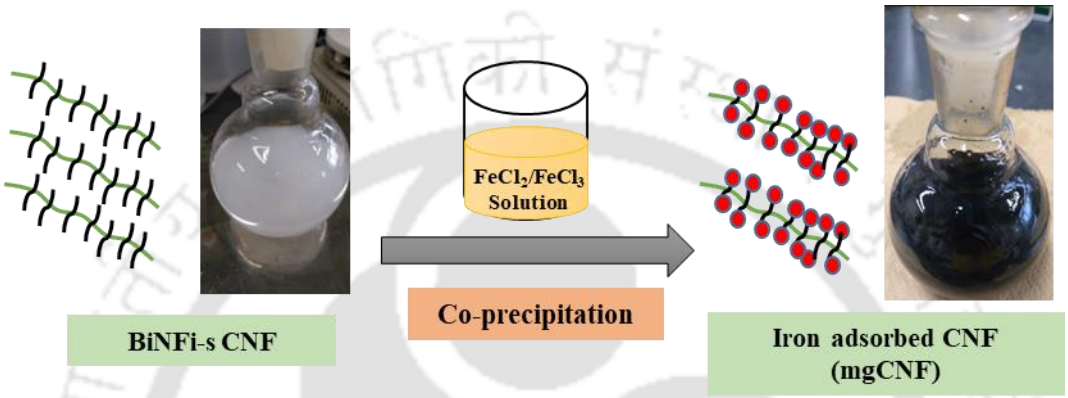


Figure 2.1 *Fabrication of Iron functionalized cellulose nanofiber via single step co-precipitation method.*

2.2.2 Fabrication of Sodium Tripolyphosphate Crosslinked Nanochitosan Through Ionic Gelation Method

Chitosan (CS) is subjected to controlled processing steps following the ionic gelation method for the fabrication of nanochitosan (NCS). Sodium tripolyphosphate (STPP) is used as a cross linking agent, where, polycation CS interacts with polyanion STPP (**Figure 2.2**). For NCS preparation, 1% w/v CS is prepared in 1% v/v acetic acid solution under stirring at 600 rpm for 3 h. After the time lapse, 0.09 wt% of STPP solution is dropped onto the above solution slowly under vigorous stirring followed by ultrasonication for 3 min. Further, the prepared solution is washed using absolute ethanol and deionized water to obtain purified NCS.

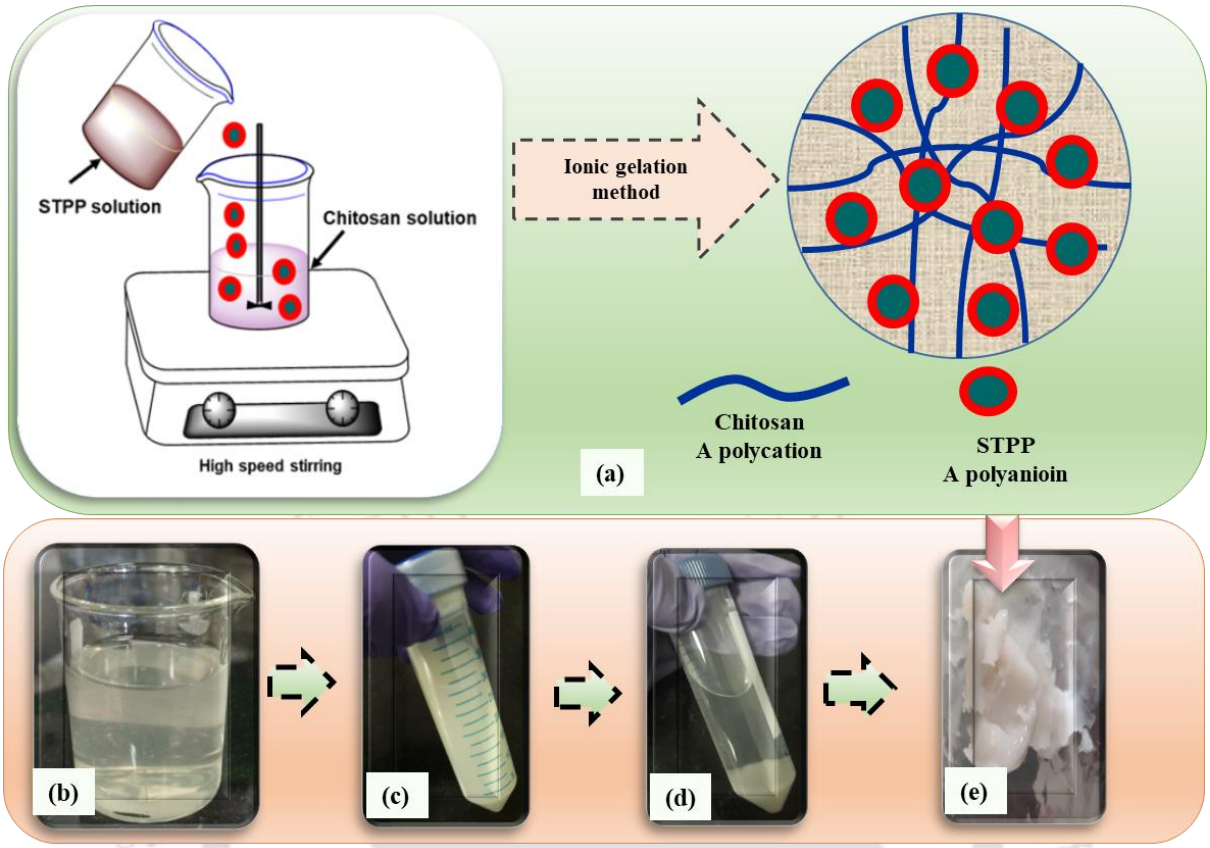


Figure 2.2 *Fabrication of Nanochitosan using Ionic Gelation method representing (a) Mechanism of action; (b) Chitosan solution in acetic acid; (c) Nanochitosan suspension after ultrasonication; (d) Nanochitosan suspension after centrifugation; (e) Nanochitosan suspension.*

2.2.3 Fabrication of Acid Functionalized Silk Nanodisc from Degummed Waste Muga Cocoons

In the current study, the WMC is designated as the source for silk fibroin (SF), which has the sericin protein as the outer layer being removed *via* degumming process. Further, the degummed silk (SF) is selected to undergo acid hydrolysis fabricating silk fibroin nanostructures (or SND) (**Figure 2.3**). For the fabrication, the collected WMC is made unblemished from plant waste matter, eggs, and other dirt materials. The cleaned WMC is degummed using 0.02 M sodium carbonate (in a boiling condition) for a period of 60 min to

remove the protective outer coat of sericin. Subsequently, the degummed fibroin is acid hydrolyzed using sulfuric acid (64 wt%) at 40 °C and 2 h to fabricate SND as mentioned. The acid hydrolysis of degummed fibroin is stopped using chilled deionized water, where the reaction mixture is kept in an ice bath to provide an utmost care to develop the nanomaterial. Afterwards, the SND suspension is centrifuged (10000 rpm for 20 min) and water washed in triplicates. The obtained centrifuged SND pellets are homogenized and sonicated to promote uniform dispersion and is further undergone dialysis to increase the pH (to ~ 7.0) *via* removing acidic ions from the suspension. Finally, the SND suspension is freeze dried and stored till further analysis.

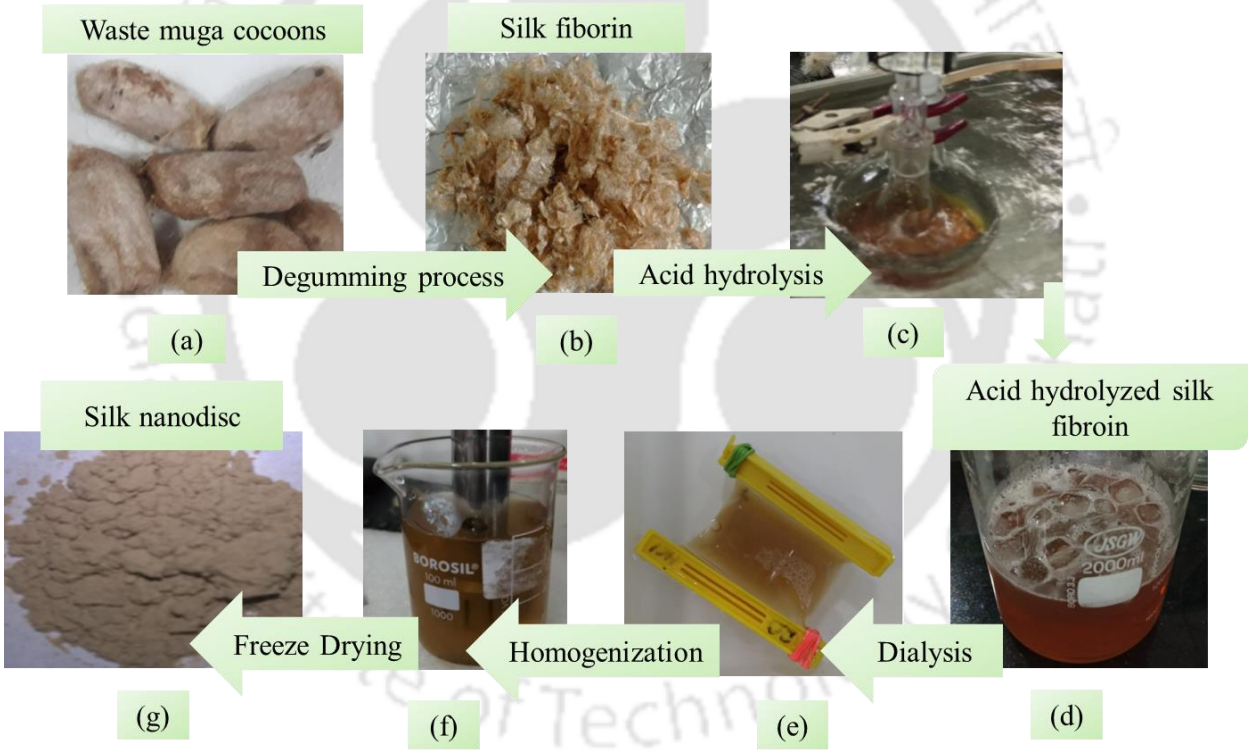


Figure 2.3 *Fabrication of silk nanodisc using acid hydrolysis method representing (a) Waste muga cocoons; (b) Silk fibroin (degummed silk); (c) Acid hydrolysis of silk fibroin; (d) Acid hydrolysed silk nanodisc; (e) Dialysis of acid hydrolysed silk nanodisc; (f) Homogenization of silk nanodisc; and (g) Freeze dried silk nanodisc.*

2.2.4 Fabrication of Curcumin Doped Iron Functionalized Cellulose Nanofibers Dispersed Chitosan Nanocomposite Based Edible Nanocoating

The preparation of coating materials is done *via* considering the various combination of targeted materials. A neat 1 % (w/v) CS solution in 1 % (v/v) acetic acid is developed by stirring at 1400 rpm for 2 h. The biocomposite based edible nanocoating preparation is done by dispersing 0.5, 1, 1.5 % (w/w) CNF and mgCNF nanofillers in CS solutions, where the solution of biofiller materials are sonicated for uniform mixing and then mixed with CS solution and stirred for another 3 h. The preparation of curcumin (Cur) loaded films has been done by mixing 0.05 % (w/v) of Cur prepared in 50% of ethanol (stock solution) followed by adding 20 mL of this solution to various combinations of CS-CNF and CS-mgCNF solution, and stirred for another 2 h. As Cur has a limited solubility in water, so ethanol is added to improve the solubility to obtain the transparent films. The above solution is then casted into films and dried for further analysis.

2.2.5 Fabrication of Nanochitosan Modified Starch and Guar Gum Nanocomposites Based Edible Nanocoating

For the development of potato starch (ST) based edible coating, potato ST in an aqueous solution containing glycerol (30 %w/w of ST) is subjected to stirring at 250 rpm under heating at 85 °C and is kept at that temperature for 10 min. Afterward, the above solution is being cooled to 60 °C followed by continuing stirring at 60 °C for 10 min. The prepared solution is solution cast in Teflon plates and is kept for drying at 40 °C. As demonstrated in **Table 2.1**, the ST-GG edible packaging is developed taking the mentioned proportions, where, the GG (guar gum) solution is subjected under vigorous stirring at 750 rpm and 25 °C for 2 h. The mixed solution of ST and GG is subjected to the processing conditions as detailed for developing ST films. Additionally, for preparing ST-GG-NCS1, ST-GG-NCS2, and ST-GG-

NCS3 based edible packaging, the NCS solution is developed in 0.2% acetic acid solution and is kept under vigorous stirring for 2 h at 800 rpm and 25 °C. Afterward, the solutions of GG and NCS are premixed with ST solutions followed by processing similar to ST solutions. Subsequently, the fabricated dried films are stored at 25 °C for 24 h for conditioning before further analysis.

Table 2.1 *Several proportions of chosen biopolymers for modified starch based edible coating preparations.*

Nomenclature	Starch (%w/v)	Guar Gum (%w/v)	Nanochitosan (%w/v)	Glycerol (%w/w of starch)
ST	2.5	--	---	30
ST-GG	2.5	0.4	--	30
ST-GG-NCS1	2.5	0.4	1	30
ST-GG-NCS2	2.5	0.4	2	30
ST-GG-NCS3	2.5	0.4	3	30

2.2.6 Fabrication of Silk Nanodisc Dispersed Chitosan Nanocomposites Based Edible Nanocoating

For developing SND dispersed CS based edible coating, both the materials are vacuum dried overnight to remove any moisture residue. The neat CS solution for film or coating preparation is done by dissolving 1% w/v of CS in 1% v/v acetic acid solution for 5 h at 1400 rpm. The SND incorporated CS based edible coatings has been fabricated using three different proportions (0.5, 1.0, and 1.5 wt%) of nanofillller and designated as CS-SND0.5, CS-SND1.0, and CS-SND1.5, respectively. Additionally, the biocomposite based edible coating have been developed by taking 0.5, 1.0 and 1.5 wt% freeze-dried SND nanomaterials. The

SND solution has been prepared *via* ultrasonication for uniform dispersion. Further, the different proportion of SND solution has been mixed to previously prepared CS solution (stirred for 2 h) and stirred further for 3 h. To avoid the bubble formations in edible coating materials, the formulated solutions are kept at stable condition before solution casting on petriplates. After the solution has become stable, a specific amount of solution is casted onto the petriplates followed by hot air drying at 45 °C for overnight. Afterwards, the dried films are peeled and stored in a dried condition till further analysis.

2.2.7 Development of Edible Nanocoating on Fruit products

Curcumin Doped Functionalized Cellulose Nanofiber Dispersed Chitosan based Edible Nanocoating on Cut Pineapple and Whole Kiwifruits

To prove the effectiveness of the edible coating materials, the developed mgCNF dispersed CS based coating materials with and without Cur are applied on cut pineapple and whole kiwifruit products. Before developing edible coating materials, uniform and non-defected kiwifruits are selected and washed by distilled water to remove any dirt from the surface. Many preliminary experiments are conducted to check the uniformity and coating efficiency of the coating materials on various fruit products through visual appearance. After that, kiwifruits are dipped into developed coating solutions for 2 min and are kept outside to drain excess material solutions. Then, a second coating layer is developed by dipping into the same coating solutions and dried in air for 15 min. Further, edible coating is developed on cut pineapple following similar route. The coated kiwifruits and cut pineapple are stored in an incubator (MIR-154-PJ, PHC Co., Ltd., Tokyo, Japan) at 10 °C for 10 days, and several quality parameters are measured to evaluate the effectivity of tested edible coating on fruits preservation.

Nanochitosan Modified Starch and Guar Gum Based Edible Nanocoating on Cut Apple Fruits

The effectivity of the fabricated edible coatings such as ST, ST-GG, ST-GG-NCS1, ST-GG-NCS2, and ST-GG-NCS3 are tested on fresh cut apple fruits and quality parameters are evaluated during storage life. The collected apple fruits are washed with hot water at 50 °C for 10 min and are kept at open environment for surface drying. The apples are then cut in dice shaped and taken for developing edible coating. The development of coating solution is done following three steps: (1) Dipping of cut apple fruits in the prepared solution for 5 min followed by drying for a time period of 5 min; (2) Dipping the coated cut apple fruits in the solution for another 5 min and air dried for 15 min; and (3) Storage of the edible coated cut apple at 25 °C for 7 days. The collected apple fruits are tested for different quality parameters such as weight loss at different time lapse, color properties, microbiological test, pH measurement, and others.

Silk Nanodisc Dispersed Chitosan Based Edible Nanocoating on Banana Fruits

The developed SND dispersed CS based edible coatings has been applied on whole banana fruits to prove the effectivity in improved quality of selected fruits during storage life. To develop uniform coating materials on the banana fruits, different preliminary experiments has been executed. The collected banana fruits are washed with hot water at 50 °C for 10 min and kept at open environment for surface drying. The development of coating solution is done following three steps: (1) Dipping of banana fruits in the prepared solution for 5 min followed by drying for a time period of 5 min; (2) Dipping the coated banana fruits in the solution for another 5 min and air dried for 15 min; (3) Storage of the edible coated banana at 25 °C for 7 days.

2.2.8 Fabrication of Oligomer Grafted Chitosan and Oligomer Grafted Gum Arabic Through Microwave Synthesis

The synthesis of MCS or oligomer grafted CS, is carried out following the method as described by Pal and Katiyar, 2016, which is being utilized as one of the reinforcements for developing composite blown films in the present study. For the preparation of MCS, the mixture of samples (CS:LA in the ratio of 1:3.33) are soaked for 12 h and synthesized using microwave under N_2 atmosphere. For better productivity and higher yield of MCS, the reaction is done through 'convection cum microwave mode' at 110 °C for 30 min at 240 W as reported earlier. Subsequently, the functionalized GA (MGA) is synthesized using previously reported method by Tripathi and Katiyar, 2016, being used as nanofillers in PLA blown film. For synthesizing MGA, the samples LA:GA in the ratio of 5:1 is mixed prior to synthesis and synthesized at 130 °C for 45 min at 240 W under 'convection cum microwave mode' in N_2 atmosphere.

2.2.9 Fabrication of Blown Films Based on Poly lactic Acid/Oligomer Grafted Chitosan and Oligomer Grafted Gum Arabic Nanocomposite

Blown films of PLA and its biocomposites are developed by considering PLA with different grafted as well as neat biopolymers such as GA, CS, MCS, MGA and additionally targeted for its influence on packaging property. Further, DCP, a radical initiator (0.5 wt % in acetone) is dissolved and sprayed on PLA granules, which is further dried at 40 °C to remove solvents. To this mixture of DCP coated PLA, functionalized CS (0.5 wt%) and functionalized GA (0.5 wt%) are mixed in separate batches and further dried to remove solvent residues. For the present study, neat PLA (NPLA), PLA with 1 wt % CS (CS-PLA), PLA with 1 wt % GA (GA-PLA), PLA with 1 wt % MCS (MCS-PLA), PLA with 1 wt % MGA (MGA-PLA), DCP coated PLA with 0.5 wt % MCS (DMCS-PLA) and DCP coated PLA with 0.5 wt % MGA

(DMGA-PLA) are taken for developing the blown films. The development of blown film is conducted using laboratory blown film extruder (Neoplast, Ahmedabad, India) having die diameter of 5 cm. The optimized temperature profiles for tool zone, adaptor zone, compression zone, metering zone, and feed zone are fixed at 185 °C, 180 °C, 180 °C, 175 °C, and 177 °C, respectively.

2.3 Analytical Instrumentations and Characterizations

2.3.1 X-ray Diffraction (XRD)

The XRD analysis of all the testing specimens is conducted using X-ray diffractometer (SmartLab9kW, Rigaku Corp. Yamanashi, Japan), where a Cu K_α radiation source generates X-rays (wavelength of 1.54 Å). Further, for the analysis, the 0.02° sampling step angle and 20° min^{-1} of scan rate are selected for a range of 2θ = 10 to 40 ° and 2θ = 10 to 80. The crystallinity index (C. I.) and degree of crystallinity of the samples are measured using the following equation.

$$C.I. = \frac{S_{crystalline}}{S_{Crystalline} + S_{Amorphous}} \quad \text{..... (2.1)}$$

Where, $S_{crystalline}$ and $S_{amorphous}$ are defined as area under crystalline and amorphous peaks, respectively.

2.3.2 Raman Spectroscopy

Raman spectroscopy analysis is recorded using Horiba Jobin Vyon (LabRam HR, Japan) connected with a 1-W, 1064 nm Nd: YAG diode-pumped laser.

2.3.3 X-ray Photoelectron Spectroscopy (XPS)

XPS is performed using Thermo Scientific Escalab XI+ XPS spectrometer to analyze CNF, mgCNF, CS, CS-CNF0.5, and CS-mgCNF0.5 samples. The detailed condition of the

performed experiment is (i) X-Ray source: Al K-Alpha X-Ray, (ii) Energy: 1486 eV, (iii) Angle between analyzer and sample surface: 90 ° and (iv) Charge neutralization: Dual beam charge neutralization (both electrons and ions).

2.3.4 Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectroscopy of developed iron functionalized CNF dispersed CS based edible nanocoating and PLA based blown films are carried out by FTIR spectrometer (IR Affinity-1, Shimazdu, Japan) with attenuated total reflectance (ATR) under dry air at room temperature. All the samples for analysis are dried at 80 °C for 2 h before analysis. The software used for the analysis is IRsolution and the spectrum is taken in a transmittance mode. The analysis is carried out by placing the small piece of each film on ATR crystal surface made up of zinc selenide for the entire wavenumber range from 4000 to 700 cm^{-1} with 20 consecutive scans at a 2.0 cm^{-1} resolutions. Additionally, the starch/guar gum/ nanochitosan based edible coating and chitosan/silk nanodisc based edible coating are carried out using FTIR spectrometer (Make: PerkinElmer, Singapore).

2.3.5 Gel Permeation Chromatography (GPC)

With the aid of GPC, the weight average molecular weight (M_w), number average molecular weight (M_n), and polydispersity index (PDI) of developed films are determined using refractive index detector (RID-10A), taking polystyrene for calibration purpose. For the analysis, the selected eluent flow rate and sample injection volumes are 1 mL/min and 40 mL, respectively. The films (30 mg) are dissolved in 1 mL of chloroform (HPLC grade) and prior to analysis, the solution is filtered through 0.45 μm filters.

2.3.6 Inductively Coupled Plasma Mass Spectrometry

The quantification of the iron element in the Cur loaded eatable packaging is done using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies 7800 ICP-MS,

USA) at 1.5 L min^{-1} of auxiliary gas flow rate; 18.0 L min^{-1} of plasma gas flow rate and 0.87 L min^{-1} of sample gas flow rate. For the analysis, the sample digestion is done using an Advance microwave digestion system (ETHOS EASY, USA). For the sample digestion process, a mixture of acid solutions is used consisting HNO_3 and H_2SO_4 in a ratio of 80:20. Further, 200 µL of the digested sample is diluted using deionized water and loaded in the autosampler (Agilent Technologies, SPS4 Autosampler, USA) for autosampling which is placed in a sample loading area. Further, the testing samples are subjected to a nebulizer for the ICP-MS analysis, where the samples form fine aerosol by mixing with argon gas.

2.3.7 Field Emission Transmission Electron Microscope

The FETEM micrographs of developed materials have been evaluated utilizing field emission transmission electron microscopy (2100F, JEOL, USA). The specimens are diluted in Millipore water followed by sonication if required to break agglomerates of the diluted suspension to avoid agglomerations. Afterward, on carbon-coated TEM grids, drop casting of the dilute suspensions is done followed by vacuum drying.

2.3.8 Field Emission Scanning Electron Microscope

The surface morphological analysis of developed edible coatings has been obtained *via* FESEM (Sigma, Zeiss™, Germany). For the analysis of the nanofiller materials, the freeze dried samples are sticked onto clean aluminum stubs using double sided carbon tape. The FESEM analysis of biocomposite based edible coating materials are done by vacuum drying overnight to remove any solvent residue. The presence of water residue generally creates problem in sample analysis. Further, the vacuum dried samples is directly fixed onto aluminum stub followed by gold coating. For developing the gold coating, a sputter coater (SC7620, Quorum) is used.

2.3.9 Energy Dispersive X-Ray Spectroscopy

To confirm the safety of the fabricated materials, the compositional analysis is evaluated using energy dispersive X-ray (EDX) spectroscopy (Oxford Instruments, UK).

2.3.10 Differential Scanning Calorimetry

The thermal properties of blown films of PLA biocomposites are studied using DSC (Model: Netzsch DSC Phonix, Germany) in the flow of the inert gas flow condition (50 mL/min). For the analysis, an amount of approximately ~8 mg samples is used and run for two thermal cycles. The first cycle is consisted of 30 °C to 200 °C at a heating rate of 10 °C/min and kept at that temperature for 2 min. After the first heating cycle, the samples are heated again to 200 °C following the above-stated heating rate. The measurement of the second heating cycle is noted after removing the history of the first heating cycle.

2.3.11 Thermogravimetric Analysis

The thermal properties of the developed materials are carried out using thermogravimetric analyzer (TGA-4000, PerkinElmer, USA) under a continuous nitrogen flow (50 ml/min). For the analysis, 5 to 10 mg of samples are placed in an aluminum crucible for a temperature range of 30 to 700 °C with a heating rate of 10 °C/min. The nitrogen flow is continuously introduced into the crucible to maintain inert atmosphere, which allow sample decomposition due to set temperature avoiding other reactions.

2.3.12 Thermogravimetric Analysis hyphenated Fourier Transform Infrared Spectroscopy

TGA (TGA-4000, PerkinElmer, USA) hyphenated FTIR (IR Affinity-1, Shimadzu, Japan) (TGA-FTIR) study of CNF and mgCNF is done using thermogravimetric analysis which is

equipped with FTIR spectrophotometer. FTIR helps to elucidate the gas evolved during thermal degradation of samples within the range of 4000 cm^{-1} to 450 cm^{-1}.

2.3.13 Ultraviolet- Visible Spectroscopy

For all the developed film materials, the transparency of developed nanocomposite films having dimensions of 20 mm × 50 mm are assessed by means of UV-Vis spectrophotometer (PerkinElmer, USA) within the wavelength of 800-200 cm^{-1}. For objective 1**,** the optical property of the nanocoating is measured using UV-Vis spectrometer (V-550, JASCO Corporation, Japan). The developed coating materials of 40 mm × 80 mm dimensions with a thickness of 15 to 40 μm are analyzed for their transparency within 700-200 cm^{-1} wave length range.

2.3.14 Universal Testing Machine

The mechanical properties of developed coating materials are determined using 5 kN electromechanical Universal Testing Machine (Zwick Roell: Z005TN) in tensile mode. Further, for objective 1, the mechanical properties of coating materials are measured using universal testing machine Autograph AGS-X 1kN (Shimadzu Corporation, Japan), which is equipped with 500 N load cell at a constant cross-head speed of 50 mm/min in tensile mode. The mechanical properties of developed blown films such as elongation at break (% E) and ultimate tensile strength are studied using the universal testing machine (KIC-2-050-C, Kalpak instruments, and controls, India), which is equipped with 500 N load cell at the constant cross-head speed of 50 mm/min in tensile mode. The preparation of the test samples for the analysis is conducted according to the ASTM D-882 protocol, which follow the dimensions of 100 mm length, 25.4 mm width and 50 mm gauge length.

2.3.15 Dynamic Mechanical Analyzer

The thermomechanical properties of the developed films are analyzed using a dynamic mechanical analyzer (DMA242, Netzsch, Germany) under tensile mode in the temperature range of 25-90 °C and heating rate of 2 °C/min (Dhar et al., 2016). The samples for analysis have dimensions of 5 mm × 5 mm × 0.1 mm, which is treated under a dynamic force of 2 N and an amplitude of 20 μm at 1 Hz frequency.

2.3.16 Tear Testing

The Elmendorf type tear resistance tests of developed films are performed through Tearing Resistance Tester (Al-1004, Angle Instruments) (Briassoulis, & Giannoulis, 2018). The analysis follows ASTM D1922, ISO 6383-2 and provides measuring force range of 0-16000 mN at 50 Hz power supply. The used specimen size is 62.0 mm ± 0.2 mm long, 50 mm± 2 mm wide, where smaller size remains perpendicular to the machine direction. For, the transverse direction, the longer side is perpendicular to the machine directions. The tearing resistance (Tres, mN), tearing Index (Tind, Nm^2/Kg), and tearing Factor (Tfact) are measured using Equations (2.2), (2.3), and (2.4) as stated below.

$$\text{Tres, } a = s \times p \quad \text{(2.2)}$$

$$\text{Tind, } x = a/w \quad \text{(2.3)}$$

$$\text{Tfact, } y = x \times 10.2 \quad \text{(2.4)}$$

Where, s, p, and w are main scale reading in the direction tested, pendulum factor, GSM (g/m^2), respectively.

2.3.17 Contact Angle

The wettability test of the developed films is accomplished using Kruss GmbH DSA25 (Germany). Small pieces of films are sticked onto glass slides using double-sided tape and

taken for wettability analysis using three types of solvents such as millipore water, glycerol, and diiodomethane (Kalita et al., 2019). For wettability analysis of developed films in contact with liquid samples are conducted by dropping 2 μL of selected liquid samples on the film surface using the syringe. The selection of syringe type varies according to the viscosity of the liquid that is taken for wettability analysis of developed films. The formed contact angle is captured by a video camera and an image analysis system is used to calculate the contact angle between the polymer composite surface and liquid taken.

2.3.18 Water Vapour Transmission Rate

The fabricated ST based biocomposite samples are analysed to measure the water vapour transmission rate (WVTR) using the cup method following ASTM standard E398-03 (Ruggeri et al., 2020). The anhydrous calcium chloride filled (0% relative humidity) cups are sealed using the sample specimens for the WVTR test. Before the analysis, the sample thickness and area are measured. The permeation cells with sample specimen are placed in a dissector maintained at 75% RH using saturated sodium chloride solutions. The increase in weight of the permeation cell is measured and water vapour transmission rate is measured.

2.3.19 Color Properties

The color coordinates and % reflectance of the developed film materials are measured using Datacolor Technology, Suzhou Co. Ltd., China (Datacolor 550) in terms of L, a* and b* values, where the subsequent terms give an overview on brightness to darkness values, red to green coloration effect and yellow to blue coloration effect, respectively (Borkotoky et al., 2019). The color values L of 0 values are perfect black, whereas, 100 value are perfect white. Further, the color values a* of positive values are red and negative values provide green coloration effect, respectively. The color values b* defines yellow and blue coloration effect for positive and negative values, respectively.

Additionally, the hue and chroma values of developed materials were determined using the equation (2.5) and (2.6).

$$Hue = tan^{-}\left(\frac{b}{a}\right) \quad \text{..... (2.5)}$$

$$Chroma = \sqrt{a^2 + b^2} \quad \text{..... (2.6)}$$

2.3.20 Optical Polarimetry

The specific and optical rotation of the developed PLA and its composite films are measured using AUTOPOL II (Rudolph Research Laboratory, USA) for a wavelength at 589 nm (Dhar et al., 2017). A self-calibrated mechanism is followed for the analysis, and an amount of 20 mg of the films is dissolved in 20 mL chloroform and the solution is taken for analysis.

2.3.21 Vibrating Sample Magnetometer

The magnetic properties of the iron incorporated samples are analysed using vibrating sample magnetometer (VSM) (Lakeshore, Model: 7410 series) at room temperature. For the analysis, ~10 mg of powdered samples are kept in Teflon tape and wrapped properly using the tape to be kept in the sample holder of VSM (Dhar, Kumar & Katiyar, 2016). Further, the developed edible coating materials are prepared in the dimensions of 10 mm× 5mm and placed onto sample holder for the analysis.

2.4 Films Property Measurement and Calculations

2.4.1 Antimicrobial property

The antimicrobial property of NCS and ST biocomposite based edible coating materials is evaluated using disc diffusion approach (Abdul Qadir et al., 2017). For the investigation, *Escherichia coli* MTCC 723 and *Staphylococcus aureus* ATCC 6538 has been taken as the

model food borne bacteria and are obtained from American type culture collection (ATCC) and Microbial type culture collection (MTCC), respectively. For the antimicrobial test, 200 µL of the microbial suspension is spread onto the nutrient agar and the solutions of 10 mg/mL are added onto the plates. Afterward, the plates are incubated at 37 °C for 24 h and the zone of inhibitions is measured in triplicates and reported.

2.4.2 In vitro Studies

The *in vitro* studies of developed materials are carried out to determine the anticancer activity of Cur (Patwa et al., 2018). HeLa (cervical cancer) cells are cultured and maintained in T25 flasks using DMEM supplemented with 1 % penicillin streptomycin and 10 % FBS in CO_2 incubator at 37 °C and 5 % CO_2. The fabricated biopolymer composites are cut into circular discs and placed in 96 well plates followed by the sterilization under UV irradiation. The cells are counted using trypan blue stain by 1:1 dilution and 1.98×10^4 cells (counted using Countess™ II FL) are seeded onto the samples along with control (polystyrene) and blank using 100 µl DMEM. Further, the tetrazolium dye MTT is used to determine the mitochondrial activity of HeLa cells at the interval of 8 h. The MTT reagent is made by dissolving 5 mg/ml of phosphate buffer saline and is added to each well (including control and blank) after 8 h followed by 3 h of incubation. This is followed by the removal of the media along with the MTT solution. Further, 100 µl DMSO is added to each well and aspirated to dissolve the formosan crystals. The 96 well plate is then placed in the 96 well plate reader to record the absorbance at 570 nm. Also, the developed CS based composites along with control is stained with acridine orange (10 mg/ ml stock solution) after dilution. The media is removed from the wells after 8 h of incubation followed by rinsing thrice with phosphate buffer saline. The acridine orange stain (after dilution) is added to the wells and is kept under incubation for 30 min followed by imaging the cells under Floid Imaging Station (Thermo Fischer Scientific, USA) at 20x resolution.

2.4.3 Gel Swelling Study

Gel swelling property of developed coating materials is measured by dropping the dried coating materials in a solution of pH 4.01, 7.00, and 9.18 for 8 h and the coating materials after subsequent time are taken out, washed with Millipore water, surface dried with blotting paper and then weighted using weighing balance (Mettler AE100, Japan) (Eroğlu, Sargon, & Öner, 2007). The time for swelling behavior is adjusted after many tests, as after the subsequent time some of the coating materials get ruptured due to obtaining the saturation in absorbing subsequent solvent, so the swelling time is fixed to 8 h.

2.4.4 Physicochemical Property

The moisture content of edible coating materials is determined by drying the testing samples at 105 °C for 24 h (Garcia-Amezquita et al., 2018; Costa et al., 2018). Further, the weight loss percentage of the dried edible coatings is calculated which designate the amount of moisture loss and further, are represented as percentage water removed. Additionally, the water solubility of the edible coating materials is determined by immersing the dried testing samples in water (50 mL) and kept under agitation for 24 h at 20 °C (Costa et al., 2018). Afterwards, the insoluble films are dried at 105 °C till constant weight has been achieved and weighted to measure the dry matter weight.

2.4.5 Blow Up Ratio and Inhomogeneity Index

The film structure and stability of blown formation are studied by measuring blow up ratio (BUR) and inhomogeneity index as described in Equation (2.7) and (2.8), respectively (Karkhanis et al., 2017; Sirisinha, & Somboon, 2012). The measurement of bubble diameter and thickness of the films are taken at 20 different locations for obtaining the accuracy in measuring BUR and inhomogeneity index of developed films.

$$\text{Blow Up Ratio (BUR)} = \frac{\text{Diameter of blown film bubbles}}{\text{Diameter of die head extruder}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.7)$$

$$\text{Inhomogeneity Index} = \frac{\text{Standard deviation of blown film thickness}}{\text{Average thickness of blown films}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2.8)$$

2.4.6 Migration Test

Overall migration tests are evaluated for the developed films having an area equivalent to 1 dm^2 of contact area per 100 mL of selected simulants such as simulant 1: 50 % ethanol (v/v), simulant 2: 3% acetic acid (v/v), and simulant 3: distilled water. The migration tests are done according to the rules of Commission Regulation EU No. 10/2011 (Dhar et al., 2017). For the analysis, rectangular strips of specified sizes are dipped in selected food simulants, which are kept in a controlled atmosphere at 40 °C for 10 days, and the rectangular films are taken out after the specific time periods and the simulants are dried and then weighted to measure the residue weights.

2.5 Shelf life Analysis

2.5.1 Respiration Rate

The changes in the headspace gas composition in terms of oxygen and carbon dioxide in the chamber (10 mm thick acrylic cylinders, 4.8 L) is analyzed using a Checkmate 3 gas analyzer **(**PBI Dansensor Checkmate3**)** **(**Ghosh, & Dash, 2018; Ghosh, & Dash, 2020**)**. Approximately, 700 g of precisely weighted kiwifruits samples are put in an airtight acrylic chamber (2 L in volume) having a gas sampling port consisting in a rubber septum at the rid. Further, for cut pineapples, a specific amount of weighted cut pineapple is kept in the chamber and the chamber has a sampling port, where, a syringe is inserted into the chamber through a septum at regular intervals of time. The headspace gas of the chamber in terms of O_2 and CO_2 are measured. Additionally, the chamber consisting the cut pineapple (control and edible

coated) and kiwifruits are kept at 10 °C. After observations, the respiration rates in terms of O_2 and CO_2 are calculated using the below equations (2.9) and (2.10).

$$RR_{o_2} = \frac{(\Delta C_{O_2}) \times V_f}{100 \times W \times \Delta t} \quad \text{..... (2.9)}$$

$$RR_{Co_2} = \frac{(\Delta C_{CO_2}) \times V_f}{100 \times W \times \Delta t} \quad \text{..... (2.10)}$$

Where, RR_{o_2} and RR_{Co_2} are the respiration rates in terms of O_2 and CO_2, respectively; ΔC_{O_2} and ΔC_{CO_2} are the difference in initial and final gaseous concentrations for O_2 and CO_2, respectively; W is the weight of sample taken for the analysis; V_f is the free headspace volume of the chamber (mL); Δt is the storage period between two consecutive observations.

2.5.2 Firmness Testing

To record the firmness of the stored coated and uncoated banana fruits, the testing is executed by conducting a penetration test using TA. XT Plus (Stable Micro Systems, UK), where a cylinder probe (P/5 5 mm diameter) is inserted into the fruit (Hao et al., 2019). The testing conditions for the tests are (i) Test Speed: 2 mm/sec and (ii) Distance: 5 mm, and (iii) Software: Exponent connect. The responses of the penetration test are recorded as force vs. time curves, where, the firmness of the fruits is recorded as the required maximum force. For objective 1, the firmness of the coated and uncoated fruit products during storage is measured using FUJIWARA Fruits Hardness Tester (KM-1). For the firmness measurement, cylindrical shaped probe is selected to press the fruit for measuring the firmness during the storage life of kiwifruit products.

2.5.3 Microbiological Analysis

The microbiological analysis of the stored coated fruit products is done in terms of mesophilic count. For the mesophilic count of the stored samples, 10 g of testing samples

is subjected to homogenization in sterilized water (90 mL) using a stomacher (Bag Mixer, Interscience, France) (Tomadoni, et al., 2018). Further, the serial decimal dilutions are prepared using the filtered homogenate of the above mentioned samples and 1 mL of this prepared testing specimens are pour plated on plate count agar (PCA) and stored at 35 °C for 2 d. After the incubation period, the colony counts are observed and reported.

2.5.4 Color Parameters

The color coordinates of the stored cut fruits are measured to evaluate the effectiveness of the coating on the stored cut fruits.

2.5.5 Total Soluble Solids

The total soluble solids (TSS) are measured using digital refractometer (PR-101α, Atago Co. Ltd., Tokyo, Japan).

2.5.6 Original Weight Loss

The weight loss analysis of fruit products is determined by measuring weight, where the weight of the coated fruit products is measured on alternative days using a weighing balance. Additionally, the fraction of original weight retained is measured by taking weight at a subsequent time lapse.

2.5.7 pH Measurement

The pH of the stored fruit products is measured using pH meter (EUTECH INSTRUMENTS).

2.6 Statistical Analysis

For shelf life analysis, the results are expressed as average values with standard deviations. Additionally, one-way analysis of variance (ANOVA) and Tukey's test are performed to test the significance of the mean values at $p<0.05$.

CHAPTER 3

Curcumin Doped Non-Toxic Magnetic Cellulose Nanofibers Dispersed Chitosan based Edible Nanocoating

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Motivation

The strategical modifications of cellulose nanofibers (CNF) to be used as edible coatings are growing interest for reduced food waste, and for improving food value. In this regards, the abundantly available surface hydroxyl groups on CNF provide an opportunity to tailor the property of CNF to be used as an edible coating. Thus, a focus has been drawn in developing edible coating materials via chemical modification of CNFs in conjugation with iron oxides delivering magnetic CNF (mgCNF). Iron is an essential nutrient for health, and can be delivered through edible nanocoating. In this regards, use of mgCNF provides a novel way to develop iron-fortified food, and can act as a carrier for active compounds.

Parts of this research work has received the scientific recognition as follows:

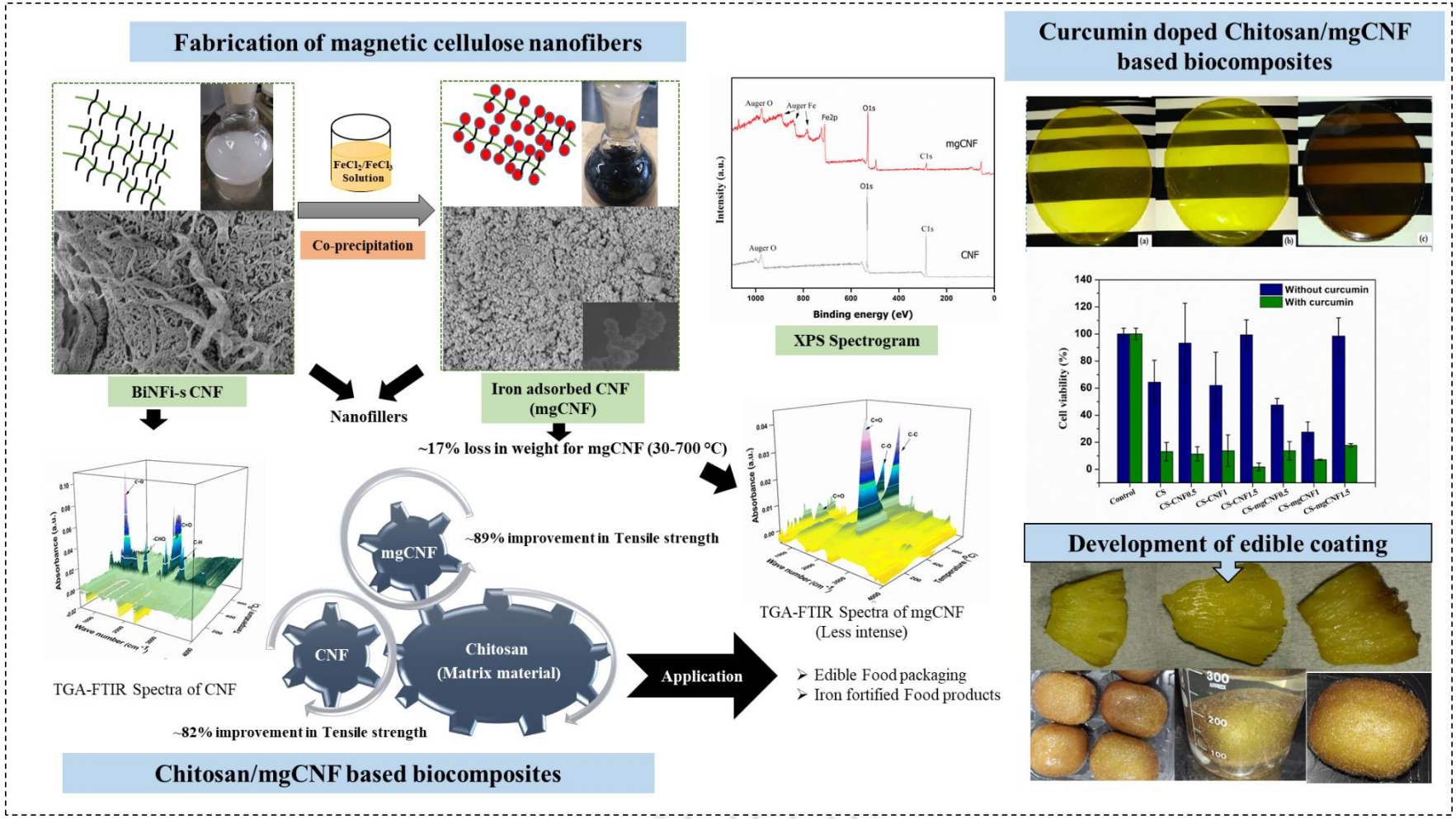
1. **Ghosh, T.,** Teramoto, Y., & Katiyar, V. (2019). Influence of Non-Toxic Magnetic Cellulose Nanofibers on Chitosan based Edible Nanocoating: A Candidate for Improved Mechanical, Thermal, Optical, and Texture Properties. ACS Journal of agricultural and food chemistry, 67(15), 4289-4299.
2. **Ghosh, T.,** Nakano, K., Mulchandani, N., & Katiyar, V. (2020). Curcumin loaded iron functionalized biopolymeric nanofibre reinforced edible nanocoatings for improved shelf life of cut pineapples. Food Packaging and Shelf life (Accepted).
3. **Ghosh, T.,** Nakano, K., & Katiyar, V. (2020). Curcumin doped Functionalized Cellulose Nanofibers based Edible Chitosan Coating on Kiwifruits. Carbohydrate Polymers (Under Revision).

Abstract

The present study demonstrates the formulation of curcumin (Cur) doped cellulose nanofiber (CNF) or magnetic cellulose nanofiber (mgCNF) dispersed chitosan (CS) based edible nanocoating with superior mechanical, thermal, optical, texture and other properties for targeted perishable fruit products. Fabrication of mgCNF is successfully achieved through a single-step co-precipitation route, where iron particles get adsorbed onto CNF as evident by FESEM, FETEM, XRD, Raman spectroscopy and XPS analysis. The thermal stability of mgCNF is improved considerably, where ~17% reduction in weight is observed, whereas CNF degrades completely under identical conditions. Further, for developed mgCNF/CS biocomposites, TGA analysis shows that there is an improvement in thermal stability in both CNF and mgCNF reinforced CS nanocoatings, where mgCNF/CS provides more heat dimensional stability than CNF/CS nanocoatings. Further, the edible nanocoatings are stable even at the temperature of heat treatment such as food sterilization. The mechanical property of mgCNF dispersed CS shows remarkable improvement in tensile strength (57.86±14 MPa) and Young's modulus (2348.52±276 MPa) compared to neat CS (6.27±0.7 MPa and 462.36±64 MPa, respectively). Further, for Cur doped CS biocomposites, it is worth mentioning that the presence of Cur with CS based biocomposites exhibited anti-cancer activity by disrupting the cell membrane of HeLa (cervical cancer) cells with cell viability of ~17 % and ~98 % for CS/mgCNF (1.5 % (w/w)) with and without Cur, respectively. The thermal properties in terms of onset degradation temperatures (10 % weight reduction) for Cur loaded CS, CS/CNF (1 % (w/w)) and CS/mgCNF (1 % (w/w)) are 78.6, 90.8, and 82.3 °C, respectively, which provides an additional approach to use these materials for heat unstable food items. The weight percentage of various elements such as carbon, oxygen, iron, and nitrogen is found to be 54.4, 38.4, 5.2, and 1.9 % (w/w), respectively, in the developed Cur loaded CS/mgCNF (1.5 % (w/w)) based materials (obtained by EDX analysis). The mgCNF (1.5 wt%) dispersed

CS edible coating assisted by curcumin provides a lamellar and heterogonous surface morphology with a hazy appearance. To recognize the developed materials as safe as food, the quantification of iron is made by using ICP-MS technique. It is noteworthy to mention that Cur loaded mgCNF coated CS help in improving texture of cut pineapples in comparison with uncoated pineapple slices. The work provides value addition to the perishable cut pineapple fruit in form of eatable packaging materials with the iron content of ~3.6 ppm, which is within permissible limit according to WHO guidelines. Therefore, the present investigation provides a novel approach towards supplementing the iron functionalized food products with anti-cancer activity and improved performance to reduce food waste. Additionally, the inclusion of mgCNF and Cur as a component of edible coating can provide a synergistic effect in maintaining the quality of kiwifruits. The used edible coating materials are effective in reducing mass loss, firmness loss, respiration rate, and microbial count of whole kiwifruits and cut pineapple during storage life (10 days at 10 °C). Additionally, color, and physiological properties of kiwifruits and cut pineapple can be tailored by using the addressed edible coating materials.

Scheme of the Chapter



3.1 Introduction

Cellulose nanofibers (CNFs) are systematically investigated biomaterial for its unique features of higher aspect ratio, better mechanical properties, more surface property, etc. in various fields of life including biomedical, and food packaging (Cherian et al., 2011; Ghaderi et al., 2014). Interestingly, the presence of hydroxyl groups onto the surface of cellulose acts as a reducing and nucleating agent for metal nanoparticles (NPs) such as Fe, Ag, Co, Cu, Ni, etc. (Dhar et al., 2015). Generally, CNF absorbed metal particles provide improved properties of mechanical, thermal, and medicinal properties with various health-promoting factors for treating diseases. Among available metal particles, magnetite acts as a medicinal herb and is also used in the preparation of Chinese medicative diets providing immense health promoting factors (Shi et al., 2010). Further, in China Erlong Zuoci decoction (ELZCD) consisting of magnetite as one of the herbs is used for the treatment of age-related hearing loss (Dong et al., 2016). Iron NPs having low toxicity, low cost can be coated with polyethylene glycol and perindopril for treating hypertension (Dorniani, et al., 2014; Gupta et al., 2017). Iron oxide NPs with added antibacterial activity by treating with *Argemone mexicana* L. leaf extract provides better antibacterial activity and renders improved property for drug delivery (Arokiyaraj et al., 2013). Additionally, iron oxide based nanomagnets have attained a focus due to internal properties, conjugation ability with bioactive molecules, colloidal stability, etc. (Meng et al., 2010). Magnetite has various medicinal property such as (1) magnetite with white quartz in rice soup is used for treating asthenia, (2) wine with powdered magnetite is used for treating impotence. Magnetic NPs with required modifications can be used to treat hyperthermia, drug delivery, tissue repair, cell repair, etc. Besides, the use of metallic nanoparticles and polysaccharide nanoparticles together impart a property of enhancing the therapeutic index of drugs with enhanced drug efficacy (Jordan et al., 2006). Based on this discussion, magnetic CNF with tunable properties can be utilized for preparing edible nanocoating on perishable

food products for improving product life with added advantages of suppressing human health diseases.

Further, the use of cellulose and its various derivatives, CNF, cellulose nanocrystals, cellulose whisker are widely utilized with other materials for formulating edible coating targeting perishable food products for improved mechanical, thermal, optical and texture properties (Park, & Chinnan, 1995; Park et al., 1993; Tesfay, & Magwaza 2017; Tesfay et al., 2017; Martelli et al., 2017; Wang et al., 2017). CNF based edible films from mango puree incorporated with CNF provide enhanced properties including mechanical, thermal and barrier for enhancing the shelf life of food products (Azeredo et al., 2009). Development of CNF based composites with chitosan offer to be a beneficial composite packaging as chitosan has antibacterial and antimicrobial property which helps in reducing the microbial degradation of CNF (Liu, & Berglund, 2012). Additionally, as discussed magnetic CNF (mgCNF) has an ability to provide additional medicinal property, which can be chosen to use as an edible material in edible food packaging. The magnetic property of mgCNF provides some inherent properties which help in improving the overall packaging properties to be used as edible food packaging materials. Additionally, iron incorporated CNFs (mgCNFs) have beneficial properties and can be used as an iron-fortified food product, and also improve the packaging properties. Iron is an essential nutrient for maintaining overall health, and there is a growing demand for iron fortified food products targeting iron deficiency anemia (Huma et al., 2007). It is noteworthy to mention that the inclusion of mgCNF as a nanofiller material in developing CS based biocomposites for edible coating materials can significantly improve the packaging properties such as thermal properties, mechanical properties, optical properties and texture properties. The plausible reasons for improved packaging property include the better interaction with the matrix materials compared to CNF. Thus, the tailored packaging properties significantly improve the shelf life of cut fruits when used as an edible coating material

including iron fortifying property and further, iron is essential for producing red blood cells (Martınez-Navarrete et al., 2002).

Additionally, chitosan (CS), being the second most widely utilized biopolymeric material after cellulose, is a potential candidate for developing edible coatings with numerous noteworthy characteristics such as an antioxidant, antimicrobial and weight reducing agent, having properties such as biocompatibility, biodegradability, non-toxicity, anti-inflammatory, etc. (Ghosh & Katiyar 2019; Sullivan et al., 2018; Hajji, et al., 2018). CS exists as a deacetylated product of chitin, and is easily available from sea animals, microorganisms, and insects (Ghosh & Katiyar 2019; El Knidri et al., 2018; Zargar, Asghari & Dashti, 2015). It is noteworthy to mention that CS based edible coating is extensively utilized to protect food products such as citrus fruit (Arnon et al., 2014), strawberries (Hajji et al., 2018; Wang & Gao, 2013), bananas (Baez-Sañudo et al., 2009), red bell peppers (Poverenov et al., 2014), etc. CS is widely used as active packaging materials in addition to various reinforcement as composites for improved food properties. In this regard, CNF and mgCNF reinforced CS provide enormous properties to be acting as edible food packaging material, which is nontoxic and health beneficial.

Additionally, the incorporation of curcumin (Cur) as an agent for edible coating has added advantages of suppressing human health hazards. The application of edible nanocoating on food products with bioactive agents can help in the suppression of human health remedies with enhanced product life. The involvement of edible medicinal coating on food materials not only protects the food from environmental degrading agents, but also provides health beneficial properties to the food matter. Cur loaded nanoemulsions/pectin based coatings provide antimicrobial properties which help in improving the shelf life of chicken fillets (Abdou, Galhoum & Mohamed, 2018). Application of Cur as an anti-carcinogenic material provides added benefit to suppress the human health remedies. In this regard, CNFs and mgCNFs

reinforced CS with added Cur agents based coating materials can be utilized for edible films and coatings.

In the recent past, fresh-cut fruit products have gained considerable attention for being convenient and high quality ready-to-eat food products (González-Aguilar et al., 2004; Issa-Zacharia et al., 2011). Among the available fresh fruit products, pineapple being a type of non-citrus fruit product is widely eaten as fresh-cut forms. The flesh of cut pineapple has a sweet texture and is a rich source of polyphenolic compounds and ascorbic acids (Mantilla et al., 2013). However, the quality deterioration in fresh-cut pineapple products is mainly caused by the microbial attack and environmental agents during storage. Specifically, the food spoilage for fresh-cut fruit products takes account of surface discoloration (browning), change in firmness, texture loss, increased respiration rate, which results in a decreased shelf life of fresh-cut fruit, limiting their availability in markets (Mantilla et al., 2013; Azarakhsh et al., 2014). Additionally, Kiwifruit is a climacteric, perishable, and seasonal fruit and further, is native to the southern China having fibrous structure all over the fruit. The texture of kiwifruit is generally influenced by various sensory attributes including firmness, soluble solid content, acidity, ripening index, etc. The application of proper food preservation techniques can prolong the storage life of fresh agricultural products (Poverenov et al., 2018). Further, the firmness of kiwifruit affects the sensory quality including aroma, acidity, sweetness, and others. The quality of kiwifruits is difficult to maintain during storage for several environmental conditions. In this context, the rapid softening of kiwifruit is a critical problem in maintaining the quality during postharvest life which makes it unacceptable for eatable purposes. Thus, the application of edible coating on kiwifruits can maintain the quality parameters of kiwifruits.

Based on the discussion, the chapter deals with the development and characterization of mgCNF to be used as a component in developing edible coating. The chapter also describes the detailed characterization of CNF and magnetic CNFs reinforced CS based biocomposite as

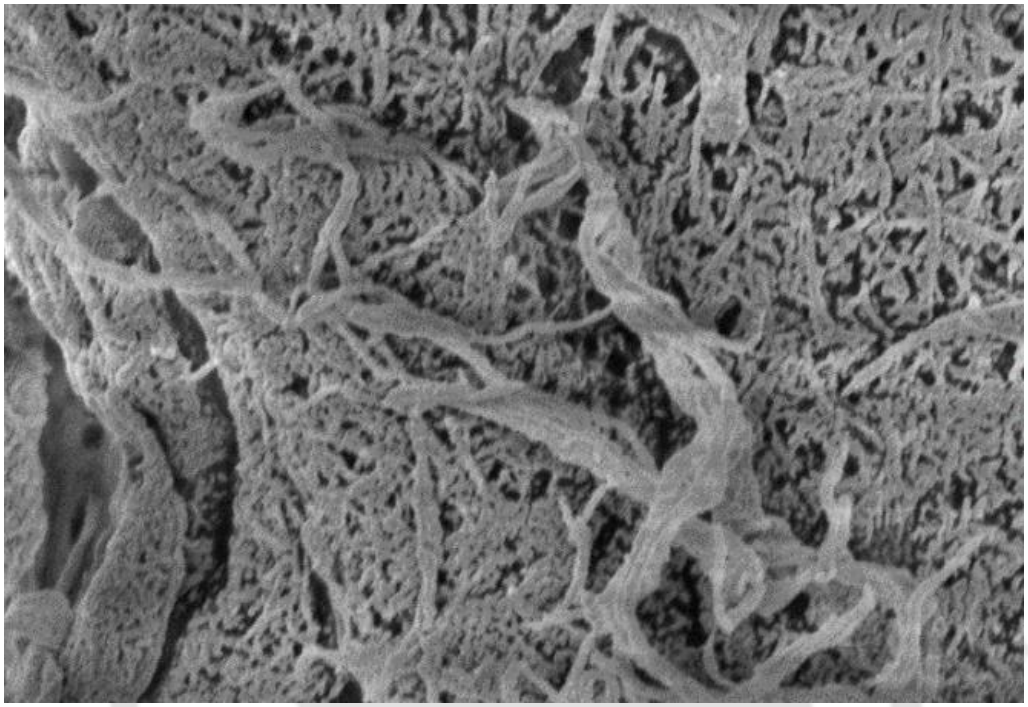
a balanced nanomaterial for edible nanocoatings and its effectiveness in packaging properties. Further, the development of Cur loaded mgCNF dispersed CS based edible nanocoating provides heat stability, anti-cancer activity, improved surface properties, leading to the making of functionalized food products, which is also described in this chapter. Further, the chapter also focuses on the application of developed edible coating materials in tailoring the properties of stored cut pineapple and whole kiwifruits for improved food value and maintained quality, respectively. The effect of coating on the firmness, weight, respiration rate, total soluble solids, and other properties during storage has been discussed.

3.2 Results and Discussions

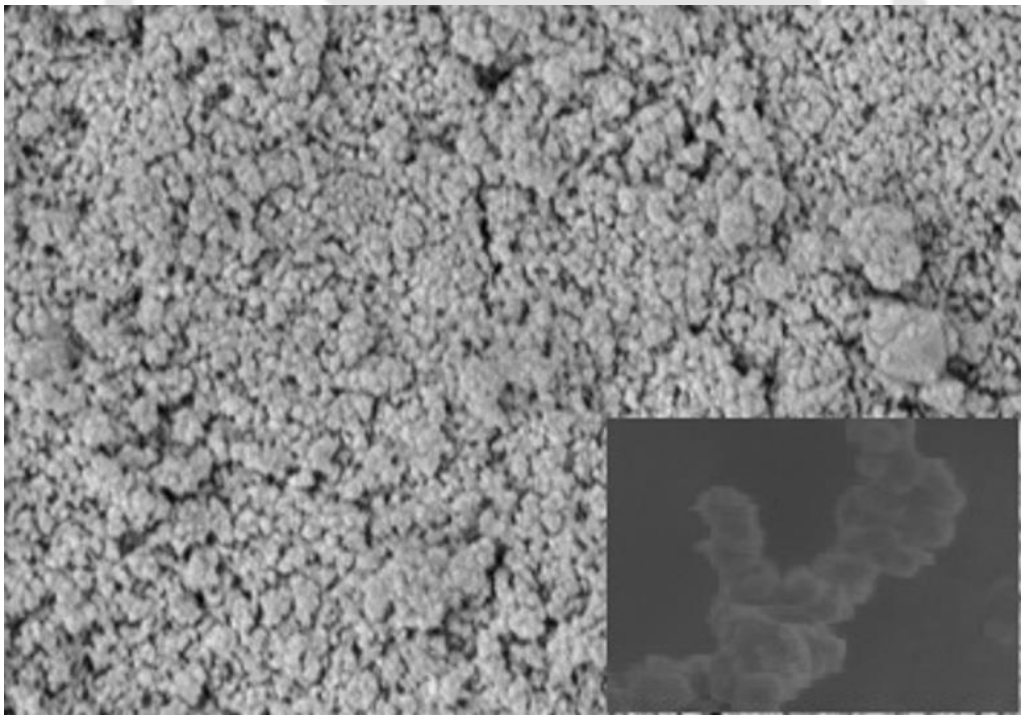
3.2.1 Characterization of Cellulose Nanofibers and Magnetic Cellulose Nanofibres

The fabrication of iron incorporated CNF (mgCNF) is carried out through a single step co-precipitation method consuming commercially available BiNFi-s CNF having dimensions of 20-50 nm (**Figure 3.1a**). Considerably, CNF possesses milky appearance in water with negligible agglomeration due to hydrophilic property, whereas drying of CNF generally causes agglomeration within fiber structure through interacting among each other by hydrogen bonding (Peng, Gardner, & Han, 2012). In addition, the synthesized nanomaterial mgCNF carries iron particles attached to CNF due to co-precipitation of iron materials onto CNF as shown in **Figure 3.1b** (Huang et al., 2016). The surface morphology of mgCNF shows that iron particles having sphere-shaped structure are adsorbed onto the CNF particles providing a cloudy cram sphere-shaped morphology (**Figure 3.1b**). The formulated mgCNF having a diameter of 110±85 nm in comparison to CNF, where variation in dimension is due to the anchoring of iron particles on the surface of CNF. The high-resolution images (**Figure 3.1b**) show that sphere shaped iron particles covered all surface areas of CNF particles and provides a uniform interaction which prevents separation of nanoparticles. Additionally, the FETEM

micrograph of BiNFi-s CNF and mgCNF has been represented in **Figure 3.2**, where it also evident the formulation of functionalized CNF.

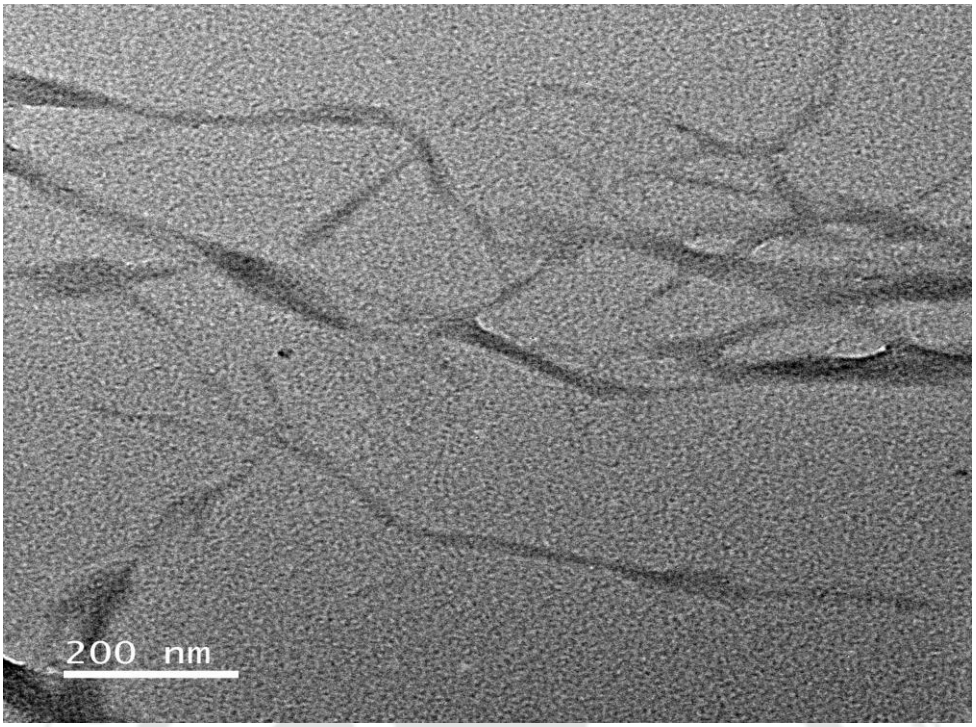


(a)

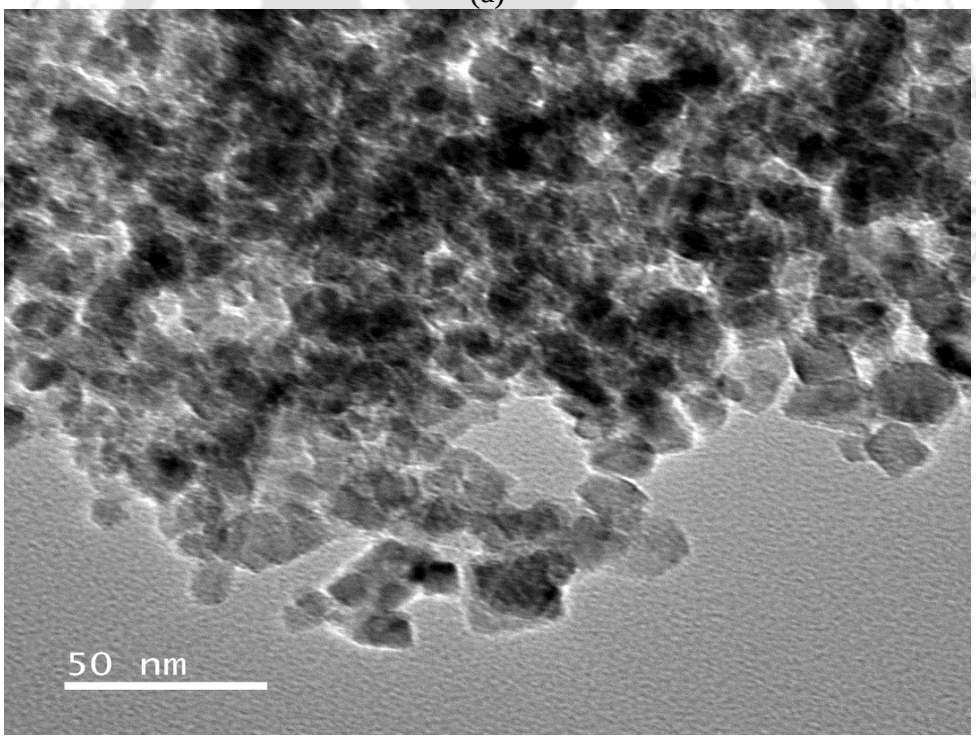


(b)

Figure 3.1 *FESEM photomicrographs of (a) BiNFi-s CNF having dimensions of 20–50 nm, and (b) mgCNF (fabricated iron incorporated CNF).*



(a)

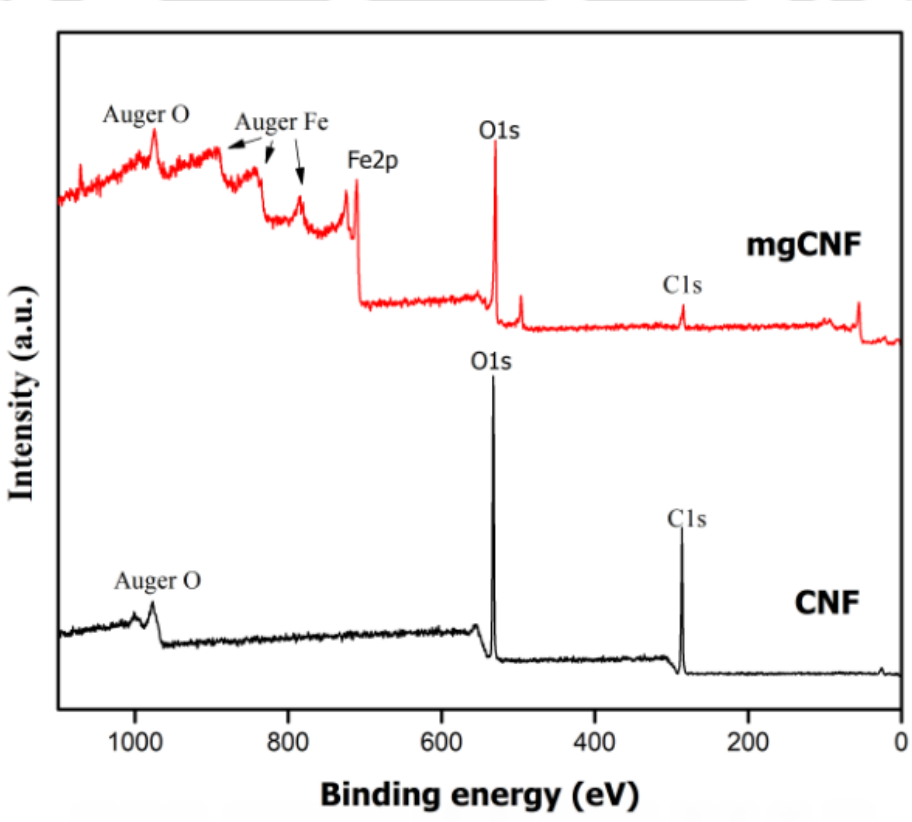


(b)

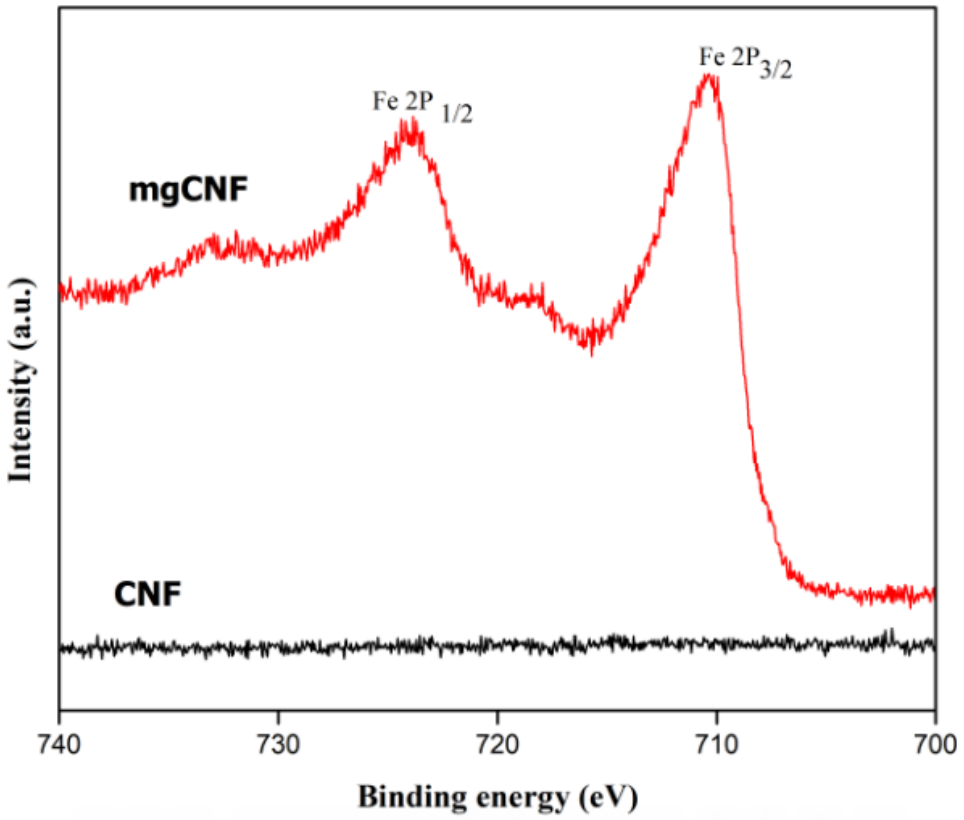
Figure 3.2 *FETEM micrographs of (a) BiNFi-s CNF having dimensions of 20–50 nm, and (b) mgCNF (fabricated iron incorporated CNF).*

XPS Analysis

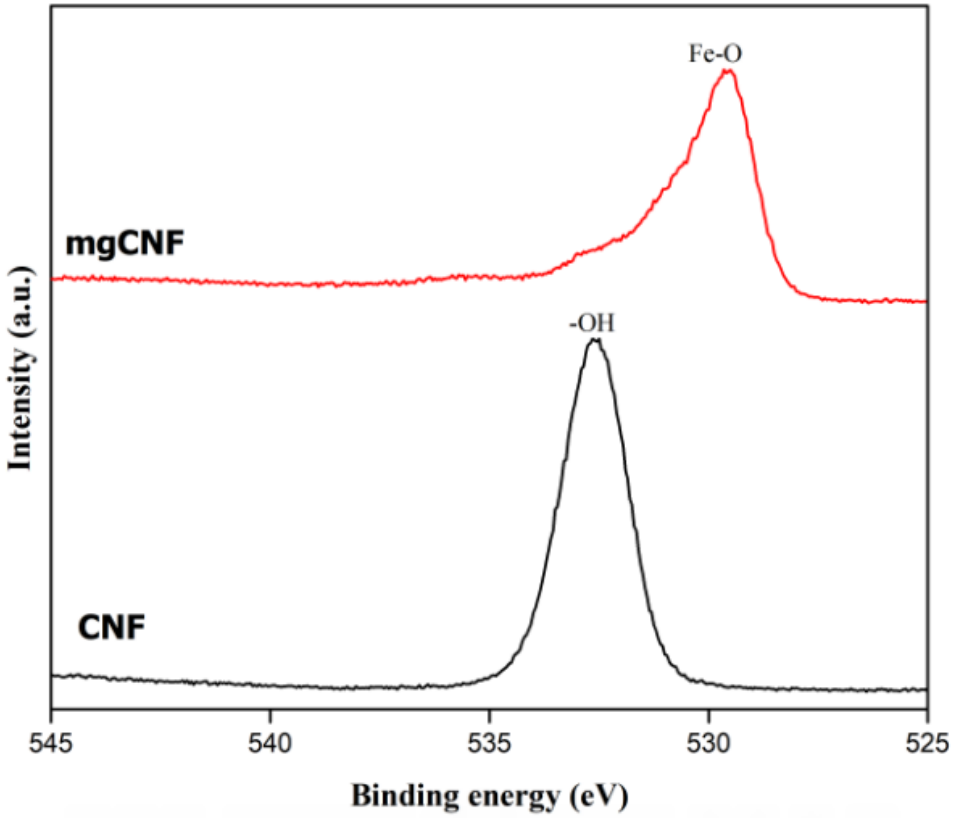
XPS analysis of CNF and mgCNF based nanostructured materials are carried out to survey the effectiveness of iron particle on the surface of CNF. As shown in **Figure. 3.3a**, photoelectron spectroscopic peaks of CNF are attained at approximately (i) 284.2 eV for C 1s, (ii) 530 eV for O 1s, and (iii) 976 eV for Auger Electron O. In case of mgCNF, an additional intense peak at around 711 eV is noticed in addition to the usual peaks of CNF, which is due to Fe 2p group confirming presence of Fe_2O_3 and Fe_3O_4 group. Moreover, photoelectron lines are observed for Auger electron of Fe, where Fe $2p_{1/2}$ and Fe $2p_{3/2}$ corresponding to binding energy line 724.1 and 710.6 eV, respectively (**Figure 3.3b**). Peaks around 530.4 and 533 eV for FeO and OH are noticed with less intensity, respectively (**Figure 3.3c**). Further, some photoelectron peaks around 284 and 286 eV are noticed representing C1s for CNF and mgCNF, respectively (**Figure 3.3d**). The obtained C, O and Fe percentage in mgCNF are obtained as ~21, 49 and 24%, respectively.



(a)



(b)



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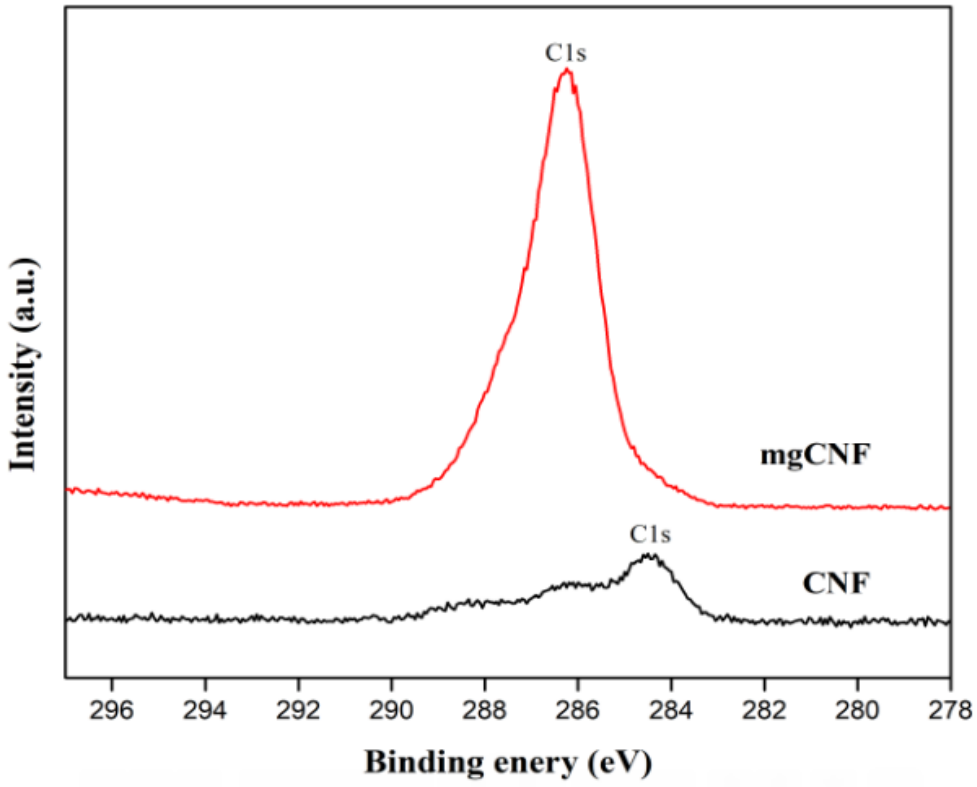


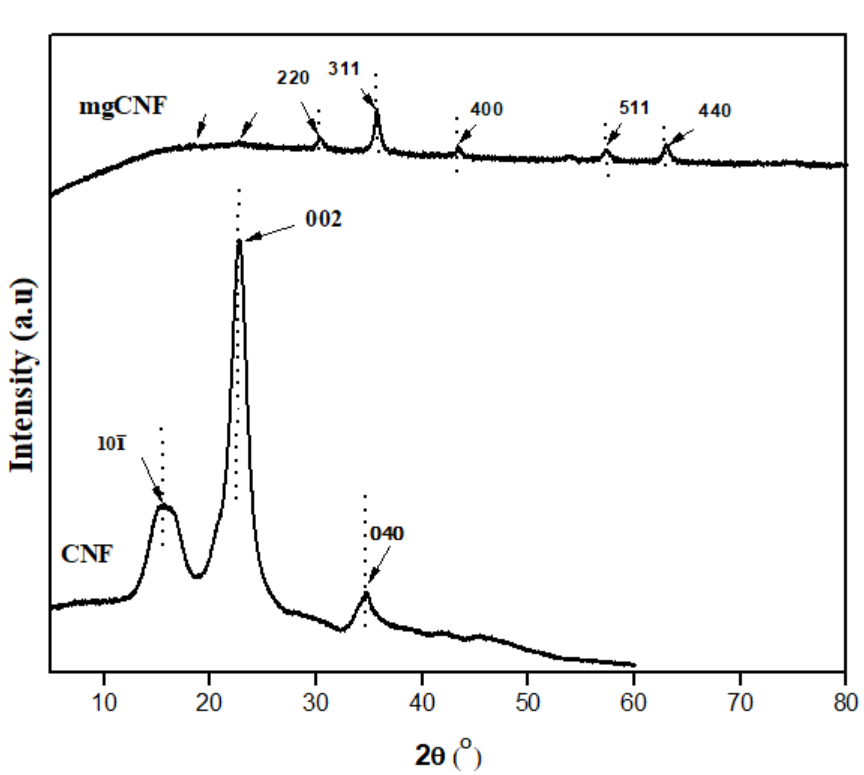
(d)

Figure 3.3 *XPS spectrum of CNF and mgCNF nanoparticles (a) wide range, (b) Fe2p spectrum, (c) O1s spectrum, and (d) C1s spectrum.*

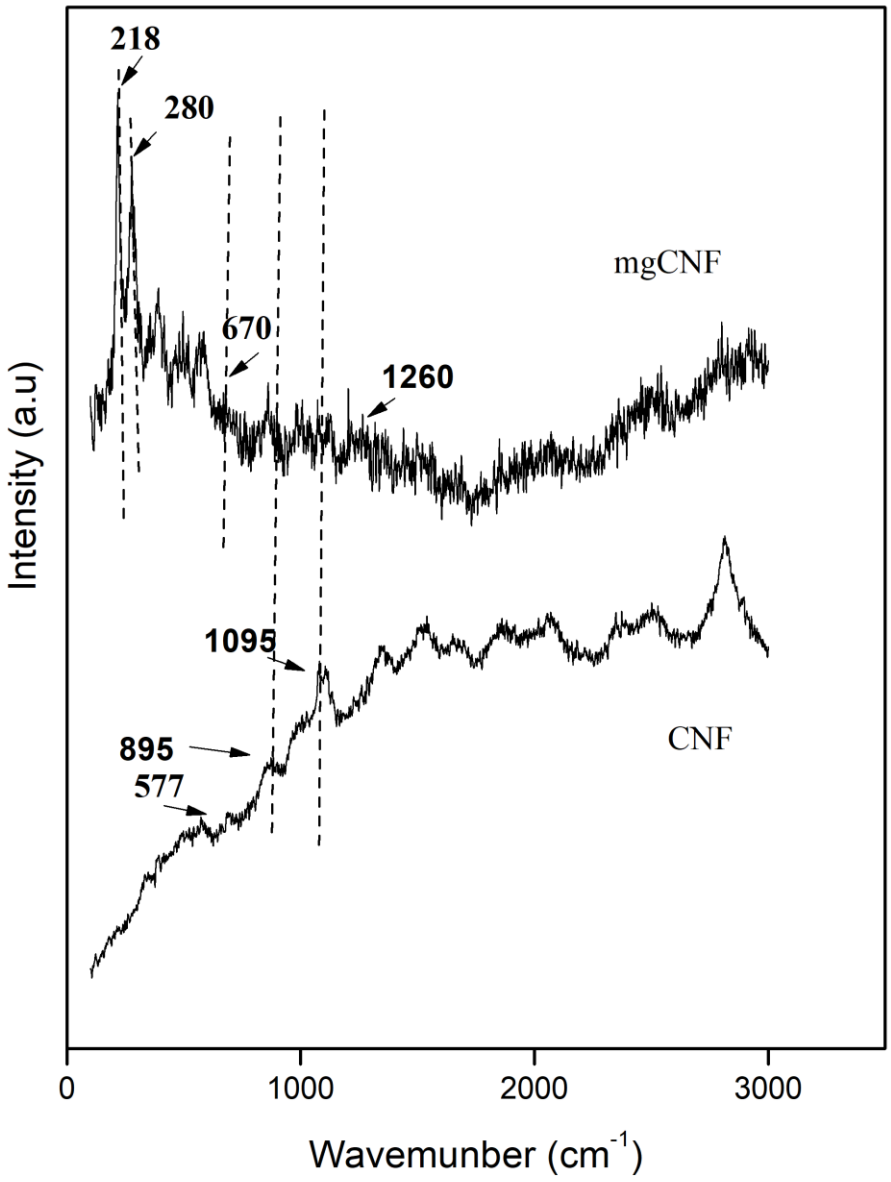
XRD Analysis

The XRD analysis of BiNFi-s CNF and synthesized iron adsorbed CNF is performed as shown in **Figure 3.4a**. The commercially obtainable CNF have characteristics peaks at 2θ = 16.2°, 22.5° and 34.5° which corresponds to the crystallographic planes (10$\bar{1}$), (002) and (040), respectively (Dhar, Kumar, & Katiyar, 2016). The mgCNF nanomaterials are obtained by decorating the iron nanoparticles on CNF *via* adopting a single step co-precipitation methods. In this regards, the XRD of mgCNF shows the clear characteristics peaks at 2θ = 30.26°, 35.62°, 43.30°, 57.26° and 62.89° for the crystallographic planes (220), (311), (400), (511) and (440), respectively (Zhu et al., 2011; Maleki, Movahed, & Ravaghi, 2017). The disappearance of the

characteristics peaks of CNF in the synthesized mgCNF provides the evidence of adsorbing iron particles onto the CNF molecules. In addition, Raman spectroscopic is also performed to ratify the attachment of iron nanoparticles on CNF by replacing surface –OH groups. Raman spectroscopic evaluation of CNF provides Raman peaks (**Figure 3.4b**) for functional groups cellulose band, C-OH (or COC) and sharp carbonyl stretch mode (C-O) bending at C-6 are obtained at 577, 895, 1095 cm^{-1}, respectively (Jordan et al., 2006; Gao et al., 2013; Agarwal, 2014). However, Raman peak at 1095 cm^{-1} is generally observed due to C-O and –C-O-C- stretching of glucose rings and glycosidic linkages (**Figure 3.4b**). Further, the characteristics Raman peaks of mgCNF are observed at 218, 280, 670, and 1260 cm^{-1}, which give the evidence of the presence of oxides of the iron particle. The noticeable Raman peak of CNF is not found in the Raman spectra of mgCNF (895 cm^{-1} is disappeared) for the functional group -COC bending at C-6, whereas Raman peak at 380 cm^{-1} gives the confirmation of absorbing on CNF **Figure 3.4b**.



(a)

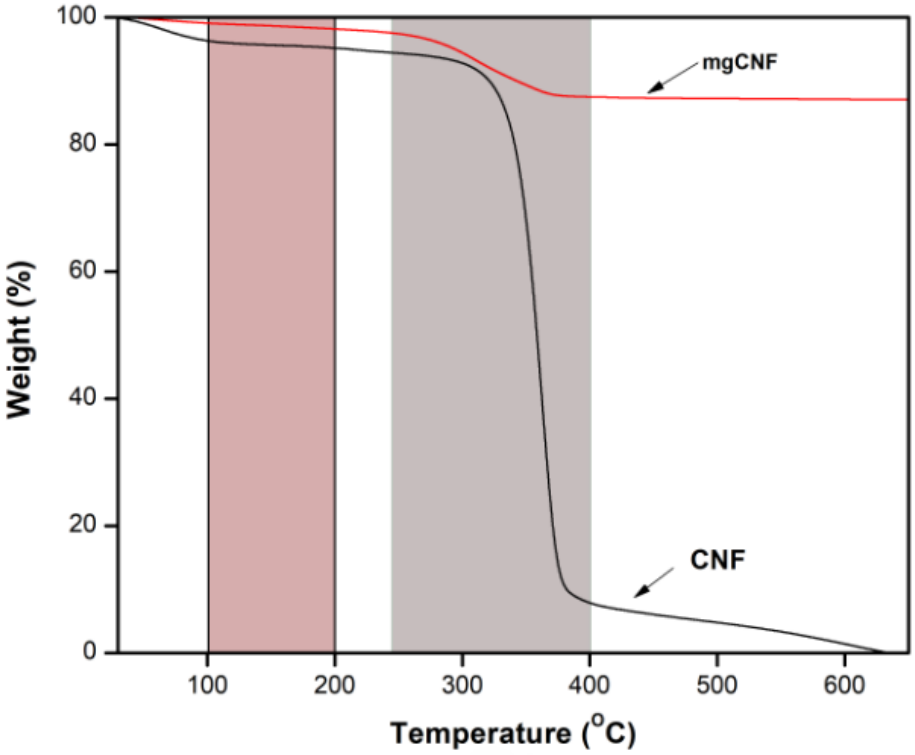


(b)

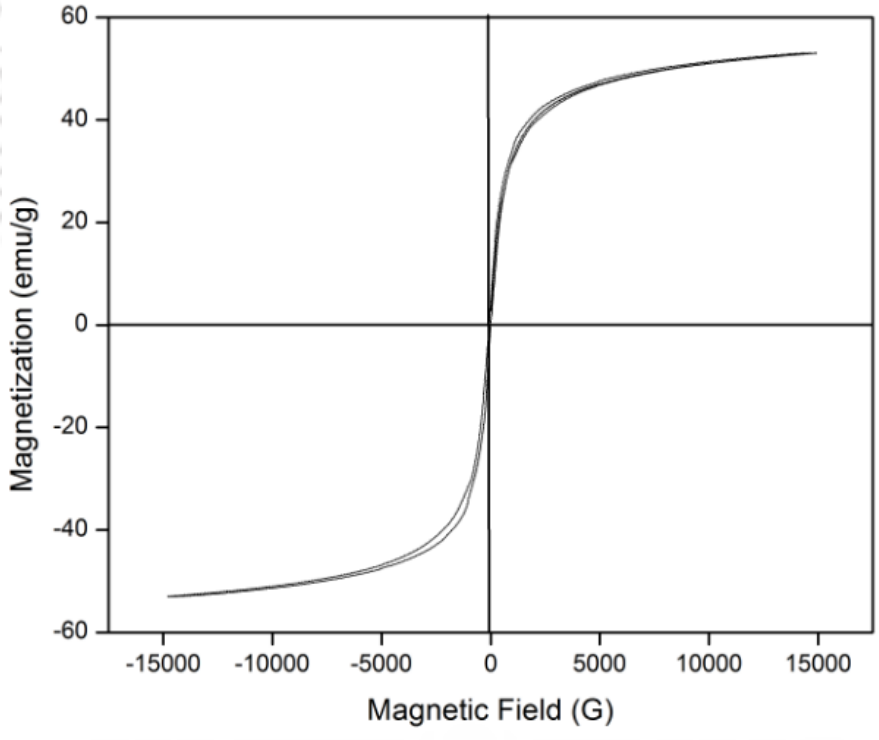
Figure 3.4 *(a) XRD Diffractrogram, and (b) Raman spectra of CNF and mgCNF.*

Thermal Property

The thermal treatment of cellulose mainly elucidates through different processes, where the rate of degradation can further be enhanced by the addition of water, the involvement of oxygen and pH conditions. The general products for cellulose thermal degradation are laevoglucose, anhydroglucoses, furan and furan derivatives, and others. The TGA thermograms show the performance of CNF and iron absorbed CNF (mgCNF) under thermal treatment (30–700 °C) as shown in **Figure 3.5(a)**). Thermal degradation profile of CNF generally follows two states of degradation such as a first and second step of degradation following the ranges of 100–200 °C and 250–500 °C representing the removal of moisture, sulphates and degradation of cellulose, respectively. TGA investigation for CNF materials is elucidated for their change in weight with respect to change in temperature, where there is only 4 and 11 wt% loss in the product occurs during temperature range of 100–200 °C and 290–380 °C, respectively. Further, cellulose generally shows a loss inbound water at a higher temperature at around 100–200 °C. Interestingly, TGA analysis of mgCNF shows that there is only a 17% reduction in weight for the temperature range 30–700 °C, where the thermal stability of the product is due to the successful incorporation of iron particle in CNF particle, where iron is present in very less amount (~24% as evident by XPS). The TGA analysis proves that the application of normal temperature will not affect the product and can easily consumable. Further, the successful fabrication of mgCNF with iron nanoparticles lead to magneto-responsive property as shown in **Figure 3.5(b)**. The magnetogram shows no distinct hysteresis loop (Dhar, Kumar, & Katiyar, 2016), however mgCNF shows response when the magnetic field is applied and the power of magnetization is obtained up to ~48 emu/g.



(a)

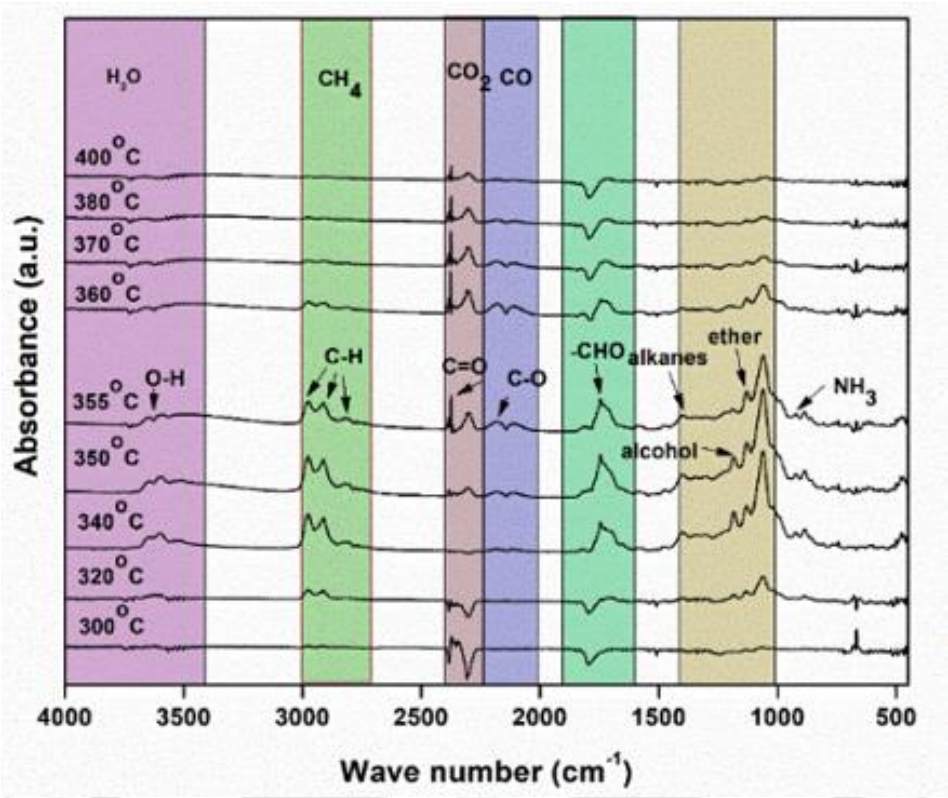


(b)

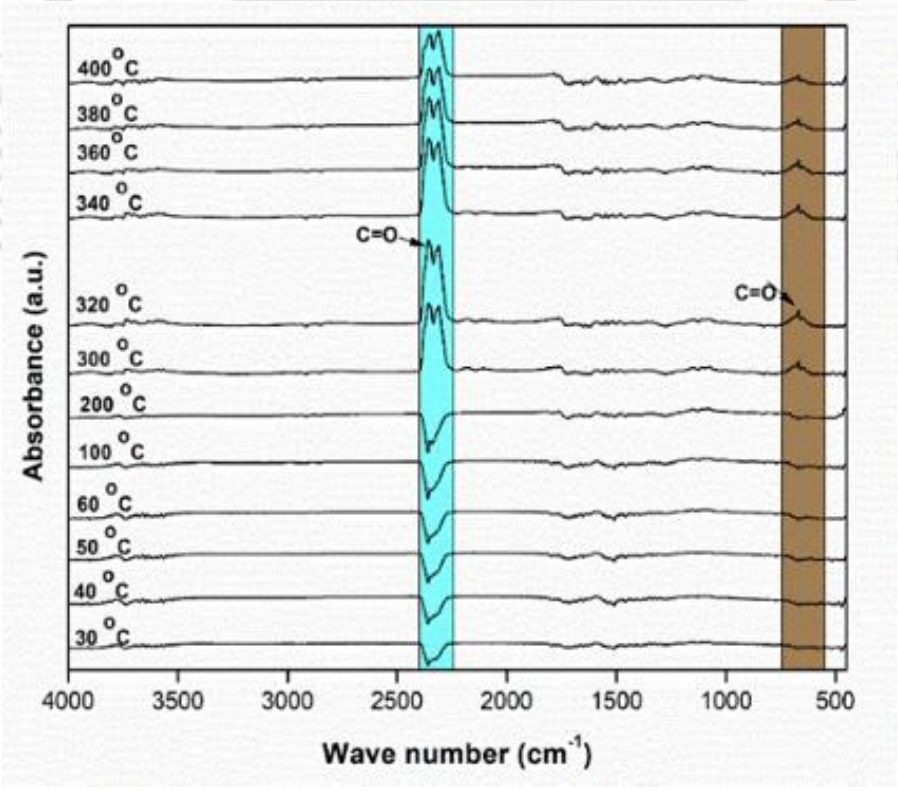
Figure 3.5 *(a) TGA thermogram of CNF and mgCNF showing two-stage thermal degradation and (b) Magnetic hysteresis of mgCNF.*

TGA-FTIR Study

TGA-FTIR study of CNF shows the development of different gaseous components at various regions of IR ranges between 4000–450 cm^{-1} (**Figure 3.6**). The evolution of various gaseous components during TGA-FTIR of CNF can be categories in different sections such as (i) IR region 4000–3400 cm^{-1} (H_2O species): O-H stretching at 3714, 3652, and 3599 cm^{-1}; (ii) IR region 3000–2700 cm^{-1} (CH_4 species): C-H stretching peaks at 2977, 2911, and 2808 cm^{-1}; (iii) IR region 2400–2250 cm^{-1} (CO_2 evolution): Peaks at 2376 and 2299 cm^{-1} for C=O stretching; (IV) IR region 2250 to 2000 cm^{-1} (CO species): Evolution peak at 2182,2116 cm^{-1} for C-O stretching (Ma et al., 2015; Cunha et al., 2016). Further, different compounds at different IR range can be obtained such as 1747 cm^{-1} for aldehydes, ketones, acid,1474 cm^{-1} for benzene, skeleton stretching, aromatics, 1399 cm^{-1} for alkanes C-C, C-H stretching, 1185 cm^{-1} for C-O stretching for alcohol, 1127 cm^{-1} for C-O stretching for ethers and 927 for amines (NH_3) as shown in **Figure 3.6**(a). Further, TGA-FTIR of mgCNF shows the evolution of 2358, 2316, and 670 cm^{-1} relate the evolution of the species CO_2. Considerably, mgCNF tends to evolve less amount of gaseous materials in comparison to CNF nanomaterials, which leads to use of these materials for developing films through melt extrusion, blown films etc. The evolution of less gaseous product during TGA-FTIR analysis of mgCNF provides another way to use during melt extrusion, blown films, and injection moulding for biodegradable films for various applications.



(a)



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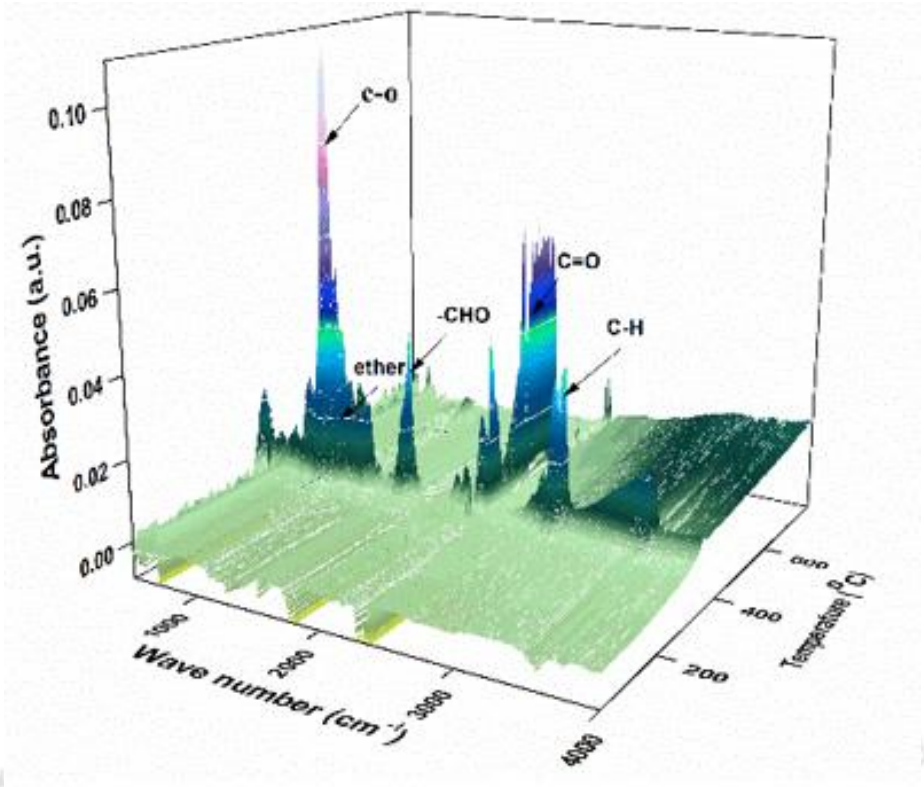

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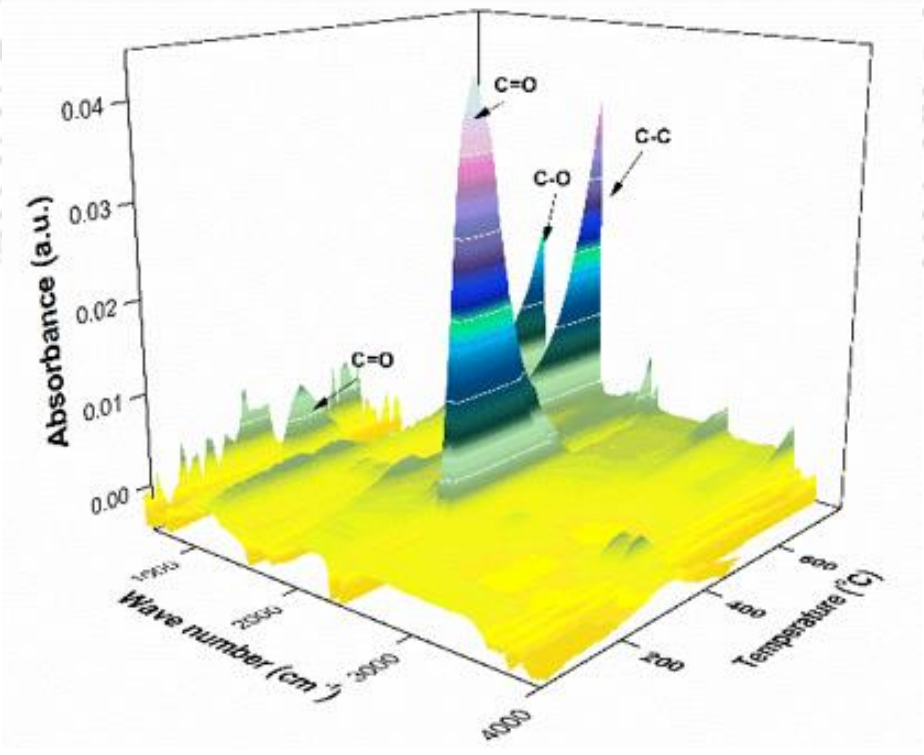


(d)

Figure 3.6 *2D stacked plots of TGA-FTIR study of (a) CNF and (b) mgCNF; 3D stacked plots of TGA-FTIR study of (c) CNF and (d) mgCNF.*

3.2.2 Cellulose Nanofibers and Magnetic Cellulose Nanofibre Dispersed Chitosan based Edible Nanocoating: Fabrication and Characterization

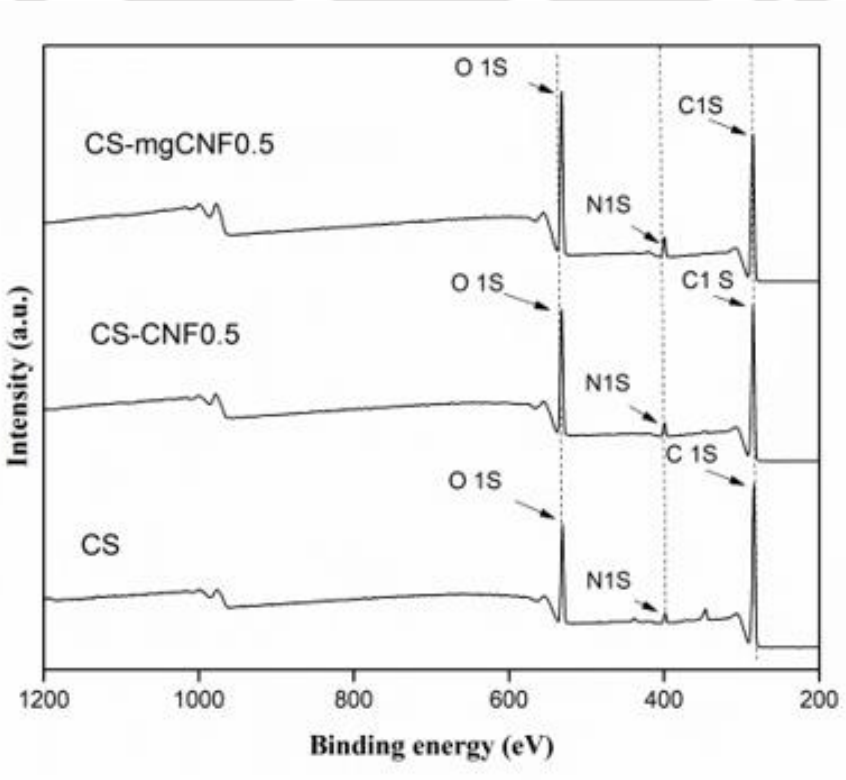
The developed mgCNF has been used in various proportions in preparing CS based biocomposites with and without Cur. In this regards, as represented in **Table 3.1**, the various proportions of the developed nanomaterials have been taken to develop edible coating materials with and without Cur. In this section, the various characterizations of the CS/CNF and CS/mgCNF include XPS analysis, FTIR spectroscopy, ICPMS analysis, magnetization characteristics, mechanical property, thermal properties, swelling property, optical property, and color measurement has been included. Further, a detailed discussion on the surface morphology, physicochemical properties, and biocompatibility study has been made in the next section.

Table 3.1 *Various combination of chitosan based edible coating materials.*

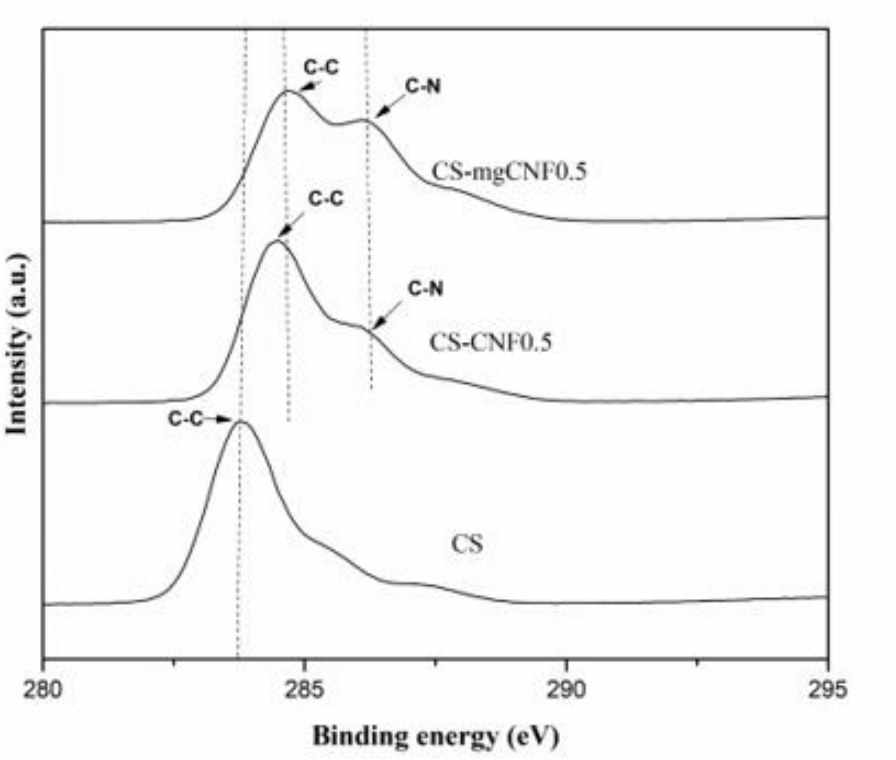
Sl. No	CS (%w/v)	CNF (%w/w)	mgCNF (%w/w)	Cur (%w/v)	Nomenclature
1.	1	--	--	--	CS
2.	1	0.5	--	--	CS-CNF0.5
3.	1	1	--	--	CS-CNF1
4.	1	1.5	--	--	CS-CNF1.5
5.	1	--	0.5	--	CS-mgCNF0.5
6.	1	--	1	--	CS-mgCNF1
7.	1	--	1.5	--	CS-mgCNF1.5
8.	1	--	--	0.01	CS-Cur
9.	1	0.5	--	0.01	CS-CNF0.5-Cur
10.	1	1	--	0.01	CS-CNF1-Cur
11.	1	1.5	--	0.01	CS-CNF1.5-Cur
12.	1	0.5	0.5	0.01	CS-mgCNF0.5-Cur
13.	1	1	1	0.01	CS-mgCNF1-Cur
14.	1	1.5	1.5	0.01	CS-mgCNF1.5-Cur

XPS Analysis

XPS spectrum of CS, CS-CNF0.5, and CS-mgCNF0.5 are represented in **Figure 3.7.** The XPS peaks for CS edible nanocoatings show binding energy 284 eV, 399 eV, and 532 eV represents C1S (C-C), N1S (NH_2 and NH) and O1S, respectively. The XPS peak for CS-CNF0.5 edible nanocoatings represents binding energy at 285 eV, 399 eV, and 532 eV representing C1S (C-C), N1S (NH_2 and NH) and O1S, respectively. In addition, the XPS peaks of CS-mgCNF0.5 for binding energy at 286 eV, 399 eV, 532 eV represents C1S (C-C), N1S (NH_2 and NH) and O1S, respectively. As represented in **Figure. 3.7b**, XPS peaks at binding energy 284 eV, 284.5 eV, 284.7 eV representing C-C linkage for CS, CS-CNF0.5, and CS-mgCNF0.5, respectively. Moreover, the peak for binding energy at 286.2 eV represents C-N linkage. The XPS peak at binding energy 531.3 eV, 532.2 eV, and 532.6 eV represents O1S for CS, CS-CNF0.5, and CS-mgCNF0.5, respectively (**Figure. 3.7**c). Considerably, after many scans of CS-mgCNF0.5 show a peak at binding energy of 711 eV as shown in **Figure. 3.7d**.



(a)



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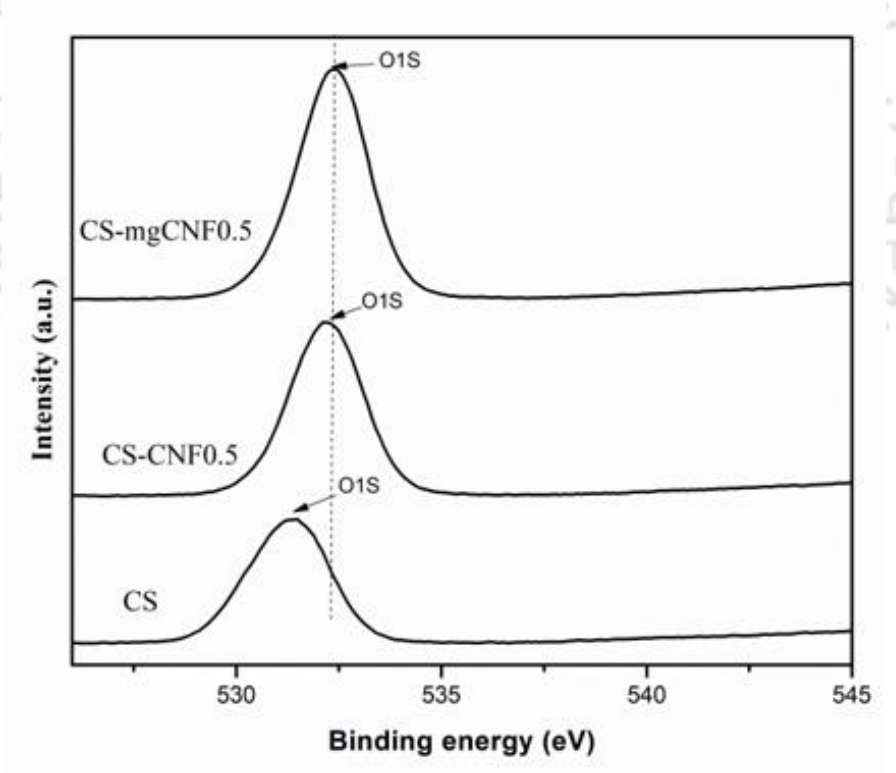

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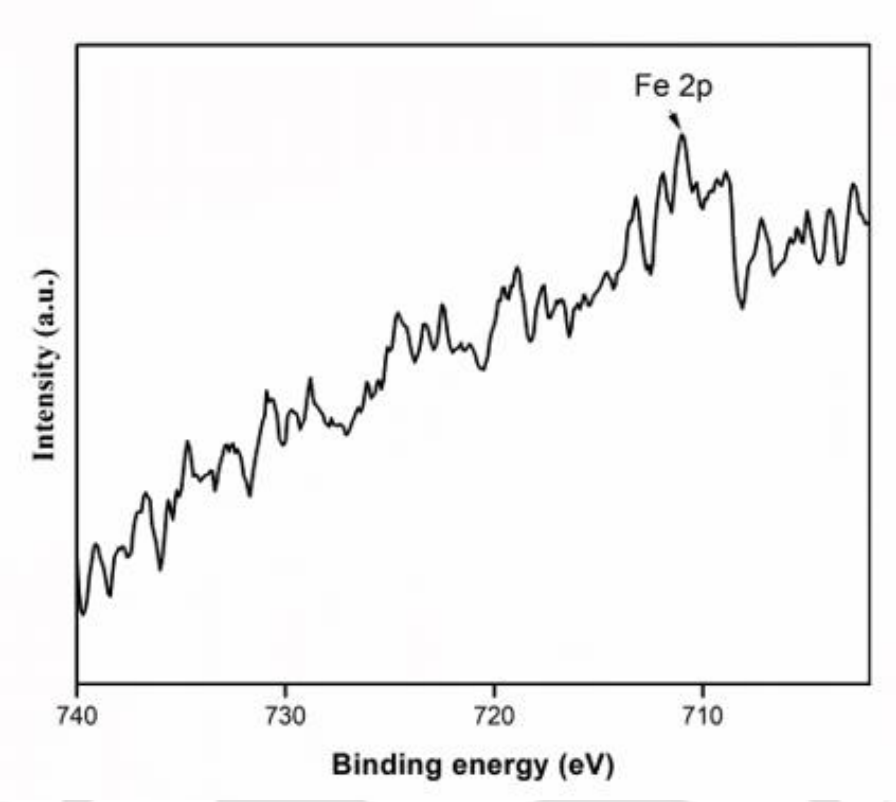


(d)

Figure 3.7 *XPS spectrum of CS, CS-CNF0.5, CS-mgCNF0.5 coating materials depicting (a) wide range, (b) C1s spectrum, (c)O 1s spectrum, and (d) Fe 2p spectrum.*

FTIR Spectroscopy

The FTIR spectroscopy of CS based composite materials is done as detailed in **Figure 3.8**. In the spectrometer, CS shows characteristic FTIR peaks at 1650 cm^{-1} for amide II, 1554 for amide I, 1419 cm^{-1} for alkane C-H stretching, 1156 cm^{-1} for C-O stretching vibration, 1079 cm^{-1} for amide I, and 891 cm^{-1} for C-O stretching vibration (Romainor, et al., 2014). A reduction in peak intensity is observed with the aid of nanofiller materials as represented in **Figure 3.8**. There is a shift in 1650 cm^{-1} characteristics peak for amide II to 1647 cm^{-1} due to the incorporation of CNF as a nanofiller for formulating CS-CNF biocomposites. The shift in peaks may be due to the overlapping of –OH bending of water present in CNF and CS (Romainor, et al., 2014). Similarly, the characteristics peak shifts to 1643 cm^{-1} from 1650 cm^{-1} for the effect of mgCNF as a nanofiller. Moreover, the characteristic peak at 1147 cm^{-1} is shifted to 1159

cm^{-1} and 1161 cm^{-1} due to the addition of CNF and mgCNF nanofillers, respectively. The shift is due to the change and extending in interaction of C-O stretching in prepared biocomposite films (Szymańska-Chargot et al., 2019). The shift in peaks may be attributed due to the overlapping the regions of the filler and matrix materials. A similar trend is obtained in the characteristic peak at 891 cm^{-1}, which is shift to 896 and 894 cm^{-1} due to the change in C-O interactions.

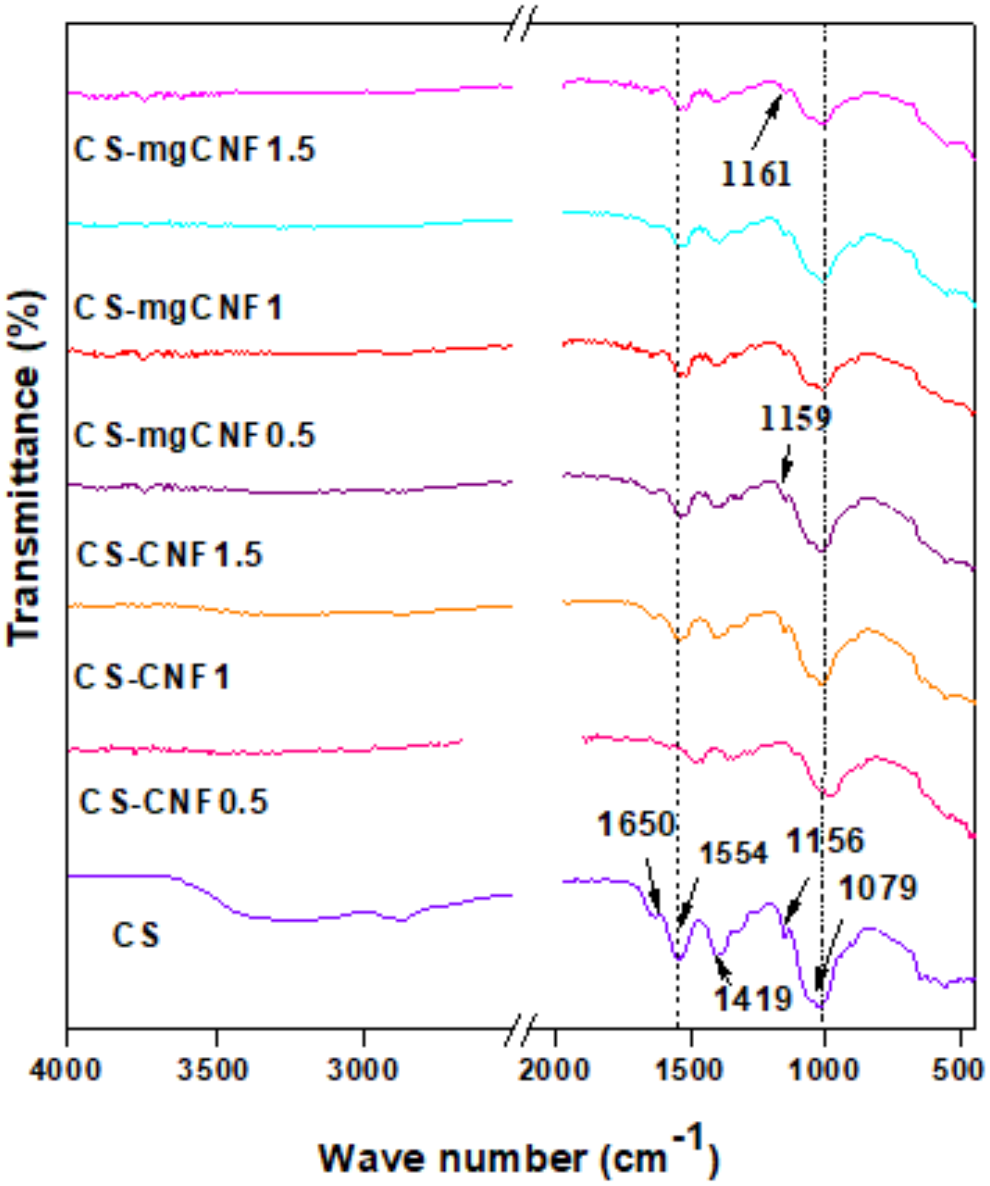


Figure 3.8 *FTIR spectroscopy of CS/mgCNF based biocomposites.*

ICPMS Analysis

Cellulose based nanofillers are widely used as filler materials for biocomposites formulations, where it helps in improving the composite property. The nanofiller mgCNF also possess some advantage in terms of packaging properties. The selection of a percentage of loading material is done based on the permissible limits of the iron component in edible food materials by WHO/FDA (**Table 3.2**). The edible nanocoating materials are prepared through solution come evaporation method, where 0.5, 1, and 1.5% of both the fillers are taken and different packaging properties are measured such as mechanical, thermal, optical, color and wettability property as discussed below. ICPMS analysis of CNF powder and mgCNF incorporated edible nanocoatings are carried out principally to quantify iron content in the developed edible coating materials. In addition, manganese (Mn), lead (Pb), Zinc (Zn), and Arsenic (As) contents are quantified to set the edible materials safe for human consumption as shown in **Table 3.2**. The quantified Fe contents in CNF, CS-mgCNF0.5, CS-mgCNF1, and CS-mgCNF1.5 are within the permissible limit as compared with some laws and regulations. The observed Fe content in CNF, CS-mgCNF0.5, CS-mgCNF1, and CS-mgCNF1.5 are ~0.13, ~1.7, ~3.7, and ~6.3 ppm, respectively, which are within the permissible limit as compared with available legislation. In addition, the utilization of developed edible materials could help in fortifying food products with iron components. The recent survey has declared iron powders as elements including electrolytic iron powders can be used as efficient iron delivery (Hurrell, 2002). Based on this discussion, the developed edible materials could act as a supplement for iron fortified food products and it could also be used as herbs for treating many diseases. To recognize the coating materials as safe, the quantification of other metals is also carried out (**Table 3.2**) (Kumar, & Puri, 2012; WHO, 1996; Buchet et al., 1983; Llobet et al., 2003), which are further compared with the available permissible limit for various food products.

Table 3.2 *Trace elements in chitosan/magnetic cellulose nanofibres based edible nanocoatings.*

Heavy metal	**ICPMS Sample name**				**Permissible limit (ppm)**	
	CNF (ppm)	**CS-mgCNF0.5 (ppm)**	**CS-mgCNF1 (ppm)**	**CS-mgCNF1.5 (ppm)**		
Fe	0.130±0.03	1.7±0.14	3.7±0.14	6.3±0.06	Anaerobic ground Water	0.5-10
					Minimum daily intake	10-50 mg/day
					Rice and many fruits and vegetables	1- 10
					Liver, kidney, fish and green vegetable	20-150
					Red meat and egg yolks	10-20
					Body iron in adult male	50mg/kg of body weight

					Body iron in adult female	34-42 mg/kg of body weight
Mn	0.004±0.00	0.002±0.00	0.0036±0.00	0.003±0.00	2-20	
Pb	0.024±0.00	0.010±0.00	0.024±0.00	0.031±0.00	93.5 ppb/kg body weight	
Zn	0.043±0.00	0.026±0.00	0.046±0.00	0.06±0.00	5-22	
As	<0.000	<0.000	<0.000	<0.000	10 ppb	

Magnetization Characteristics

The magnetization characteristics of mgCNF reinforced CS nanocoating materials are measured using a Vibrating sample magnetometer (VSM) (**Figure 3.9**). The depicted figure provides a clear view that the edible nanocoating materials have created a magnetic hysteresis, which is supposed to a loss in their superparamagnetic property. Further, the reinforcement of mgCNF at various loadings in CS edible nanocoating materials has kept their integrity and maintained their magnetic property.

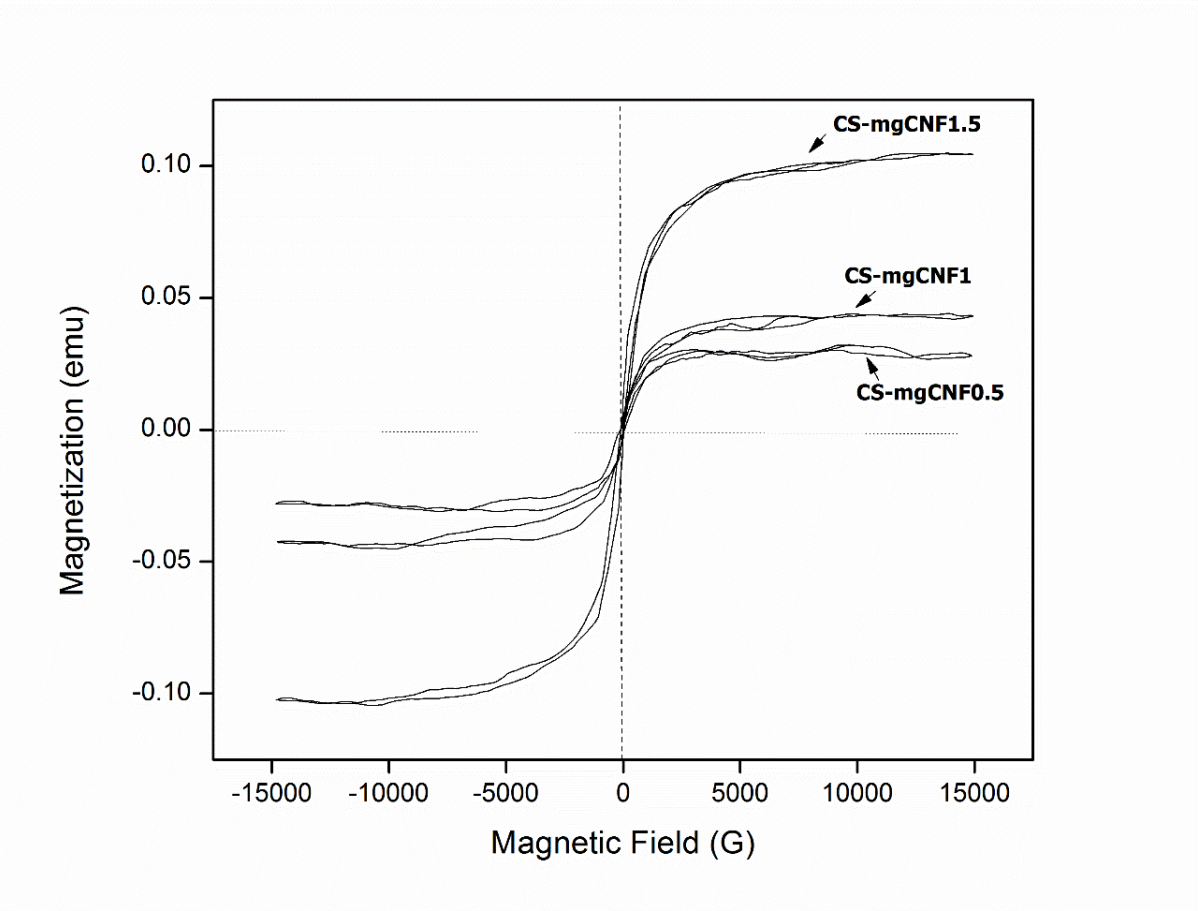
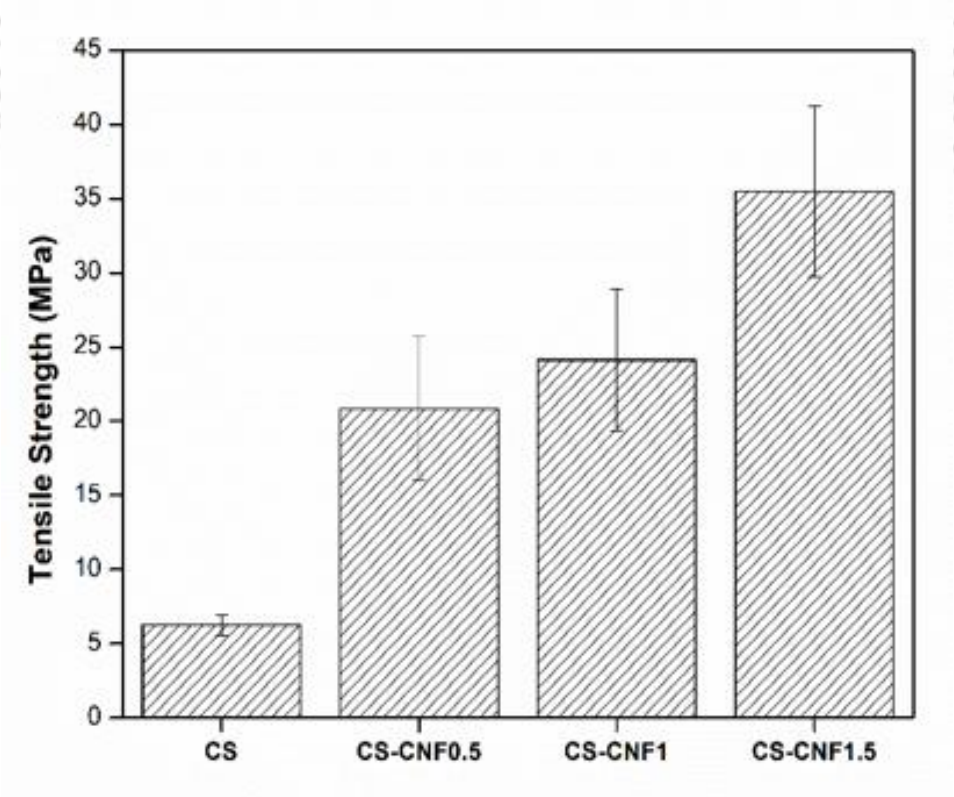


Figure 3.9 *Magnetic hysteresis loop of CS-mgCNF based biocomposites with loading 0.5, 1, and 1.5 wt%.*

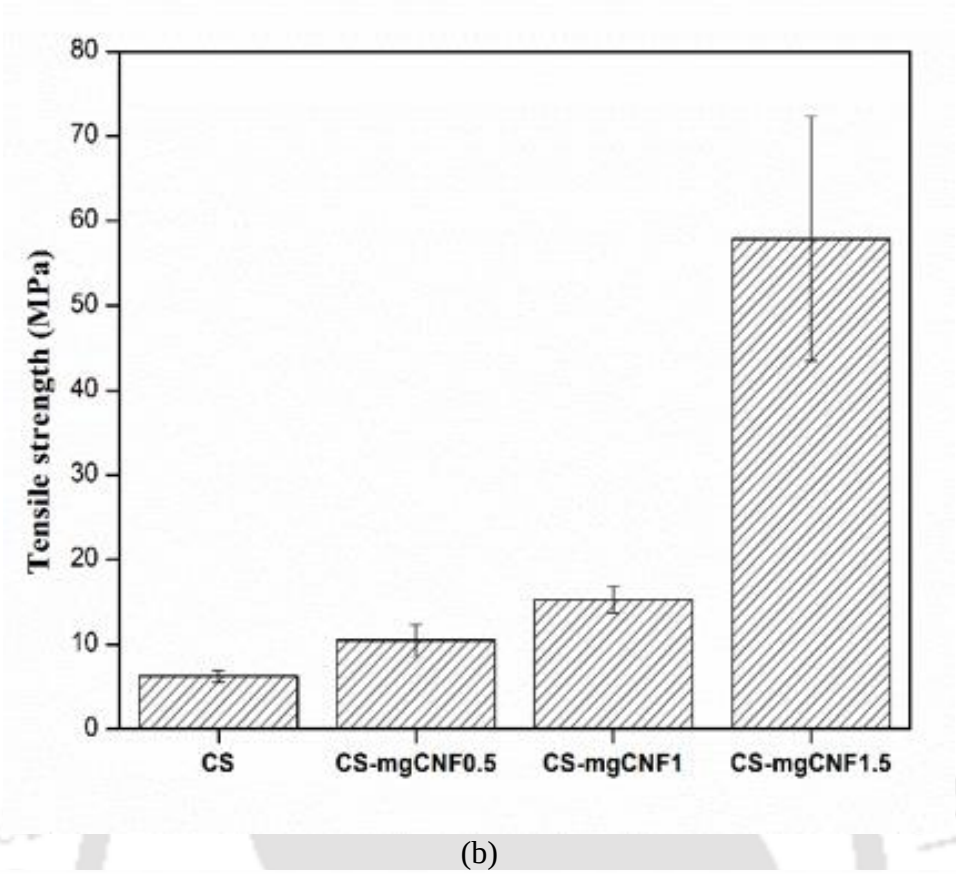
Mechanical Property

The mechanical properties of CS based biocomposites in terms of tensile strength and Young's Modulus are represented in **Figure 3.10**. Generally, the remarkable emphasizing effect of CNF and mgCNF as reinforcement materials are attained due to forming a percolating network in various polymer grounds for assembling composite materials. In addition, CNF having a fibre like structure can form a connecting network in chitosan molecule and CNF bearing hydroxyl sites can interact with hydrophilic sites of CS molecules. The observed tensile strength and Young's modulus of neat CS materials are 6.27±0.7 MPa and 462.36±64 MPa, respectively. Further, CNF having a fibre like structure can easily make different arrangements

in the matrix structure. In this regard, the tensile strength for CS-CNF0.5, CS-CNF1, and CS-CNF1.5 is improved by ~69%, ~74%, and ~82%, respectively (**Figure 3.10a**). The increase in tensile strength may be due to better interaction between CNF and CS molecule. Moreover, the observed Young's modulus of CS-CNF0.5, CS-CNF1, CS-CNF1.5 is found to be improved by ~32%, ~45% and ~51%, respectively (**Figure 3.10**b). The loading of CNF materials through percolating networks can positively transfer the mechanical property to matrix materials. The tensile strength of CS-mgCNF0.5, CS-mgCNF1 and CS-mgCNF1.5 are improved by ~40%, ~58%, and ~89%, respectively (**Figure 3.10**c). In addition, Young's modulus of CS-mgCNF0.5, CS-mgCNF1 and CS-mgCNF1.5 are improved by ~61%, ~69% and ~80%, respectively in comparison to neat CS coating materials (**Figure 3.10**d). The filler material mgCNF can possibly intercalate within the polymer matrix by increasing interaction and provide better dispersion due to negligible agglomeration.



(a)



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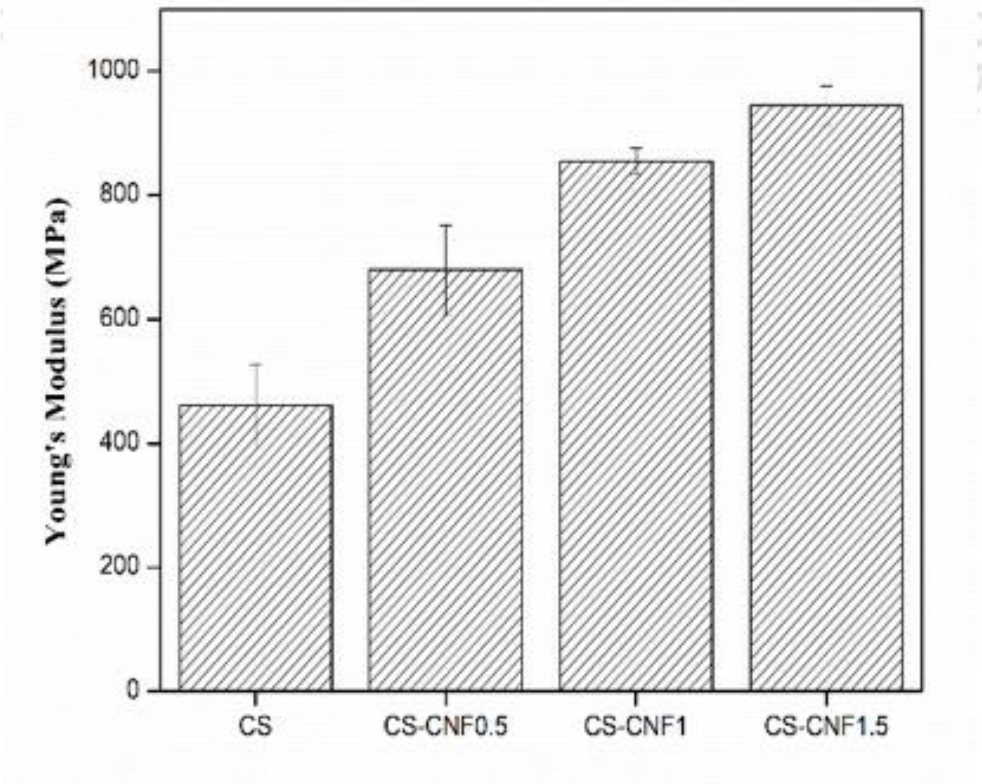

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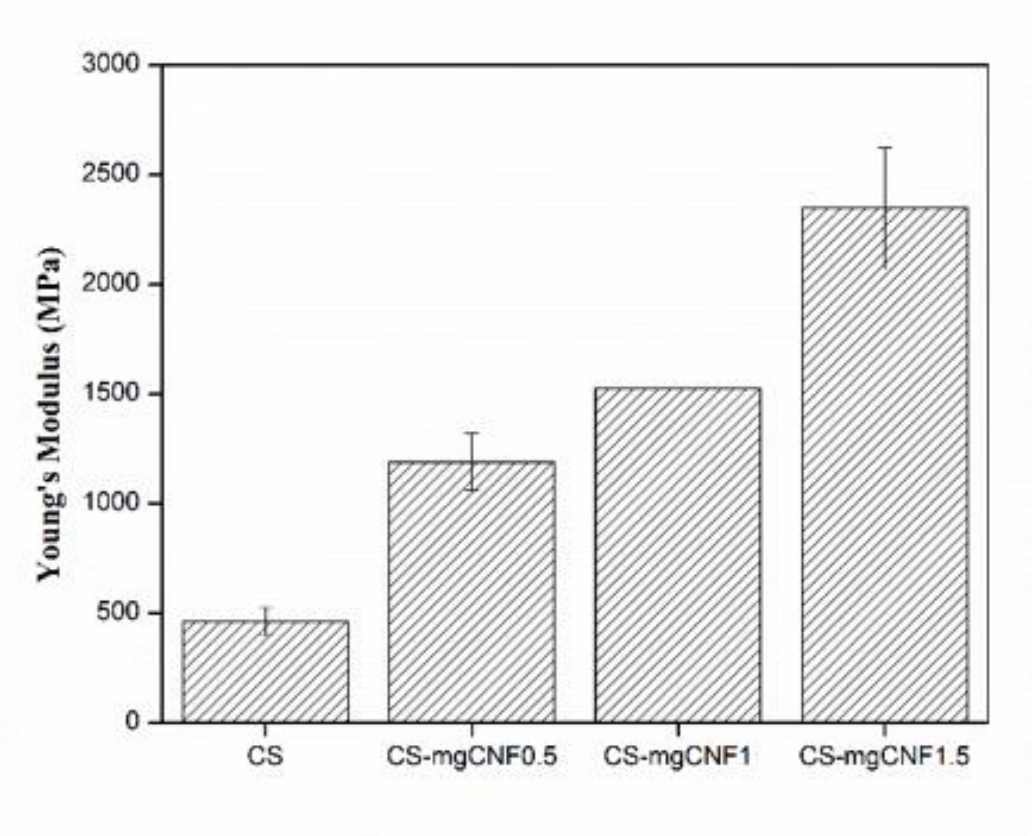


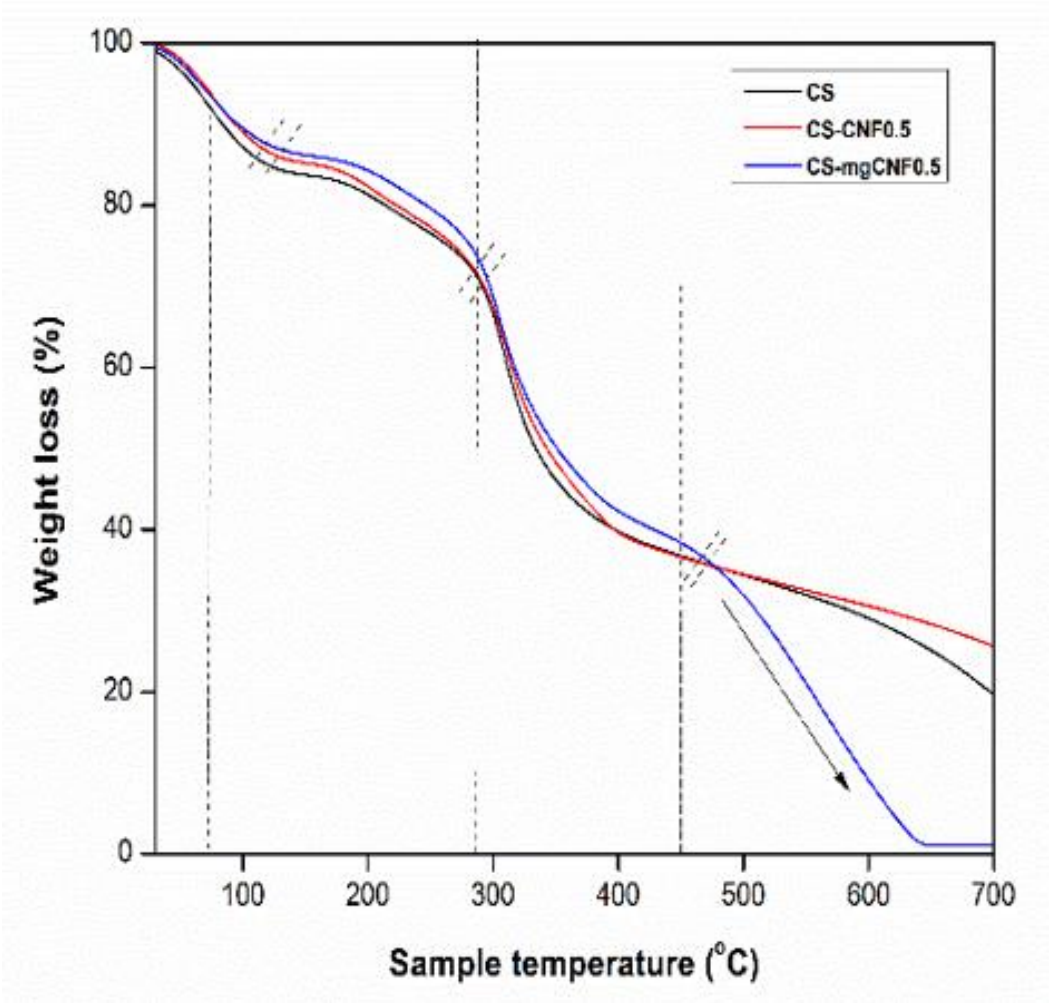
(d)

Figure 3.10 *(a) Tensile strength, (b) Young's modulus of CNF reinforced CS edible materials, and (c) Tensile strength, (d) Young's modulus of mgCNF reinforced CS edible materials.*

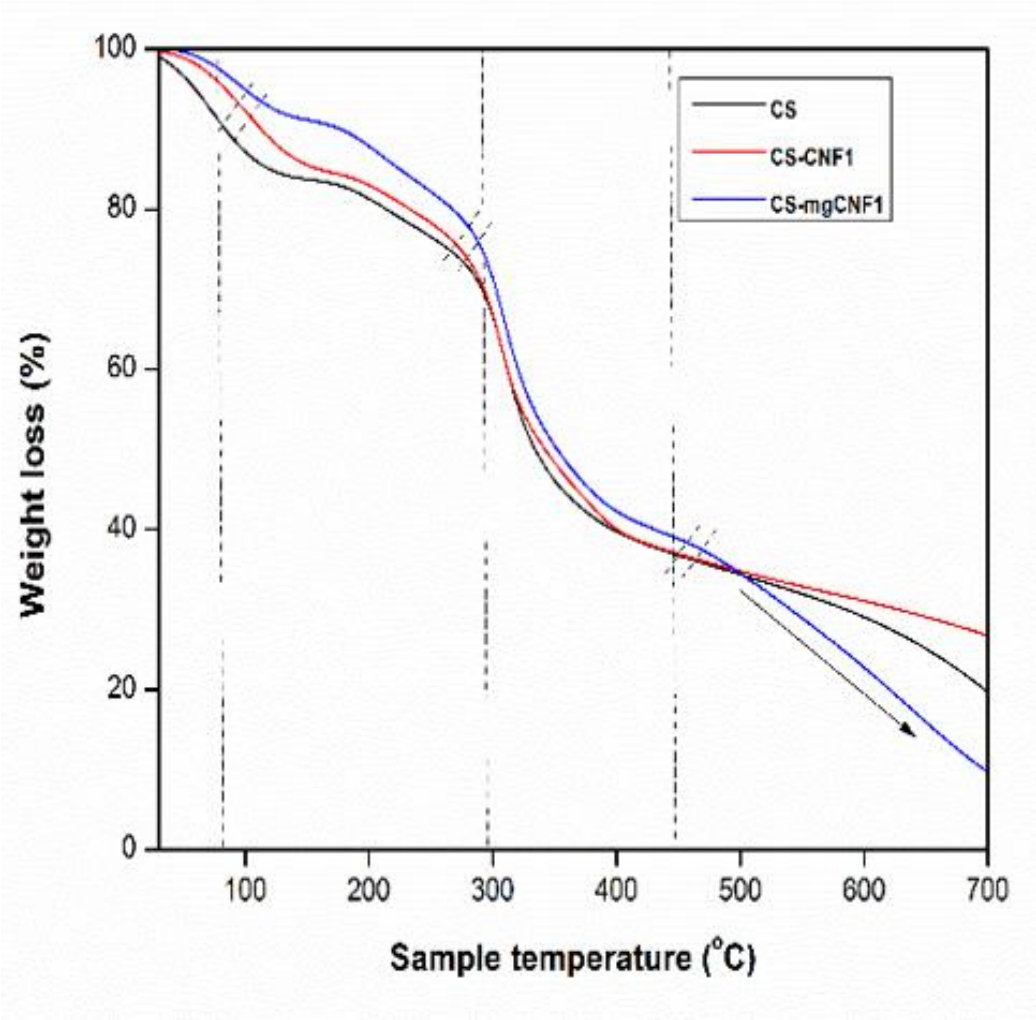
- ***Thermal Properties***

TGA analysis is conducted to understand the effect of reinforcement CNF and mgCNF on the property of CS. The TGA thermogram of CS based biocomposite materials with 0.5 and 1% loadings are outlined as shown in **Figure 3.11**. The TGA analysis of CS generally follows two steps degradation in all cases, which can be categorized in two different temperatures such as (1) 75 to 155 °C and (2) 280 to 480 °C. The first step thermal degradation involves the removal of free and bound water that remains in chitosan and cellulose based materials. The second step degradation involves the degradation of major material including matrix and filler materials. As mentioned earlier, CNF has interacted with CS through hydrophilic sites, which

helps in acquiring improved thermal stability of CNF reinforced CS composite materials. Further, from previous discussion mgCNF is a thermally stable material which improves the thermal property of CS edible coating materials when used as reinforcing materials as shown in **Figure 3.11**. Weight loss percentage at various temperatures varies widely such as 10%, and 50% weight loss temperature, which is denoted as T_{10}, and T_{50}, respectively. The weight loss% of CS coating materials is observed at T_{10}=80 °C, T_{50}=334 °C, which are improved to T_{10}=116 °C, T_{50} =342 °C for CS-CNF0.5, and T_{10}=95 °C, T_{50} =351 °C for CS-mgCNF0.5.



(a)



(b)

Figure 3.11 *TGA thermogram of chitosan/magnetic cellulose nanofibres based nanocomposite materials at (a) 0.5% and (b) 1% loading.*

Swelling Property

The swelling effect of CS based composites is studied at three different pH (pH 4, pH 7 and pH 9) as represented in **Figure 3.12**. Gel swelling study can provide an idea relating to the interaction of packaging materials with different food materials. As it can be depicted from **Figure 3.12** that tendency of gel swelling of edible materials get increased with increase in pH. Further, at acidic pH medium, edible nanocoatings generally tend to shrink due to protonation effect. Addition of filler materials put a decrease in swelling effect of composite material which

is due to formulating percolating network between the matrix and reinforcing agents. The increase in CNF content in CS tends to decrease %gel swelling of coating materials due to forming a cross-linking network between CS and CNF, which improves, and can be correlated with mechanical properties of CS-CNF based coating materials. Swelling property of the edible coating follows the same trend with CS-CNF. The pH condition effects swelling property for the available interacting surface area, protonation effect of materials. The observed %gel swelling of CS is observed approximately at ~300%, 356%, and 388% for pH 4, pH 7 and pH 9, respectively. The %gel swelling of CS-CNF0.5, CS-CNF1 and CS-CNF1.5 is reduced by ~10, 24, and 38%, respectively at pH 4. At acidic pH, there is available protonation lead to ionic attraction making lesser absorption of water molecules. However, though CS and CNF are hydrophilic in nature, the strong interaction between hydroxyl groups of CNF and hydrophilic sites of CS help to reduce the swelling property at different pH condition in comparison to neat CS. Further, %gel swelling of CS-CNF0.5, CS-CNF1, and CS-CNF1.5 is reduced by ~16, 26, 40%, respectively at pH 7. The %gel swelling of CS-CNF0.5, CS-CNF1, and CS-CNF1.5 is reduced by ~5, 15, 20%, respectively at pH 9. The noticeable improvement in %gel swelling of composite at basic pH is observed due probable less attraction between CS and CNF at pH 9, which lead to available space for the solvent. Similar trends have been noticed in case of CS and mgCNF based composite, where with an increase in pH of solution lead to an increase in swelling effect of the biocomposite. The observed %gel swelling of CS-mgCNF0.5, CS-mgCNF1 and CS-mgCNF1.5 is reduced by ~6, 20, and 38%, respectively at pH 4.

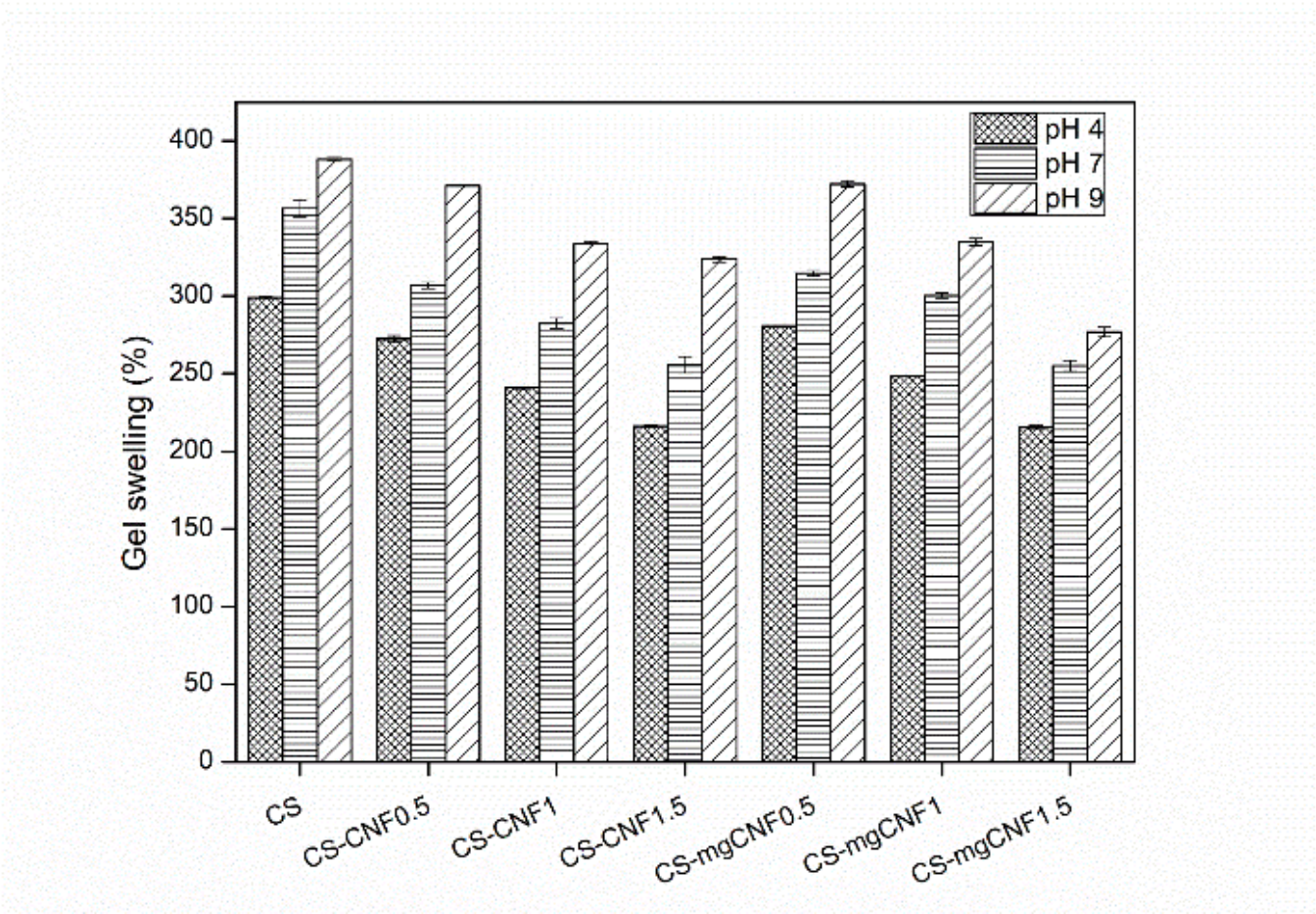


Figure 3.12 *Swelling property of chitosan/magnetic cellulose nanofibres based nanocomposite materials.*

Optical Property

The optical property of packaging materials is a very crucial factor in the field of food packaging application. Optical properties of food products generally involve transparency and color coordinates (L, a*, b*). The transparency of packaging materials is generally determined for the region of Ultra-violet (200–400 nm) and Visible (400–700 nm) ranges. There are various food products which can be degraded by the exposure to UV radiation and visible range. Food components such as protein, lipid, and fat undergo oxidation when exposed to light, so reduce transparency can put an advantageous effect on the packaging of food products. As

shown in **Figure 3.13,** the transparency of neat CS is reached to ~85%, which is reduced to ~71%, 69%, 64%, 42%, 33% and 18% for CS-CNF0.5, CS-CNF1, CS-CNF1.5, CS-mgCNF0.5, CS-mgCNF1, and CS-mgCNF1.5 respectively in UV region. In visible region, there is also a reduction in transparency in comparison to neat CS due to filler effect. Further, the dark brown coloration of mgCNF fillers put a considerable effect on acting as a safeguard against UV and visible rays. The transparency of composites is reduced to ~81%, 76%, 71%, 67%, 55%, and 45% in comparison to neat CS (89%), respectively. Further, the color properties of edible nanomaterials are detailed in supporting information (**Table 3.3**).

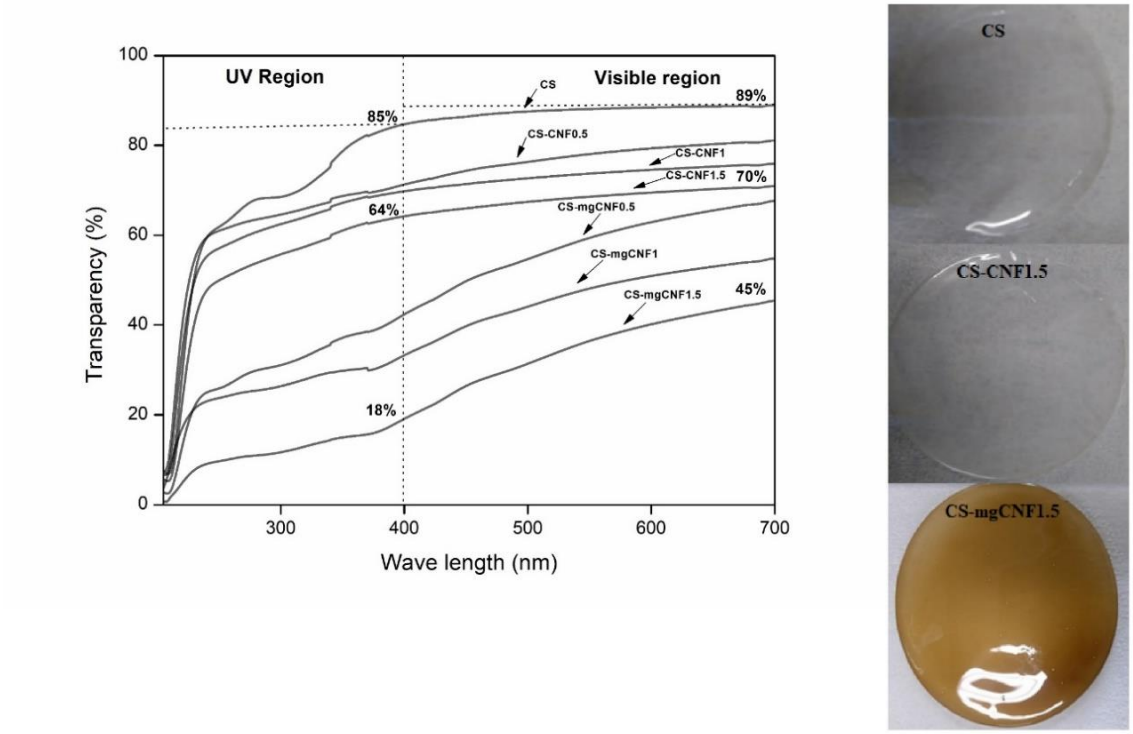


Figure 3.13 *Optical properties of cellulose nanofibres and magnetic cellulose nanofibers dispersed chitosan edible nanomaterials.*

Color Measurement

Color coordinates including L, a*, b* and hue value in food division commonly describes the quality, property, and competence of food produces. In addition, color parameters provide a safety tool for determining product adulteration, the stability of pigment materials, browning effect, and others. The color factors are greatly affected by incorporation of filler materials in the polymer matrix as shown in **Table 3.3**. The observed L value of neat CS film is approximately observed at ~89, which is reduced by ~ 0.3%, 0.5%, 0.8%, 12%, 37%, and 210% for CS-CNF0.5, CS-CNF1, CS-CNF1.5, CS-mgCNF0.5, CS-mgCNF1, and CS-mgCNF1.5, respectively. The decrease in L provides an idea about the loading of filler materials in the composite films. Further, less L value can provide a barrier against light, which helps further reducing lipid based oxidation and a considerable reduction in L value is noticed for CS-mgCNF1.5 which is due to coloring effect of mgCNF filler material. In addition, CS has a "a*" value of ~0.8, which is altered by 3.6, 15, 76, 74, 86, and 89% for CS-CNF0.5, CS-CNF1, CS-CNF1.5, CS-mgCNF0.5, CS-mgCNF1, and CS-mgCNF1.5, respectively. The increase in a* values can be related to increasing filler materials which impart red coloration effect to films. Similarly, "b*" values for neat CS film is ~8 which is further altered by ~1, 10, 13, 52, 42, and 524% for CS-CNF0.5, CS-CNF1, CS-CNF1.5, CS-mgCNF0.5, CS-mgCNF1, and CS-mgCNF1.5, respectively.

Table 3.3 *Color properties of chitosan/magnetic cellulose nanofibres based edible nanocoatings.*

Sample name	**L value**	**a* value**	**b* value**
CS	89.16±0.02	0.8±0.02	8.08±0.10
CS-CNF0.5	88.93±0.04	0.83±0.03	8.16±0.16
CS-CNF1	88.70±0.04	0.69±0.02	9.1±0.14
CS-CNF1.5	88.49±0.05	0.45±0.01	10.54±0.14
CS-mgCNF0.5	78.94±0.54	3.08±0.14	22.07±0.58
CS-mgCNF1	64.87±2.62	6.13±1.09	15.47±1.77
CS-mgCNF1.5	28.69±0.38	7.34±0.84	2.48±0.66

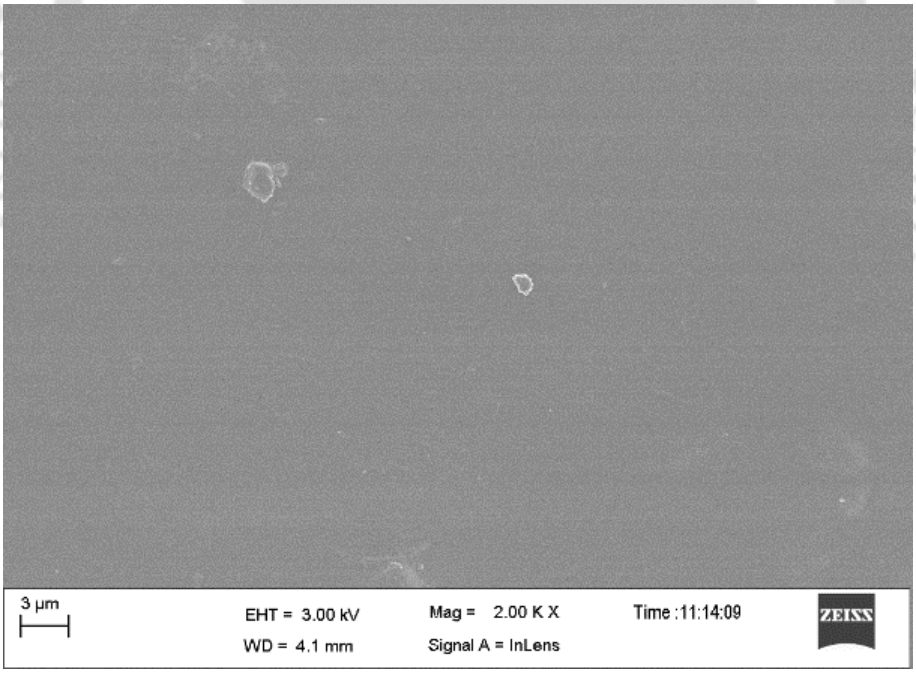
Further, the effectiveness of the developed nanofiller in surface morphology, physicochemical property, *in vitro* studies of the biocomposite based edible coating has been discussed in the next section.

3.2.3 Curcumin Doped Cellulose Nanofibers and Magnetic Cellulose Nanofibre Dispersed Chitosan based Edible Nanocoating: Fabrication and Characterization

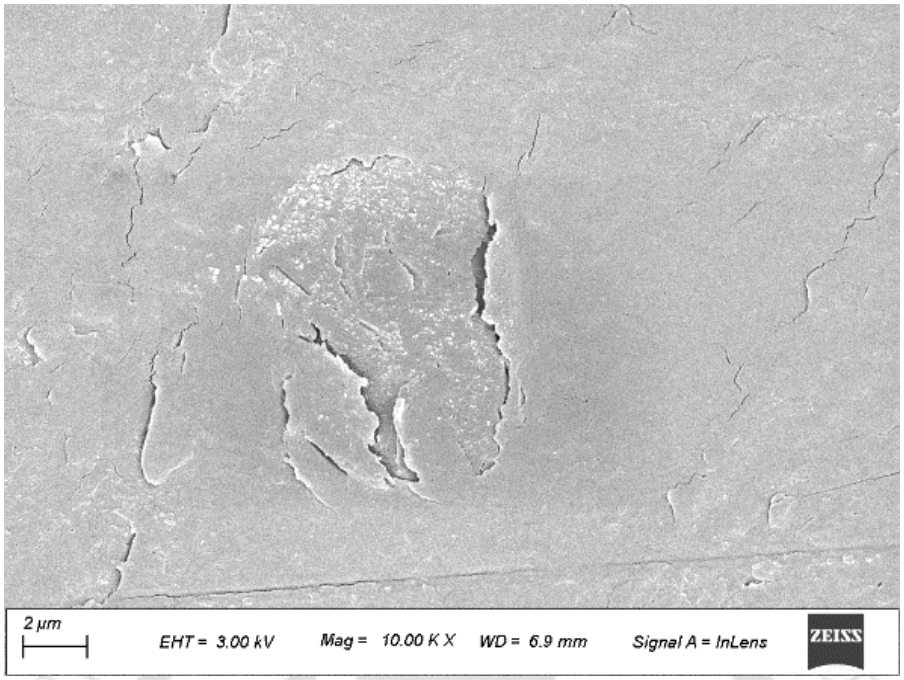
Surface Morphology

As shown in **Figure 3.14,** the FESEM micrographs of different film specimens are taken to observe the changes in the surface morphology of the edible coating materials. The CS based films have homogeneous, and relatively smooth surface morphology without any crack and micro-pore (**Figure 3.14a**). The addition of Cur provides a different type of surface texture such as an uneven surface of the CS film matrix with some cracks on the film morphology (**Figure 3.14b**). Further, the incorporation of CNF into the CS based matrix emerges a percolation network type structure with an uneven surface (**Figure 3.14c** and **c´**). The addition of Cur in CNF reinforced CS based films show an aligned arrangement of Cur on the CS surface unlike the CS-Cur based coating materials. Further, the incorporation of mgCNF provides a

lamellar and heterogonous surface morphology with a cloudy appearance. Interestingly, in comparison to the CS-CNF1.5-Cur based films, the CS-mgCNF1.5-Cur edible coating materials has more aligned structure. The added Cur is found to be superimposed with the nanofiller materials in the matrix which is clearly visible from the FESEM micrographs. Further, in all the FESEM micrographs, no visible agglomeration has been observed on the surface of the films, which proves the uniform distribution and interactions of the matrix and nanofiller materials. In this regards, the better dispersion and networking and aligned microstructure have been found in the developed edible coating materials with and without the aid of Cur.



(a)



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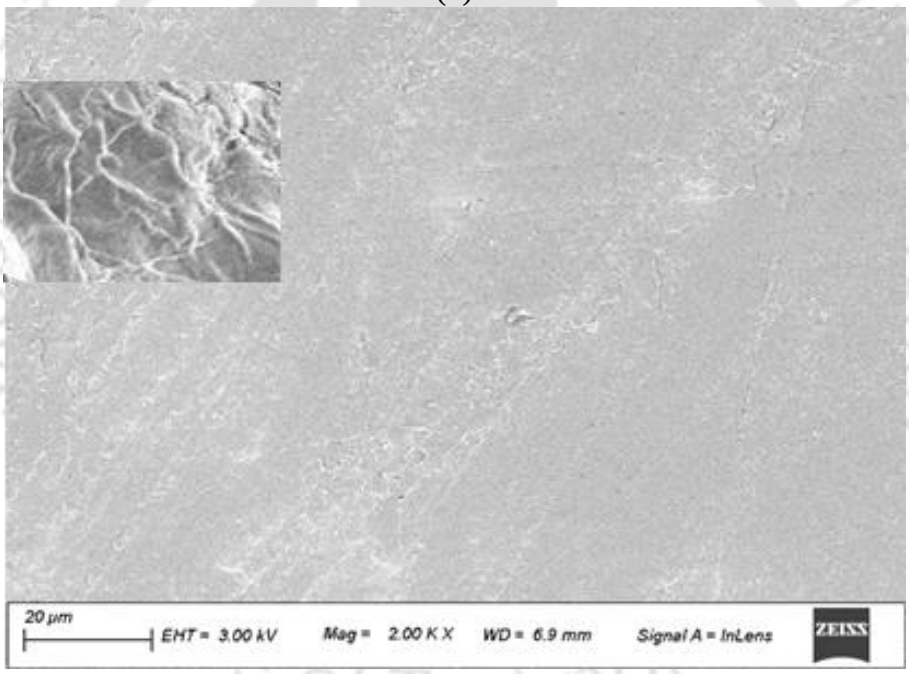
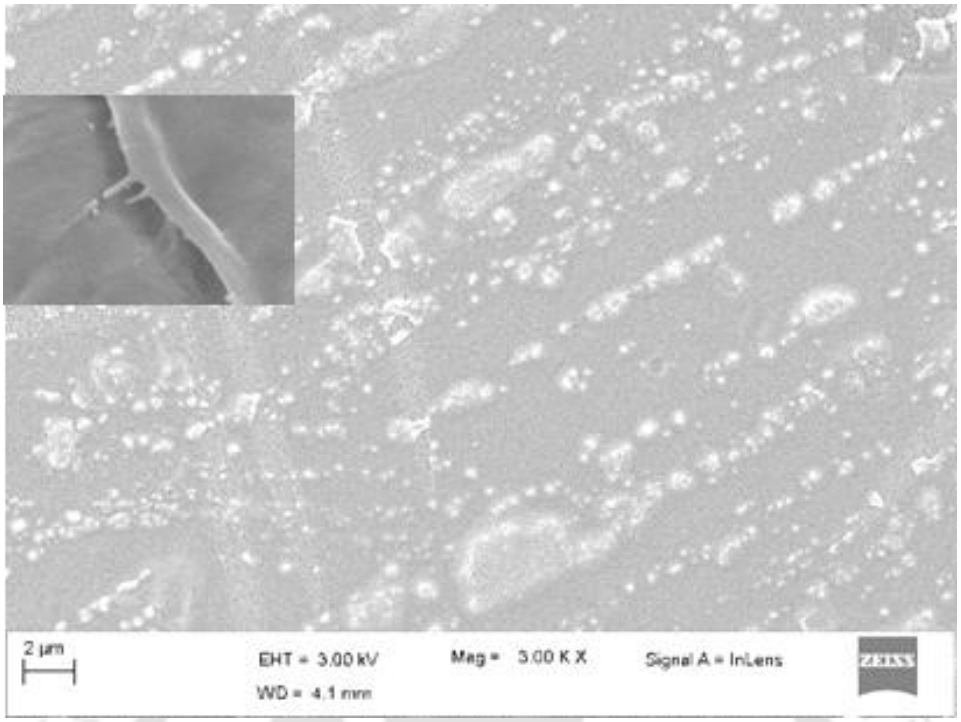
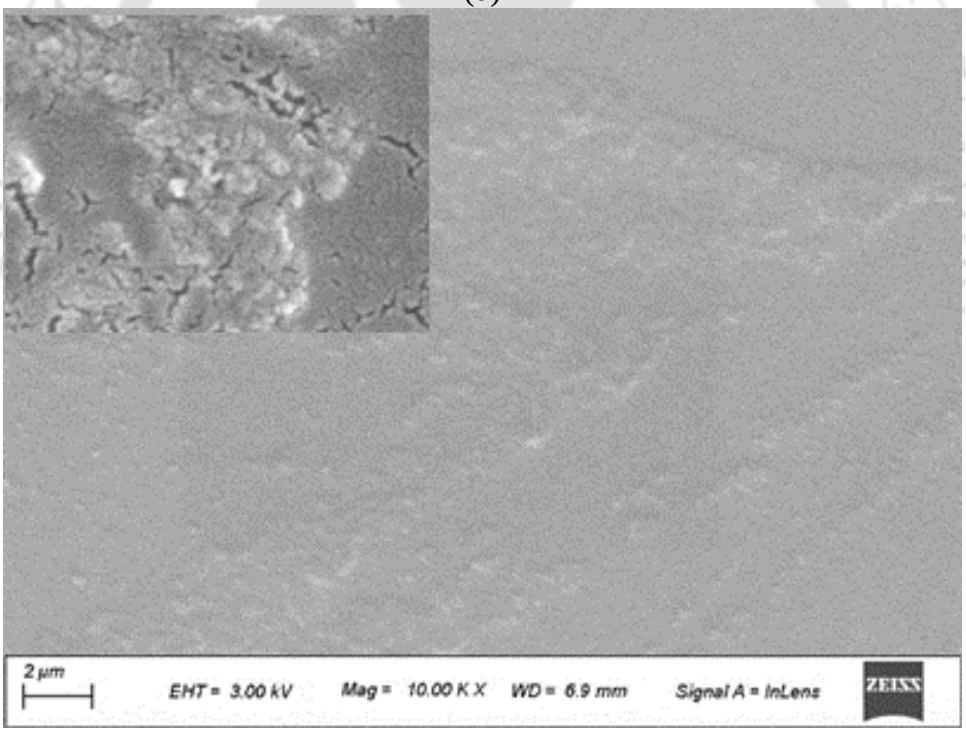


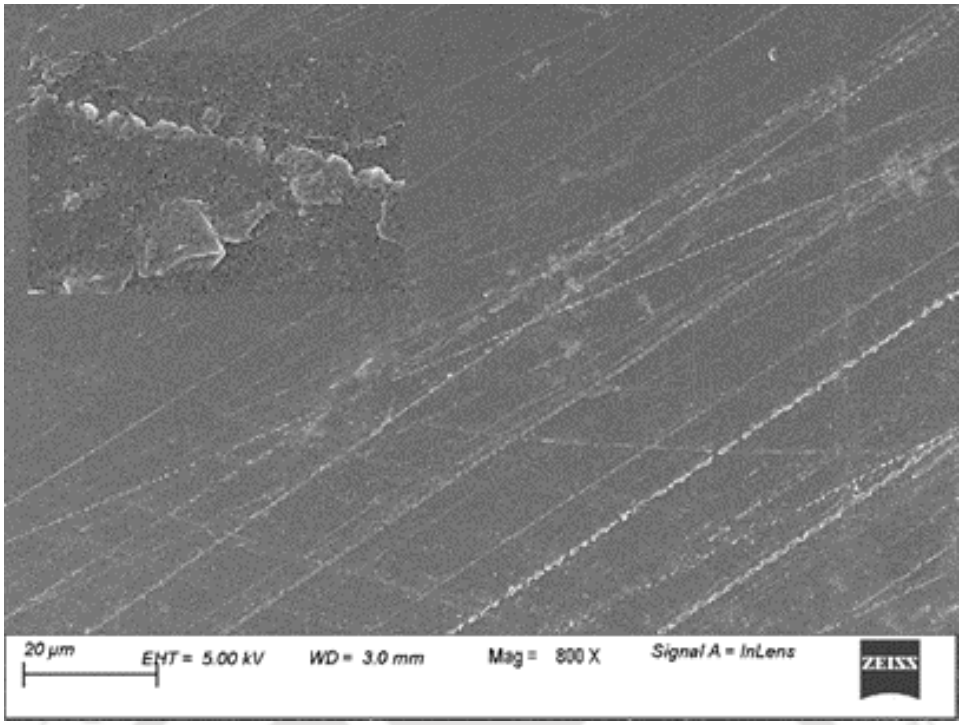
(c)



(d)



(e)



(f)

Figure 3.14 *FESEM micrographs of edible coating materials (a) CS; (b) CS-Cur, (c) CS-CNF1.5, (d) CS-CNF1.5-Cur, (e) CS- mgCNF1.5, and (f) CS- mgCNF1.5-Cur.*

Physicochemical Properties

The incorporation of Cur may significantly affect the physicochemical properties of various films (Musso, Salgado & Mauri, 2017). The addition of Cur loading, CNF, and mgCNF significantly affect the physicochemical properties of the edible nanocoating in terms of thickness, moisture content, and water solubility as shown in **Table 3.4**. The addition of both nanofillers such as CNF and mgCNF has increased the thickness of CS based biocomposites. There is no significant difference in thickness of CS based biocomposites without Cur, however, the incorporation of Cur has observed to increase the thickness of the films. Further, the moisture content for all the CS based biocomposite films are almost in the similar ranges, however, the films with Cur has a reduced moisture content. Similarly, the moisture content of

the developed films is found to be in the range of 18 to 25 % with and without Cur. Thus, the fillers and Cur addition do not provide much effect on the moisture content of the films. However, the water solubility of the films decreased with Cur loading, which may be due to less soluble nature of Cur in water. A significant reduction is observed in the water solubility values of the Cur loaded CS based biocomposite films than the other biocomposite films.

Table 3.4 *Thickness, moisture content, and water solubility of curcumin based films with or without curcumin using water and hydroalcoholic solution. Data sets are represented as mean ± SD (n=5). Different letters define the significant differences between samples (p<0.05)).*

Sample name	Thickness (µm)	Moisture content (%)	Water solubility (%)
CS	13.1 ± 1.3^{c}	24.6 ± 0.1^{a}	34.8 ± 0.6^{a}
CS-CNF0.5	15.9 ± 1.9^{c}	22.9 ± 0.3^{ab}	31.9 ± 0.2^{bc}
CS-CNF1	18.1 ± 4.3^{c}	22.4 ± 0.8^{ab}	30.3 ± 0.1^{bc}
CS-CNF1.5	17.9 ± 3.3^{c}	20.9 ± 1.2^{bc}	29.1 ± 0.3^{c}
CS-mgCNF0.5	16.7 ± 1.1^{c}	22.5 ± 0.6^{ab}	32.4 ± 1.2^{b}
CS-mgCNF1	14.9 ± 1.7^{c}	21.9 ± 0.5^{bc}	30.8 ± 1.0^{bc}
CS-mgCNF1.5	16.9 ± 2.1^{c}	20.8 ± 0.9^{bc}	28.7 ± 1.5^{cd}
CS-Cur	28.7 ± 1.2^{b}	20.7 ± 1.6^{bc}	30.7 ± 0.9^{bc}
CS-CNF0.5- Cur	38.0 ± 4.3^{a}	20.2 ± 1.6^{bc}	29.5 ± 2.3^{bc}
CS-CNF1- Cur	28.7 ± 1.1^{b}	19.2 ± 1.5^{d}	27.6 ± 1.8^{cd}
CS-CNF1.5- Cur	32.9 ± 2.2^{ab}	18.7 ± 1.5^{d}	24.9 ± 0.5^{d}
CS-mgCNF0.5- Cur	37.3 ± 3.8^{a}	20.2 ± 1.9^{bc}	28.2 ± 2.7^{cd}
CS-mgCNF1- Cur	30.5 ± 3.5^{b}	18.9 ± 2.1^{d}	25.9 ± 2.2^{d}
CS-mgCNF1.5- Cur	31.1 ± 1.6^{b}	18.5 ± 1.9^{d}	25.2 ± 2.4^{d}

Thermal Properties

The thermal properties of Cur loaded CS/CNF nanocomposites with various proportions of biofiller materials are studied using TGA analysis at a heating rate of 10 °C/min. The TGA thermogram for the samples Cur, CS-Cur, CS-CNF0.5-Cur, CS-CNF1-Cur, CS-mgCNF0.5-Cur, and CS-mgCNF1-Cur are represented in **Figure 3.15**. The thermal stability behavior of the developed samples is investigated to predict the onset degradation temperatures of the Cur loaded films. Further, the various thermal parameters in terms of T_{10} (°C): Onset temperature at 10 % weight loss, T_{50} (°C): Onset temperature at 50 % weight loss, and W_{700} (%): Weight residue at 700 °C are also determined as shown in **Table 3.5**. The Cur powder shows a single step thermal degradation with a T_{10} and T_{50} of 307.6 °C and 423.9 °C, respectively. However, Cur is a heat stable component showing a weight residue of 32.27 % at 700 °C. An approximately similar kind of behavior has also been observed for Cur in some reported studies (Luo et al., 2012). The thermal decomposition of Cur within the range of 30 to 700 °C occurs in a single stage mass loss, where the final mass weight of 32.27 % is obtained as black colored material. The Cur may contain other components with it such as sugar and sucrose molecules, which turned into the formation of carbon in the final product attributing to the ash components. However, cur loaded nanocomposites of CS show further improvement in the thermal properties, where a two stage thermal degradation has been observed. The cur loaded CS based nanocomposite films have been observed to improve the thermal properties, which is an advantageous property for food packaging. The materials can further be used for processing at high temperatures to provide different forms of inedible packaging materials. However, an increase in mgCNF based filler improves the thermal properties, however, there has been observed a decrease in the values of T_{10} and T_{50} with an increased biofiller percentage which may be due to the formation of agglomerates in between the filler materials. The onset degradation temperatures in terms of T_{10} for CS-Cur, CS-CNF0.5-Cur, CS-CNF1-Cur, CS-

mgCNF0.5-Cur, and CS-mgCNF1-Cur are 78.6, 86.3, 90.8, 88.5 and 82.3 °C, respectively. The nanofiller added CS composites with Cur have a significant improvement in the thermal stability of the films, which can be utilized for heat sterilization food items. Thus, the use of biofillers such as CNF and mgCNF at various proportions through solution casting approach provide an improvement in thermal stability of CS based materials. Additionally, the onset degradation temperature of CS-Cur, CS-CNF0.5-Cur, CS-CNF1-Cur, CS-mgCNF0.5-Cur, and CS-mgCNF1-Cur at T_{50} are 323.6, 336.9, 336.7, 346.7, and 342.8 °C, respectively. There has been found an increase in thermal properties when nanofiller materials are added. The Cur loading has improved the properties of biocomposites, which shows a good interaction within the components. Thus, the thermal stability of the cur loaded CS films has been found to improve with the addition of nanofiller materials, which is a desirable property for developing Cur loaded nanocomposite films.

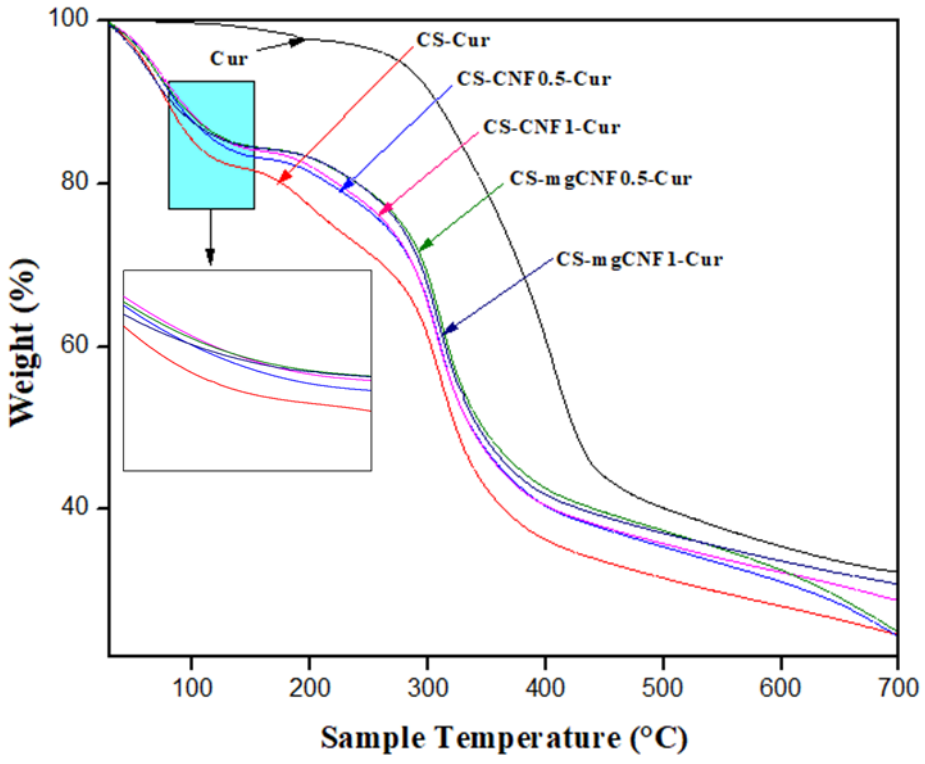


Figure 3.15 *TGA thermogram of curcumin loaded chitosan based biocomposite films.*

Table 3.5 *Thermal properties of curcumin loaded chitosan based nanocomposites.*

Sample name	**T_{10} (°C)**	**T_{50} (°C)**	**W_{700} (%)**
Cur	307.6	423.9	32.3
CS-Cur	78.6	323.6	24.6
CS-CNF0.5-Cur	86.3	336.9	24.4
CS-CNF1-Cur	90.8	336.7	28.8
CS-mgCNF0.5-Cur	88.5	346.7	24.7
CS-mgCNF1-Cur	82.3	342.8	30.8

Appearance and Optical Properties

The Cur loaded biocomposites of CS based edible nanocoating materials are developed using aqueous dispersion which provides flexible film materials. The visual appearance of the films is shown in **Figure 3.16**, where the addition of Cur provides yellow colorants effect to the materials. However, the use of hydroalcoholic solution for the preparation of Cur based films helps to make them soluble in water providing more transparent appearance (**Figure 3.16**). Further, it is noteworthy to mention that the more intense yellow coloration in the developed coating materials with transparent nature may be obtained due to Cur's solubility in the medium with proper dispersion of filler materials.

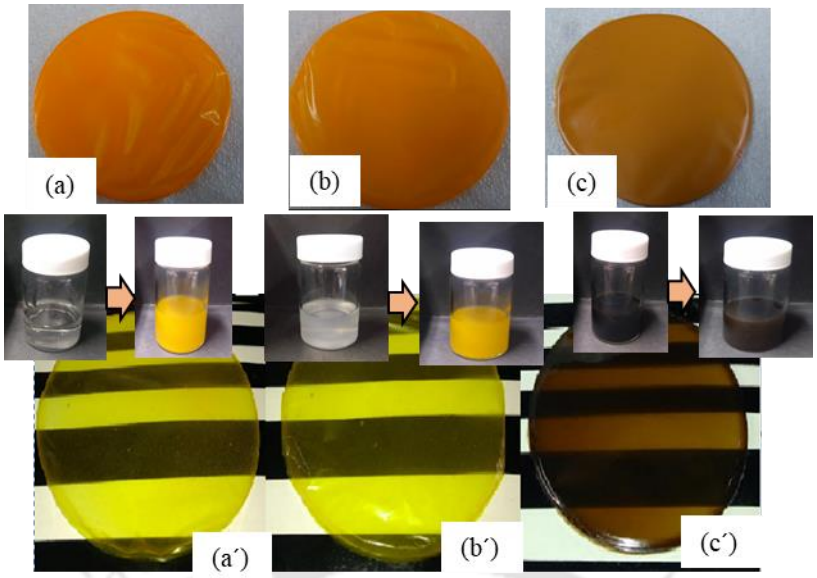


Figure 3.16 *Visual Appearance of CS-Cur (a), CS-CNF1-Cur (b), CS-mgCNF1-Cur (c) films prepared using water as a solvent; CS-Cur (a´); CS-CNF1-Cur (b´); CS-mgCNF1-Cur (c´) films prepared using ethanol-water mixture as a solvent; CS-Cur (a"); CS-CNF1-Cur (b").*

Color Properties

Additionally, the color parameters such as L, a*, b*, hue and chroma values are represented in **Table 3.6**. The use of various filler materials affects the color parameters, where the use of mgCNF significantly reduces the L in comparison to the neat Cur loaded CS films as shown in **Table 3.6**. CS films with various proportions of biofillers have very less significant differences in L values. The L values provide the darkness (L values less than 50) to brightness (L values more than 50) of the edible nanocoating materials. In this regard, it is observed that all the film samples are bright for having L values of more than 50. There has been found a discontinuity in the color values of the various composites, which may be due to the agglomeration of the used fillers in the preparation of biocomposites. However, the L values are found to decrease with the addition of mgCNF as a filler material. On the other hand, the addition of CNF improves the brightness of the films, however, there has been shown a slight

improvement in the L values with CS-CNF1-Cur and CS-CNF1.5-Cur by 1.1 and 0.8 %, respectively, in comparison to CS-Cur films. Further, all the Cur loaded CS based biocomposites show a red coloration effect which can be known from their positive a* values. All the a* values of the films are in close proximity, however, CS-mgCNF1.5-Cur has shown an intense increment of a* values in comparison to others, which is due to the coloration effect by mgCNF loadings. The changes in the color coordinates do not show a consistent value with the filler loadings of same kind, which may be due to agglomeration within the filler materials. Further, the hue and chroma values are also within the close proximity. The cur loaded films provide a red and yellow colorant effect which can be well known from the values of a*, and b* values.

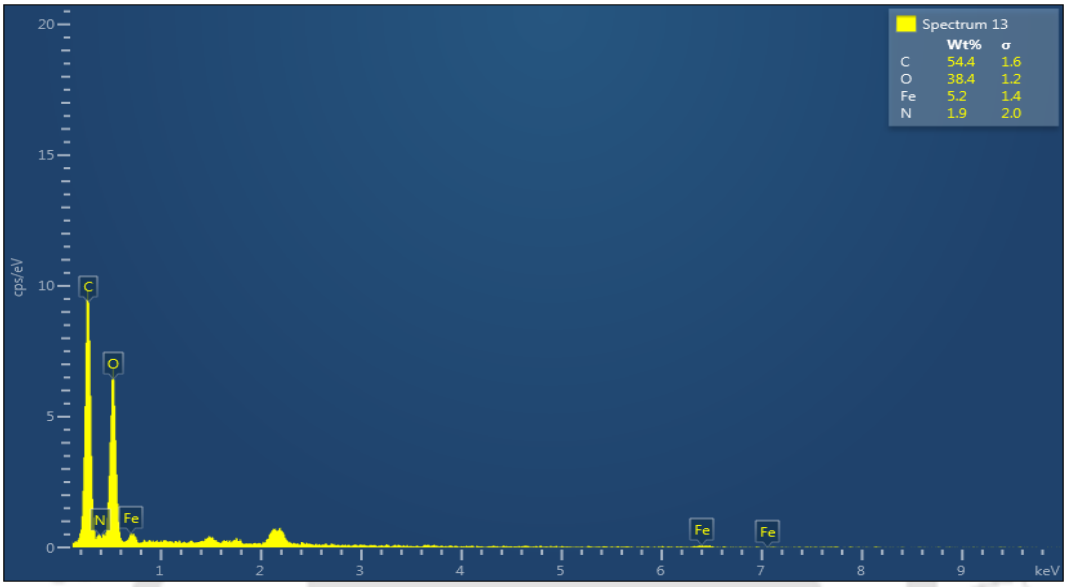
Table 3.6 *Color properties of curcumin loaded chitosan biocomposites. Data sets are represented as mean ± SD (n=5). Different letters define the significant differences between samples ($p<0.05$)).*

Sample name	L	a*	b*	Chroma	Hue angle
CS-Cur	83.6 ± 0.1^{a}	3.2 ± 0.1^{d}	44.8 ± 0.9^{c}	44.9 ± 0.9^{c}	85.9 ± 0.1^{b}
CS-CNF0.5-Cur	79.9 ± 1.3^{b}	7.2 ± 1.1^{b}	54.5 ± 1.5^{a}	54.9 ± 1.6^{a}	82.5 ± 0.9^{d}
CS-CNF1-Cur	84.7 ± 0.3^{a}	1.4 ± 0.1^{e}	40.9 ± 0.3^{d}	40.9 ± 0.3^{d}	88.0 ± 0.2^{a}
CS-CNF1.5-Cur	84.4 ± 0.1^{a}	2.0 ± 0.1^{de}	42.0 ± 0.6^{c}	42.1 ± 0.5^{d}	87.2 ± 0.1^{a}
CS-mgCNF0.5-Cur	78.4 ± 0.5^{bc}	5.7 ± 0.2^{c}	50.7 ± 0.4^{b}	50.9 ± 0.4^{b}	83.6 ± 0.2^{c}
CS-mgCNF1-Cur	77.9 ± 0.5^{c}	4.6 ± 0.4^{c}	41.6 ± 1.7^{bc}	41.9 ± 1.7^{d}	83.7 ± 0.3^{c}
CS-mgCNF1.5-Cur	69.9 ± 1.8^{d}	10.5 ± 1.4^{a}	51.5 ± 1.9^{b}	52.6 ± 2.1^{ab}	78.5 ± 1.1^{e}

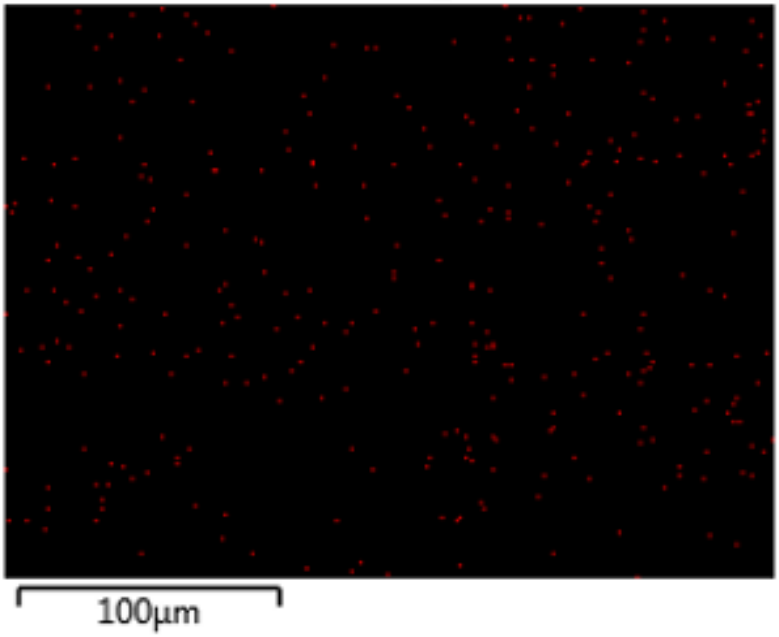
EDX Spectra

The EDX spectra and mapped FESEM images of CS-mgCNF1.5-Cur are represented in **Figure 3.17**, where, the presence of carbon, oxygen, nitrogen and iron components at various percentages are obtained. The EDX spectra further confirms the presence of iron in uniformly

dispersed form in the developed films (**Figure 3.17**). The estimated amounts of C, O, Fe, and N in the developed edible nanocoating materials are 54.4, 38.4, 5.2, and 1.9 %, respectively.



(a)



(b)

Figure 3.17 *EDX spectrum of CS-mgCNF1.5-Cur (a), and elemental mapping of iron (b) in the developed films.*

Biocompatibility Test

Cur is a very effective anticancer, antioxidant, anti-inflammatory, antitumor agent and generally appears as a yellow-orange dye (Refat, 2013; Chen et al., 2020; Hamzehzadeh et al., 2018). Cur is widely used in various types of food products such as instant rice, bread and noodles for its remarkable health beneficial properties. Thus, in the present study, the effectiveness of Cur to be used as an edible coating material is tested by the biocompatibility test before using it as edible nanocoating on food products. The HeLa cell viability on the surface of fabricated biopolymer composites along with control in the presence and absence of Cur drug is determined by MTT assay (**Figure 3.18**). A significant decrease in cell viability is observed in the presence of Cur which indicates the anticancer effect of the drug. This clearly suggests that the biopolymer composites without the addition of drug support the cell growth and the addition of Cur (anti-cancer drug) suppresses the cancer cell viability while releasing the drug during 8 h. Additionally, as viewed from **Figure 3.18**, the CS-mgCNF0.5 is also effective compared to the CS-CNF0.5, where the effectivity can be attributed due to the presence of iron complexes. In this regard, the iron complexes form reactive oxygen species leading to DNA damage (oxidative) (Turner et al., 2005; Wani et al., 2016). Thus, the fabricated materials may thus be considered as candidates for applications involving anti-cancer activity. The adhesion and proliferation of HeLa cells on the surface of the biopolymer composites in the presence and absence of Cur along with control is analysed (**Figure 3.19**). The green color shows the cell morphology after staining with acridine orange stain. The cell adhesion and proliferation are evident on the surface of the biopolymers in the absence of Cur. The cells in the presence of biopolymers with Cur showed altered morphology in terms of disruption of cell membrane, rounding and detachment possibly indicating the cell death in the presence of drug. The mechanism of cell death in presence of Cur has been reported by inhibiting the STAT3 and NF-κB signaling pathways (Vallianou et al., 2015). The combined

use of iron complex and curcumin also provide improved anticancerous activity compared to neat nanofibers for delivering better chemical interactions between iron and curcumin. Further, Cur (a mixture of curcumin, demothoxycurcumin and bisdemothoxycurcumin) is a naturally available β-diketone ligands, which is suitable to be acted as a metal chelating ligand providing several biological properties (Wanninger, et al., 2015). The metal and curcumin complexes always provide several advantages such as anticancerous property (Kareem et al., 2018; Yallapu, et al., 2012). In this context, the iron fortified cur films provide improved anticancer properties and synergistic effects. Further, similar results have been found to be obtained by Patwa et al., (Patwa et al., 2018) which strongly support the present results of providing synergistic effects.

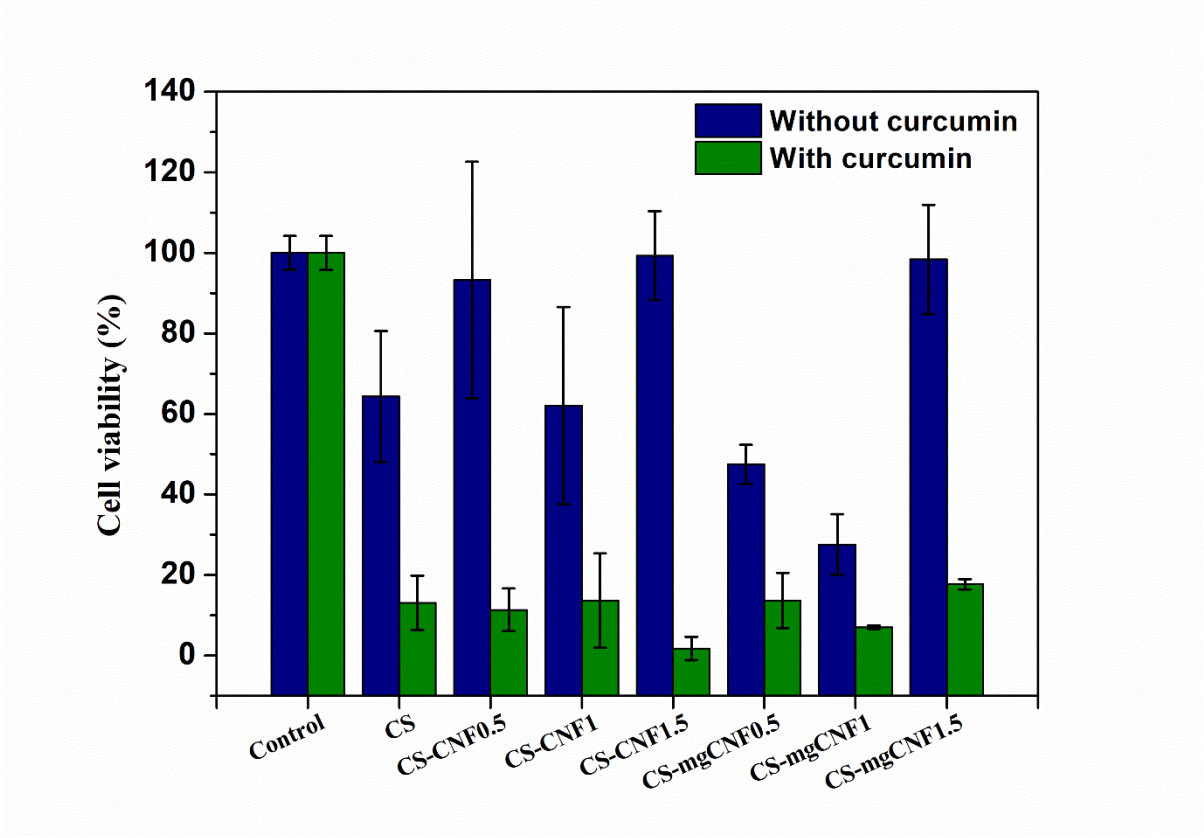


Figure 3.18 *Effect of Cur on the cell viability of HeLa cells on the surface of fabricated CS based biocomposites. Data sets are represented as mean ± SD (n=3).*

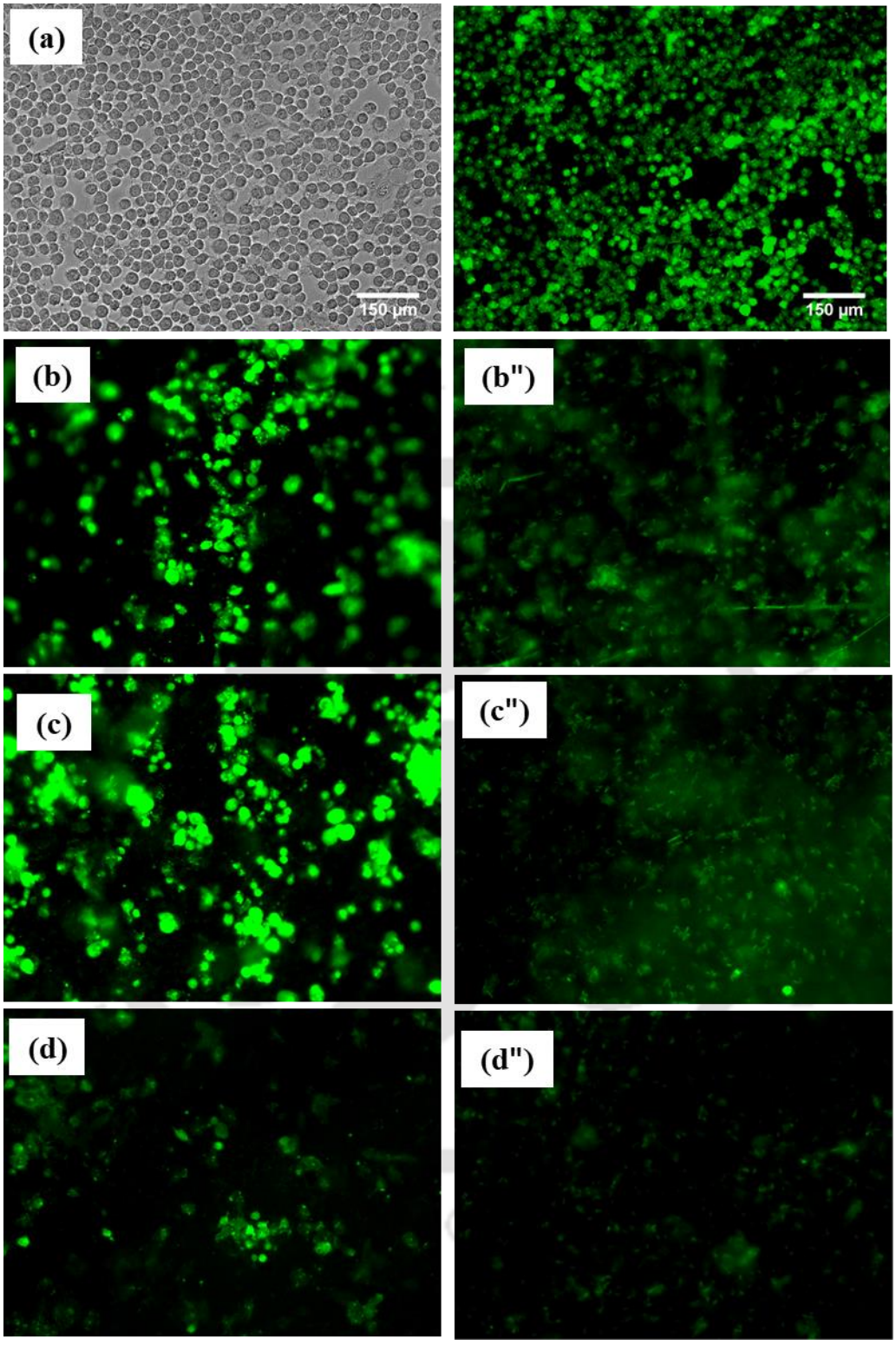


Figure 3.19 *Adhesion and proliferation of HeLa cells on the surfaces of control (a and a"), CS (b), CS-Cur (b"), CS-CNF0.5 (c), CS-CNF0.5-Cur (c"), CS-mgCNF0.5 (d), and CS-mgCNF0.5-Cur (d").*

ICPMS Analysis

The iron contents of CS-mgCNF0.5-Cur, CS-mgCNF1-Cur and CS-mgCNF1.5-Cur are ~1.1 ppm, ~2.2 ppm and ~3.6 ppm as determined using ICP-MS technique, which is within permissible limit (in comparison to the iron content of water) as per WHO guidelines.

3.2.4 Shelf life Analysis of Edible Coated Cut Pineapple

From very early days, pineapple is one of the most eaten tropical fruit products having a pale, and yellowish flesh (Larrauri, Rupérez & Calixto, 1997; Das, Bhat & Gowda, 1997; Brat et al., 2004). Pineapple in the form of slices are stored at frozen conditions, however, it is also consumed as fruit salads, juices, canned slices, etc. (Larrauri, Rupérez & Calixto, 1997). In this regard, the storage life of slices can be tested with different edible coating materials to check the effectiveness of the edible coating materials with or without Cur at low temperature storage. However, at normal environmental condition, the fresh cut food products are very prone to weight loss due to their juice leakage phenomena, thus, low temperature storage can help to reduce the juice leakage from cut pineapple products when coated with edible coating materials. As shown in **Figure 3.20**, the non-coated cut pineapple has a weight loss of ~68 % during its 10 d of storage at 10 °C. However, at an early stage of the storage, coated and control samples maintain the weight which shows the quality maintenance in the food products. The reduction in weight are also related with the environmental degradation of the fruit products. Generally, inclusion of microbial attacks increases the weight loss phenomena in fruit products. In comparison to control cut pineapple samples, all the coated cut pineapple has a reduced weight loss values. However, the application of edible nanocoating materials provide some weight stability and significant effect ($p<0.05$) with increase in storage period. It is noteworthy to mention that the addition of Cur has a synergistic effect in improving the shelf life of cut pineapple fruit products. Among all the coated samples, CS-Cur, CS-CNF1.5-Cur and CS-

mgCNF1.5-Cur coated cut pineapples have a weight loss % of 55, 57 and 56 %, respectively, which provides an ability in reduced weight loss of cut pineapples due to the improved antimicrobial effect. The microbial attack in cut fruit products is a very crucial factor for increased weight loss, where the use of active agents may have a capability in reducing the weight loss phenomena in fruit products. In this regard, the antimicrobial based coating materials can help to prolong the storage life of fruit products, especially perishable products.

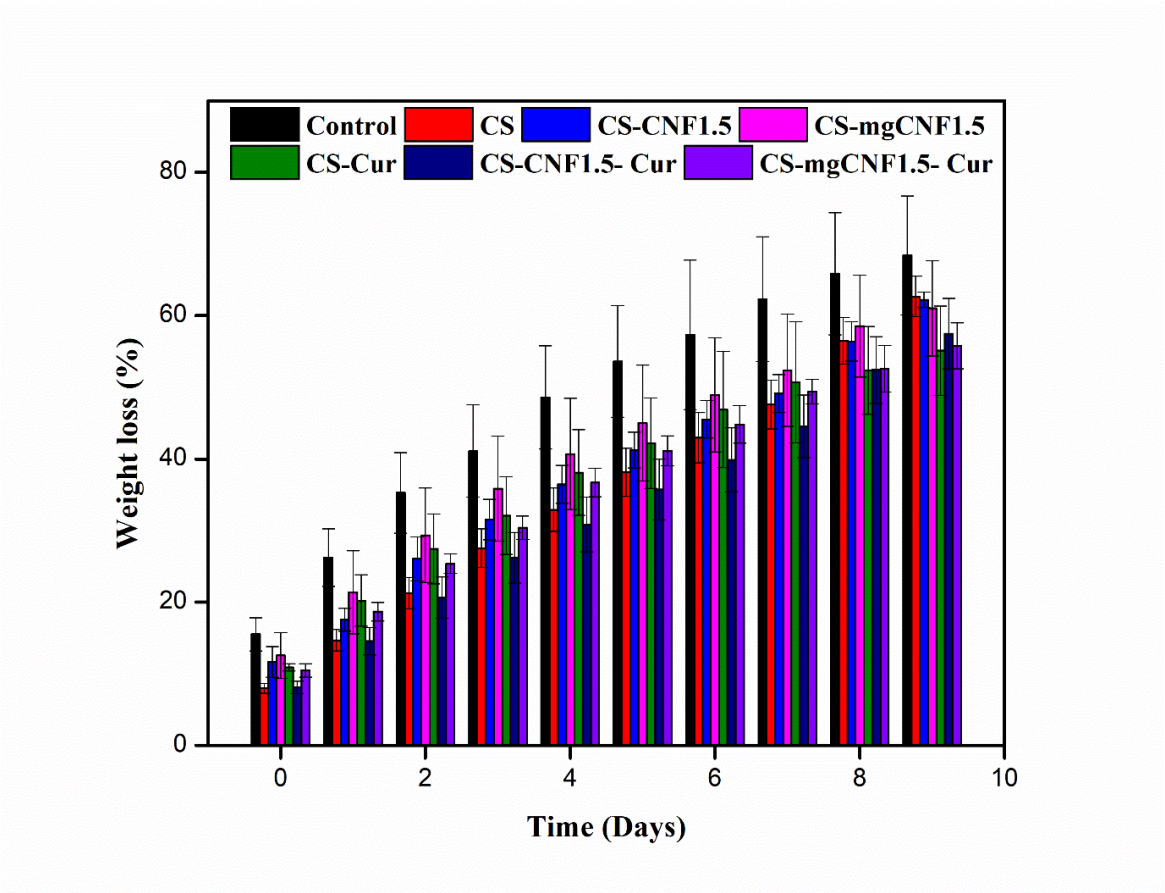
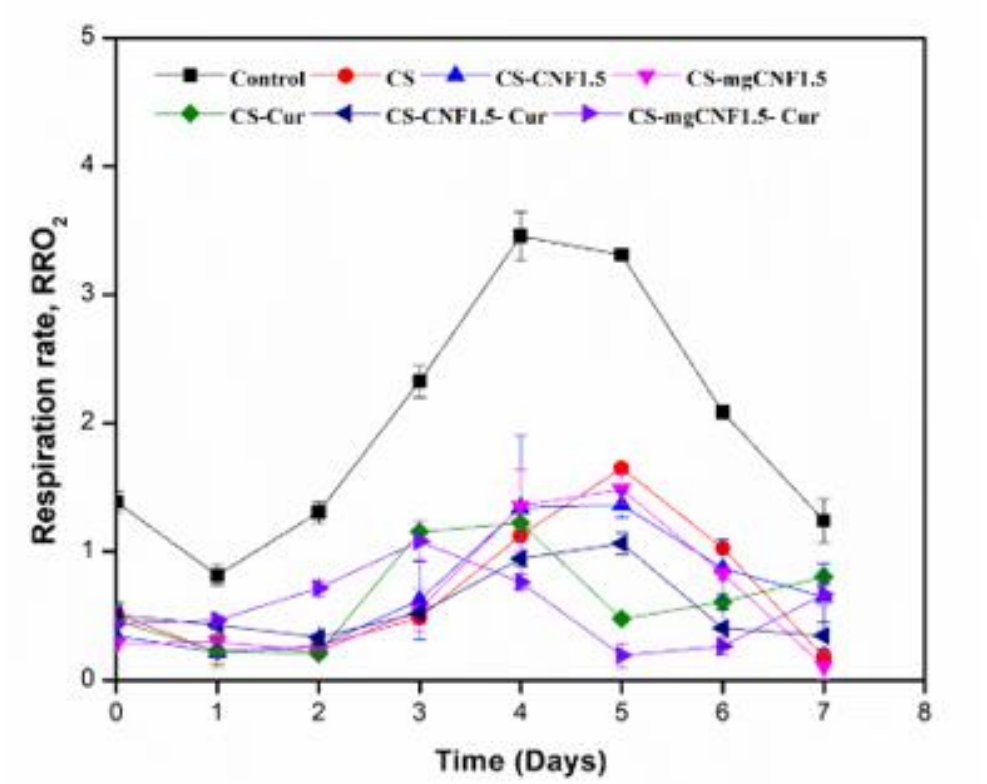


Figure 3.20 *Changes in properties of cut pineapple in terms of Weight loss analysis. Data sets are represented as mean ± SD (n=5).*

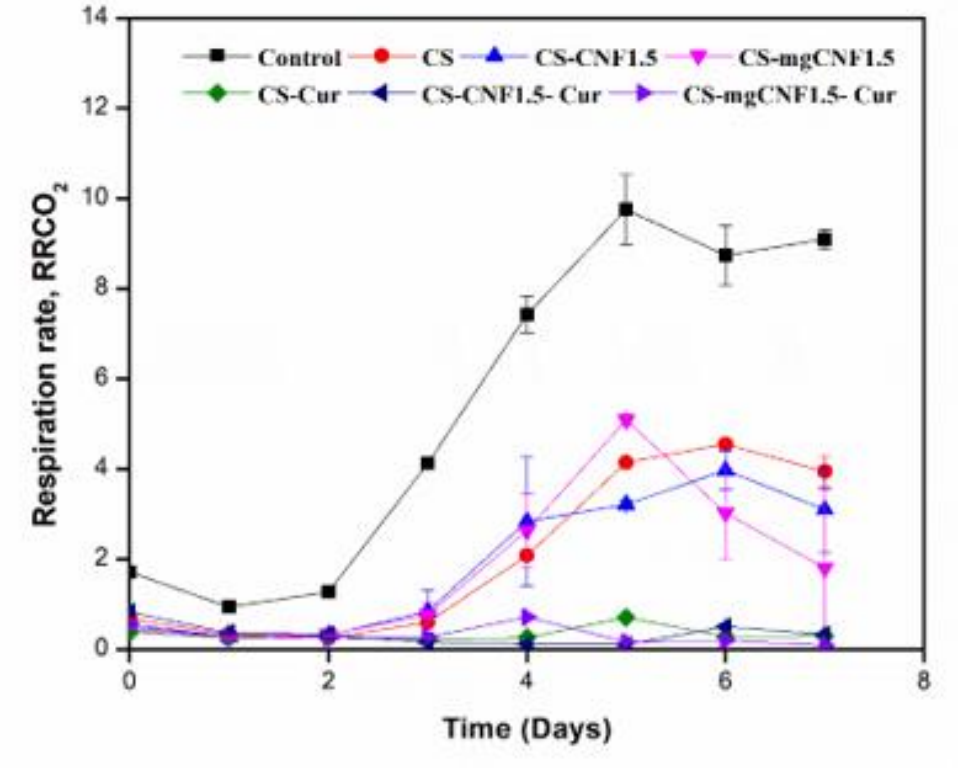
Respiration Rate

Respiration rate in fruit and vegetables is considered as one of the most crucial phenomenon responsible for fruit losses. In **Figures. 3.21a and 3.21b**, the respiration rate levels at $p< 0.05$ in terms of O_2 and CO_2 in the headspace during storage are represented. The

respiration rate in terms of O_2 concentration is found to be decreased in all the edible coated fruit products including the control. However, the results obtained in the present investigation are found to have lower respiration rates for all the coated fruit products in comparison to the uncoated one during the storage conditions. In this regard, the selected coating materials help to reduce the respiration rates for all the fruit products. The decreased respiration rate of coated fruit products is due to the creation of a modified atmosphere, which further decreases the gaseous interchange in terms of oxygen and carbon dioxide between storage environment and edible coated fruit products. The results obtained in the current research shows that the respiration rates for the coated pineapple sample with Cur-CS-mgCNF1.5 are reduced in terms of carbon dioxide at later stages also. The use of bioactive components Cur is very effective with neat CS and mgCNF dispersed CS films, where the respiration rate in terms of carbon dioxide is less in comparison to other coated food products. The microbial degradation in food products may increase the metabolic activity which in turn increases the respiration rate of stored cut pineapple. As represented in **Table 3.7**, the mesophilic count has been found to reduce due to the addition of Cur, which may help in reduced respiration rate (**Table 3.7**). In this regard, the combination of Cur loaded mgCNF reinforced CS films have provided positive limiting factors in comparison to the other coating materials. However, the used edible coating developed using mgCNF is found to be more effective compared to CNF incorporated composites in reducing the mesophilic count during storage. The plausible reason may include the modified bionanointerfaces between mgCNF and matrix materials compared to other biocomposites. The pineapple tissue respirations are continuing throughout the respiration, which results in decreasing oxygen concentration and an increasing carbon dioxide concentration inside the headspace of all the stored samples.



(a)



(b)

Figure 3.21 *Respiration rate of cut pineapple in terms of oxygen (a); and carbon dioxide (b). Data sets are represented as mean ± SD (n=3). Different letters define the significant differences between samples (p<0.05)).*

Table 3.7 *Mesophilic count of stored edible coated cut pineapple. Data sets are represented as mean ± SD (n=5). Different letters define the significant differences between samples (p<0.05)).*

Sample name	1 day Log CFU/mL	6 day Log CFU/mL	10 day Log CFU/mL
Control	2.90±0.04^{a}	3.79±0.05^{a}	5.47±0.04^{a}
CS	2.49±0.13^{bc}	3.57±0.05^{ab}	4.34±0.04^{bc}
CS-CNF1.5	2.55±0.05^{b}	3.48±0.11^{b}	4.44±0.09^{b}
CS-mgCNF1.5	2.30±0.03^{cd}	3.25±0.04^{cd}	4.23±0.04^{cd}
CS-Cur	2.17±0.09^{d}	3.19±0.07^{d}	4.02±0.05^{e}
CS-CNF1.5-Cur	2.24±0.03^{d}	3.19±0.09^{d}	4.15±0.05^{de}
CS-mgCNF1.5-Cur	2.34±0.03^{cd}	3.08±0.03^{d}	4.14±0.03^{de}

Pineapple being a non- climacteric fruit maintains a total soluble solid content during the complete life cycle. However, at the prolonged storage life, pineapple may be found to have an increase in TSS content. As shown in **Figure 3.22**, the TSS content is found to vary within 12 to 18 %. The uncoated pineapple fruits are found to have more TSS content at prolong storage in comparison to coated cut pineapple fruit products. **Table 3.7** reports the microbial count of mesophilic bacteria at 0, 6, and 10 days of sampling for edible coated and uncoated samples stored at 10 °C. At the initial storage period, all food materials maintain a similar microbial count, however, involvement of Cur show a significant reduction of microbial count in comparison to the other fruit products (Khorshidi et al., 2018; da Silva et al., 2017). The mesophilic count generally occurs at a medium temperature, thus, when the fruit products are allowed to stay at medium temperature, and then there may be chances of growing mesophilic count.

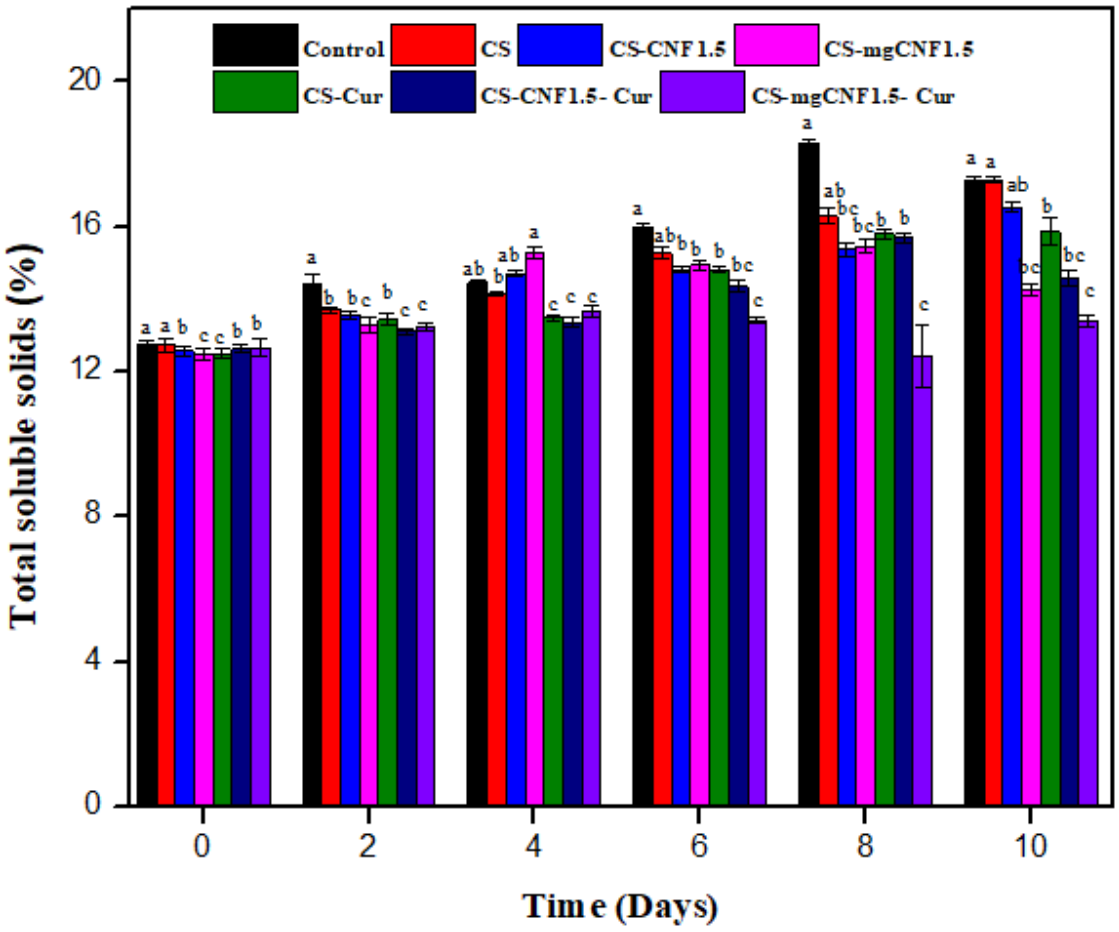


Figure 3.22 *Changes in properties of cut pineapple in terms of total soluble solids. Data sets are represented as mean ± SD (n=5). Different letters define the significant differences between samples (p<0.05)).*

3.2.5 Shelf life Analysis of Edible Coated Whole Kiwifruit

The various quality parameters of kiwifruits are affected due to the storage conditions. The weight of fresh fruit products reduces due to the degradation in property such as environmental affecting agents such as gas concentration, microbes, temperature, etc. The TSS and pH of fruit products increase due to ripening as ripening converts starch to sucrose, and further, decreases the acidity of fruit products. The development of CS based biocomposite can provide the quality of kiwifruits during the storage life.

Firmness of Edible Coated Kiwifruit

The firmness of uncoated and coated kiwifruits during the storage is represented in **Figure 3.23**. Interestingly, the firmness of kiwifruits without edible coating (control) are drastically decreased by 80% to 4.9 N at 10 days of storage, on the other hand, that of the fruits coated by CS-CNF1.5 or CS-mgCNF1.5 mixed material is declined by only 20% to around 14 N at the end of storage. The softening of kiwifruits flesh may occur due to several reasons including microbial attack, biochemical changes, mechanical damages, respiratory behaviour, storage conditions, and others. Additionally, in the control sample, the underlying mechanism of softening may include rapid conversion of water insoluble polysaccharides (starch and others) to water soluble polysaccharides such as fructose, sucrose, glucose. The other plausible reason for kiwifruit ripening may include conversion of the water insoluble pectin present in cell wall to water soluble pectin form. However, the ethylene production is a crucial reason for rapid conversion of fruit cell wall components. Thus, the firmness of the kiwifruits can be maintained using developed edible coating, which act as a barrier from the environment. The maintained firmness of the stored kiwifruits may be obtained due to the possible percolation network created by the CS-CNF1.5 and CS-mgCNF1.5 biocomposites on the surface of kiwifruits due to edible coating application. In case of the fruit coated by CS, the decrease of the firmness is more rapidly than the fruit coated by CS-CNF1.5 or CS-mgCNF1.5 mixed material, but it is less than non-coated fruit. These results show that inclusion of CS-CNF1.5 in coated material provided more positive effect in preserving the firmness of stored kiwifruits than the single application of CS coating which has been studied well as a fruit coating material. Additionally, the incorporation of Cur has also shown positive effectiveness in retaining the firmness of the food products which can be caused due to both the superimposition property and its crystallinity property obtained due to Cur doping. In this regards, the application of CS based composites has helped in reduced microbial load and maintaining biochemical changes

during the storage conditions. Additionally, the firmness of fruit products is considered as an important quality parameter which describes about the fruit consistency (Drevinskas et al., 2017).

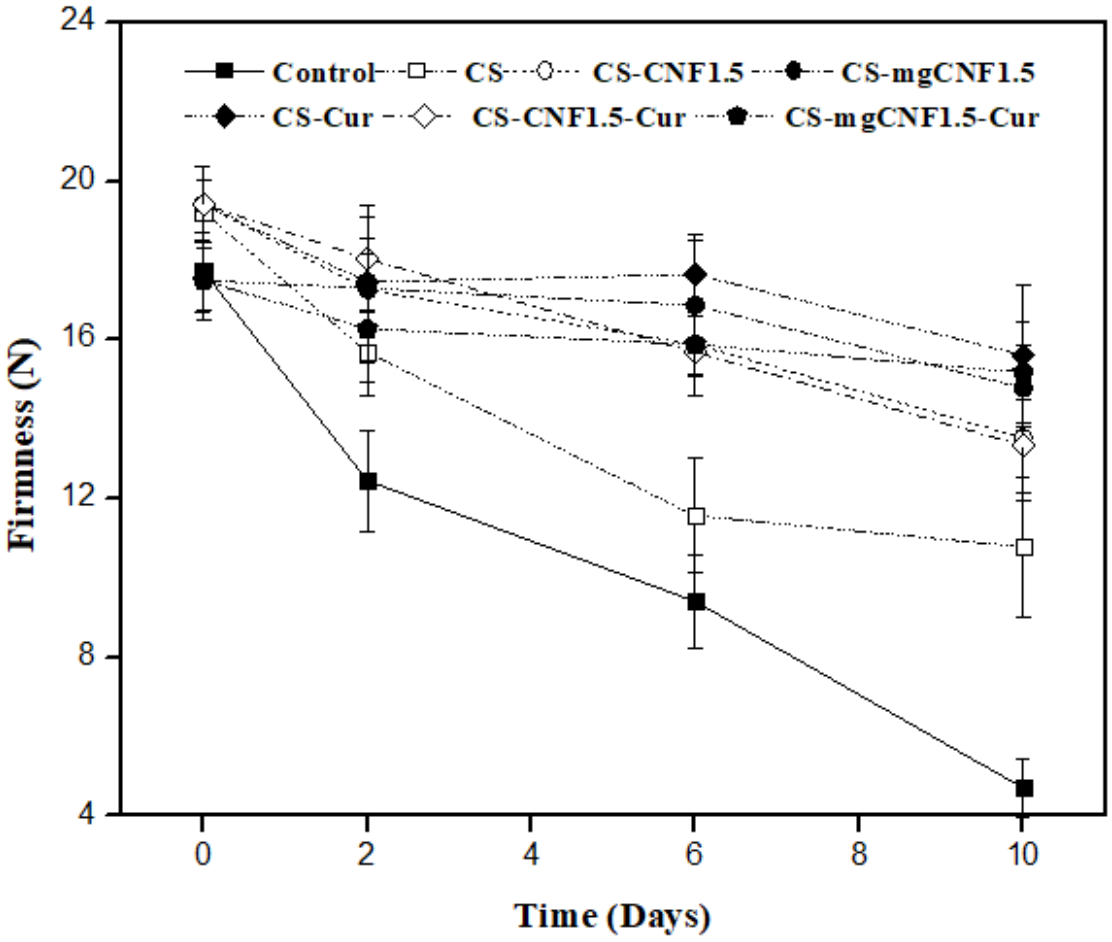


Figure 3.23 *Texture analysis of edible coated kiwifruit products at 10 °C (Data sets are represented as mean ± SD (n=5)).*

Respiration Rate Analysis of Edible Coated Kiwifruits

As shown in **Figure 3.24**, the application of different types of edible coating has a significant effect on the storage life of kiwifruits products. The CO_2 respiration rates of CS biocomposite based coated kiwifruits with and without Cur was found to decrease during the whole storage period, however, the CS associated edible coated kiwifruits are found to have

higher respiration rates at 0 days of storage (**Figure 3.24**). Subsequently, the respiration rates in terms of CO_2 concentration of Cur assisted edible coated kiwifruits such as CS-Cur, CS-CNF1.5-Cur, and CS-mgCNF1.5-Cur retain a sharp decrease at 0 days. However, the inclusion of CS to the edible coating materials provide an increase in the respiration rate at 0 days of the storage, which may occur due to activation of some enzymatic reaction on the kiwifruit surface for CS layers. Some investigation reports enhanced respiration rate coated carrot sticks in comparison to the uncoated carrot sticks (Simões et al., 2009). Additionally, the respiration rate datasets are found to be in line with the firmness and other physicochemical properties of the kiwifruit products.

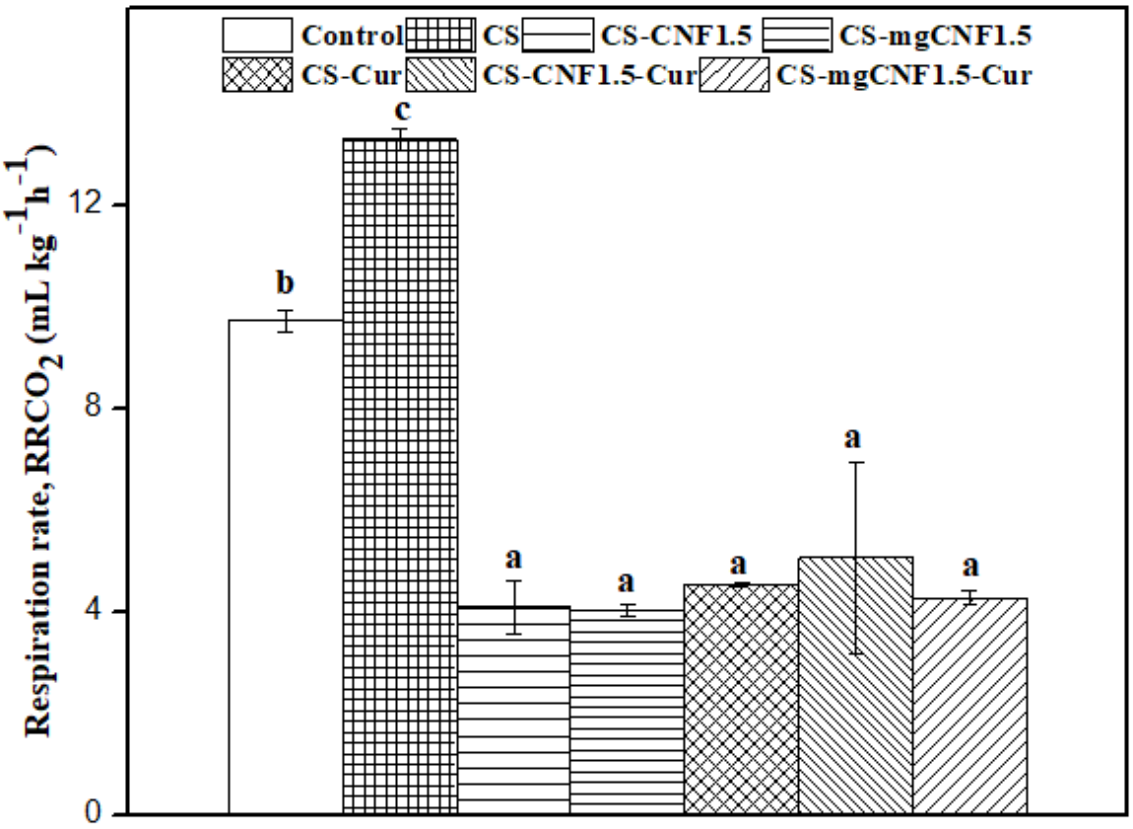


Figure 3.24 *Respiration rate of whole kiwifruits in terms of carbon dioxide gas composition inside close chamber. Different letters define the significant differences between samples ($p<0.05$)).*

Weight Loss Analysis of Edible Coated Kiwifruits

The weight loss analysis of edible coated Kiwifruit products during storage are represented in **Figure 3.25**. The weight loss for all the sample varies within 2.5%, which shows that kiwifruit can maintain the weight stability at a lower temperature. However, the application of edible coating materials provides some weight stability with an increased storage period. However, at an early stage of the storage, control and coated samples maintain the weight, which shows the quality maintenance in the food products. Further, the several reports suggest maintained keeping quality of fruits and vegetables in terms of maintaining weight more than 7 days in comparison to the uncoated fruit products (Joshy et al., 2020a; Salama, Aziz, & Alsehli, 2019; Joshy et al., 2020b). Generally, the inclusion of microbial attack increases the weight loss phenomena in fruit products. In this regards, the antimicrobial based coating materials can help to prolong the storage life of fruit products, especially perishable fruit products. The weight loss of kiwifruits is found to be lowest with CS-Cur based edible coating. However, till 8 days of storage, the kiwifruits with and without application of edible coating have found to maintain the weight. However, at 10 days of storage, the % weight loss of uncoated fruit has been found to increase in comparison to other edible coated food products. In this regards, CS biocomposites is a promising agent in reducing the weight loss of fruit products and others. From very early days, the edible coating is found to be effective when applied on fruits and vegetables for maintained quality and reduced weight loss during storage.

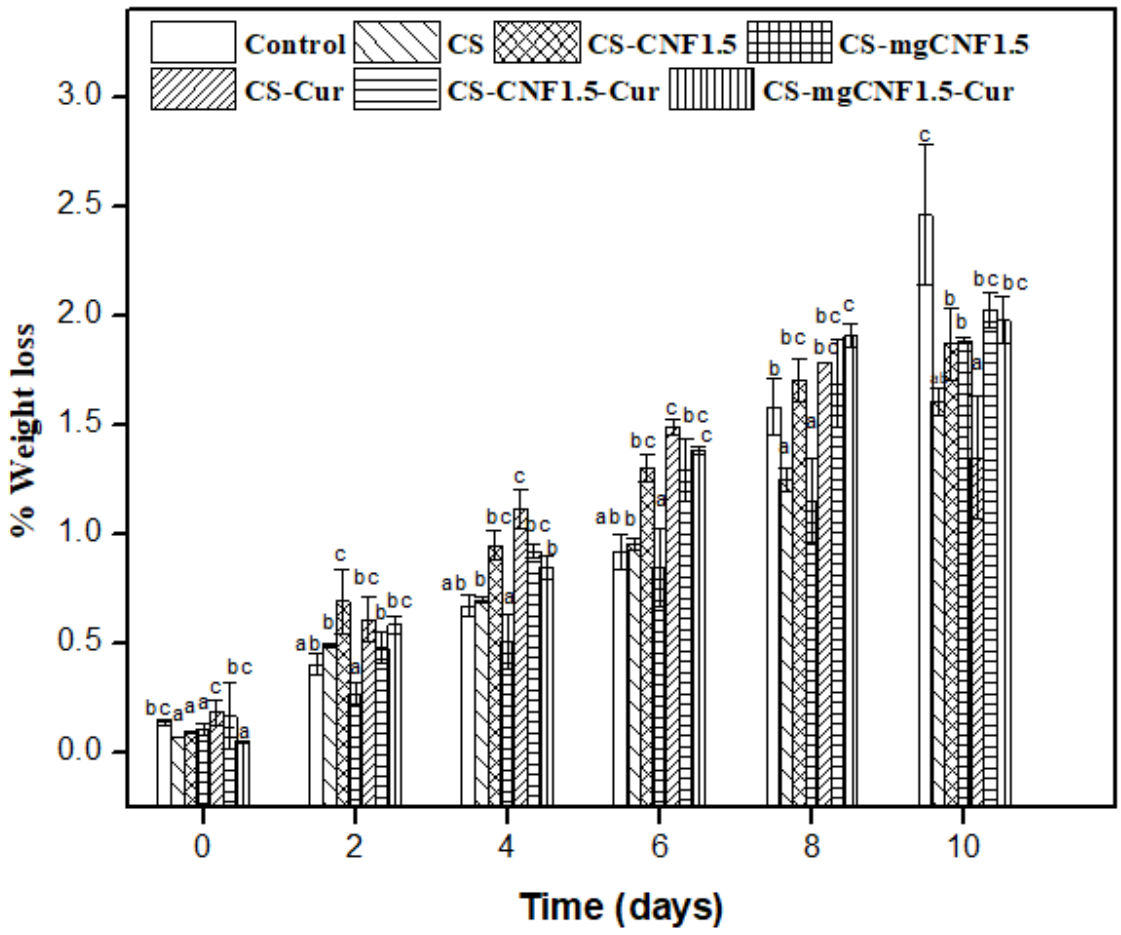


Figure 3.25 *Weight loss analysis of edible coated kiwifruit products at 10 °C (Data sets are represented as mean ± SD (n=5)). Different letters define the significant differences between samples (p<0.05)).*

Color Evaluation of Edible Coated Kiwifruit Products

The various coloration effects can be found in the developed edible coated kiwifruits compared to control samples (uncoated kiwifruits), where, the total color difference (ΔE) for the samples has been measured for all the testing samples at 0 days as shown in **Figure 3.26**. There has been noticed a variation in ΔE values for the coated fruit products. The color difference in CS-mgCNF1.5 occurs mainly due to reduced brightness compared to control sample. Further, the changes in ΔE values for CS-Cur, CS-CNF1.5-Cur and CS-mgCNF1.5-Cur are attained mainly due to the yellow coloring effect by Cur doping. In this way, the color

parameters affect the consumer acceptance of edible coated food products, and further, the quality of food products is greatly affected by the application of the edible coating.

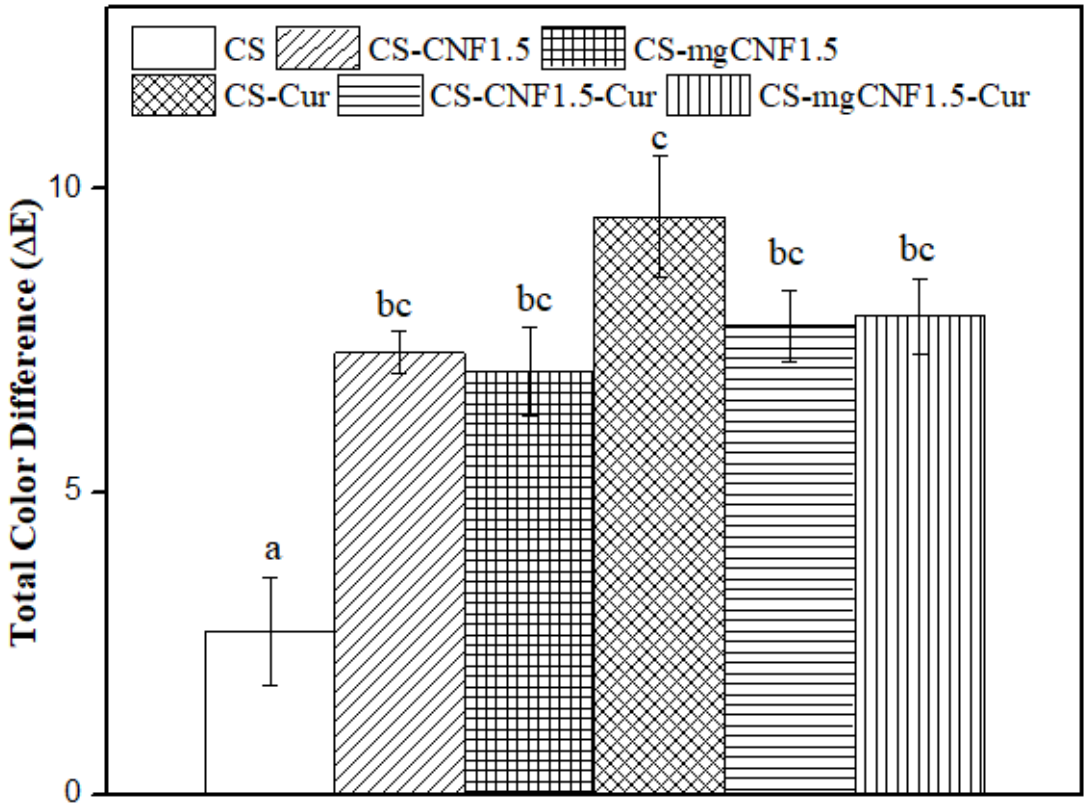


Figure 3.26 *Evaluation on total color difference values of edible coated kiwifruit products at 0 day (Data sets are represented as mean ± SD (n=5)). Different letters define the significant differences between samples (p<0.05)).*

Microbiological Analysis of Edible Coated Kiwifruit Products

For the microbiological analysis, **Table 3.8** represents the microbial count of mesophilic bacteria at 0, 5, and 10 days of sampling for edible coated and uncoated samples stored at 10 °C. The initial mesophile count in uncoated and edible coated kiwifruits is found to be in the range of 1.49±0.03 and 1.69±0.00 Log CFU/mL. At 5 days of storage, the microbial count is found to be highest for uncoated kiwifruits (2.96 ± 0.33 Log CFU/mL), however, the application of various formulated edible coating is very helpful in maintaining the property during storage

conditions. The investigation shows that at the initial storage period, all food materials maintain a similar microbial count, however, involvement of Cur shows a significant reduction of microbial count in comparison to other fruit products. The mesophilic count generally occurs at medium temperature, thus when the fruit products are allowed to stay at medium temperature, then there may be a chances of growing mesophilic count. Further, the observed reduced microbial growth with the application of specified edible coating materials possibly helps to retain the firmness of the kiwifruit products during storage. As observed from the firmness and mesophilic count that the incorporation of Cur alongwith CS bionanocomposites materials has better retained the firmness and interestingly, reduced the microbial count. In this regard, the application of CS-mgCNF1.5-Cur based edible coating on kiwifruits has maintained the microbial count at 3.38±0.31 Log CFU/ mL ($p<0.05$) at 10 days of storage which is reduced considerably compared to control kiwifruit samples. Similarly, the addition of CS-mgCNF1.5-Cur has better preserved the firmness as compared to other coated and control samples. Similarly, in case of TSS, the CS-mgCNF1.5-Cur coated kiwifruit has maintained reduced TSS values compared to others during the storage conditions. Thus, the addition of Cur in CS biocomposite has better quality retention property of kiwifruits as compared to other coating materials.

Table 3.8 *Analysis of Mesophile count of Edible Coated Kiwifruit products stored at 10 °C (Data sets are represented as mean ± SD (n=5). Different letters define the significant differences between samples (p<0.05)).*

Treatment	Days of storage		
	0 day	5 day	10 day
	Log CFU/mL	Log CFU/mL	Log CFU/ mL
Control	1.68±0.02^{a}	2.96±0.00^{a}	3.69±0.01^{a}
CS	1.65±0.02^{a}	2.62±0.02^{b}	3.50±0.00^{b}
CS-CNF1.5	1.62±0.02^{ab}	2.72±0.01^{ab}	3.54±0.03^{ab}
CS-mgCNF1.5	1.56±0.02^{bc}	2.53±0.00^{c}	3.50±0.06^{b}
CS-Cur	1.58±0.02^{b}	2.56±0.51^{bc}	3.39±0.42^{c}
CS-CNF1.5-Cur	1.62±0.03^{ab}	2.58±0.75^{bc}	3.43±0.39^{bc}
CS-mgCNF1.5-Cur	1.49±0.03^{c}	2.49±0.45^{c}	3.38±0.31^{c}

3.3 Conclusions

The inclusion of renewable bioresources and their nanoforms in developing edible coating materials are considered as a potential candidate for maintaining the quality attributes of fruits and vegetables. The current research studies the effectiveness of CNF based biofiller in CS based edible nanocoating materials, which is further facilitated by the incorporation of Cur. The chapter confirms the anti-cancerous effect of the developed nanocoating with Cur and further delivers the coating materials with active nutraceuticals and added fortified food materials with iron components for cut pineapple products. The iron adsorbed CNF has been obtained from a single step co-precipitation method, which is used as a nanofiller material in the present study to develop edible nanocotaing materials with and without Cur. The CS based edible nano-coated pineapple fruit product is developed using dip-coating method. The combined effect of Cur and nanofillers has a significant impact on quality maintenance of cut pineapple products. In addition, the use of the coating materials can help to maintain the quality

of the product to prolong the shelf life of cut pineapples and also make the availability of "ready to eat" pineapple products with seasonal availability. Additionally, the application of mgCNF reinforced CS based edible coating assisted with Cur on kiwifruits are effective in maintaining different food properties in terms of maintaining weight, texture, reduced microbial count, physicochemical properties (total soluble solids, pH), and color parameters during the storage period.

CHAPTER 4

Nanochitosan Incorporated Starch/Guar gum Nanocomposites Based Edible Nanocoating

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Motivation

The inclusion of guar gum (GG) in developing starch (ST) based biocomposites for targeted edible food packaging has attained significant attention for delivering improved inherent characteristic attributes of ST based edible coatings/films. The existing shortcomings of ST based edible films and coatings include hydrophilicity, poor mechanical property, no antimicrobial activity, which has reduced its commercialization. Further, the inclusion of GG in developing ST based biocomposite materials can significantly improve the inherent properties of ST based food packaging in terms of crystallinity, and mechanical property. Interestingly, ST and GG (ST-GG) biocomposites based edible coating/ films are used to maintain the food properties during storage life. However, the increased hydrophilicity and reduced transparency of ST-GG biocomposites have limited its use, thus requires strategic modifications to overcome the existing shortcomings. In this regards, the inclusion of nanochitosan (a food based nanomodifier) can overcome the existing shortcomings of ST-GG biocomposites.

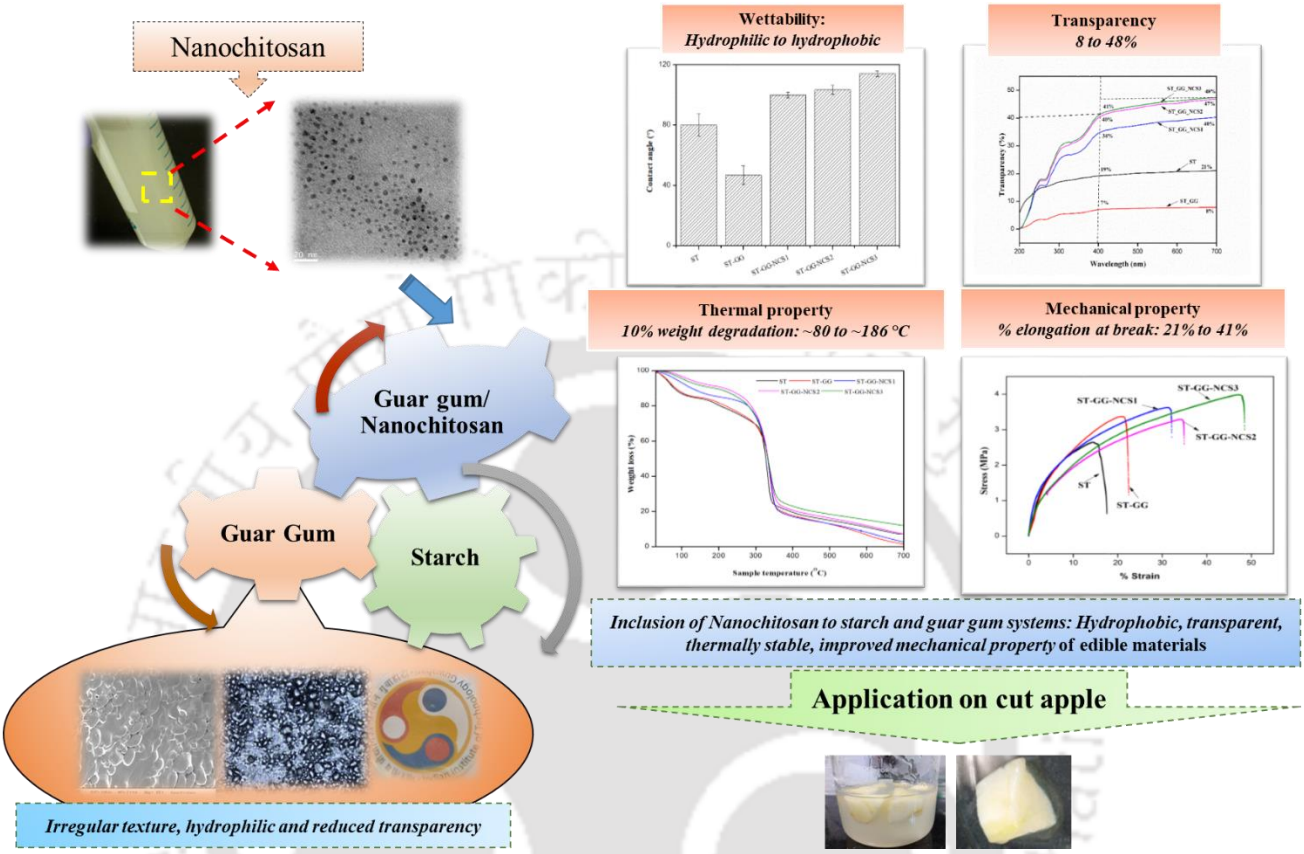
Research Output

1. *Vimal Katiyar and Tabli Ghosh, "Process for Preparing Nanochitosan Aided Starch and Guar Gum Based Biocomposites Based Edible Packaging Material" (Patent in process).*
2. ***Ghosh, T.,** Mondal, K., Giri, B. S., & Katiyar, V. (2021). Nanochitosan Incorporated Starch/Guar gum Biocomposites Based Edible Coating: A Potential Candidate with Hydrophobic, Transparent, Thermally stable, Ductile Materials for Fresh cut Apple. (Ready for submission).*

Abstract

This study reports a new approach of utilizing nanochitosan (NCS) for modification of starch (ST)/guar gum (GG) biocomposite with superior packaging properties targeting stringent edible coating applications on fresh cuts. The effectiveness of NCS in terms of structure-property-performance analysis of ST/GG biocomposites has undergone to develop an edible coating on fresh cut apple fruits. The incorporation of NCS at various proportions delivers a more homogeneous dispersion of GG in the matrix with tailored features and surface properties of ST-GG biocomposites. The inclusion of NCS to the biocomposites of ST/GG converts its hydrophilic surface nature to hydrophobic (contact angle of ~114°) by modifying the surface features. Further, the crystallinity of ST-GG biocomposites is improved by introducing NCS to the biocomposites. The addition of NCS improves the thermal stability, where the observed 10% weight degradation of ST biocomposites are ~79.36, ~80.49, and ~186.89 °C for neat ST, ST/GG biocomposites, and ST/GG/NCS (3% w/v) (ST-GG-NCS3), respectively. Thus, NCS can be included in the ST-GG biocomposites for developing heat stable food products. The observed transparency of ST, ST-GG, and ST-GG-NCS3 are 21, 8, and 48%, respectively in the visible region suggesting consumer preference for transparent packaging materials. The wt% of O, C and N elements in ST-GG-NCS3 as observed by EDX spectra are ~50.2, ~47.6, and ~2.2%, respectively, which confirm the safety of the materials. Additionally, it is noteworthy to mention that the storage quality in terms of microbial growth, pH change, color attributes, and weight loss are better preserved for cut apple fruits when edible coatings are developed with the aid of NCS. In this study, a novel approach to offer tunable properties to ST and GG biocomposite based packaging materials by addressing NCS as a nanofiller material, where it performs as a quality improver for the edible coating material targeting fresh produces.

Scheme of the Chapter



4.1 Introduction

Over the past few years, considerable attention has been given in developing biodegradable food packaging in the academia and industrial sections to elucidate the environmental challenges associated with perto-based packaging materials including biological incompatibility and increased carbon footprint tending towards amplified global warming (Borkotoky et al., 2019; Ghosh et al., 2019). Therefore, an emphasized interest has been shifted in the rigorous utilization of renewable, biodegradable, and non-toxic biomaterials. The existing focused biopolymers for fabricating edible coatings include cellulose, chitosan (CS), starch (ST), protein, lipids, and others. However, among available, ST is one of the most investigated biopolymeric materials for fabricating edible packaging materials exhibiting various remarkable attributes such as non-toxicity, biodegradability, biocompatibility, easy availability, film forming properties, cost-effective, and others. ST based edible coatings are one of the emerging packaging techniques to provide increased food quality and shelf life. ST based edible coatings and films exhibit physical properties similar to the synthetic polymers (Saberi et al., 2016). Additionally, ST based films having properties of transparency, tasteless, semi-permeable to gaseous environment, odorless, and low cost has fascinated the researcher to utilize as a remarkable ingredient to develop edible coatings. To date, ST as an edible coating material has been widely applied to improve the shelf life of various fruit products such as plum fruit (Thakur et al., 2018), tomato fruit (Nawab et al., 2017), banana (Thakur et al., 2019), pomegranate fruit (Oz et al., 2012), and others. However, ST based edible films exhibiting some of the limitations such as hydrophilicity and poor mechanical properties, which has led to include some other biopolymers to tailor the existing shortcomings. In this context, biocomposite system based edible coatings or films can tailor the inherent properties of ST materials for enhanced product life.

Guar gum (GG) is a type of galactomannan polysaccharide obtained from the endosperm of *Cyamopsis tetragonoloba* L. (Gresta et al., 2013). GG is a high molecular weight biopolymer consisting of linear chains of β 1,4-linked mannose residues as the backbone and side chains of 1,6-linked galactose residues (Das et al., 2011; Chauhan et al., 2009). GG has many health benefits such as cholesterol lowering effects, glucose lowering effects, obesity prevention, and also provide significant reductions in cholesterol level, diabetes control, prevention of constipation, and others. Additionally, GG delivering different physicochemical properties including emulsification, interfacial, surface activities, excellent stiffness and non-toxicity has many versatile applications. Thus, GG has wide applicability in different food sectors for several health beneficial properties and noteworthy attributes. In food applications, GG is used as a thickening and viscosity controlling agent, manage syneresis, and deliver water binding properties, which make it a potential agent to be used for versatile food application in ice cream, beverages, processed cheese, dairy products, processed meat, and other food sectors (Mudgil et al., 2014). Interestingly, GG is considered as a potential candidate to be used in developing edible coating for different food products. Additionally, different reports studied the use of GG with different biopolymers for developing edible coatings on food products such as GG and CS based composite for mushroom (Huang et al., 2019), GG and ginseng extract coatings for sweet cherry (Dong and Wang 2018), mung bean ST and GG based edible emulsion coatings on rice cakes (Lee et al., 2020), pea ST and GG edible coating on valencia oranges (Saberi et al., 2018), GG with glycerol based edible coating on fried potato chips (Yu et al., 2016); and others. Therefore, GG is a potential candidate in improving the inherent properties of different biopolymers. However, the biocomposite of GG and ST provide increased crystallinity and mechanical properties, however, exhibit reduced water resistance property and transparency. So, the incorporation of different other nanofillers such as nanochitosan (NCS) can modify the existing shortcomings of ST and GG biocomposites.

CS, the deacetylated product of chitin, is the second most remarkable biopolymer and has attained a great application in developing green packaging materials for various noteworthy functional attributes. However, the attributes of NCS include improved functionality compared to bulk CS such as non-toxicity, surface chemistry, higher surface area, compactness, which has made it a notable candidate for the present market in multifaceted advanced packaging application to provide tailored packaging property. The NCS is a kind of versatile biopolymeric materials to intensify the ready-to-eat healthy food demands. Thus, the fabrication of NCS aided edible coated food products can fulfill the consumer demands which has directed the researcher to develop CS nanostructure aided edible coated food products. NCS can be fabricated by following a simple ionic gelation method, where, the strategical use of NCS in modifying ST-GG biocomposites can be a new route. However, NCS is extensively used in developing edible coating on various food products including cucumber (Mohammadi et al., 2016), strawberry (Eshghi et al., 2014), apple (Gardesh et al., 2016), cherry (Arabpur Mohammad Abadi et al., 2020), and others.

Based on the discussion, the current investigation focuses on the fabrication of NCS modified ST and GG biocomposites based edible coating with tailored properties. The present study develops NCS following the ionic gelation method, where the formation of NCS has been confirmed by FETEM, FTIR, and XRD analysis. Further, NCS incorporated edible coating materials have been analyzed for evaluating various coating properties such as thermal properties, mechanical properties, surface morphology, wettability, and others. Besides, the chapter further aims to develop an edible coating on fresh cut apples and evaluated for the fruit properties such as weight loss at time lapsed, color properties, microbiological study, and others. Additionally, to date no study has been reported targeting ST, GG and NCS based edible coating on cut apple products.

4.2 Results and Discussions

The CS, being one of the most notable biopolymers, having noteworthy attributes including non-toxicity, biodegradability, biocompatibility, edibility, and others are focused to be used in developing edible coatings for fresh produces. To date, several research reports the use of CS as an ingredient to maintain the quality of fresh produces such as strawberry (Wang and Gao, 2013), citrus fruits (Arnon et al., 2014), banana (Baez-Sañudo et al., 2009), carrot sticks (Simões et al., 2009), tomato (Amarillas et al., 2018), apricot (Zhang et al., 2018), peach fruits (Jiao et al., 2019), etc. However, several studies confirm the beneficial attributes of NCS over bulk CS including improved solubility and functionality of edible coated materials for the increased surface area due to nanodimensionality (Sivakami et al., 2013; Gardesh et al., 2016). Therefore, the present study aims to utilize NCS as a component of edible coating materials with ST-GG based biocomposites which may be appealing to overcome the shortcomings associated with ST-GG biocomposites.

4.2.1 Fabrication and Property Analysis of Nanochitosan

In developing NCS using the ionic gelation method, sodium tripolyphosphate (STPP) is used as a cross linking agent to obtain the desired nanostructure after optimizing the suitable processing conditions. The CS and STPP possessing cationic amino groups and anionic phosphate groups, respectively, interacts with each other forming intermolecular and intramolecular links which reduce the dimensions of CS formulating NCS as shown in **Figure 4.1**. However, the dimensions of NCS are significantly influenced by the CS and STPP concentrations. However, the NCS may form aggregations delivering micron-scale particles through van der Waals force. The morphology and dimension of the fabricated NCS are evaluated by FETEM analysis, where, the fabricated nanoparticles possess a size range of 3 to 9 nm with a smooth surface and spherical shape.

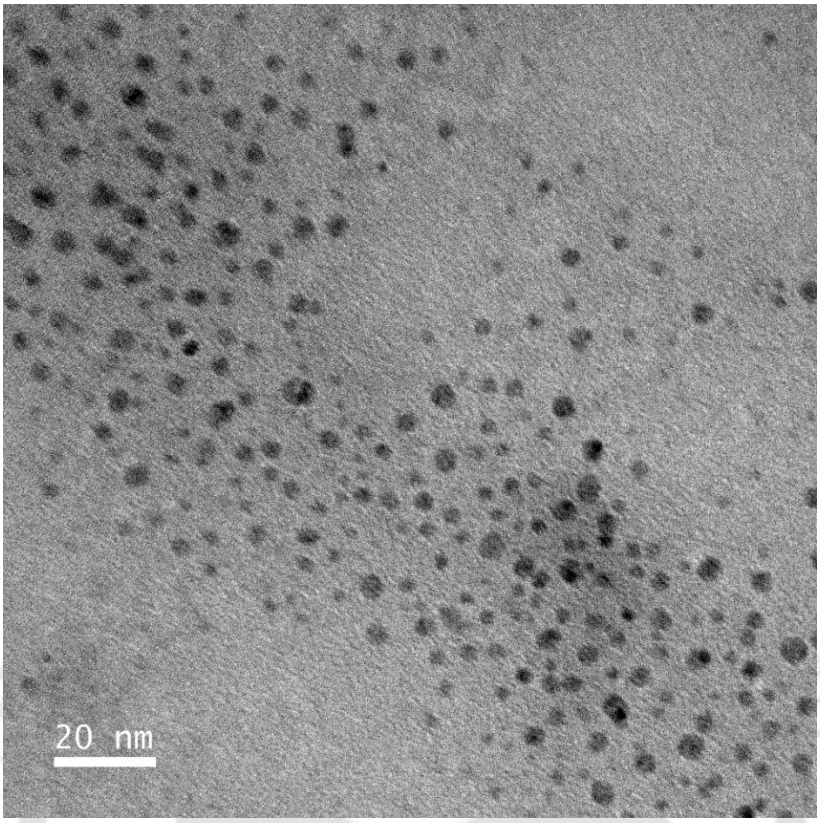


Figure 4.1 *FETEM micrograph of nanochitosan fabricated using ionic gelation method.*

The TGA of NCS shows the weight loss pattern during thermal treatment for a range of 30 to 700 °C. As observed from **Figure 4.2**, there has been observed two step degradation process of the developed nanomaterials. During the temperature range, the first and second stage degradation occurs between 45 to 140 °C and 240 to 330 °C, respectively. The first stage thermal degradation occurs due to the removal of water content, whereas, the second stage thermal degradation occurs for NCS degradation. Additionally, NCS has retained 38% of its original weight at 700 °C suggesting increased thermal stability of NCS compared to CS (~16% weight retain). The increased thermal stability of the NCS for the studied temperature region may be due to probable cross linking between CS and STPP.

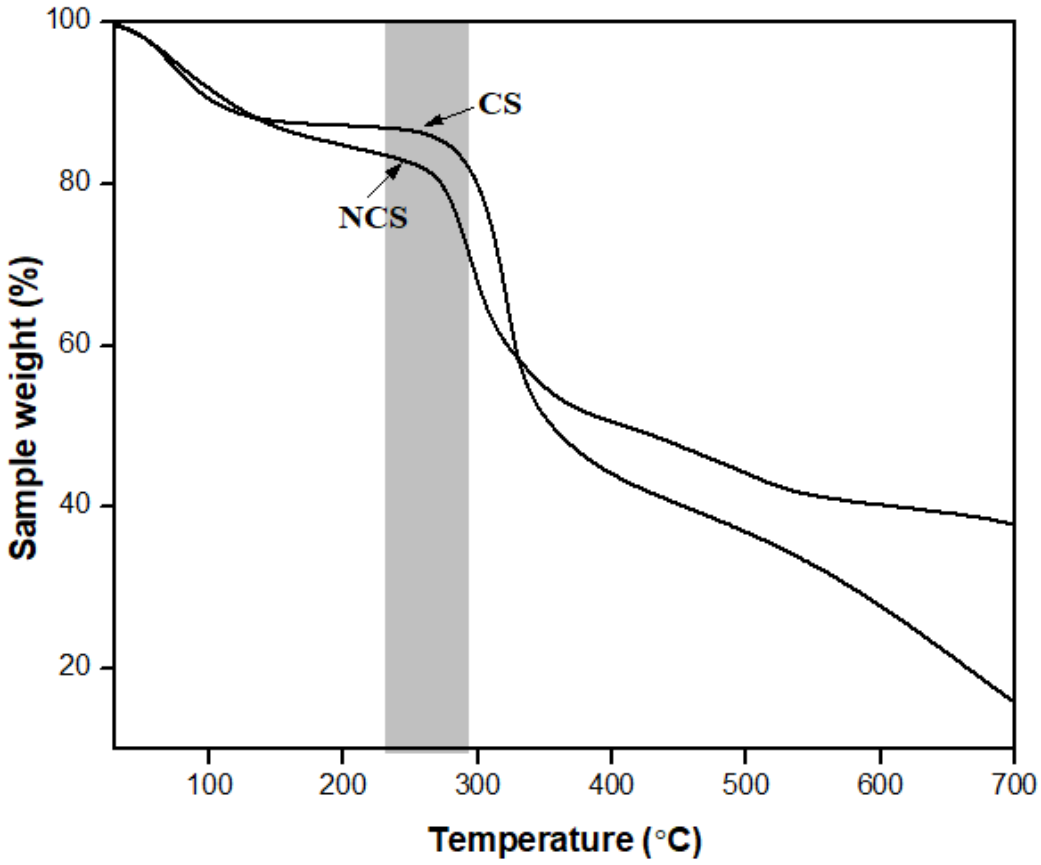
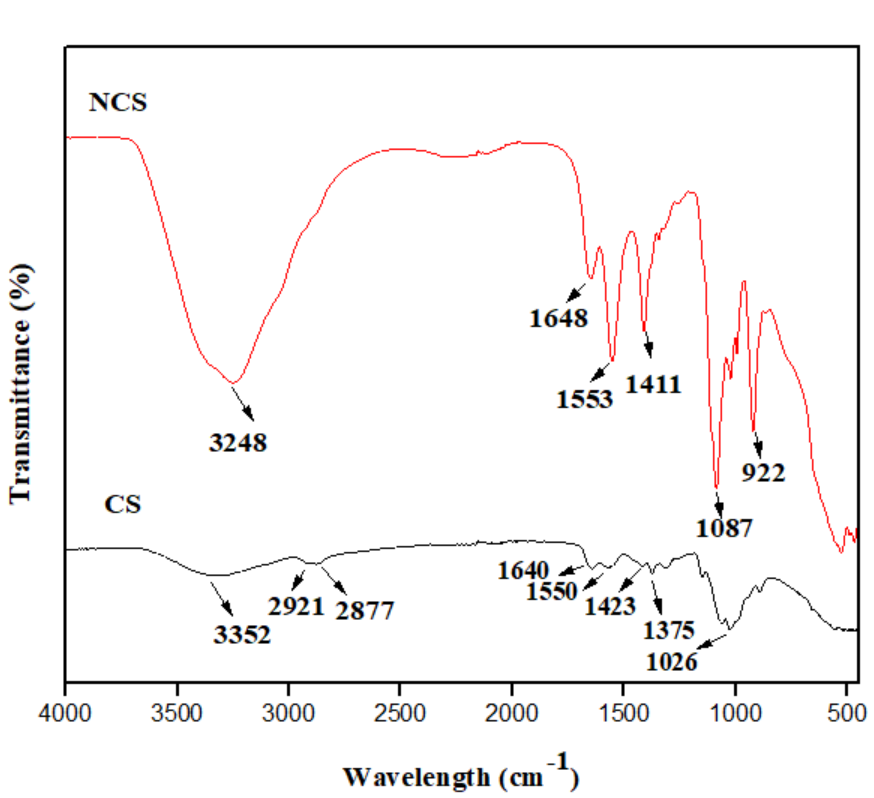


Figure 4.2 *Comparative thermal analysis of chitosan and fabricated nanochitosan biomaterials representing TGA thermograms.*

The FTIR analysis is executed to analyze the chemical IR bands as represented in **Figure 4.3a**. In the FTIR images, the FTIR peaks of CS powder at 1550 and 1640 cm^{-1} are attributed to the –NH bending vibration (secondary amides) and stretching vibrations of –C=O present in primary amides, respectively (Fernandes Queiroz et al., 2015; Pal and Katiyar, 2016; Kumar Pal et al., 2016). Additionally, the FTIR peak at 3352 cm^{-1} is attributed to $–NH^2$ and –OH stretching vibrations in CS; (Fernandes Queiroz et al., 2015; Pal and Katiyar, 2016; Pal et al., 2016). The FTIR peak at 2921 and 2877 cm^{-1} are attributed due to symmetric –CH stretching and asymmetric –CH stretching, respectively (Malarselvi et al., 2020), The FTIR peak at 1423 and 1377 cm^{-1} are obtained due to –NH stretching amide bonds and NH stretching of amide III band (Douglas et al., 2017). Additionally, the FTIR peaks at 1028 and 1067 cm^{-1}

are attributed to -CO based IR bands (Buraidah & Arof, 2011). For NCS, the shift in peaks to 1648 and 1553 from 1640 and 1550 cm^{-1} occurs due to the interactions between cationic group of CS and phosphate group of STPP. The FTIR peaks at 3352 cm^{-1} are shifted and become sharpen for NCS formation due to enhanced hydrogen bonding.

As represented in the XRD patterns (**Figure 4.3b**), the bulk CS shows two characteristics diffraction peaks at 2θ value of 10° and 20°. However, the formed cross linking between CS and STPP delivers NCS, which reduces the crystallinity of NCS confirming the formation of dense network structure between the opposite charges. The reduced crystallinity of NCS has the advantages of increased surface area providing faster dissolution rate, better dimensional stability, physical stability, chemical compatibility and further extent the exposure of bioactive components. Due to this, the dissolution rate of NCS increases delivering increased antimicrobial properties in comparison to CS.



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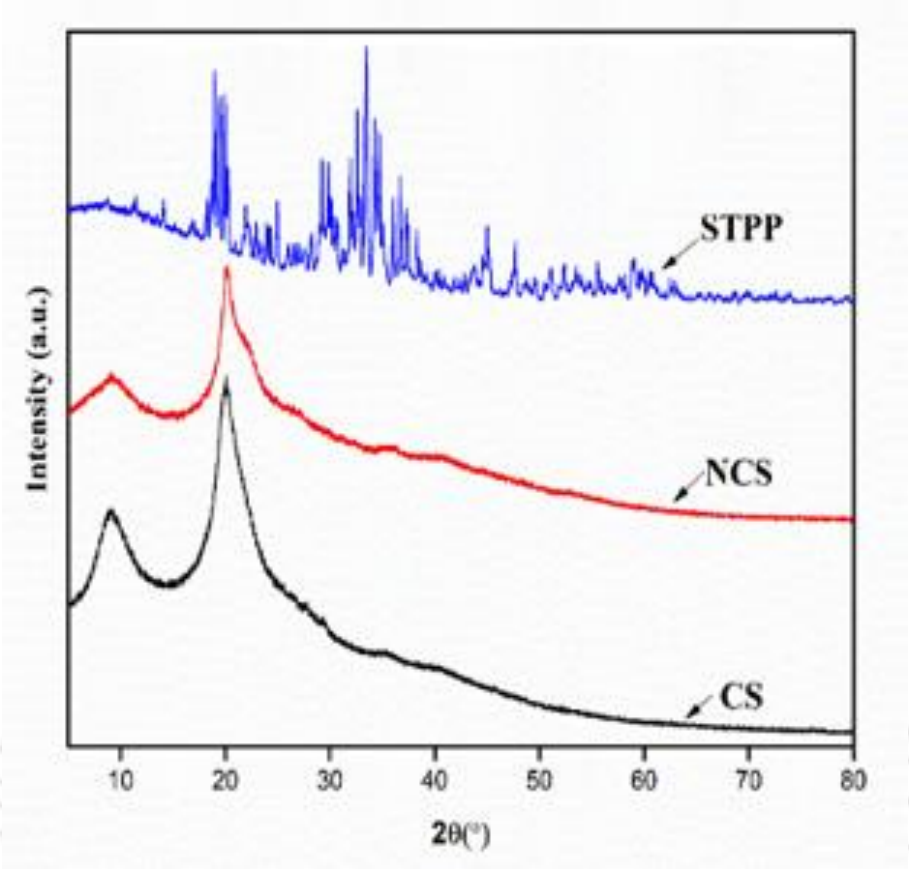


(b)

Figure 4.3 *Chitosan and fabricated nanochitosan biomaterials representing FTIR spectrum (a) and XRD analysis (b).*

4.2.2 Characterization of Nanochitosan Modified Starch/Guar Gum Nanocomposite Based Edible Coating

NCS, being fabricated from CS (medium molecular weight) adopting single step ionic gelation methods with the aid of food grade cross linking agents, can be considered as a potential candidate to be used as a reinforcement to modify the existing limitations in ST-GG based biocomposite materials. However, the ST-GG based biocomposite is found to deliver improved packaging properties in terms of increased crystallinity, and mechanical property as reported earlier (Nandi & Guha, 2018). On the otherhand, the addition of GG to develop ST based biocomposite can reduce the transparency due to compact network formation and increased water absorption property (Saberi et al., 2016; Nandi & Guha, 2018). Therefore,

modifying the ST-GG biocomposite using edible NCS materials delivers a novel approach to overcome the existing shortcoming in terms of transparency, wettability, water vapor permeability, microstructure property, antimicrobial property and others. Further, the properties of ST based films are affected by various factors including ST types, processing condition of ST film formation, type of plasticizer used, storage conditions and others. Therefore, the processing conditions for developing the ST based nanocomposite films has been fixed after some trial and error methods. The prepared film forming suspension has been cast in petriplates after sonication to avoid bubble formations and dried.

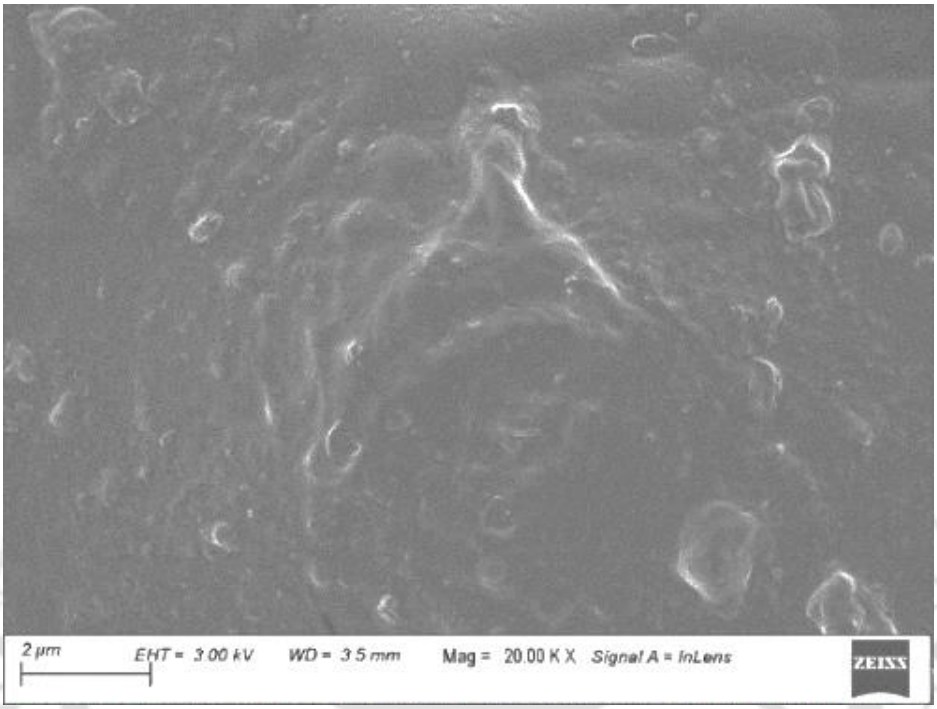
Morphological Properties of ST/GG/NCS Nanocomposite based Edible Coating

The surface micrographs of the fabricated ST, ST-GG, and ST-GG-NCS3 based edible coating materials *via* simple solution casting process are observed using FESEM micrographs, FETEM micrographs, and optical microscopy as represented in **Figure 4.4 and 4.5**, respectively. The interactions between film forming components directly affect the attributes of the films. The surface morphology of ST based edible coating materials shows some irregularities and non-uniform texture due to the gelatinization process. Further, the pores in the fabricated ST films are commonly formed for incorporating glycerol in the matrix which creates some channels (Nandi & Guha, 2018). However, the non-uniform fragments observed on the FESEM micrograph of ST surface occur due to the processing effects. In this regards, the incorporation of GG significantly affects the morphology of ST based edible coatings, where, GG has a spherical morphology in the matrix of ST with different size ranges. The dispersion of GG in the matrix of ST reduces the transparency due to continuous intactness. However, some microcracks are observed on the surface of the ST-GG biocomposites which may affect some of the properties. The FESEM image confirms the fabrication of GG incorporated ST based biocomposites with continuous enactment of GG having spherical shapes and are compact in nature. The uniform dispersion of the reinforced GG in the matrix

of ST can be observed from the surface (**Figure 4.5b**) and fractured cross sections (**Figure 4.5b´**) of ST-GG biocomposites. Additionally, the incorporation of NCS at different proportions influences the surface morphology by altering the GG morphology making it more dispersed in the matrix. The cross sectional images of the film materials provide layered structure and uniform distribution in ST-GG-NCS1. The incorporation of NCS at various proportions helps to disperse the GG in the base matrix more homogeneously which improves the various features of ST-GG biocomposites. It is suggested that the incorporation of NCS can successfully make the ST-GG film more stable with enhanced dispersibility and compatibility in ST base materials. As it is evident from the FETEM that NCS has a nanodimensional range, which significantly influences the microstructural morphology of the modified biocomposite materials. Thus, the inclusion of NCS may create a large surface area and functional attributes to GG material by modifying the property of ST. The NCS may adhere to GG materials by modifying ST based biocomposite properties.

The observed dissimilarity in the morphology of fabricated ST biocomposites is possibly due to the several dissociation degrees obtained for incorporating NCS which changes the morphology of ST-GG biocomposites. Further, to understand the granule dimensions and changes in the features of the biocomposites, high resolution TEM images are captured for ST-GG-NCS3. The ST-GG biocomposites provide non-uniform dispersions with formed agglomerations of GG in the matrix of ST with distinct spherical size ranges. Interestingly, the addition of NCS to the matrix of ST-GG provides the uniform dispersibility of GG with reduced dimensions. Therefore, the FETEM micrographs suggest that the fabricated biocomposites show significant variations in the morphology in terms of dimension, dispersibility and other factors, which could be tailored using different filler/nanofiller materials. Further, the photographs from optical microscopy clear that agglomeration effects of GG in the ST matrix have been found to reduce due to the introduction of NCS in ST-GG biocomposites. Visually,

ST-GG-NCS3 offers better dispersions of the fillers than ST-GG materials. In this way, the inherent features of ST based films in terms of functional and physical attributes can be tailored by incorporating nanofiller materials.



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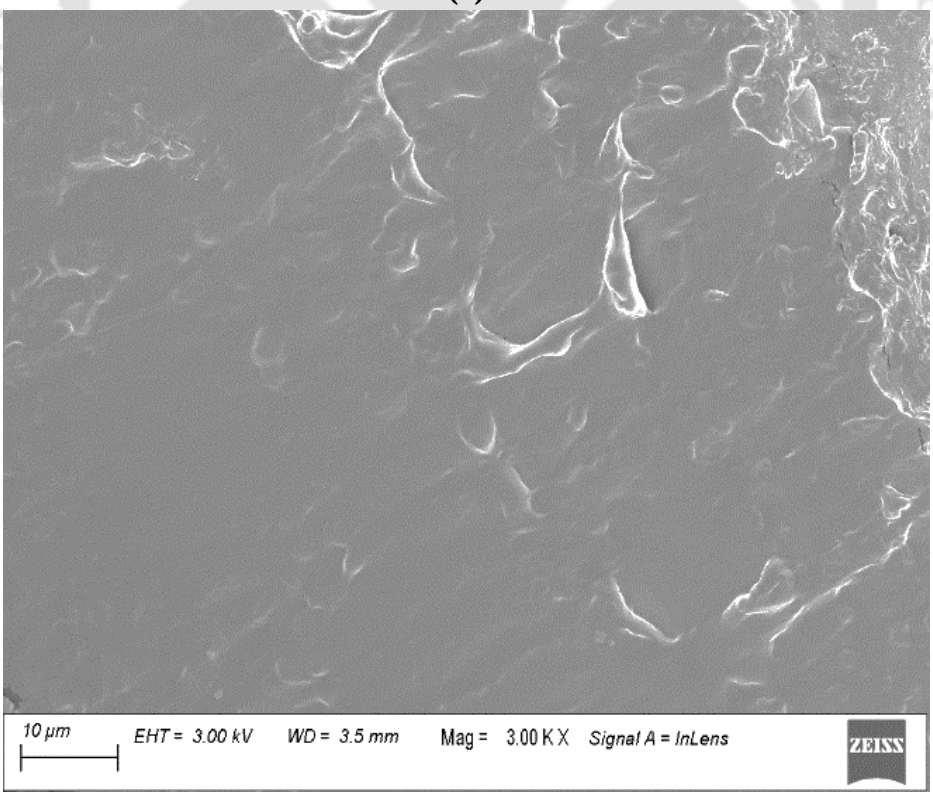

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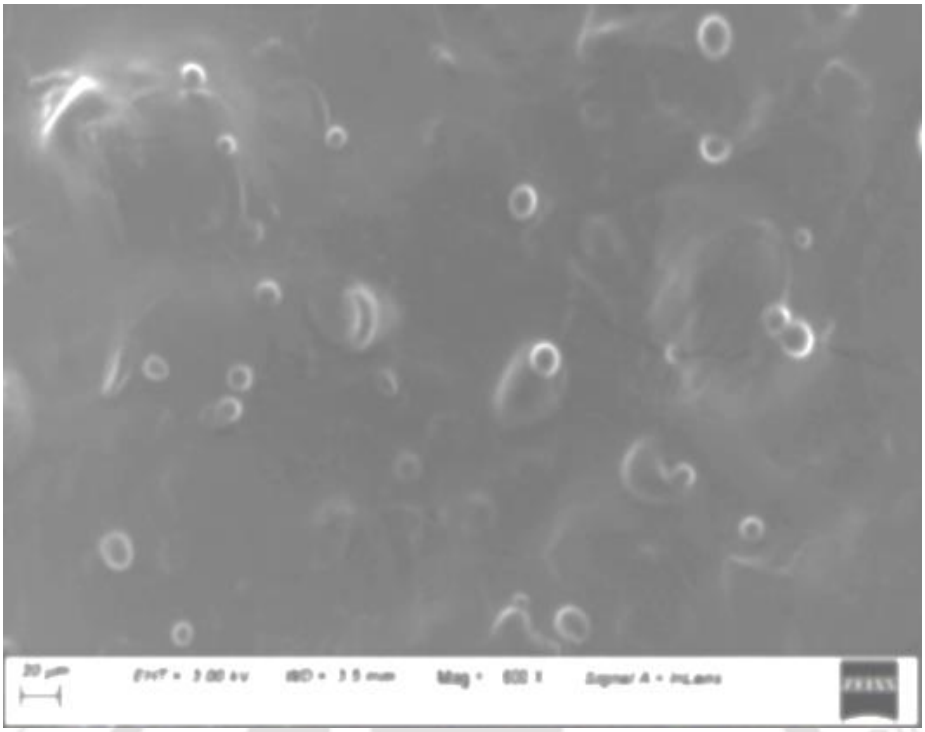

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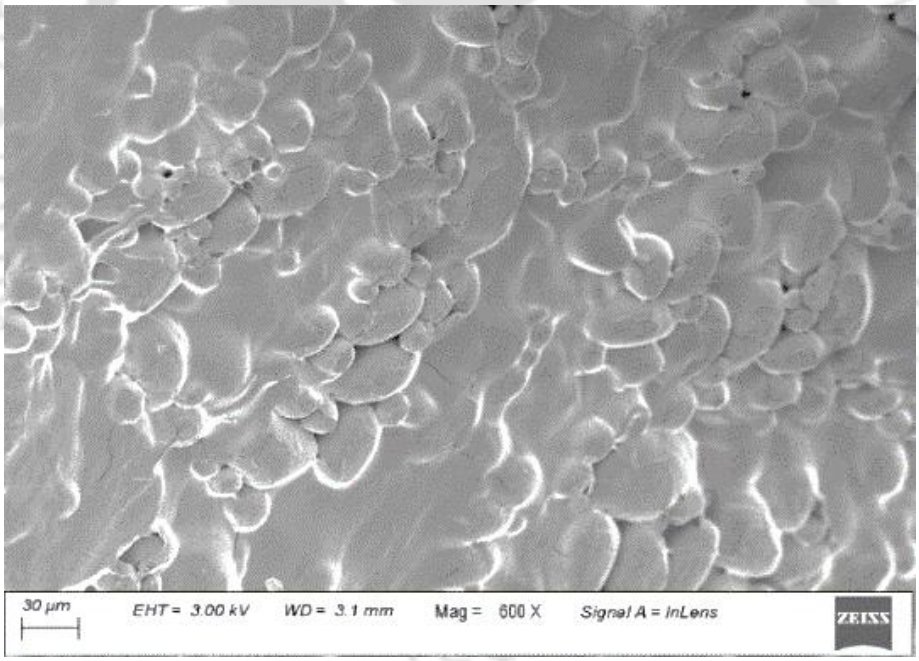

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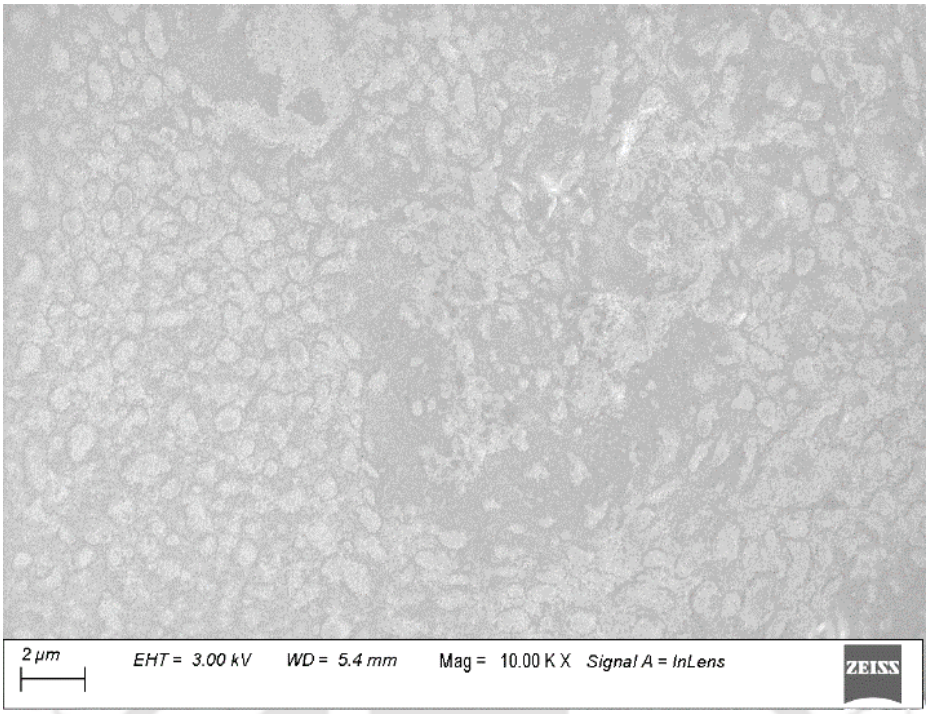

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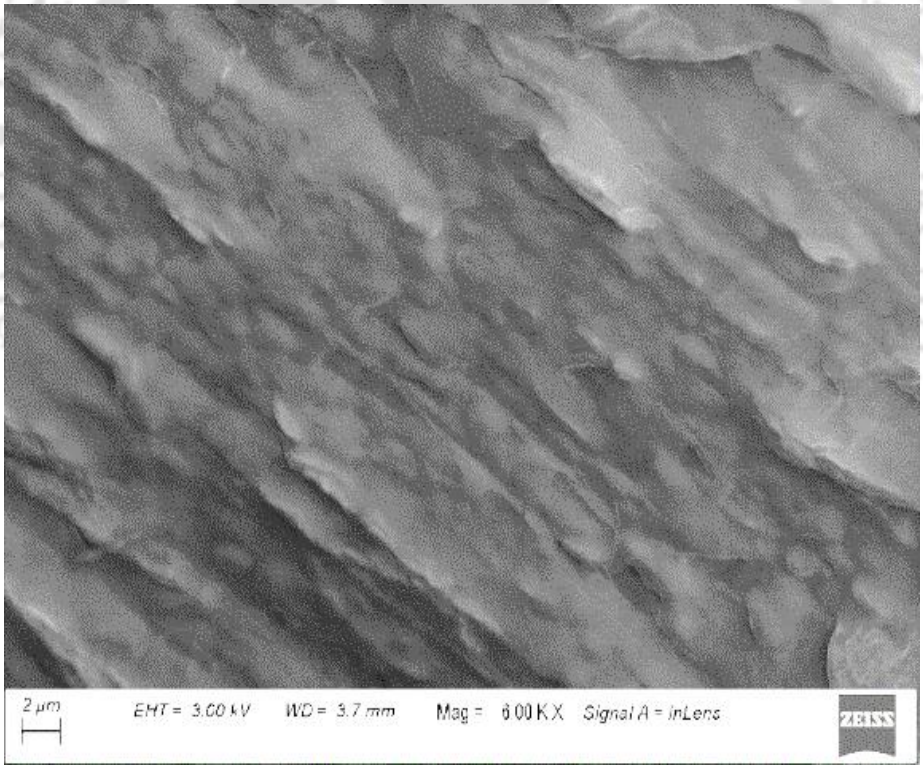
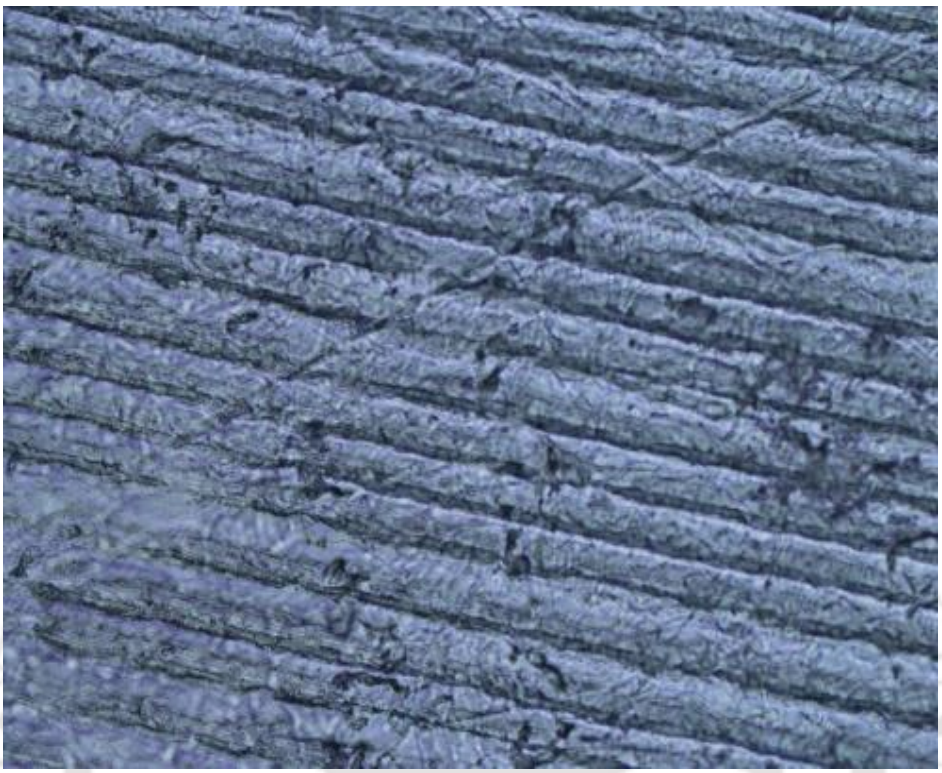


(c')

Figure 4.4 *FESEM micrographs of starch surface (a), cross sectional fractured surface of starch (a'), starch-guar gum biocomposites (b), cross sectional fractured surface of starch-guar gum biocomposite (b'), starch-guar gum-nanochitosan (3 wt%)*

surface (c), cross sectional fractured surface of starch-guar gum-nanochitosan (3 wt%) (c').



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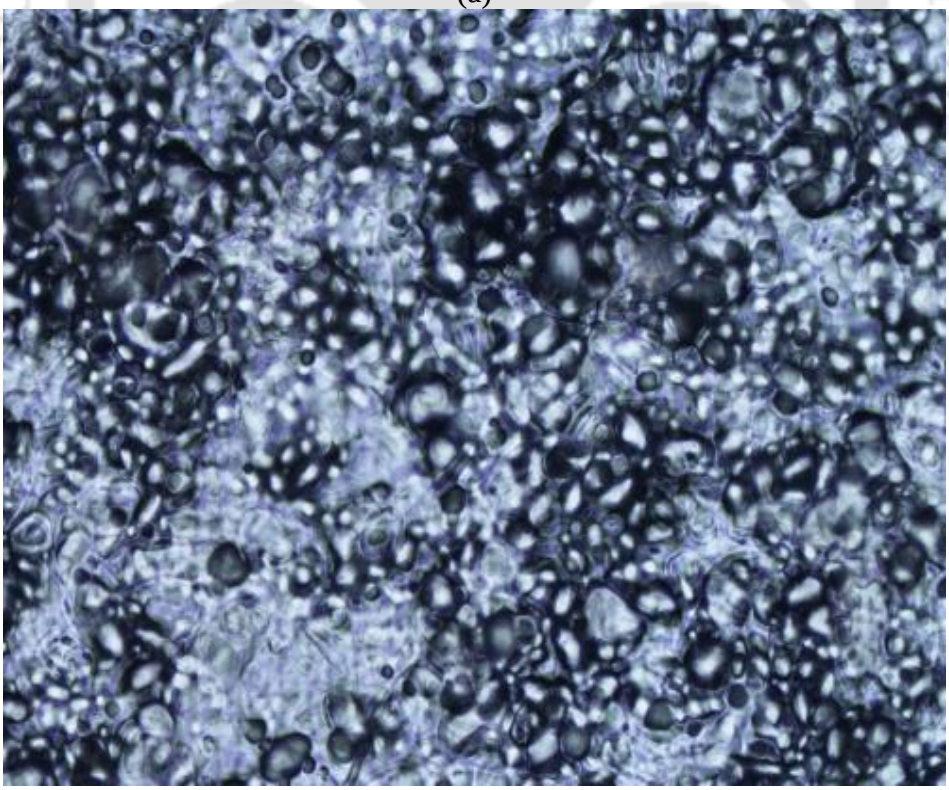

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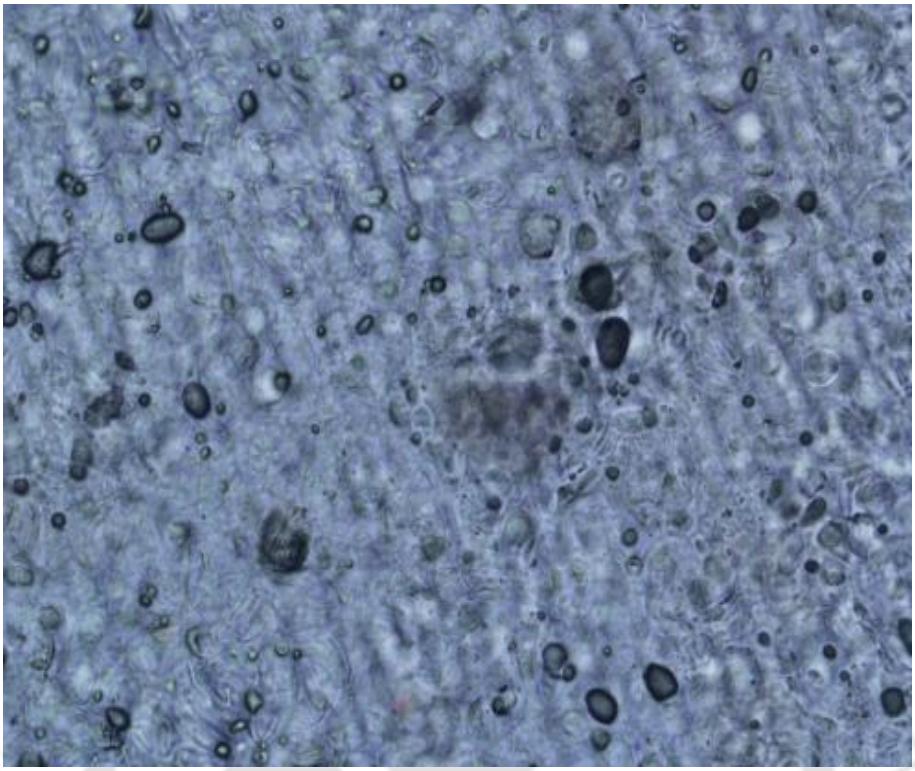

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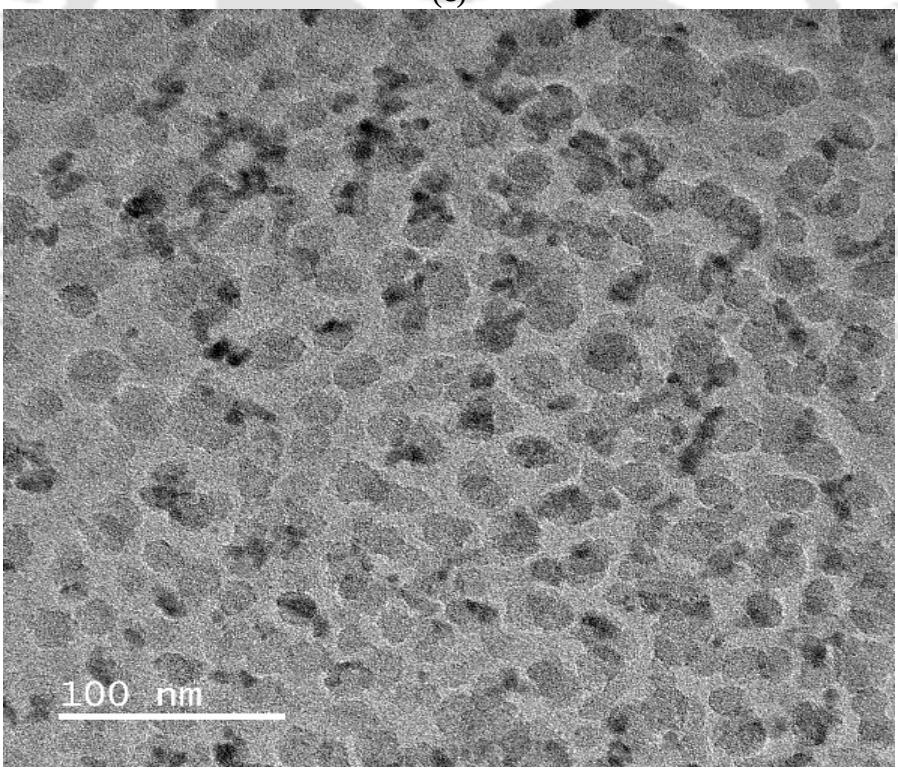
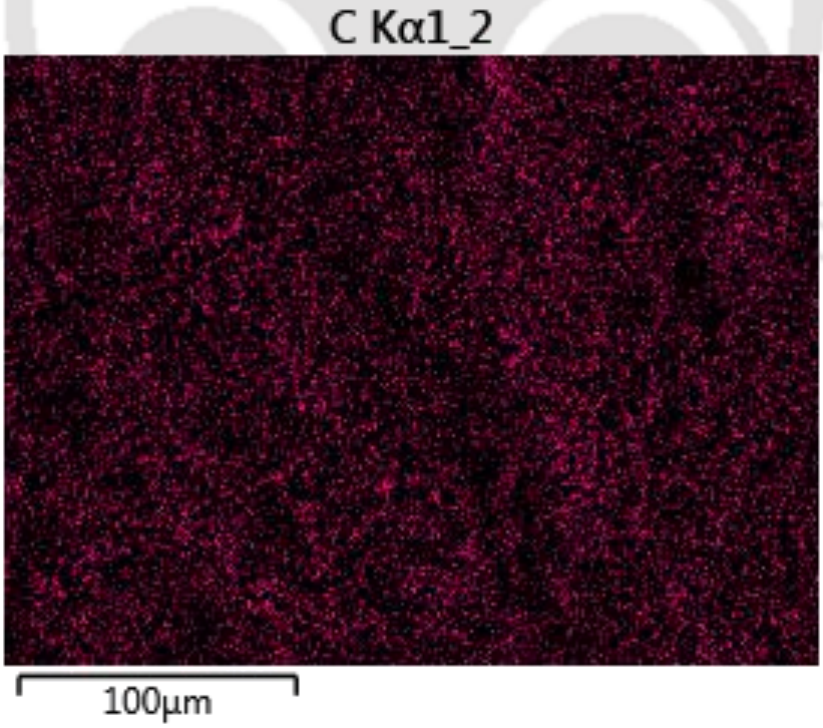


(d)

Figure 4.5 *Optical microscopy images of edible coating representing starch (a), starch-guar gum (b) and starch-guar gum-nanochitosan (3 wt%) (c) and FETEM micrographs of starch-guar gum-nanochitosan (3 wt%) (d).*

EDX Spectra

The EDX spectra and mapped images of fabricated ST-GG-NCS3 biocomposites including corresponding elemental compositions in wt% have been represented in **Figure 4.6**. The analysis has been executed to confirm the absence of any metal component in the fabricated coating materials to be used as an edible material to coat fresh cut apples. As given by EDX spectra, the wt% of O, C and N in ST-GG-NCS3 are ~50.2, ~47.6, and ~2.2%, respectively, confirming the safety of the materials. Additionally, as observed from **Figure 4.6,** the uniform dispersion of N components has been noticed which further confirms the distribution of NCS in the biocomposites of ST-GG as CS consists of (1→4)-linked 2-amino-2-deoxy-β-D-glucan based linear chains having N elements in the amine structures. Further, according to the chemical structure of ST and GG, both the components do not contain N elements in the structure suggesting the uniform distribution in the matrix.



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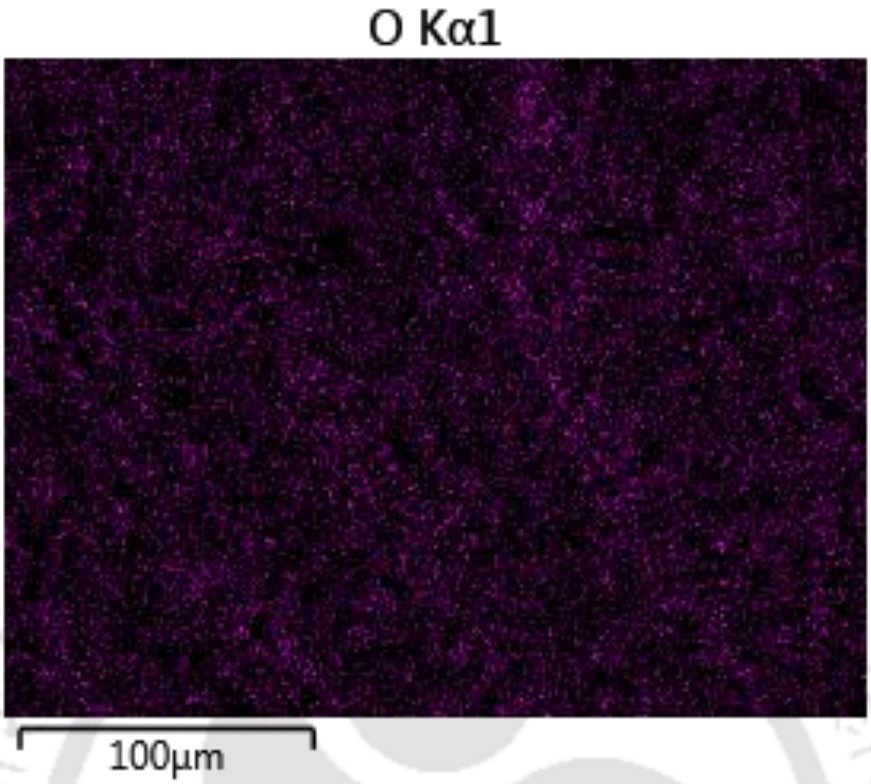

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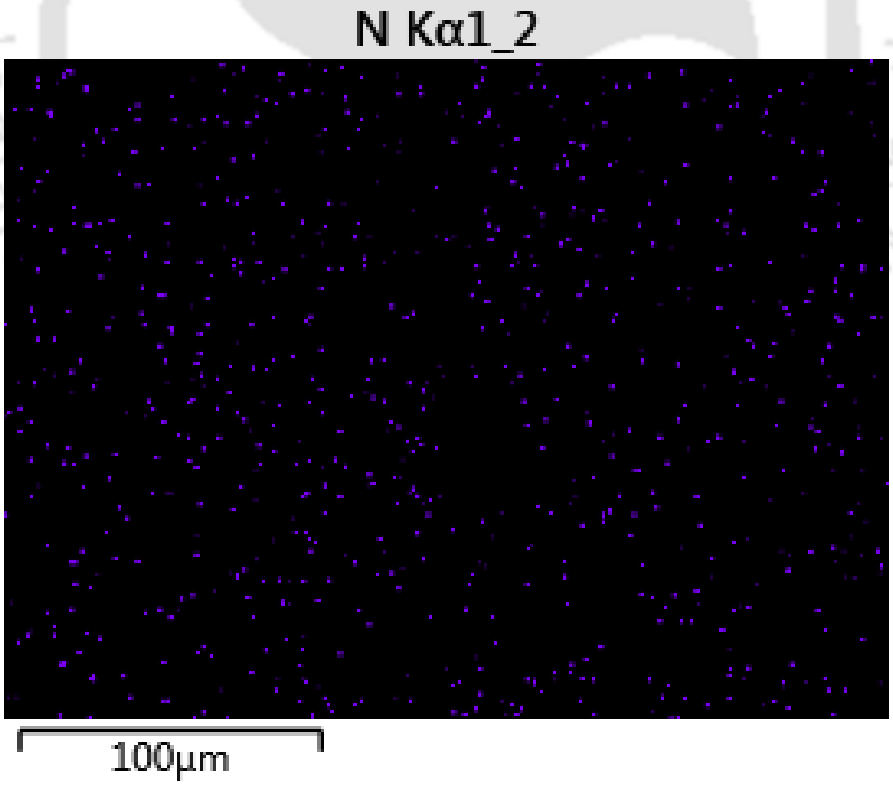

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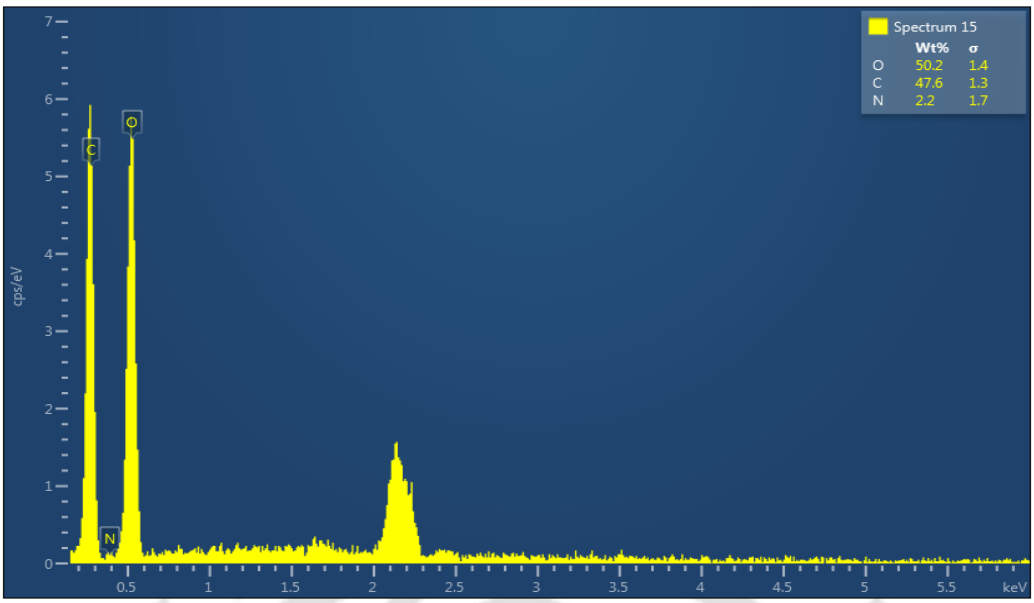


(d)

Figure 4.6 *EDX results of starch-guar gum-nanochitosan (3 wt%) representing mapping of elements C (a); O (b); N (c) and spectrum of different components representing weight percentage (d).*

XRD Analysis

The XRD diffractograms of fabricated ST based biocomposite films are represented in **Figure 4.7,** where the characteristics XRD peaks have been pointed confirming the presence of ST as a base material and the effectiveness of using GG and NCS materials on the crystallinity property of the coating materials. In the ST based edible coating materials, three distinct sharp intensity crystalline peaks have been observed at 2θ = 16.8°, 19.3° and 22.7° (Amran et al., 2016). The specified diffraction peaks define the crystalline nature of the ST edible coating materials. Additionally, the granules structure in potato ST is composed of large (lenticular A-type) and small (spherical B-type) granules, where the observed characteristics diffraction peaks correspond to A-type pattern. Additionally, in the diffraction peaks of all ST based biocomposite edible coating materials, all the characteristics peaks have been noticed with altered intensity. It is noteworthy to mention that the addition of GG has considerably

improved the characteristics of potato ST materials at 2θ = 16.8°, and 22.7°, which further help to enhance the crystallinity of the ST-GG. On the otherhand, the incorporation of NCS has shown its effectiveness at several levels due to various proportion of NCS in the ST-GG materials. The incorporation of NCS at 3 wt% has been observed to increase the crystallinity of ST-GG biocomposite at a significant level, where the plausible reason includes interfacial interactions between the functional groups of the components. Thus, it is noteworthy to mention that, the crystallinity of ST-GG biocomposites can be modified by introducing NCS to the biocomposites.

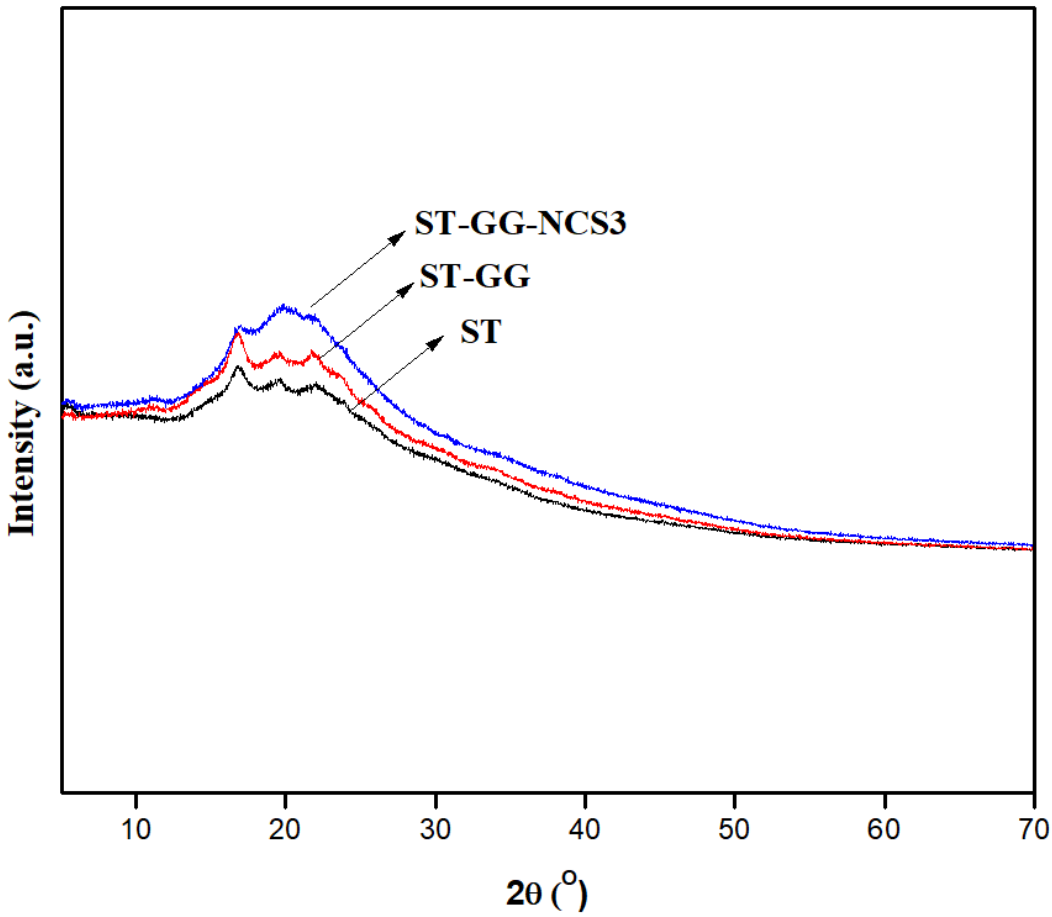


Figure 4.7 *XRD patterns representing starch (ST), starch and guar gum biocomposites (ST-GG), and starch, guar gum and nanochitosan (3 wt%) biocomposites (ST-GG-NCS3).*

FTIR of Starch based Biocomposites

The FTIR spectra of the ST based biocomposites has been represented in **Figure 4.8**. The interaction between ST, GG and NCS can be identified using FTIR spectra, and the hydrogen bonding intensity plays an important role in defining the interactions between used molecules. In this regards, the alternations in peak frequency, strength, and bandwidth deliver the information related to intermolecular hydrogen bonding in the developed biocomposites. The FTIR peaks at 3293 cm^{-1} and 2929 cm^{-1} are obtained due to –OH stretching vibrations and –CH stretching in the molecules, respectively. The ST band peak at 1645 cm^{-1} provides the formed hydrogen bonds with presence of bound water. The FTIR peaks at 1416 and 858 cm^{-1} are obtained due to the vibrational CH_2 groups. The observed FTIR peaks at 1150 and 999 cm^{-1} are observed due to -CO bond stretching in C-O-C groups associated with anhydroglucose ring. The observed FTIR peaks are in good agreement with the available literature (Aydın, & Ilberg 2016; Vu, & Lumdubwong, 2016). Additionally, the characteristics FTIR peaks have been noticed in the developed ST based biocomposite materials such as ST-GG, ST-GG-NCS1, ST-GG-NCS2, and ST-GG-NCS3 with altered intensity and further, there has been noticed some shift in the FTIR peaks for the developed biocomposites. The FTIR spectrum of ST-GG biocomposites has reduced peak intensity and shift in characteristics ST peaks. Further, the FTIR peak at 1103 cm^{-1} of ST is obtained due to vibration of C-O-C pyranose ring or attributed to crystalline region of ST, which is found to absent in other biocomposites (Wang et al., 2017).

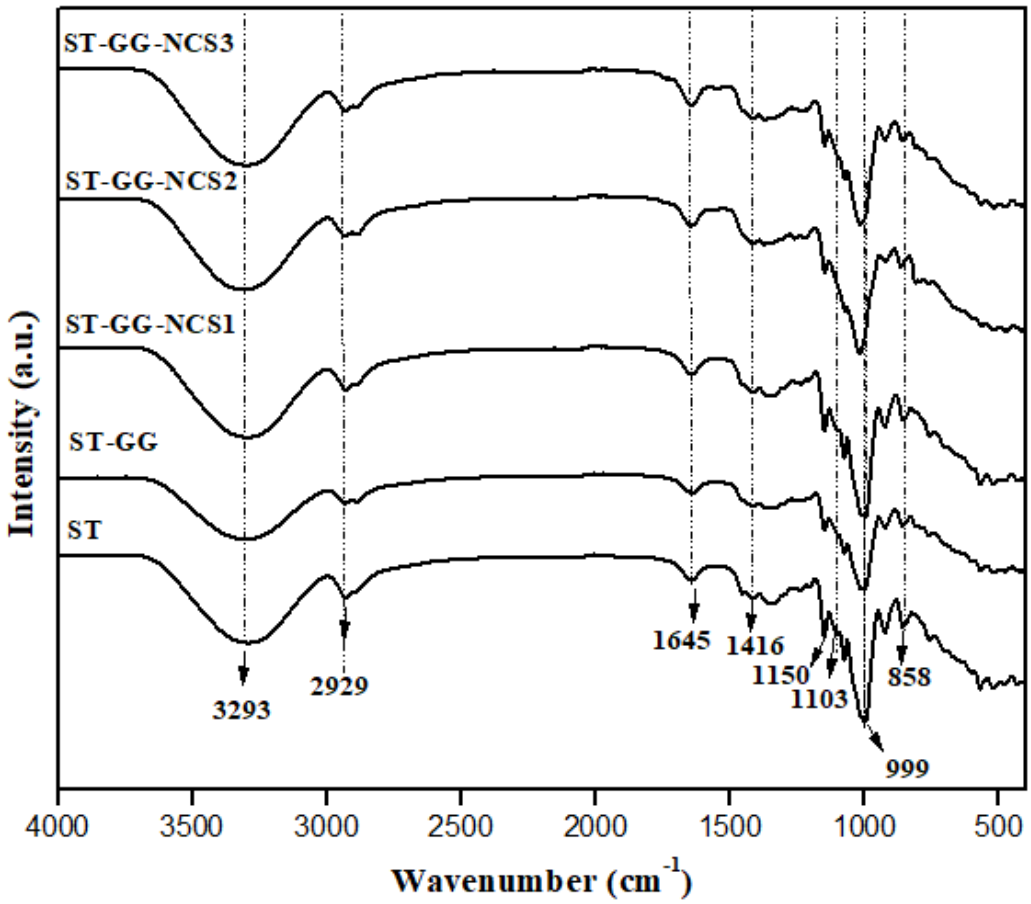


Figure 4.8 *FTIR spectrum of ST/GG/NCS based biocomposites.*

Thermal Property

The thermal properties of developed materials have been represented in **Figure 4.9** and **Table 4.1**. The comprehensive study on the thermal stability of modified ST biocomposites delivers the information on the effectiveness of used biofillers against storage temperature to be used in food based application as processing of ST films include different heat treatments (**Soares et al., 2005**). In this study, the thermal stability behavior of the fabricated edible coating samples is executed to predict the effectiveness of incorporating GG and NCS in the matrix of ST. The several parameters including temperatures at which thermal degradation of 10 and 50% weight of the subsequent testing samples are determined and denoted as T_{10} **(**°C**)** and T_{50} **(**°C**)**, respectively. Additionally, the offset thermal degradation temperature (T_{offset}) of

the samples has also been determined. All the testing samples provide two step degradation at two different temperature ranges. However, the first step degradation of the edible coating materials occurs due to the moisture residues present in the edible coating materials at different temperature ranges. For the sample specimens ST and ST-GG, there is observed a drastic first stage thermal degradation in the ranges of 40 to 140 °C due to adsorbed and bound water removal. On the otherhand, the edible coating materials such as ST-GG-NCS1, ST-GG-NCS2 and ST-GG-NCS3 have a first stage temperature degradation range of 60 to 140 °C. Further, the second stage degradation occurs due to depolymerization, dehydration, and breakdown of main chains of ST. Additionally, the observed T_{10} values for the ST biocomposites are ~79.36, ~80.49, ~126.62, ~214.82, ~186.89 °C for ST, ST-GG, ST-GG-NCS1, ST-GG-NCS2 and ST-GG-NCS3, respectively. The observed T_{10} values define that the incorporation of NCS for 1, 2 and 3 wt% has significantly improved thermal stability by ~47.26, ~135.46, and ~107.53 °C, respectively compared to ST based coating materials. It is noticeable that the incorporation of NCS at a percentage of 3 wt% to the ST-GG biocomposite materials has reduced the thermal stability compared to ST-GG-NCS2 based edible coating, which may occur due to the agglomeration effect of the nanofiller decreasing the thermal stability. The improved thermal stability may be obtained due to the probable good interactions between the ST components, GG and NCS nanofillers. Further, as represented in **Table 4.1,** the T_{50} values of the fabricated modified ST based biomaterials are ~327.36, ~332.88, ~332.95, ~333.79, and ~333.35 °C for ST, ST-GG, ST-GG-NCS1, ST-GG-NCS2 and ST-GG-NCS3, respectively. After incorporating the GG as a reinforcement material in ST has improved the thermal stability in terms of T_{50} values by ~ 5.52 °C. Additionally, the inclusion of NCS at 1, 2 and 3 wt% improves the thermal stability by ~ 5.59, ~6.43 and ~ 5.59 °C, respectively. The T_{offset} values of ST based edible coatings are ~346.36, ~357.82, ~359.65, ~362.73, and ~366.67 °C for ST, ST-GG, ST-GG-NCS1, ST-GG-NCS2 and ST-GG-NCS3, respectively. This also suggests the obtained

improved thermal stability of the edible coating materials. Therefore, from the conducted thermal stability study, it can be concluded that the inclusion of NCS as a strategy to modify the existing properties of ST-GG biocomposite can successfully improve the thermal attributes of the biomaterials which are required for using the developed edible coating for thermally sensitive food products.

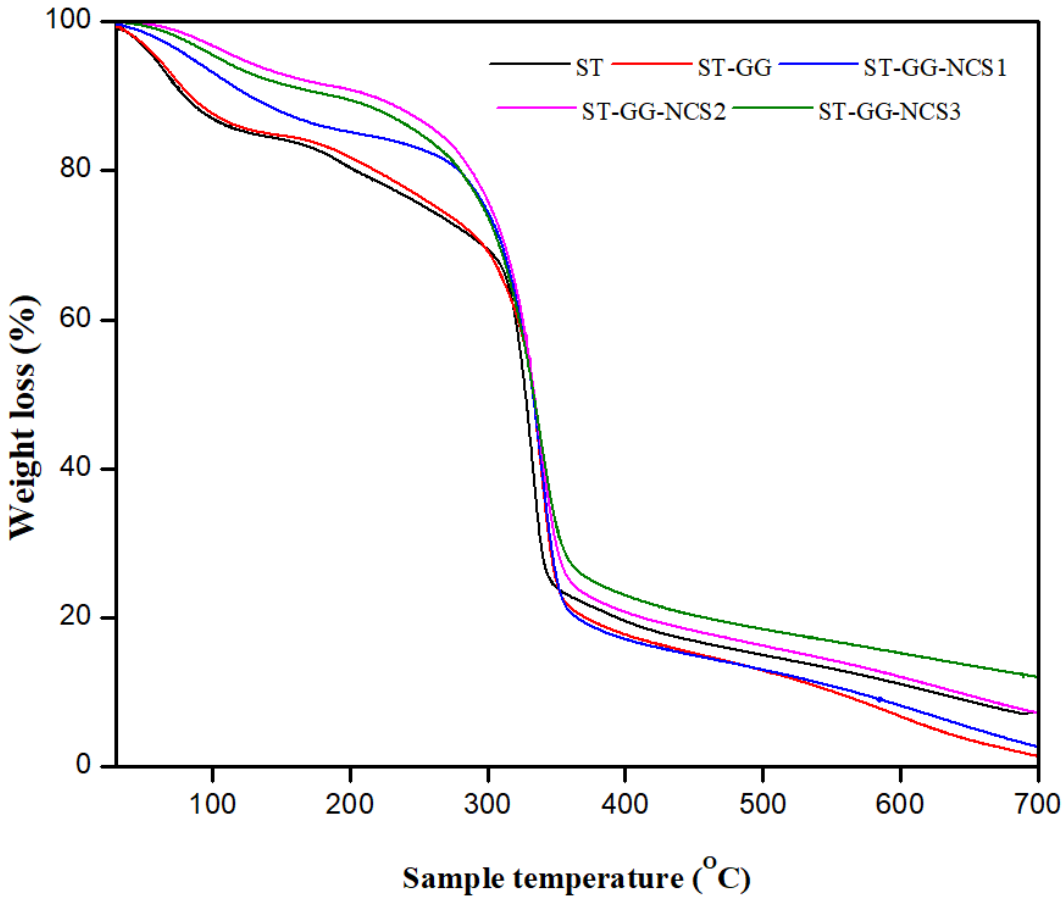


Figure 4.9 *TGA thermograms of nanochitosan added starch and guar gum based biocomposites.*

Table 4.1 *Thermal properties of nanochitosan added starch and guar gum based biocomposites.*

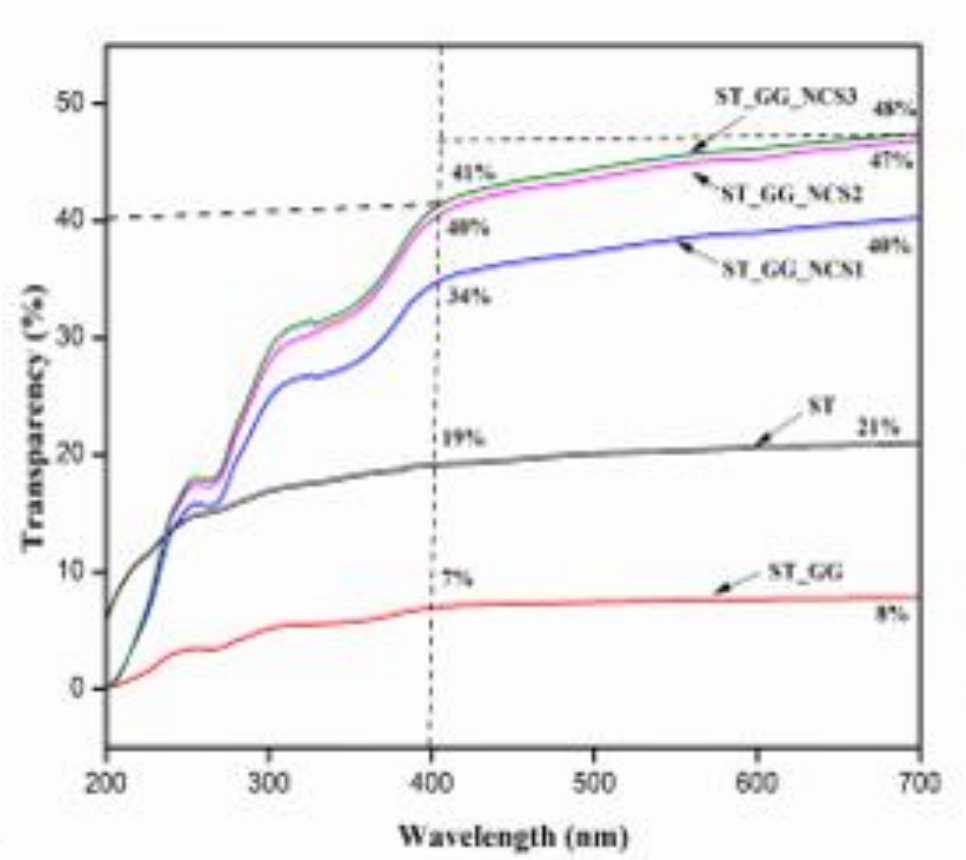
Testing samples	T_{10} (°C)	T_{50} (°C)	T_{offset} (°C)	W_{res} (%)
ST	79.36	327.36	346.36	7.24
ST-GG	80.49	332.88	357.82	1.47
ST-GG-NCS1	126.62	332.95	359.65	2.75
ST-GG-NCS2	214.82	333.79	362.73	7.28
ST-GG-NCS3	186.89	333.35	366.67	12.08

Optical Property

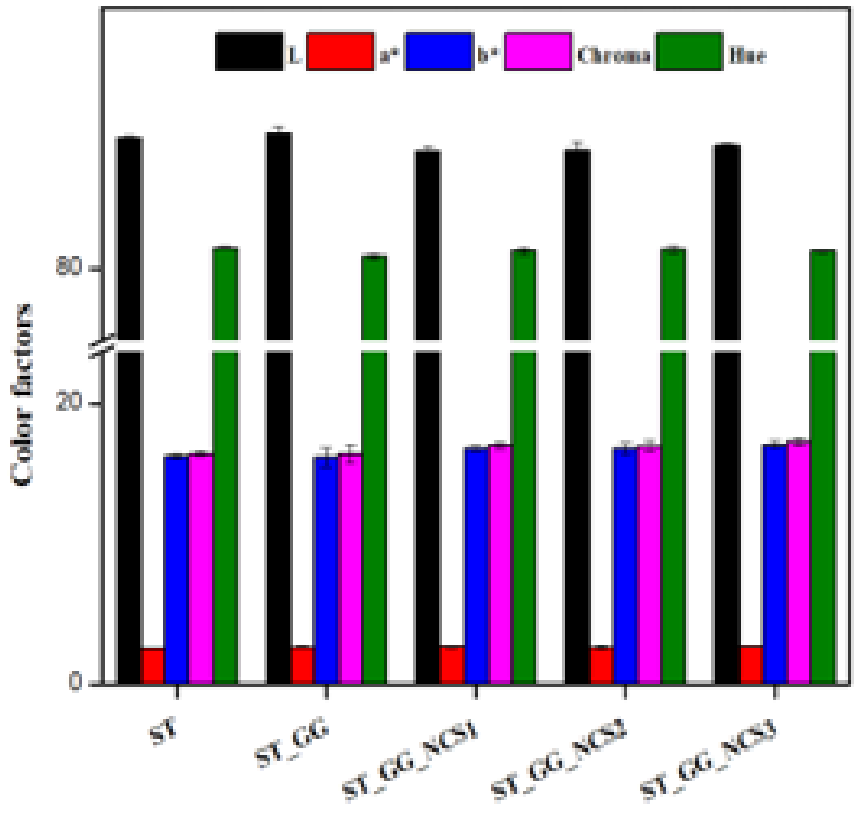
The optical properties of ST based edible coating materials have been investigated based on transparency and color factors as shown in **Figure 4.10**. As represented in **Figure 4.10a**, the fabricated films have a transparent and homogeneous texture as viewed from the images. The transparency of ST based edible coating has been varied significantly due to altered microstructural features caused due to incorporating various nanofiller materials. Additionally, the transparency effect of the various formulations can depict the dispersion effect of the reinforced materials physically. As shown in **Figure 4.10a**, the transparency of the packaging materials has been determined for the ultraviolet and visible regions. The edible coating materials has the transparency of 21%, 8%, 40%, 47%, and 48%, for ST, ST-GG, ST-GG-NCS1, ST-GG-NCS2, and ST-GG-NCS3, respectively. As represented in **Figure 4.10a**, the incorporation of GG in ST based films have a negative effect on transparency property due to obtained higher polymeric chain compaction which restricts the passage of light through the ST biocomposite materials. Similar type of result has been found by Saberi et al., 2016 for developing pea ST and GG based edible films. The GG cause an interaction with ST components such as amylose and amylopectin *via* non-covalent hydrogen bonding and other

network structures. The transparency of films depends on homogeneity, dispersion, molecular weight, plasticizer effect, and others. The reduced transparency of ST-GG films occurs due to white coloration effect by GG in the base materials. In the visible range, the transparency of the materials are 21%, 8%, 40%, 47%, and 48% for ST, ST-GG, ST-GG-NCS1, ST-GG-NCS2, and ST-GG-NCS3, respectively. Additionally, the transparency of the films are 19%, 7%, 34, 40, 41% for ST, ST-GG, ST-GG-NCS1, ST-GG-NCS2, and ST-GG-NCS3, respectively. Based on this, the incorporation of NCS delivers noteworthy improvement in the transparency suggesting better dispersion and compatibility among the edible coating materials. The ST-GG-NCS3 films have relatively similar transparency to the ST-GG-NCS2 edible materials which suggest the formation of possible agglomerates within the nanofillers due to strong interactions by reducing the effectivity. The increased transparency due to incorporating NCS suggests the formation of better uniformity of GG by reducing the light scattering property through interfacial sites. In this way, the reduced transparency of ST-GG based edible coating can be tailored-made by incorporating the NCS based nanofiler materials for changing the molecular behavior of the matrix.

The color factors have received a remarkable interest in consumer acceptability of the packaging materials. The color parameters in terms of lightness, hue coordinates (a*, b*), hue and chroma of the developed edible nanocoating materials such as ST, ST-GG, ST-GG-NCS-1, ST-GG-NCS-2, and ST-GG-NCS-3 has been represented in **Figure 4.10b**. The visual differences in the edible nanocoating materials have been investigated using L, a*, b*, hue and Chroma values. From the figure, it can be observed that the color parameters have maintained the color feature. The L values of the films vary from 86.6 to 87.56 suggesting brightness, a* varies from 2.5 to 2.7 suggesting red coloration effect, b* varies from 16.14 to 17.05 suggesting yellow coloration effect, chroma value varies from 16.3 to 17.2, hue value varies from 80.6 to 81.1 suggesting yellow coloration effect (Ghosh et al., 2020).



(a)



(b)

Figure 4.10 *Optical properties of developed ST based biocomposites representing transparency (a); color factors (b) and visual images of the developed ST based biocomposites for ST (c), ST-GG (d), ST-GG-NCS1 (e), ST-GG-NCS2 (f), and ST-GG-NCS3 (g).*

Antimicrobial property

The antimicrobial property of CS, NCS and developed ST biocomposites is tested against *Staphylococcus aureus* ATCC 6538 (Gram positive bacteria) and *Escherichia coli* MTCC 723 (Gram negative bacteria) using disc diffusion technique as represented in **Table 4.2 and Figure 4.11**. Interestingly, the results indicate that NCS has more antimicrobial activity against both the pathogens, where NCS is found to be more effective against *E. coli* (25.33 ± 1.24 mm) than *S. aureus* (24 ± 0.82 mm). The fabricated NCS has more antimicrobial property than CS for both *E. coli* and *S. aureus.* The ST and ST-GG based edible coating materials have no antimicrobial activity against both the tested microbes. Similar reports have been found that sago ST and GG based bi-phasic edible films provide no antimicrobial activity against *E. coli* and *Bacillus cereus* (Dhumal et al., 2019). The incorporation of NCS provides antimicrobial property to the ST-GG biocomposite coating. Considering the fabricated edible coating solutions, the incorporation of NCS to the ST-GG based biocomposite provides antimicrobial activity against food borne pathogens which is beneficial for reducing the microbial growth on food products during storage. Additionally, CS is reported to be used in combination with GG as a composite film to provide antimicrobial property (Rao et al., 2010). However, there has

been observed a decrease in the diameter of zone of inhibition in NCS incorporated edible coating materials which may be due to the electrostatic interactions between the ST, GG and NCS, which may reduce the availability of active amine group in NCS, and is supposed to be responsible for antimicrobial property.

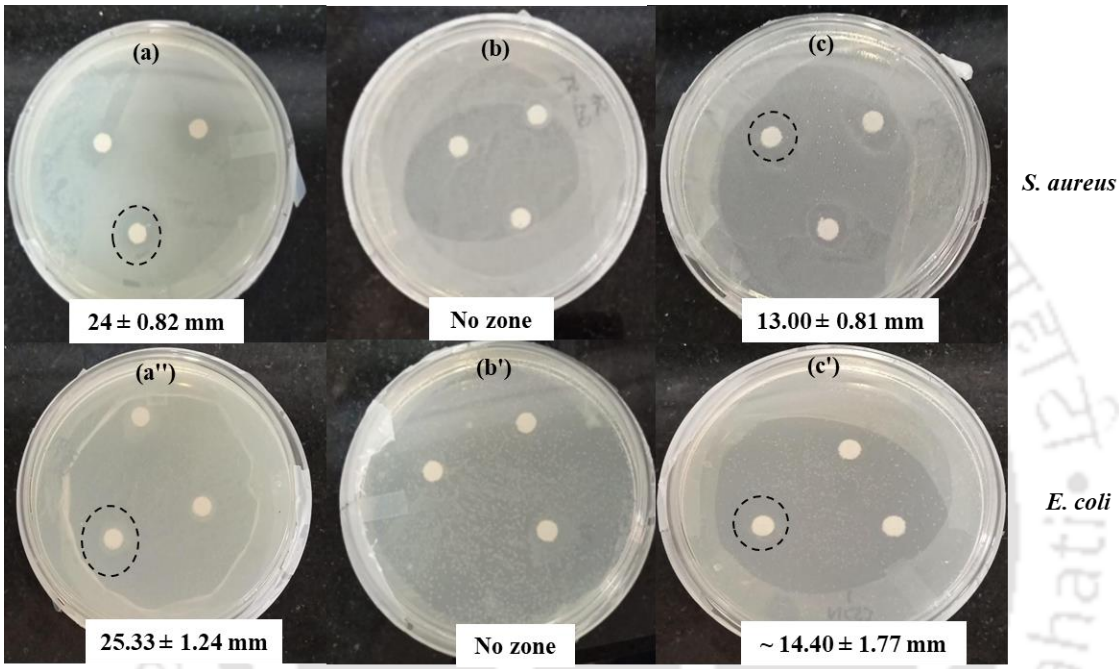


Figure 4.11 *Antimicrobial activity of NCS(a), ST-GG(b), ST-GG-NCS3(c) based biocomposites against S. aureus and NCS (a'), ST-GG (b'), ST-GG-NCS3(c') based biocomposites against E. coli.*

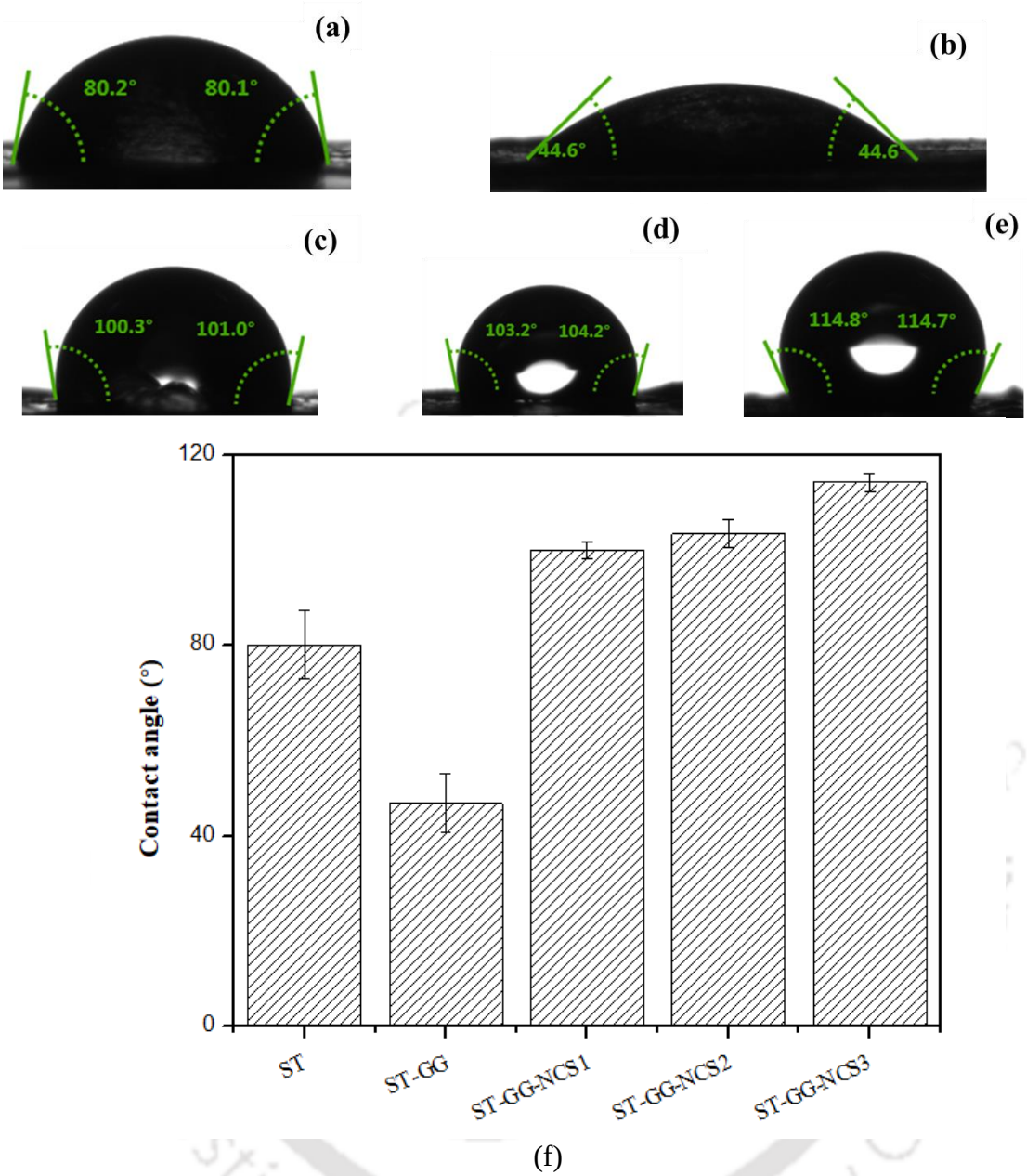
Table 4.2 *Zone of inhibition for fabricated NCS, and ST based biocomposite against S. aureus and E. coli. Different letters define the significant differences between samples ($p<0.05$)).*

Sample Name	Zone of Inhibition (mm)	
	S. aureus	***E. coli***
CS	18.67 ± 2.05^{b}	20.00 ± 0.82^{b}
NCS	24 ± 0.82^{a}	25.33 ± 1.24^{a}
GG	0^{d}	0^{d}
ST	0^{d}	0^{d}
ST-GG	0^{d}	0^{d}
ST-GG-NCS1	10.00 ± 0.81^{c}	11.67 ± 0.47^{c}
ST-GG-NCS2	11.33 ± 1.24^{c}	14.33 ± 0.94^{c}
ST-GG-NCS3	13.00 ± 0.81^{c}	14.40 ± 1.77^{c}

Note: Chitosan (CS); Nanochitosan (NCS); Guar gum Powder (GG); Starch (ST); Starch-Guar Gum (ST-GG); Starch-Guar Gum-NCS-1% (ST-GG-NCS1); Starch-Guar Gum-NCS-2% (ST-GG-NCS2); Starch-Guar Gum-NCS-3% (ST-GG-NCS3)

Wettability Analysis

The wettability of different edible coatings is evaluated by determining the contact angles of the developed edible coating materials as represented in **Figure 4.12**. The surface wettability of the fabricated ST-GG based biocomposites is greatly influenced by the incorporation of NCS based nanofiller materials. The observed surface wettability of ST based materials is 80.1 ± 7.2°. Similarly, a research reports the contact angle of potato ST to be ~ 85° (Morán et al., 2013). The inclusion of GG in the ST based materials has been found to increase the hydrophilic nature with decreased contact angle (46.8 ± 6.2°), which is an undesirable property for a food packaging material. In this regards, the inclusion of NCS has sharply increased the contact angle directing improved hydrophobicity of packaging materials to 114.3 ± 2.0° due to the altered surface property of the biocomposites. The decreased hydrophilicity of biocomposites can be correlated with this, where the incorporation of NCS significantly alters the surface morphology. Additionally, the improved transparency has been found to improve due to the inclusion of NCS in ST-GG biocomposites.



(f)

Figure 4.12 *Static contact angle of starch based biocomposites at 27 °C representing ST (a); ST-GG (b); ST-GG-NCS1 (c); ST-GG-NCS2 (d); ST-GG-NCS3 (e) and comparative analysis (f).*

Water Vapour Transmission Rate

The measurement of water vapour transmission rates (WVTR) are done to study the effectiveness of GG and various proportions of NCS on the barrier properties of ST based nanocomposite materials as depicted in **Figure 4.13**. The ST-GG biocomposite films have shown increased WVTR values, the plausible reason may be due to increased hydrophilicity of

the materials as mentioned in the previous section. Additionally, the ST-GG-NCS biocomposite based edible coating materials provides a significant drop in WVTR values in all nanocomposites. As observed from the WVTR values, there has been observed that with increased concentration of NCS, there have been a subsequent reduction in the WVTR values. The observed trend can be correlated with the wettability property of the biocompoites, where the incorporation of NCS significantly improves the surface wettability of the ST-GG biocomposites.

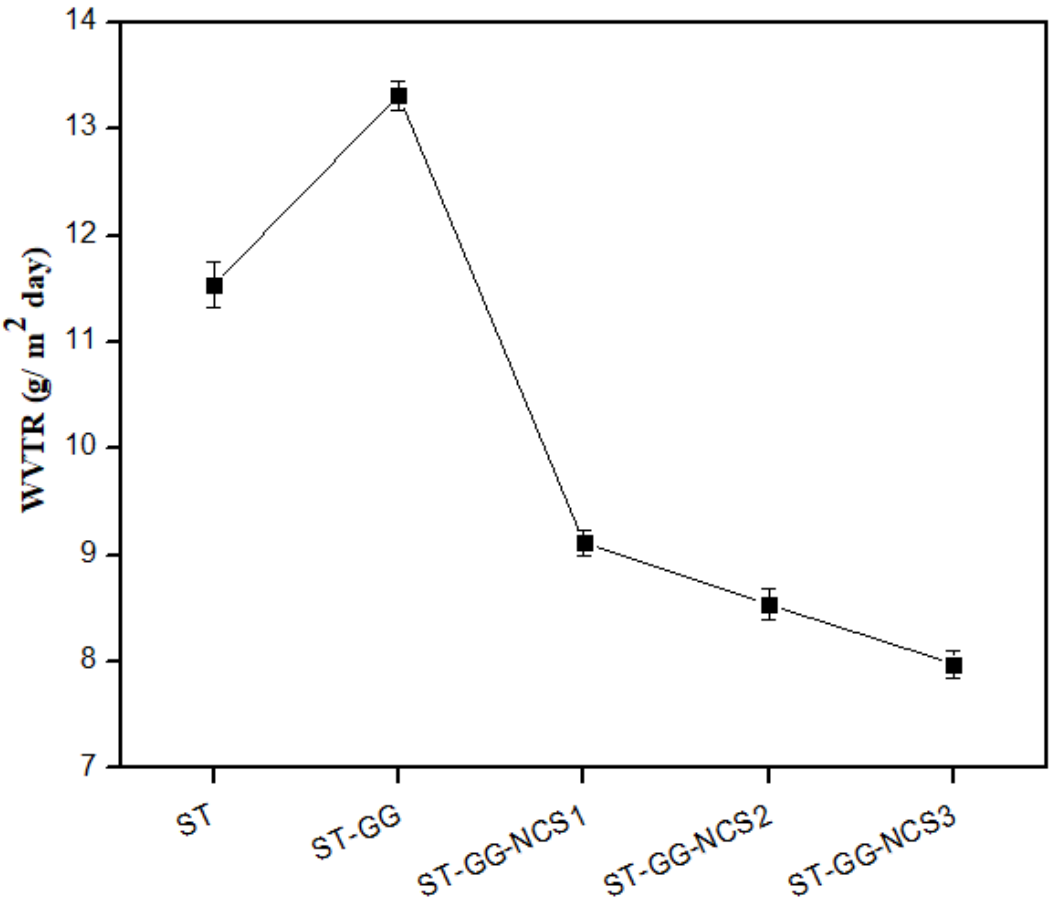
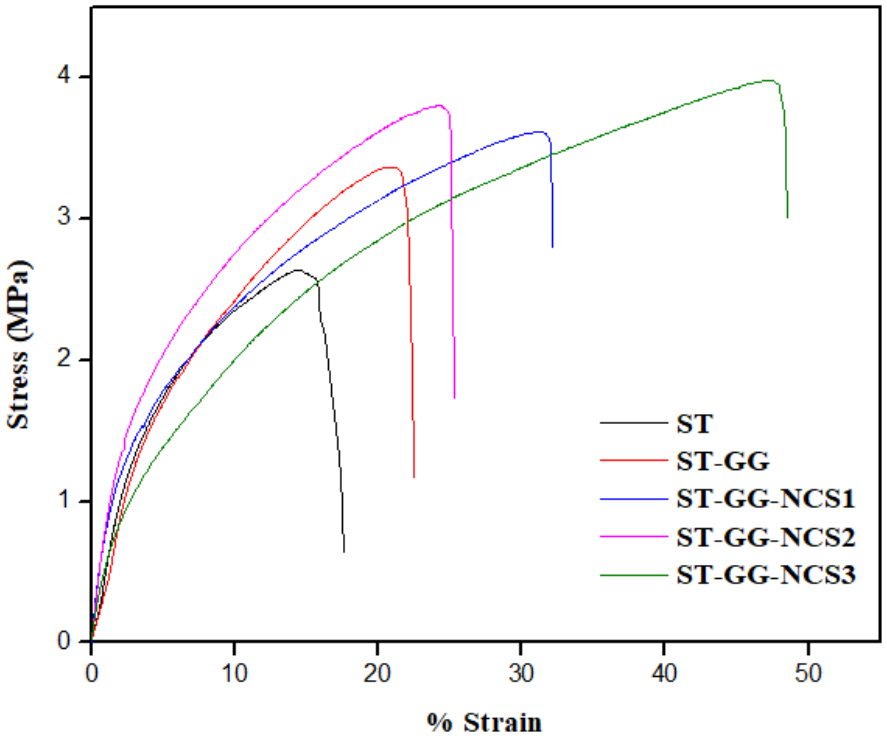


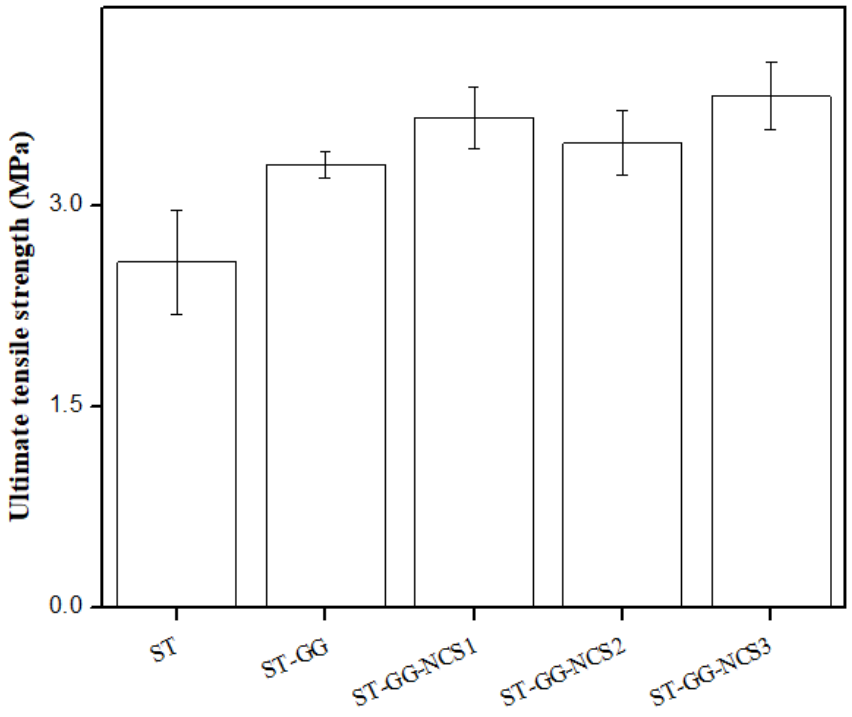
Figure 4.13 *Water vapour transmission rate of modified starch based biocomposites for edible coating applications.*

Mechanical Property

The obtained stress-strain curves of the developed NCS incorporated ST-GG based edible coating materials have been represented in **Figure 4.14a,** which define that the fabricated materials provide flexible materials **(**Menzel, et al., 2019**)**. Additionally, the mechanical properties in terms of tensile strength, and elongation at break of the developed edible coating materials are determined in tensile mode as represented in **Figure 4.14 (b & c)**. The tensile strength of ST and its biocomposites is found to be in the range of 2.50 to 3.85 MPa. The tensile strength has been found to improve consecutively. However, the % elongation at break of ST was 18.21 % which is improved to 41.06 % for ST-GG-NCS3. The % elongation at break of ST-GG biocomposites is found to increase significantly due to the incorporation of NCS. Additionally, the % elongation at break of ST-GG is increased by increased concentration of NCS. The increasing % elongation at break is attributed due to increase ductility of the materials. Additionally, as viewed from **Figure 4.14a,** the increased ductility of the materials can be seen due to increased NCS content in ST-GG biocomposites. Thus, it is confirmed that incorporation of GG in ST matrix improves the mechanical properties in terms of % elongation at break. Additionally, an increased proportion of NCS further increases the ductility and % elongation at break of ST-GG based biocomposites. The mechanical properties of developed ST based biocomposites materials is measured which is an essential property to retain food products from mechanical injury.



(a)



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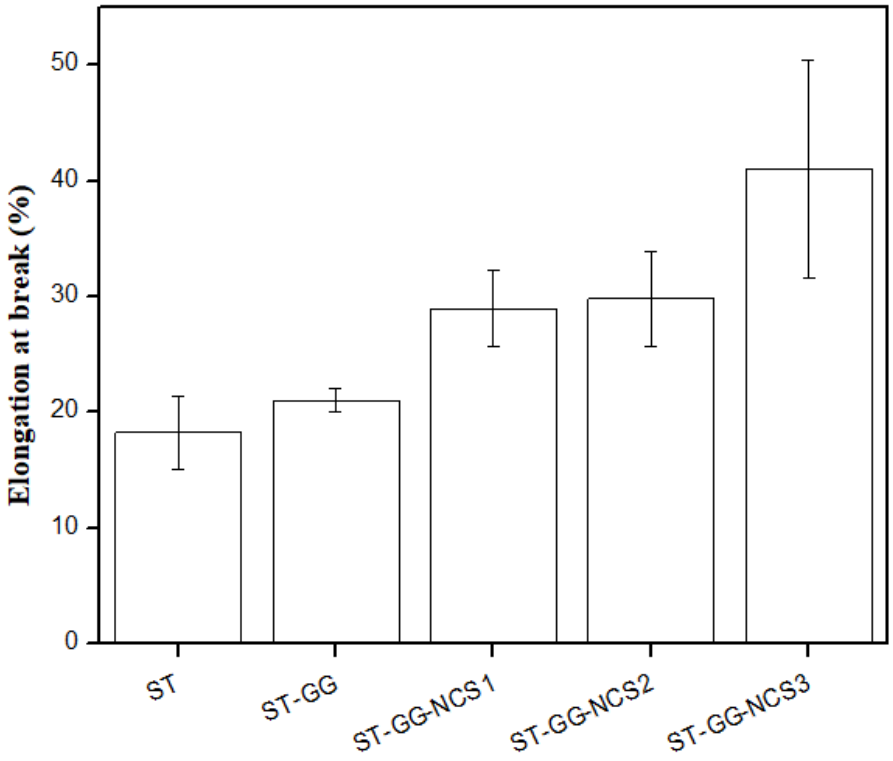


(c)

Figure 4.14 *Representative curves for Stress vs %strain (a); ultimate tensile strength (b); and (%) elongation at break of starch, guar gum and nanochitosan based biocomposites.*

4.2.3 Application of Nanochitosan Modified Starch/Guar Gum Biocomposites Based Edible Coating on Cut Apple

ST as an edible coating is used in combination with other biomaterials and nanofillers as blends and biocomposites are used to improve the shelf life of fresh produces, respectively. In this context, several research reports the effectivity of NCS over conventional CS in improving the quality of stored fruits and vegetables as an edible coating. The application of edible coating based on ST-GG-NCS improves the storage quality of cut apples compared to uncoated apples (**Figure 4.15**). Additionally, it is noteworthy to mention that the storage quality in terms of microbial growth, pH change, color attributes, and weight loss are better preserved for cut apple fruits when edible coatings are developed with the aid of NCS.

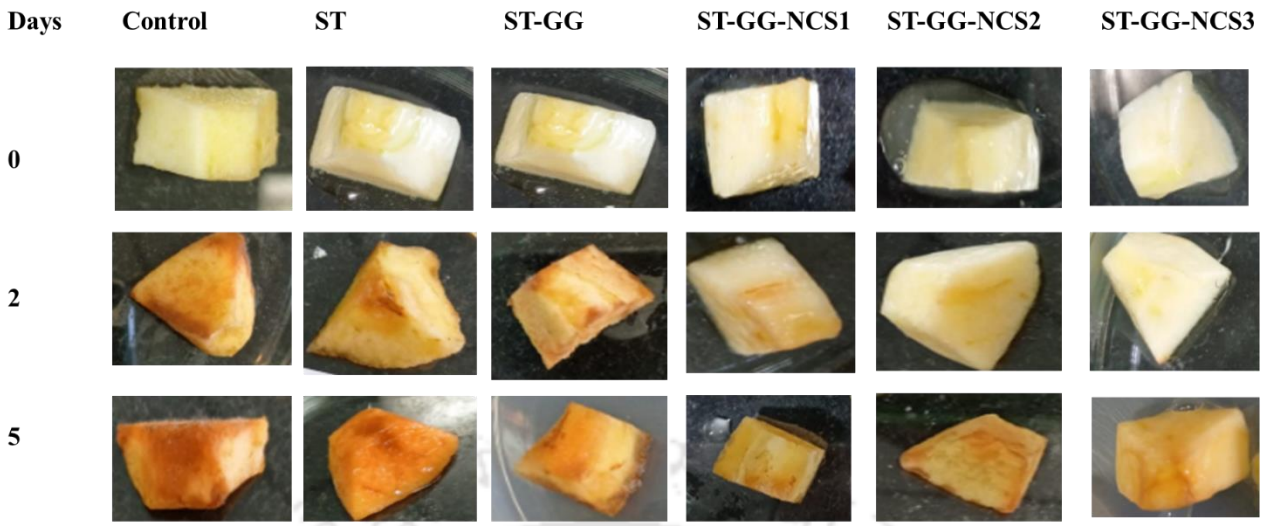
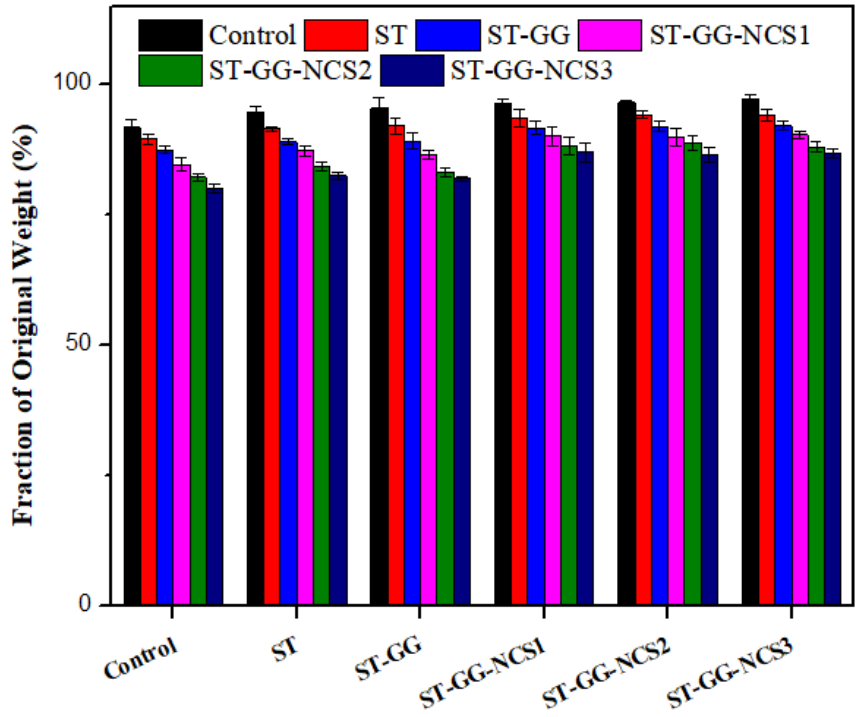


Figure 4.15 *Photographs of stored uncoated and edible coated Cut apple with ST, ST-GG, ST-GG-NCS1, ST-GG-NCS2, and ST-GG-NCS3 at different time lapse.*

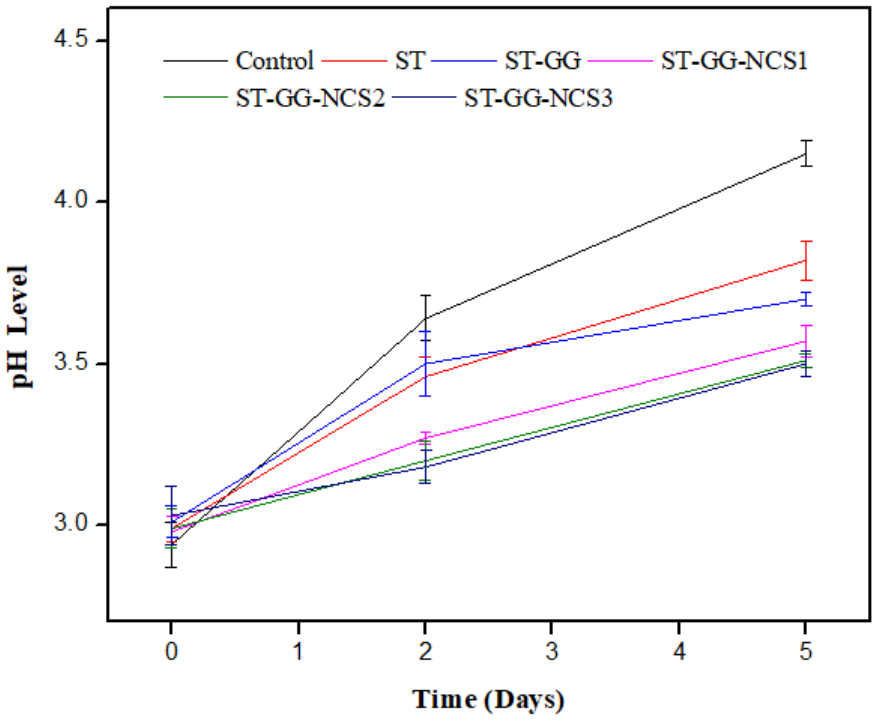
Original Weight Fraction

The effectivity of the coating materials in maintaining the original weight as a function of storage time is observed at 25 °C for a duration of 5 days as represented in **Figure 4.16.** The fresh fruits and vegetables continue weight loss during storage life due to several environmental conditions such as water loss, microbial attack, inhibition of water supply from plant part, continue respiration, and others. In this regards, the weight loss phenomena of cut apples increase with storage time due to the mentioned natural phenomena**.** The reduced weight loss of fruit products can severely affect the economic condition, where the application of proper coating materials can help to preserve the weight of the materials. As observed from the **Figure 4.16**, the original weight for edible coated cut apple fruits are retained significantly ($p<0.05$) using the developed edible coating. However, the control cut apple samples are found to have a weight loss upto ~20% during 5 days of storage. The weight loss of cut apple held mainly due to higher moisture loss from the fruit products, which may get reduced due to the application of edible coating materials. ST based edible coating is effective in maintaining the

weight of plum fruit (Thakur et al., 2018), banana fruits (Thakur et al., 2019), tomato fruits (Nawab et al., 2017), strawberries (Pinzon et al., 2020), etc. In this regards, the hydrophilic nature of ST-GG may be prone to absorb moisture and acts as a poor barrier for water vapour, which increase the water loss phenomena in coated cut apple compared to ST based edible coating. In this regards, the edible coating with ST-GG-NCS1, ST-GG-NCS2, and ST-GG-NCS3 have more positive effectiveness in reduced weight loss due to hydrophobic nature, antimicrobial property and water vapour permeability. The NCS addition delays weight loss of cut apple in an effective way as compared to control cut apple fruits. The weight loss has been found to decrease due to application of ST and ST-GG based edible coating material by 3% and 2% at 5 days of storage compared to control, respectively. During the complete storage life, the effectiveness of ST in maintaining original cut pineapple weight is more than ST-GG biocomposites. The possible reason may be due to increased hydrophilicity behavior of ST-GG biocomposites compared to ST, which reduce the weight of cut apple. Additionally, the microbial attack may have increased for ST-GG edible coated products. On the otherhand, the inclusion of NCS is effective in maintaining the hydrophobicity, water vapour permeability, antimicrobial property, which helps in keeping quality of cut apple fruits. Cut apple in ST-GG-NCS based edible coating has reduced weight loss than the other type of edible coating materials. However, CS as an edible coating material is widely used to preserve the water content in several perishable fruits and vegetables such as strawberry, banana, mango and others. The use of NCS aided coating delivers improved cut apple quality with postharvest storage life by altering product quality. In addition to this, our formulation is more effective in maintaining the weight loss phenomena of cut apples during storage. Similarly, the application of fabricated ST based edible coating is found to be efficient in maintaining pH of the cut apple as represented in **Figure 4.16b** at $p<0.05$.



(a)



(b)

Figure 4.16 *Original weight retained (a) and level of pH (b) for the control and edible coated cut apple fruits at 25 °C for 5 days. Data sets are represented as mean ± standard deviations.*

Microbiological Study

The microbiological analysis of the control and edible coated cut apple fruits at 0, 2 and 5 days are done by measuring the mesophilic count as represented in **Table 4.3**. The mesophile count at 0 days is found to be between 2.15 ±0.02 Log (CFU/mL) and 1.97±0.01 Log (CFU/ mL). In this regards, the initial counts for mesophiles for the food products are found to reduce significantly due to the application of NCS aided edible coating materials for antimicrobial property. Additionally, the mesophile count is found to be highest for control sample of 3.46 ± 0.05 and 4.07 ± 0.03 Log (CFU/ mL) at 2 and 5 days of storage, respectively. The observed mesophile count for ST-GG-NCS3 based edible coated cut apple is found to be 3.17 ± 004 Log (CFU/ mL) ($p<0.05$) which is significantly reduced compared to the control samples. Additionally, there has been observed a significant reduction in the mesophilic count due to the application of edible coating materials. The results are in line with the observed antimicrobial property of the edible coating materials, where ST-GG-NCS3 has been found to be most effective among the used edible coating materials. In this way, the incorporation of NCS is found to be effective in reducing the microbial count of cut apple fruit products during storage.

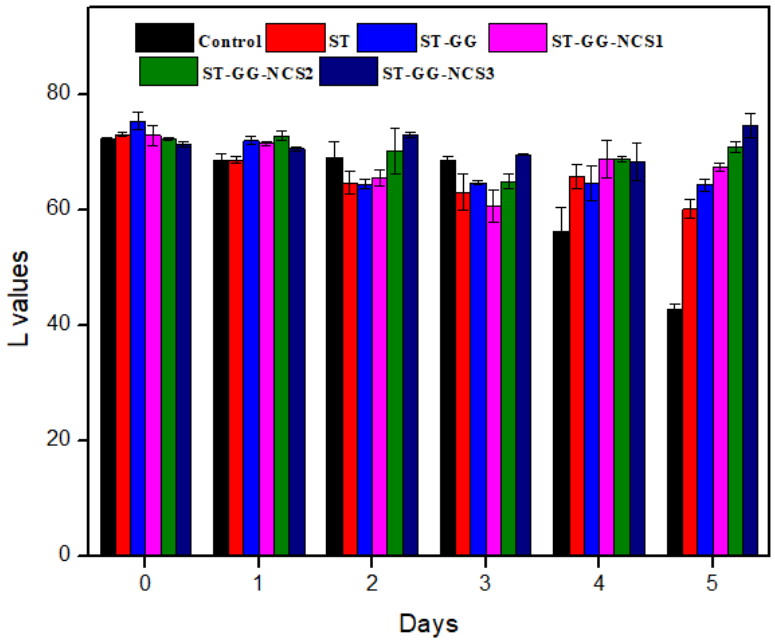
Table 4.3 *Mesophile count of stored cut fruit products. Data sets are represented as mean ± standard deviations. Different letters define the significant differences between samples (p<0.05)).*

Sample	0 Day Log CFU/mL	2 Day Log CFU/mL	5 Day Log CFU/mL
Control	2.15 ±0.02^{a}	3.46 ± 0.05^{a}	4.07 ± 0.03^{a}
ST	2.08 ± 0.01^{b}	3.30 ±0.03^{b}	3.99 ± 0.01^{a}
ST-GG	2.11 ±0.01^{ab}	3.32 ±0.03^{b}	3.99 ±0.01^{a}
ST-GG-NCS1	2.02 ± 0.01^{bc}	3.11 ± 0.05^{c}	3.54 ± 0.04^{b}
ST-GG-NCS2	1.98±0.01^{c}	2.91 ± 0.04^{d}	3.33 ±0.04^{bc}
ST-GG-NCS3	1.97±0.01^{c}	2.62 ±0.14^{e}	3.17 ± 004^{c}

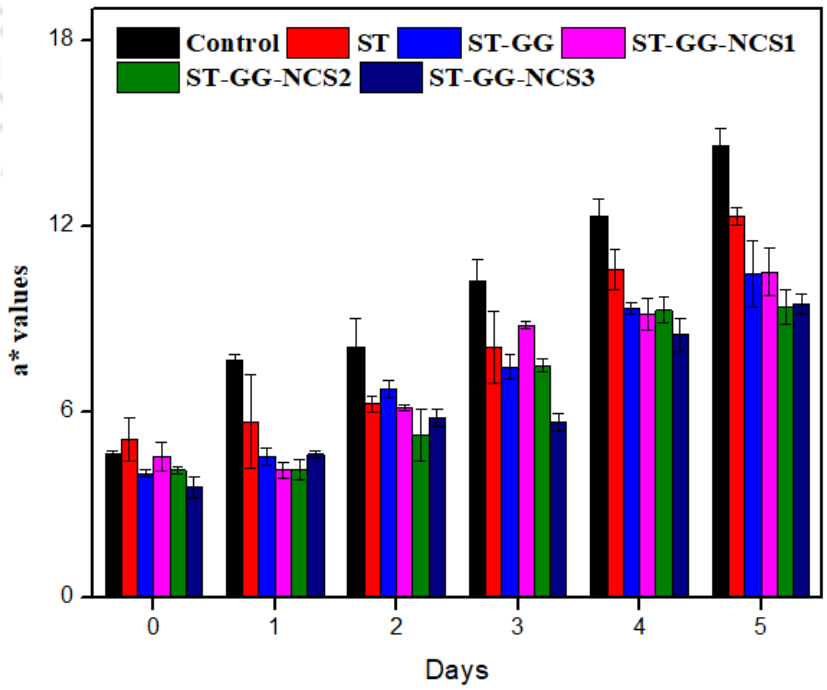
Color Parameters

The various color factors such as L, a*, b*, hue and chroma values has been represented in **Figure 4.17** and **Figure 4.18**. The color properties of food products are considered as an essential factor in determining the attributes of food products. The observed original color values of cut apple in terms of L, a*, b*, hue and chroma values are ~72.14, ~4.64, ~26.78, ~80.11, and ~27.18, respectively. As shown in Figure 4.18 and 4.19, the L values defining brightness (L values> 50) to darkness (L values> 50) color has been shown to maintain the bright coloration effect in the stored edible coated cut apple fruits. However, in the uncoated cut apple fruit products, there has been noticed a sharp decrease in the brightness values. The a* values of the stored cut apple fruits define that the stored fruit has a red coloration effect (positive values), where, there has been observed sharp increase in a* values for control sample. Additionally, the positive b* values define the yellow coloration effect, which is better

maintained during storage life due to development of edible coating. Additionally, the hue angle also suggests the yellow coloration effect of edible coated food products (**Figure 4.18**). On the otherhand, chroma also signifies the effectiveness of hue color during storage.



(a)



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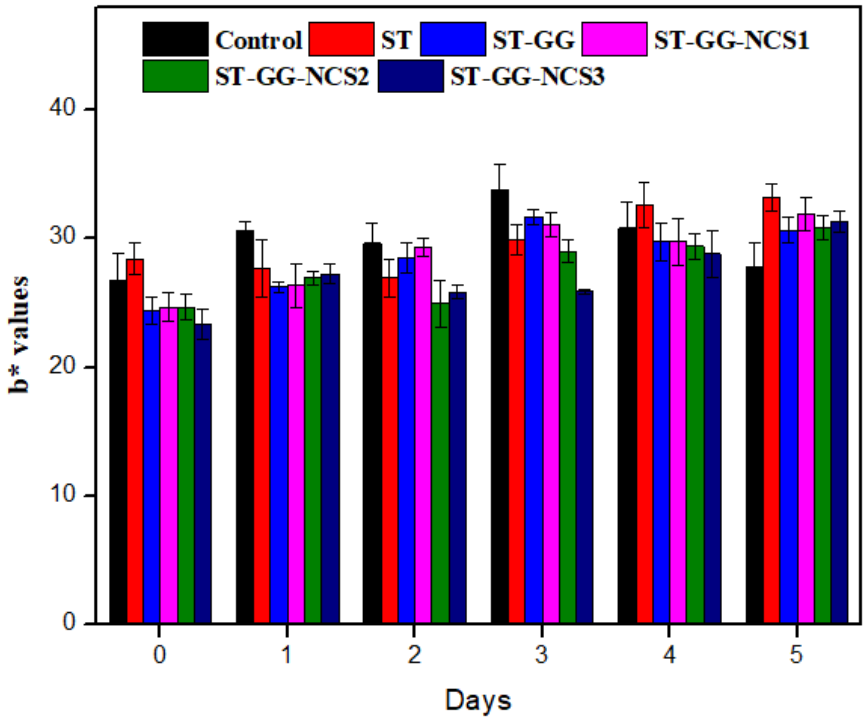
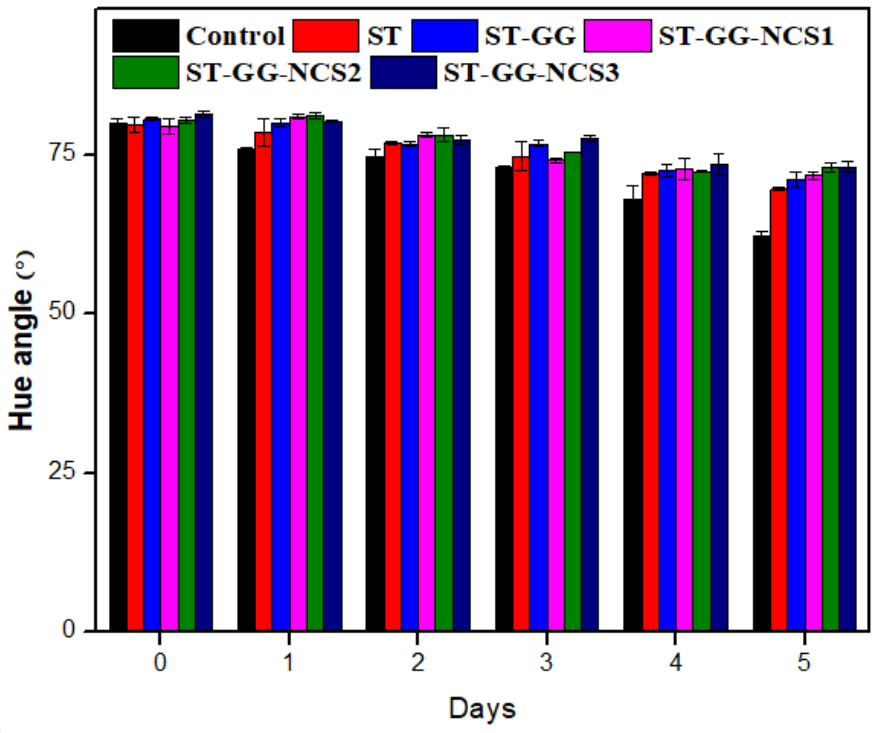
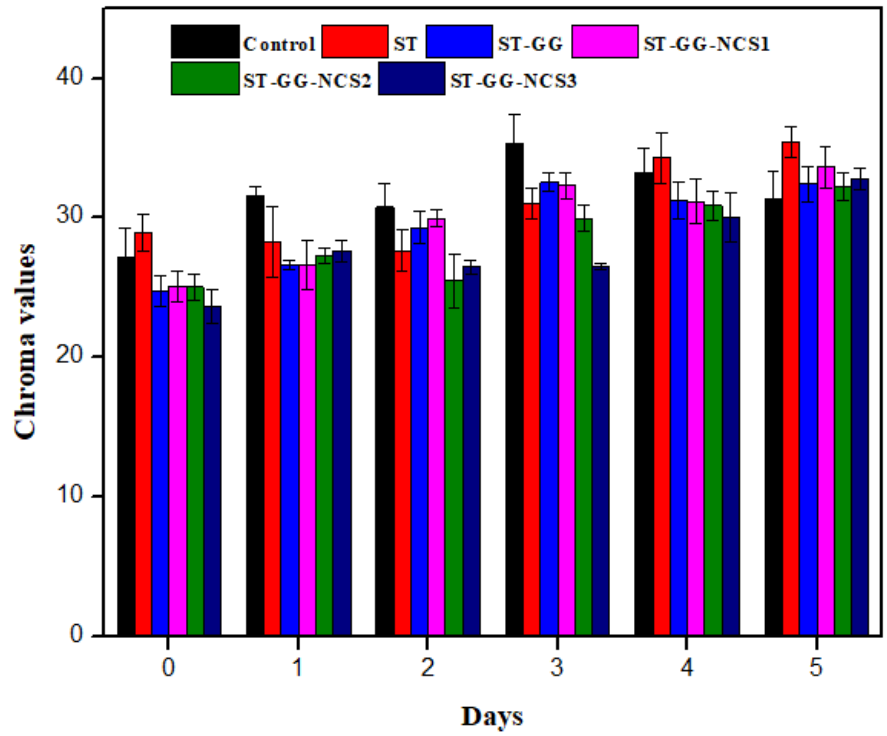


(c)

Figure 4.17 *Color parameters of stored edible coated cut apple products representing L values (brightness to darkness) (a); a* values (+ve a* defines red coloration effect) (b); and b* values (+ve b* defines red coloration effect) (c).*



(a)



(b)

Figure 4.18 *Color parameters of stored cut apple products representing (a) Hue angle and (b) Chroma values.*

4.3 Conclusion

The fabricated NCS possessing a size range of 3 to 9 nm as evident by FETEM analysis is obtained following a single step ionic gelation method. The investigation addresses the use of NCS, a food based modifier, in tailoring the packaging properties of ST and GG based biocomposites for targeted edible food packaging. The addition of NCS in ST-GG biocomposites significantly improves the surface property altering wettability properties, surface morphology (more homogeneous), optical properties (improved transparency), mechanical properties and others. Further, the developed edible coating materials act as remarkable renewable and available sources to be used as edible packaging materials on several different types of food products. The inherent features of ST are focused to be improved by including GG as a reinforcement material. In this context, the inclusion of GG may reduce the transparency and increase the hydrophilic nature of ST. Thus, the current investigation can be a new route to overcome the existing limitations of using ST-GG biocomposites as edible coating materials using NCS as a food based nanomodifier system. NCS aided edible coating materials such as ST-GG-NCS1, ST-GG-NCS2, and ST-GG-NCS3 has been found to decrease the weight loss in a better way. The developed coating materials having multidimensional benefits including biodegradability, edible, non-toxic and easy availability can be targeted for edible coating preparation.

CHAPTER 5

Silk Nanodisc Based Edible Chitosan Nanocomposite Coating for Fresh Produces

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Motivation

Silk is a natural protein (mainly composed of silk fibroin), which is extracted from cocoons of larvae. Further, the derivatives of silk such as degummed silk (silk fibroin), its nanoforms (silk nanodisc, silk nanoparticles), and its biocomposites are extensively used in developing edible food packaging. It is noteworthy to mention that silk fibroin can be an emerging material in developing edible food packaging. Further, edible coating based on silk fibroin can significantly maintain the keeping quality of perishable fresh produces. However, the silk nanoparticles can be beneficial for improved inherent properties of available biopolymers. Thus, the current chapter focuses on the development of one of the remarkable nanoproteins reinforced chitosan based edible coating for improved shelf life of perishable products. Additionally, the inclusion of chitosan and silk nanodisc materials can provide antimicrobial properties which is beneficial for reduced microbial growth on food products during storage life. The silk nanodisc incorporation to chitosan matrix can be promising alternatives for preservation of perishable food products.

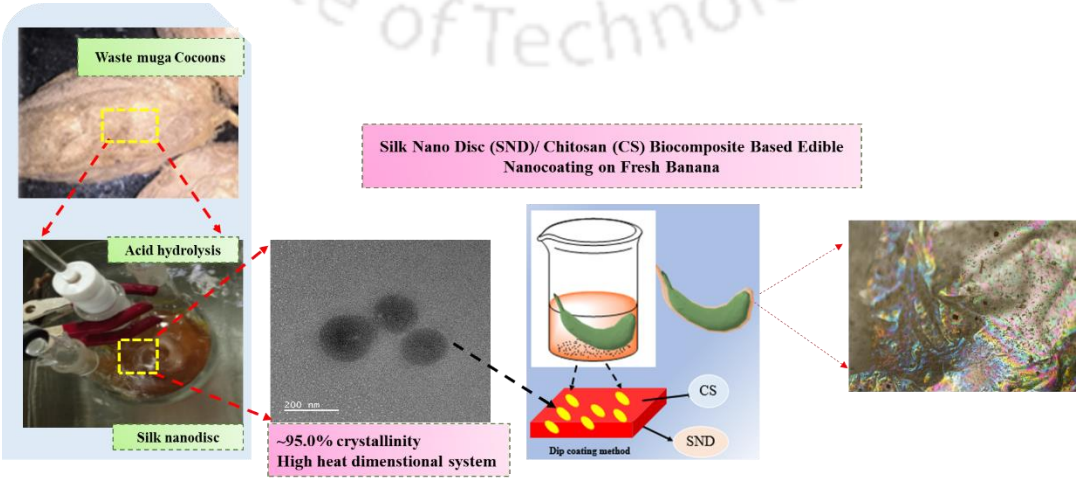
The work in this chapter originated from:

Ghosh, T., Mondal, K., Giri, B. S., & Katiyar, V. (2021). Silk Nanodisc Based Edible Chitosan Nanocomposite Coating for Fresh Produces: A Candidate with Superior Thermal, Hydrophobic, Optical, Mechanical and Food Properties. Food Chemistry (Under Review).

Abstract

This chapter demonstrates the fabrication of silk nanodisc (SND) (a type of nanoprotein) dispersed chitosan (CS) based new edible coating as a candidate for superior thermal, surface hydrophobicity, optical, mechanical, and physicochemical properties, which further provide remarkable storage quality with preservative action for fresh banana fruits. Fabrication of SND is attained following acid hydrolysis of silk fibroin (SF) (source: waste muga cocoons), where the successful nanostructures formulations are confirmed by FESEM, FETEM and XRD analysis delivering disc shaped morphology with amplified crystallinity (~95.0%). The effectiveness of SND materials has been investigated on the packaging properties of CS biocomposites including thermal property, wettability, mechanical property, color, surface morphology, and others. The thermal property of SND (1.5 wt%) dispersed CS has been found to be improved by 9 °C (~275 °C) as compared to CS (~266 °C). Additionally, the FESEM studies evident the intactness of SND in the matrix of CS which is helpful in improving the inherent attributes of CS. The surface wettability of SND dispersed biocomposites enhance by ~10° suggesting improved hydrophobicity. Further, the fabricated edible coatings are a new candidate to improve the shelf life of fresh bananas over 7 days at 25 °C in terms of prevailing original weight loss, optical property, firmness, and others.

Scheme of the Chapter



5.1 Introduction

Silk nanostructured materials are recognized as one of the potential bionanomaterials in the current century, being derived from natural protein fibers, and exhibiting tremendous application in miscellaneous sectors including packaging materials, biomedical engineering, and drug delivery aiding technological advancements (Chen et al., 2018; Wang et al., 2015; Kundu et al., 2010; Shao et al., 2016). Silk nanostructures are the nanoforms of silk fibroin generally obtained by adopting several strategical extraction methodologies from available natural resources including Mulberry (*Bombyx mori*), Tasar (*Antheraea mylitta*), Muga (*Antheraea assamensis*), and Eri (*Philosamia Cynthia ricini*) which has the protein components such as sericin and fibroin protein (Mehrotra et al., 2019; Silva et al., 2016). In a broad spectrum, silk is industrialized in the nanosize ranges assisting functional moieties through controlled designing of the ingredients such as beta nanocrystals and beta nanofibrils. Remarkably, biocompatibility, biodegradability, non-toxicity, tunable physical property, and most considerably thermal stability, and edibility are the favorable characters of silk nanostructured materials to be employed in fabricating edible food packaging. Interestingly, in 1993, silk fibroin has been recognized as the biomaterials by US food and drug Administrations and afterwards is considerably utilized as a remarkable biomacromolecule for several noteworthy applications as mentioned. Recently, investigations based on the development of silk nanostructures based composites for advanced applications have been enhanced in food sectors. To date, some research reports the use of silk fibroin and its nanoforms as a potential candidate in food sectors, specifically in edible and non-edible food packaging area, respectively (Marelli, et al., 2016; Kuchaiyaphum et al., 2013; Mondal et al., 2019). Additionally, the inclusion of silk fibroin has been shown to improve the storage life of perishable fruits such as strawberry and banana up to a notable extent (Marelli et al., 2016).

In modern society, the present demand for food grade or eatable biobased packaging has been increased by food packaging industries and consumers. However, the edible packaging should have the capability to prolong the shelf life of food items specifically perishable fruits and vegetables. By considering these existing conditions, there is an increasing trend in using biopolymeric constituents for emerging eco-friendly packaging. Food packaging ingredients have been known as the principal source for generating solid waste and environmental pollution. In this regards, the extensively utilized biopolymeric materials for biobased packaging comprise cellulose, chitosan (CS), starch, agar, carrageenan, and others. Additionally, the mentioned biopolymers are also used for the fabrication of edible food packaging as edible films or coatings. Among available, CS is one of the most promising biomaterials for developing edible food packaging as a base or reinforcement material (Ghosh & Katiyar, 2019**)**. However, the individual use of the biopolymers may exhibit some limiting properties, where the incorporation of nanofillers can provide tailored-made intrinsic properties (Ghosh, Monika & Katiyar, 2020). In this regards, the properties of CS are reported to be modified using several nanomaterials including organic and inorganic nanomaterials (Ghosh, & Katiyar, 2019**)**. Some research reports the combined use of biopolymeric materials as edible packaging including microcrystalline cellulose nanoparticle/hydroxypropyl methylcellulose based edible films (Bilbao-Sáinz et al., 2010), CS /hydroxypropyl methylcellulose (Möller, et al., 2004), cellulose nanofibers/CS as edible coatings (Ghosh et al., 2019), CS/gelatin/nanoencapsulated tarragon essential oils (Zhang et al., 2020), CS/gelatin nanofibers (Ebrahimi, et al., 2019), etc. Various research reports the successful development of edible coating on fresh produces, where the modified interior atmosphere can help to maintain the quality of fresh produces by reducing microbial count, weight loss, maintaining firmness, and preserving color attributes (Ghosh, & Katiyar, 2019). Interestingly, color attributes play a crucial role in customer acceptance of the food products. The inclusion of edible coating is a route for food waste management

specifically for perishable fruits and vegetables, which helps in the economic development of the society (Ghosh, Teramoto, & Katiyar, 2019). Several perishable fruits and vegetables have a high degradation rate due to environmental conditions such as microbial degradation, color degradation, thermal degradation, off-flavor, and others. The edible coating should be safe for consumptions and have biodegradable nature similar to food products. The several fruits and vegetables including banana, apple, strawberry, grapes, and mango are considered as highly perishable food products and can be preserved applying edible packaging. Edible food packaging works primarily to protect food products from the environment with enhanced shelf life of perishable fruits. In this regard, banana has a very short life, which is a stimulating factor for industrialized divisions. Banana fruits are a category of highly perishable produces which is easily degradable by the environmental condition. The quality preservation of banana using edible coating has been done by developing different coating formulations such as starch coating (Thakur, et al., 2019), gum Arabic/CS (Maqbool, et al., 2011), silk fibroin (Marelli, et al., 2016), carrageenan coating (Bico et al., 2009), shellac/gelatin composite film (Soradech et al., 2017), pullulan based edible active coating (Ganduri, 2020**),** etc. Thus, the current research reports the use of some unique and new edible coating for enhanced product quality. Among available, silk fibroin nanoparticles can be considered as promising nanomaterials to combat the present need of developing improved properties of edible food packaging. Further, the thermally stable silk nanoparticles can be used to develop heat dimensional food products. The several research reports the use of biopolymer composites as a food packaging material for providing some inherent characteristics attributes.

In the present investigation, the waste muga cocoons (WMC) has been utilized to fabricate silk nanodisc (SND) by removing the sericin proteins followed by acid hydrolysis methods, where the nanostructure formation has been confirmed by FESEM, FETEM, and XRD analysis. Additionally, the SND has been employed to develop biopolymer nanocomposite based edible

coating materials with tunable properties. Due to various synergistic properties of silk nanostructures in terms of thermal properties, surface properties, optical properties, mechanical and physicochemical properties, the silk nanostructures have gained a significant attention to be used in developing edible food packaging. The nanofillers are dispersed in CS matrix to develop edible coating on fresh banana produces and the influence of filler materials on the firmness, color properties, weight, and physicochemical properties, has been studied. To the best of our knowledge, the use of SND in developing edible coatings has not been done till date. Further, SND dispersed CS based edible food packaging for improved packaging attributes has not been reported. In addition, the developed edible coating materials are nontoxic, biocompatible, and edible, which can be used in multiple directions for considerable applications as edible films, and coating for enhanced product life and quality.

5.2 Results and Discussions

5.2.1 Fabrication and Characterization of Silk Nanodisc from Waste Muga Cocoons

The surface morphological analysis of WMC (the way it is received without any further purifications), SF, and SND (produced *via* acid hydrolysis) has been represented in **Figure 5.1**. The degumming of silk involves the removal of silk gum/sericin, where the gummy substances provide a protective layer to muga silk. The observed diameter of WMC is 24.9 ±4.3 μm, whereas, the diameter of SF is 20.5± 5.2 μm (**Figure 5.1**). The morphology of WMC as evident by FESEM provides the tightly packed fiber structures or nonwoven compact structure (Zhang et al., 2013**)**. Similar results have been reported by several researchers displaying the packed fiber structure for different types of cocoons (Kaur et al., 2013). The degumming of WMC shows no destruction and damage to the fabricated SF and further, the fibers have a smooth surface property with longitudinal fibrillary structure. A research reports the fabrication of degummed silk fiber using soap solution and citric acid which also deliver similar kind of morphology and surface free form destructions (Khan et al., 2010). The morphological study

of SND illustrates the average dimensions and surface morphology using FESEM analysis as displayed in **Figure 5.1**. The dimensions of SND, i.e., distribution of average diameter are measured using FESEM analysis. The acid hydrolyzed SND shows disc shaped morphology with an average diameter of 50 to 120 nm. However, the acid concentration, acid hydrolysis time and reaction temperature affect the final dimension of the developed nanostructured silk (Hu et al., 2019).

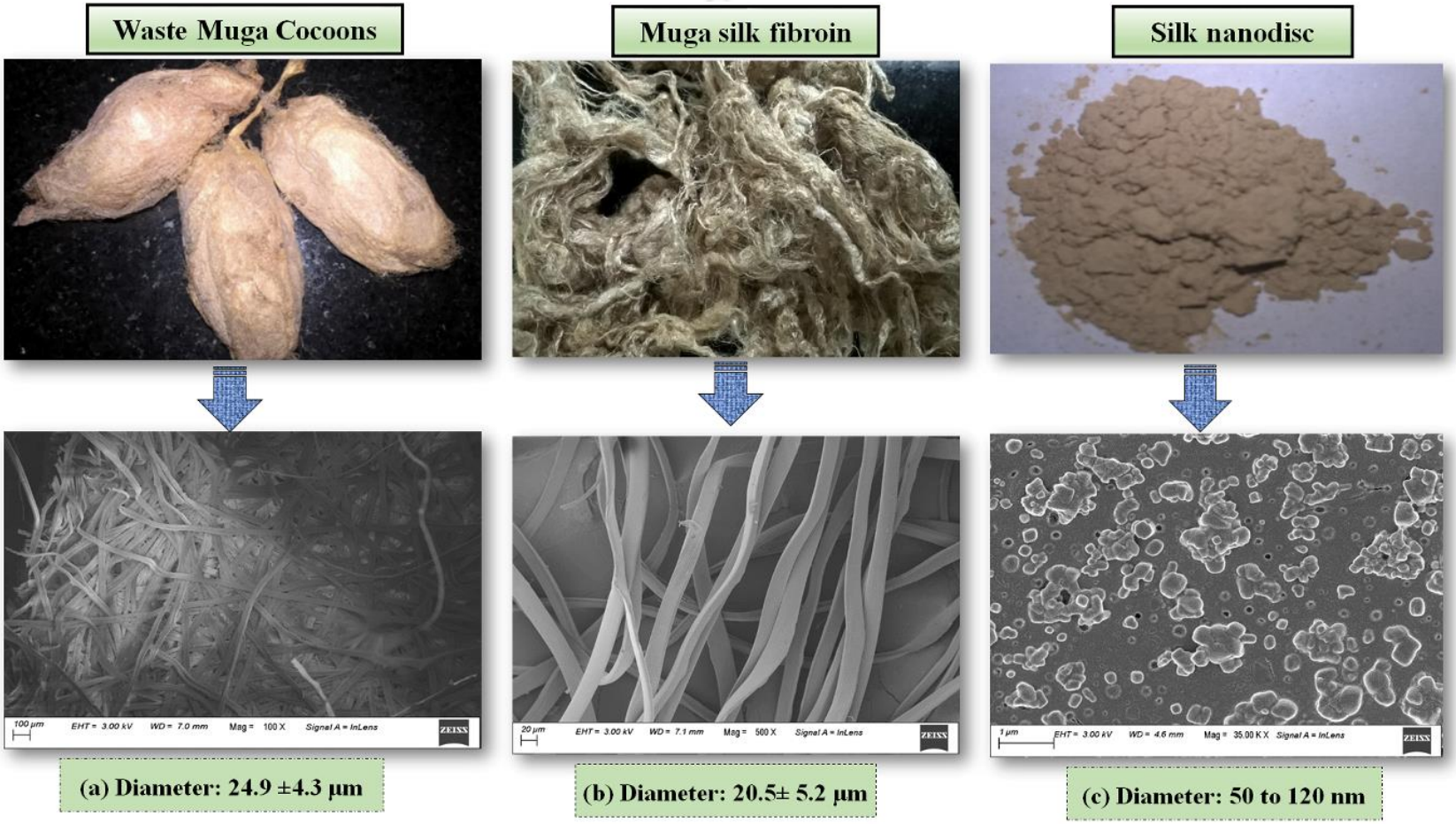
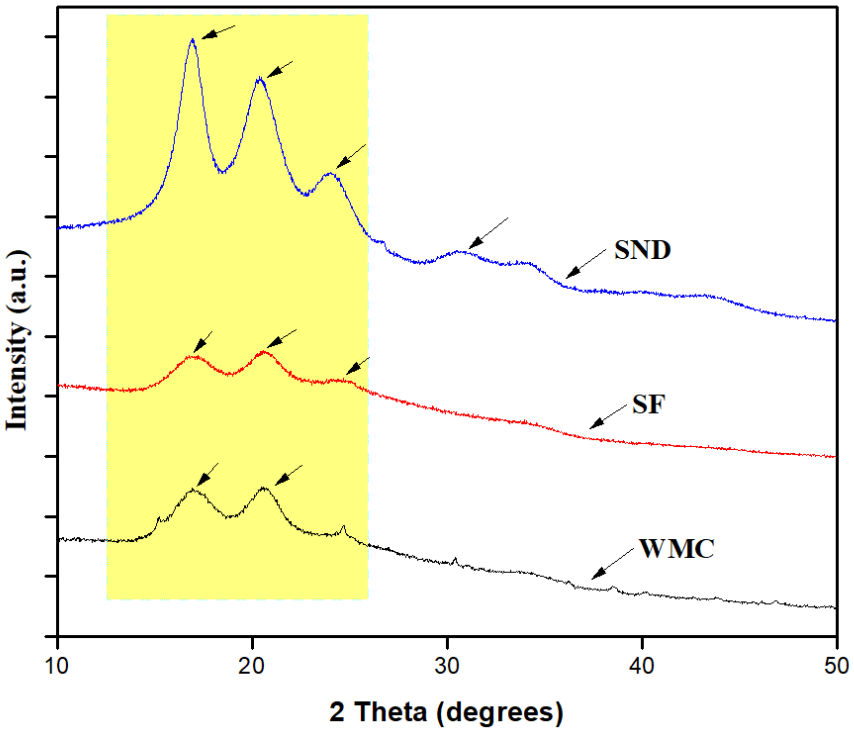
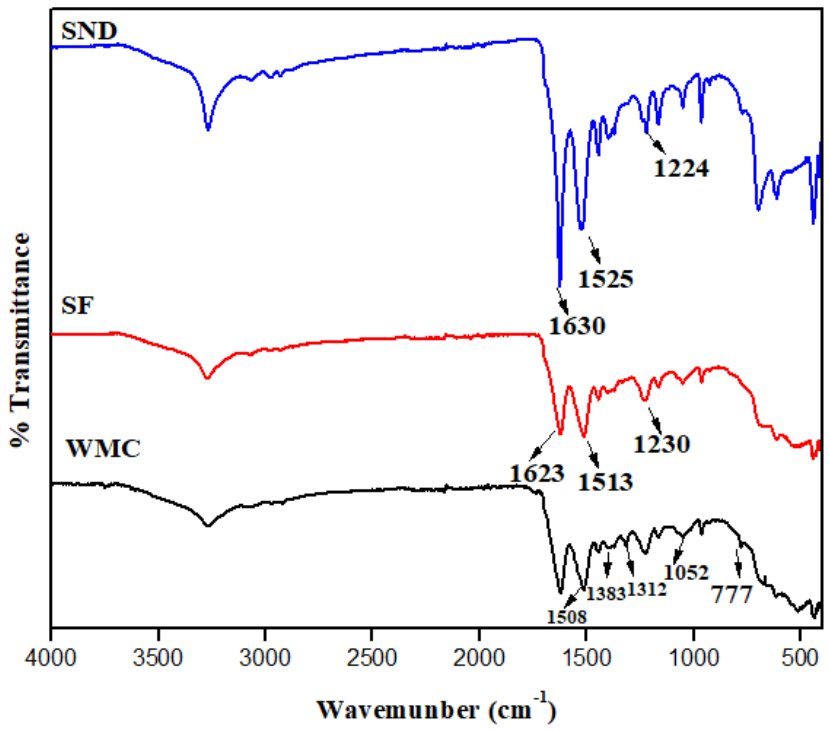


Figure 5.1 *FESEM Micrographs of silk resources, purified and processed silk materials representing waste muga cocoons (a); Purified Silk Fibroin (b) and Silk Nanodisc (c).*

The XRD analysis of fabricated SND using acid hydrolyzed degummed silk (source: waste Muga cocoons) has been done as represented in **Figure 5.2a**. The XRD analysis is executed to comprehend the crystallite physical properties and to examine the crystallinity behavior of the biomaterials. In this regards, the WMC has the characteristics XRD peak at 2θ= 16.6°, and 20.2°, which are obtained due to the crystalline nature (Andiappan et al., 2016). However, the extracted SF has sharp XRD peaks at 2θ= 16.9°, 20.5°, and 23.8° confirming the β-pleated sheet structures and α-helix structure. The silk fibroin has increased peak intensity in comparison to the WMC. SND has sharp XRD peaks at 2θ=16.9°, 20.38°, 24.02°, and 30° indicating the β-pleated sheet structures (Patwa et al., 2018; Chomachayi et al., 2020). Additionally, the observed percentage crystallinity of SF and SND are 58% and 95%, respectively. The high crystallinity of SND is obtained due to the acid hydrolysis of degummed silk, which removes the amorphous domains. The FTIR analysis of WMC, SF and SND are represented in **Figure 5.2b**, where, the chemical IR bands of the specimens are analyzed. The IR peaks for amide I, amide II and amide III are obtained at 1625 to 1630 cm^{-1}; amide II at 1520 to 1530 cm^{-1}; and amide III at 1265 to 1270 cm^{-1}. The IR peaks of WMC representing (i) 1315, and 779 cm^{-1} for calcium oxalate; (ii) 1620 (amide I β-sheets), 1508, 1221, and 961 cm^{-1} for β-sheets crystallinity; (iii) 1052 cm^{-1} for sericin C-O stretching; (iv) 1508 cm^{-1} for amide II β-sheets; **(v)** 1383 cm^{-1} obtained due to bending of CH_2 and polyalanine glycine (Boulet-Audet et al., 2015). Additionally, the FTIR peaks at 1052 and 1315 cm^{-1} are absent in defining efficient degumming of WMC producing SF and efficient removal of sericin and calcium oxalate. The IR peaks of SF at 1623, 1513, and 1230 cm^{-1} confirm β-sheets absorption peaks (Zhang et al., 2012). Additionally, the characteristics IR peaks 1630, 1525, and 1224 cm^{-1} confirm the β-sheets absorption peaks.



(a)



(b)

Figure 5.2 *XRD diffractograms (a) and FTIR analysis (b) of waste muga cocoons, silk fibroins, and silk nanodisc.*

The thermal properties of WMC, SF, and SND in terms of TGA thermograms have been represented in **Figure 5.3**. Further, the TGA thermograms represent the thermal performance of WMC, SF, and SND. To study the thermal properties of the used materials, the percentage mass reduction at different temperatures has been represented in **Table** 5.1, where, T_5, T_{10}, and T_{50} denote sample temperature at 5, 10, and 50% mass reduction during the thermal analysis. Additionally, W_{650} describes the obtained weight residue during the thermal treatment at 650 °C. As represented in the **Table 5.1**, the T_5 for WMC, SF and SND are ~71, ~68, and ~193 °C, respectively. Further, the observed T_{10} for WMC, SF and SND are ~178, ~140, and ~305 °C, respectively. By observing the thermal properties, the fabricated SND has been found to provide improved thermal stability compared to WMC, and SF. Further, in all the samples, the weight loss at ~100 °C occurs due to the moisture removal phenomena for heat application. On the otherhand, at ~210 °C, the second stage thermal degradation occurs for WMC, and SF due to chemical transformation caused by thermal treatment. However, the second stage chemical transformations for SND occurs at a later stage (~ 300 °C) due to improved thermal stability. Further, the weight residue W_{650} (%) obtained for WMC, SF, and SND are ~30%, ~20%, and ~1.2%, respectively which corresponds to the presence of inorganic materials in the samples.

Table 5.1 *Thermal properties of waste muga cocoons, silk fibroins, and silk nanodisc representing sample temperatures at 5% (T_5), 10% (T_{10}), and 50% (T_{50}) mass reduction during the thermal analysis and weight residue at 650 °C (W_{650}).*

Sample	T_5 (°C)	T_{10} (°C)	T_{50}(°C)	W_{650} (%)
WMC	71	178	421	30
SF	68	140	405	20
SND	193	305	387	1.2

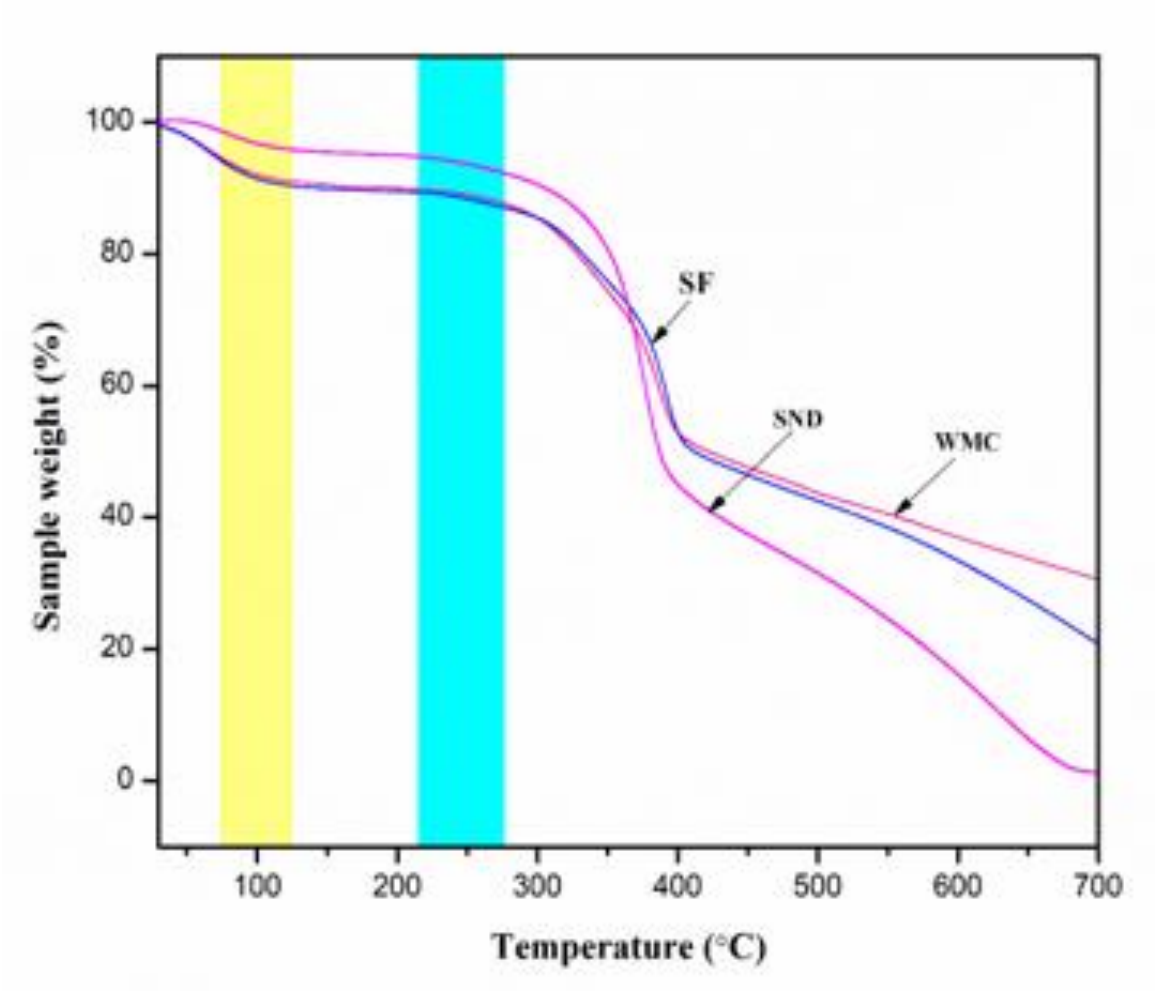
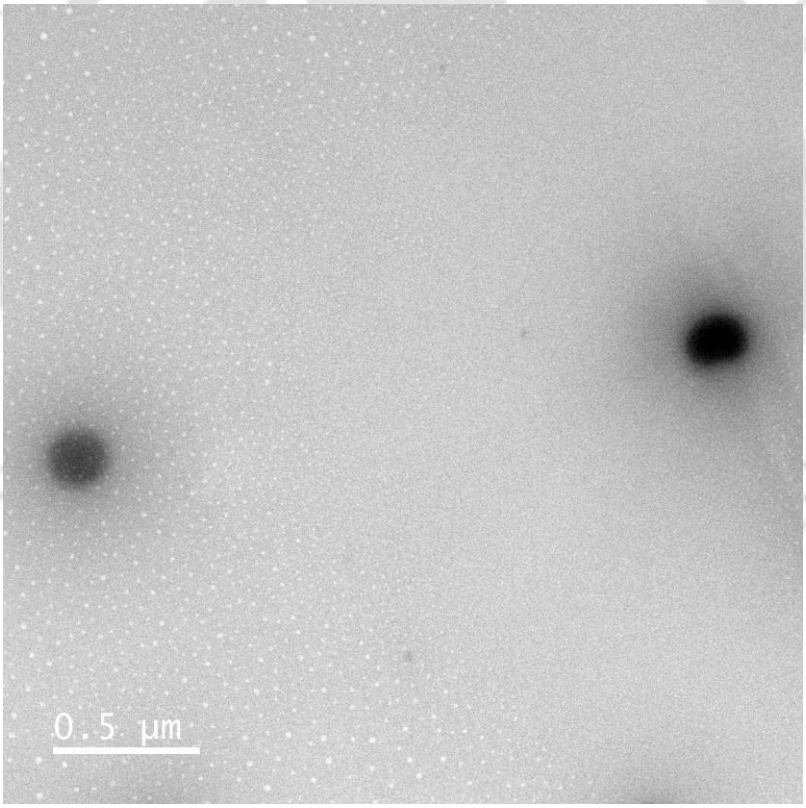


Figure 5.3 *Thermal analysis (at a heating rate of 10°C min^{-1}) representing the thermograms for waste muga cocoons, silk fibroins, and silk nanodisc.*

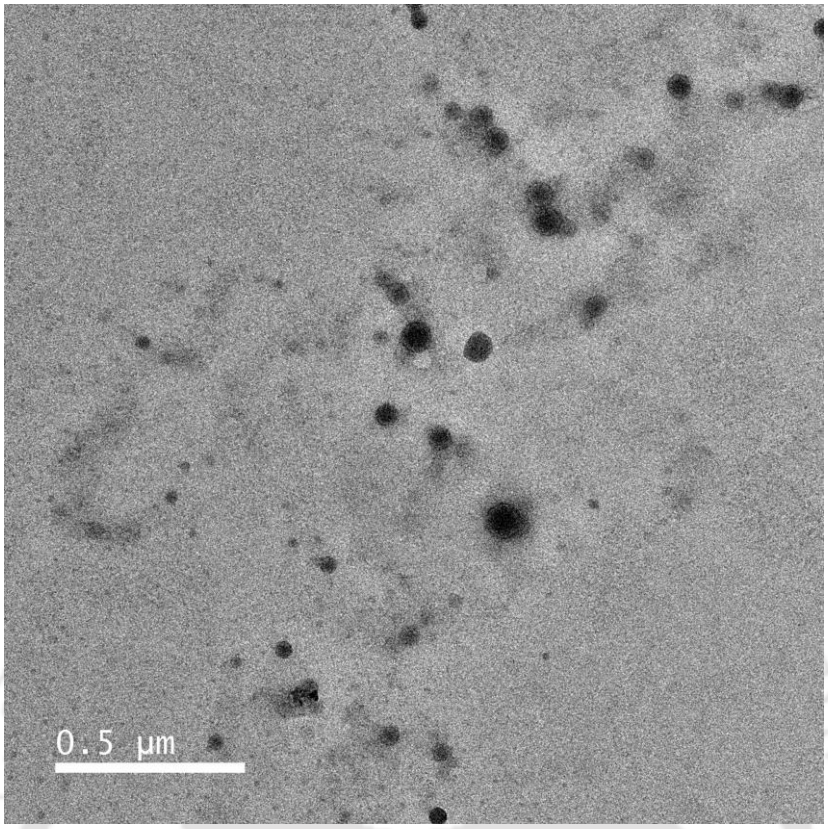
5.2.2 Characterization of SND/CS Biocomposites based Edible Coating Materials

The morphological study of SND illustrates the average dimensions and surface morphology using FETEM analysis as displayed in **Figure 5.4a**. Additionally, the FETEM analysis shows the presence of SND in the matrix of CS (CS-SND1.5) and confirms the morphological dimensions of the nanofiller in the base materials (**Figure 5.4b**). The surface morphology of developed CS/SND bionanocomposite has been examined using FESEM to observe the intactness (**Figure 5.4c and d)**. A comparisional analysis of the surface appearance of fabricated CS-SND0.5, and CS-SND1.5 developed by simple solution evaporation process

as represented in **Figure 5.4c and d**. The incorporation of SND at different proportion shows dispersion of nanofiller materials at different levels. The cross sectional and surface based FESEM micrograph provides clear visibility of the presence of SND. The study on surface morphology provides the homogeneous dispersion of SND in the matrix materials. The FESEM micrograph of CS-CND1.5 provides spontaneous disc shaped aggregates of the SND in the matrix of CS. The micrographs have a smooth surface texture with reduced filler content, whereas, the surface with higher loading has a rough and cloudy appearance of the surfaces held by the effectiveness of the SND materials.



(a)



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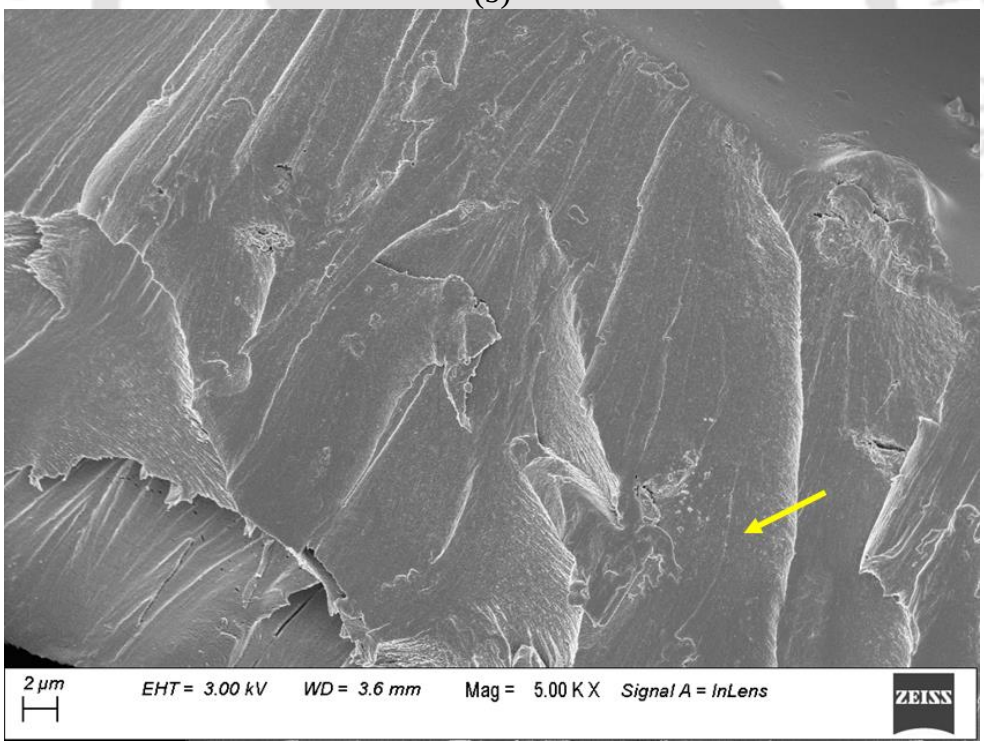

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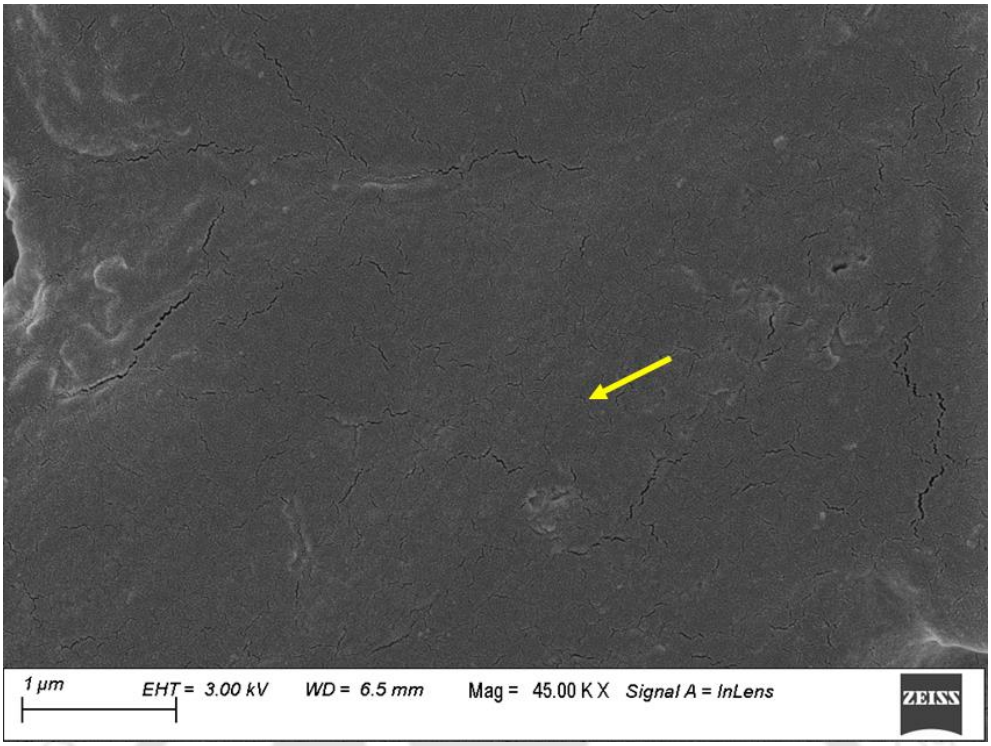

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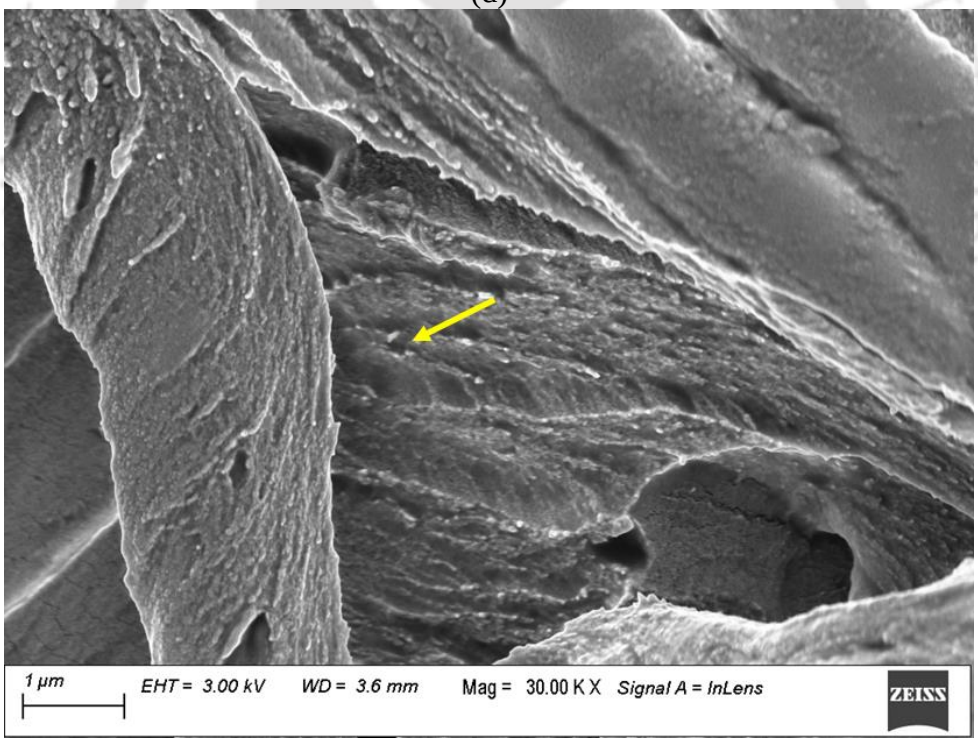

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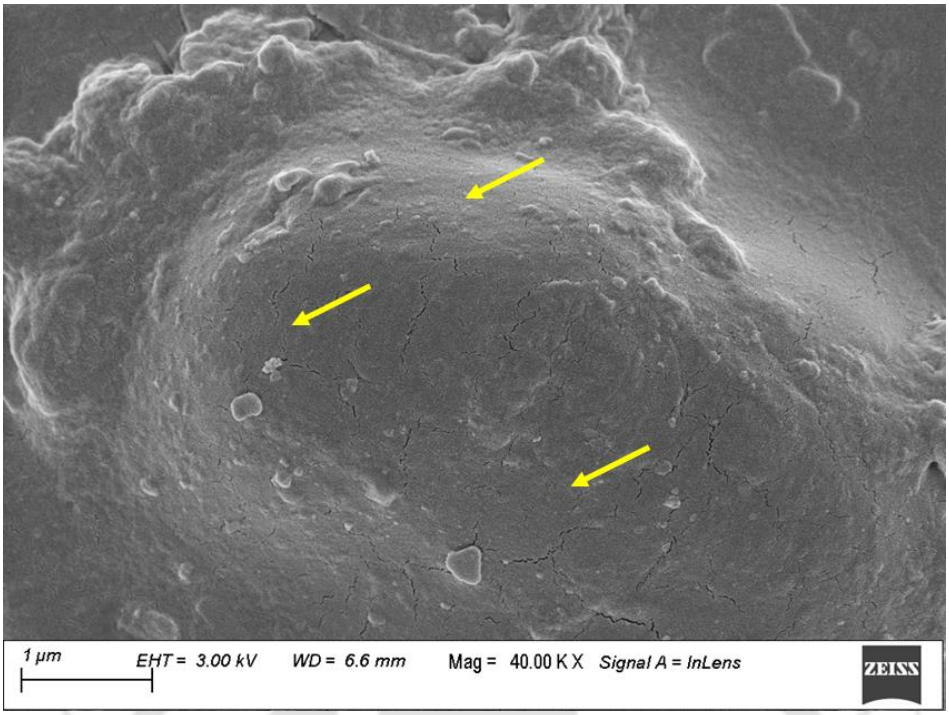
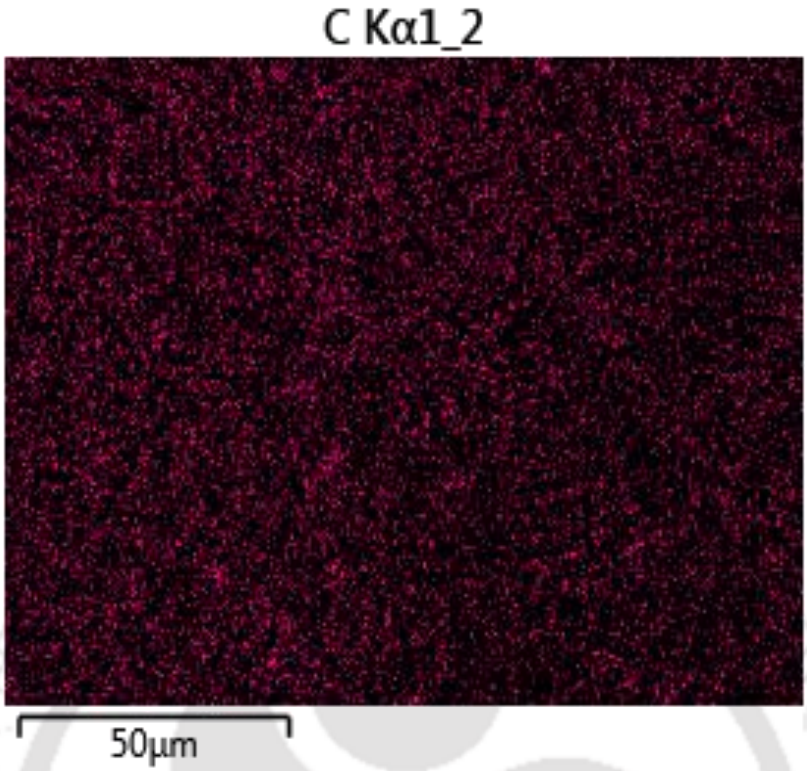


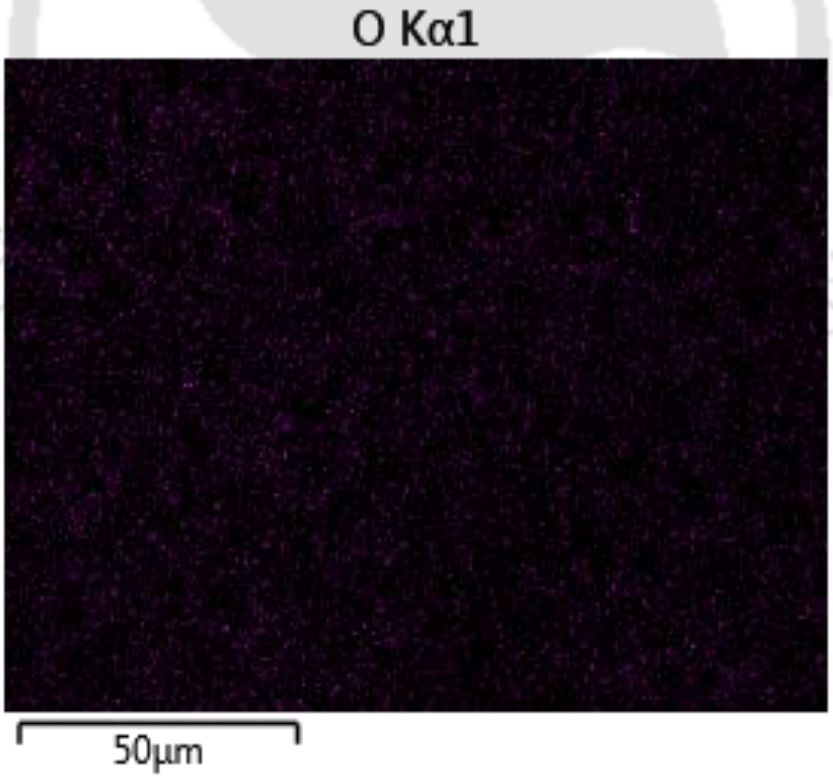
(f)

Figure 5.4 *FETEM Micrographs of silk nanodisc (SND) fabricated via acid hydrolysis techniques (a), and CS-SND1.5 (b); FESEM morphology of CS-SND0.5, cross sectional view (c), surface view (c´); FESEM morphology of CS-SND1.5, cross sectional view (d), surface view (d´).*

The EDX spectra and FESEM mapping of CS-SND1.5 biocomposite based edible materials have been done as represented in **Figure 5.5**. The components of C (~50.1 wt%), O (~41.8 wt%), and N (~8.1 wt%) in the materials can be visualized from the figures. The EDX analysis provides the component analysis confirming the safety of the coating materials. Interestingly, the compositional analysis of the edible coating materials confirms the absence of metallic components and other minerals confirming the safety of the materials. Similarly, the distribution of elements has been confirmed by the mapping of the fabricated coating materials.



(a)



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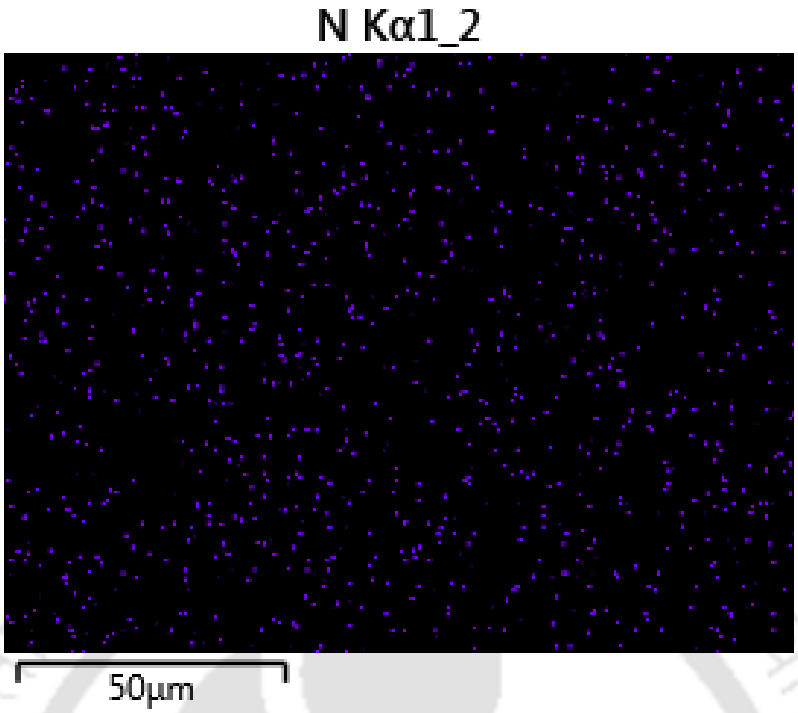
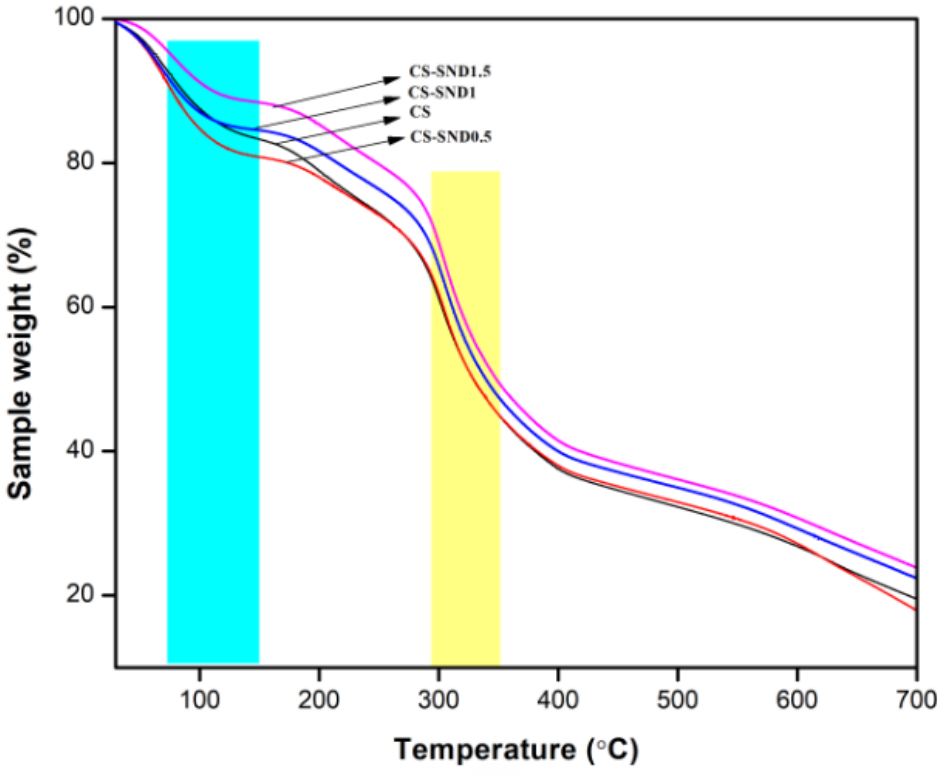


(c)

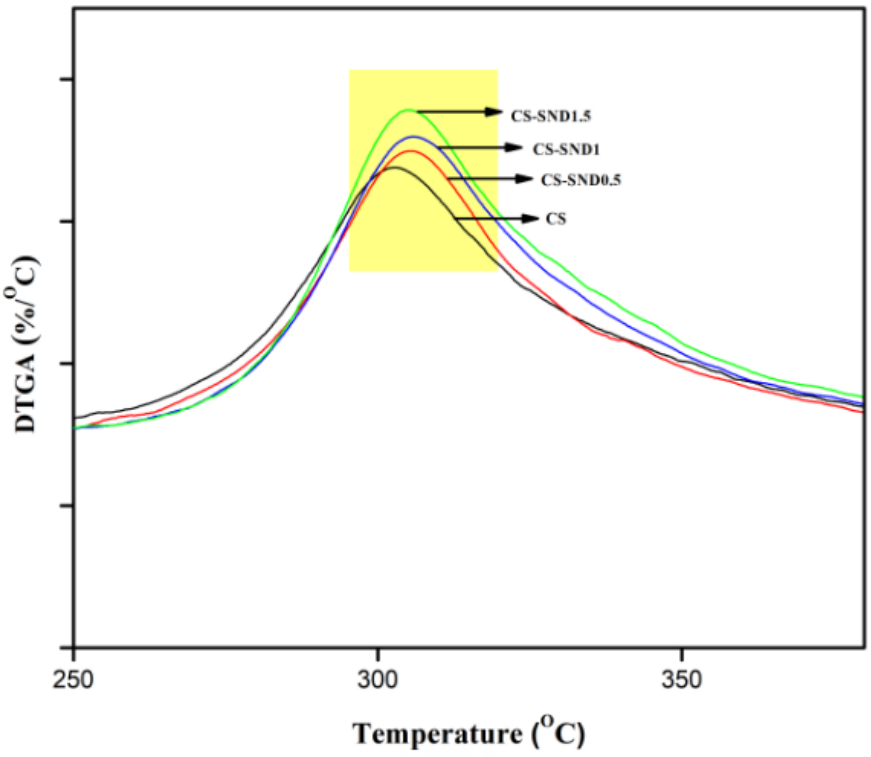
Figure 5.5 *EDX mapped micrography of CS-SND1.5 biocomposites depicting elemental analysis for carbon, oxygen and nitrogen.*

The three proportions of SND has been used to develop CS based biocomposites and the thermal properties of the edible materials have been analyzed for a temperature range of 30 to 700 °C as shown in **Figure 5.6a** and **5.6b**. The weight loss during 100 °C occurs due to the presence of solvent and moisture residue in the biocomposite films. Additionally, as shown in **Table 5.2**, the onset degradation temperatures (T_{onset}) for the SND dispersed CS films are 266.49 °C, 271.20 °C, 272.55 °C and 275.15 °C for CS, CS-SND0.5, CS-SND1, and CS-SND1.5, respectively. The thermal property of the CS-SND1.5 has been found to be improved by 9 °C (~275 °C) compared to CS (~266 °C). Besides, the maximum thermal degradation temperatures for the film materials are 302.87 °C, 304.57 °C, 305.65 °C, and 305.23 °C for CS, CS-SND0.5, CS-SND1, and CS-SND1.5, respectively. As represented in **Figure 5.6a** and **5.6b**, the edible coating material *viz.* CS-SND1.5 has improved thermal stability compared to others.

In this regards, the thermal degradation properties can be tailored based on the processing temperature for storage food products requiring heat dimensional stability. The % weight loss at maximum degradation temperatures are 59.97, 59.73, 63.02, and 65.35 % for CS, CS-SND0.5, CS-SND1, and CS-SND1.5, respectively. Further, the residual weights for CS, CS-SND0.5, CS-SND1, and CS-SND1.5 are 19, 17, 22, and 23%, respectively. Thus, it can be concluded from the observed thermal properties that incorporation of SND (1.5 wt%) has significantly improved the thermal stability of the edible materials and can be used at heat sterilization temperatures (Ghosh, Termaoto, & Katiyar, 2019). However, use of SND at higher concentrations may form agglomerates which can reduce the thermal stability.



(a)



(b)

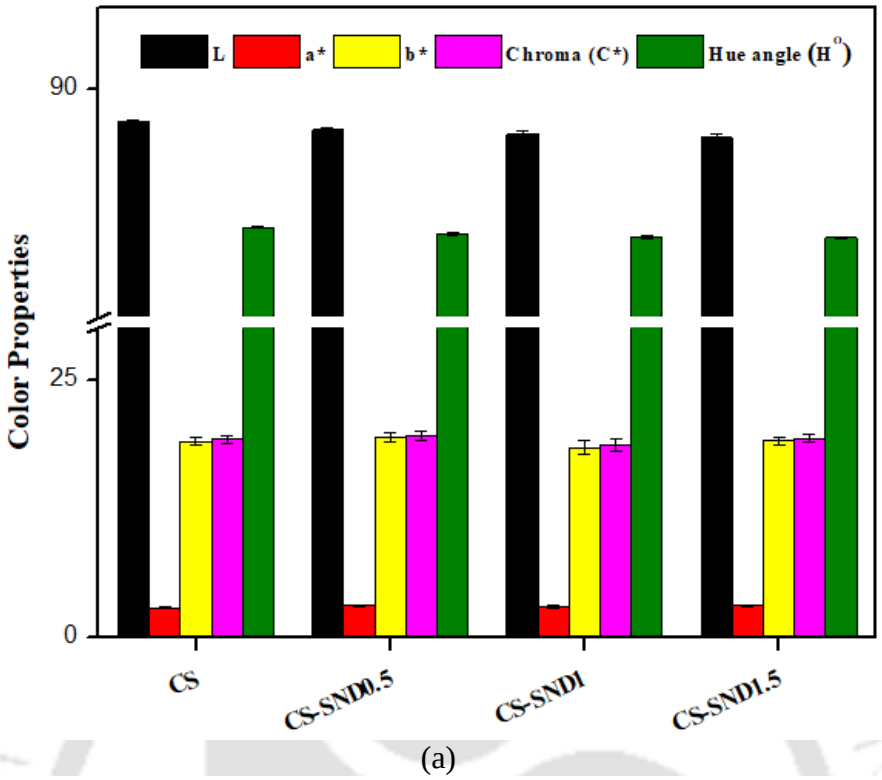
Figure 5.6 *TGA thermogram (a) and DTGA (b) of chitosan/silk nanodisc based biocomposites.*

Table 5.2 *Thermal properties of CS/SND nanocomposite based edible coatings observed by TGA analysis.*

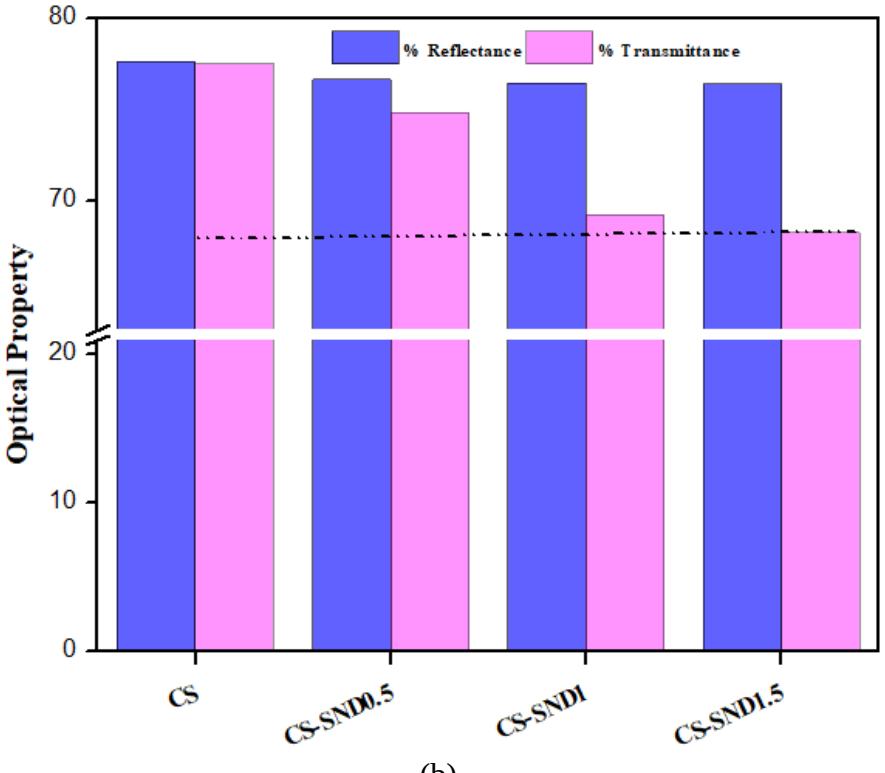
Sample	**T_{onset} (°C)**	**T_{max} (°C)**	**W_{max} (°C)**	**W_{res} (%)**
CS	266.49	302.87	59.97	19
CS-SND0.5	271.20	304.57	59.73	17
CS-SND1	272.55	305.65	63.02	22
CS-SND1.5	275.15	305.23	65.35	23

In edible food packaging materials, the optical properties in terms of color coordinates and transparency are considered as crucial aspects for selecting proper packaging materials considering the end users. In the present investigation, the observed color properties and transparency have been represented in **Figure 5.7(a)** and **5.7(b)**. The color properties in terms

of L, a*, b*, hue, and chroma of CS, CS-SND0.5, CS-SND1, and CS-SND1.5 edible coating materials have been represented in **Figure 5.7(a)**. The color parameters of edible coating materials are a crucial factor for evaluating the color effectiveness of nanofiller materials on base polymers, which on the otherhand significantly influences the end users (Ghosh, Bhasney, & Katiyar, 2020). The L based color factors for CS, CS-SND0.5, CS-SND1, and CS-SND1.5 has the values of ~87.98, ~87.49, ~87.24, ~87.04, respectively, which define the brightness of the targeted samples. The minor decreasing trends of L values in the samples occur due to the increased nanofiller content of the edible coating materials. Additionally, the color factor "a*" values of the edible coating materials have redness effect which indicated by the positive values of the edible coatings (**Figure 5.7a**). Similarly, the "b*" values of color factors provide yellow coloration effect of the edible coating materials. The hue values depict the combined effect of all coloring factors, where hue angle of 90° provides yellow coloration effect and the fabricated films have hue angles of ~81°. Additionally, the chroma values (~19) define the saturation of color values of edible coating materials. Thus, as observed from the **Figure 5.7a**, the edible coating materials has the properties of yellow coloration effect mainly with brightness effect. Additionally, as represented in **Figure 5.7b**, the transparency of film materials for CS films (medium molecular weight, deacetylated chitin) is ~77.53 %, which is found to get reduced to ~74.84%, ~69.20%, and ~68.22% for CS-SND0.5, CS-SND1, and CS-SND1.5, respectively in the visible region. The attained decreased transparency of the films in the visible region are caused due to incorporated SND, which is beneficial for light sensitive food components. Additionally, the reflectance at 700 nm are ~77.64, ~76.65, ~76.47, and ~76.39% for CS, CS-SND0.5, CS-SND1, and CS-SND1.5, respectively.



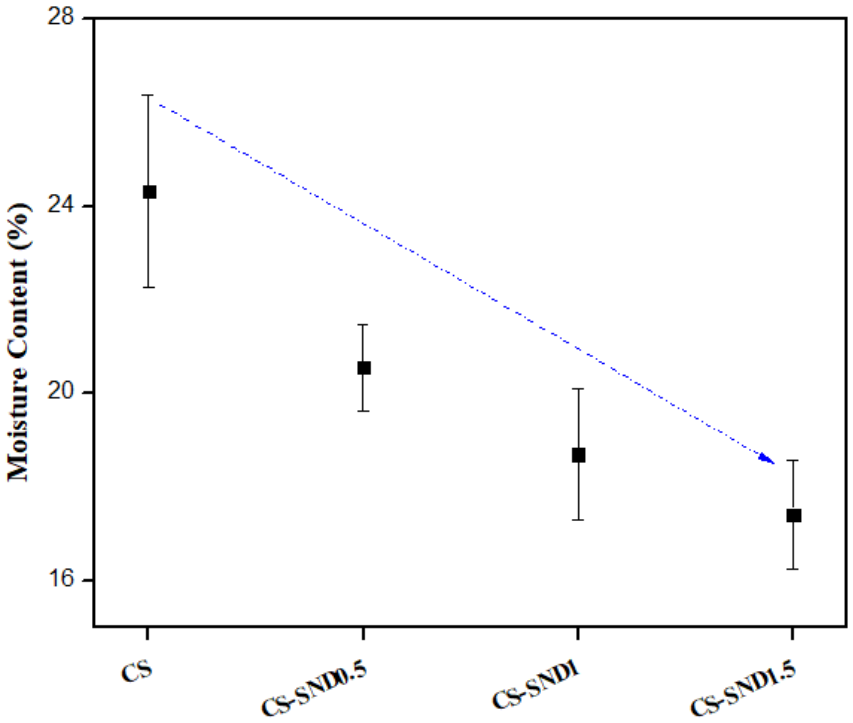
(a)



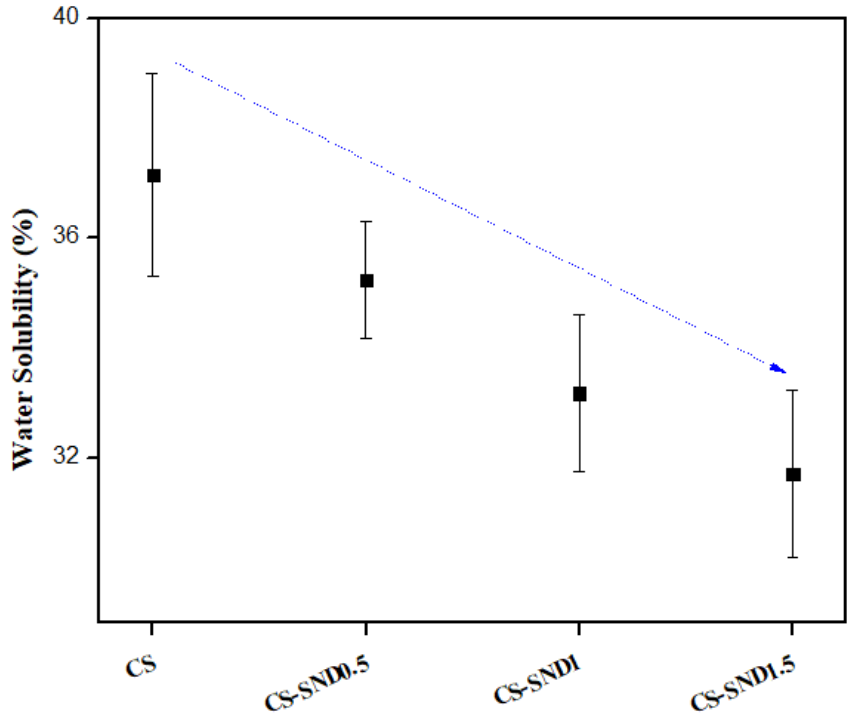
(b)

Figure 5.7 *Color properties (a) and optical properties (b) of chitosan/silk nanodisc based biocomposites.*

The moisture content and water solubility of edible coating materials are a crucial parameter to know the water affinity of the fabricated edible coating materials while introducing SND in fabricating CS based biocomposites to be used as edible coating materials. The bound moisture and moisture affinity can significantly affect the coated food products in terms of preserving food quality. However, the hygroscopicity of edible coating is beneficial for packing fresh food products, however, the dry food products may get degraded due to the application of hygroscopic packaging materials. Additionally, the moisture content of packaging materials may affect the other packaging properties of CS coating including surface properties, thermal properties, mechanical properties and others (Aguirre-Loredo et al., 2016). The introduction of SND to CS based edible coating at various proportion significantly affects the physicochemical properties in terms of moisture content and water solubility as represented in **Figure 5.8a** and **5.8b**. The moisture content of CS/SND based biocomposite coating materials significantly gets reduced due to the incorporation of SND materials. However, the moisture content and water solubility provide a direct indication of the water resistance property of the specified materials. Similarly, the water solubility of edible coating materials also found to be decreased due to the application of SND nanofillers. Additionally, moisture sensitivity of the packaging materials significantly affects the stored food products. The water solubility indicates the resistance of edible coating material against water, where, the water solubility is found to be decreased with the application of SND materials ($p<0.05$).



(a)



(b)

Figure 5.8 *Physicochemical properties of CS/SND biocomposite edible coating depicting moisture content (a) and water solubility (b) of chitosan/silk nanodisc based biocomposite materials.*

The observed tensile strength of CS/SND based biocomposites is measured in tensile mode as represented in **Figure 5.9**. The tensile strength of the developed biocomposites has been found to increase on introducing SND in CS biocomposites as observed in **Figure 5.9**. However, the increased loading of SND in CS biocomposites significantly reduces the tensile strength, where the plausible reason may be the possible agglomeration of SND in CS matrix. Further, it is noteworthy to mention that the increased tensile strength of CS/SND biocomposite may obtain due to crystalline nature of SND.

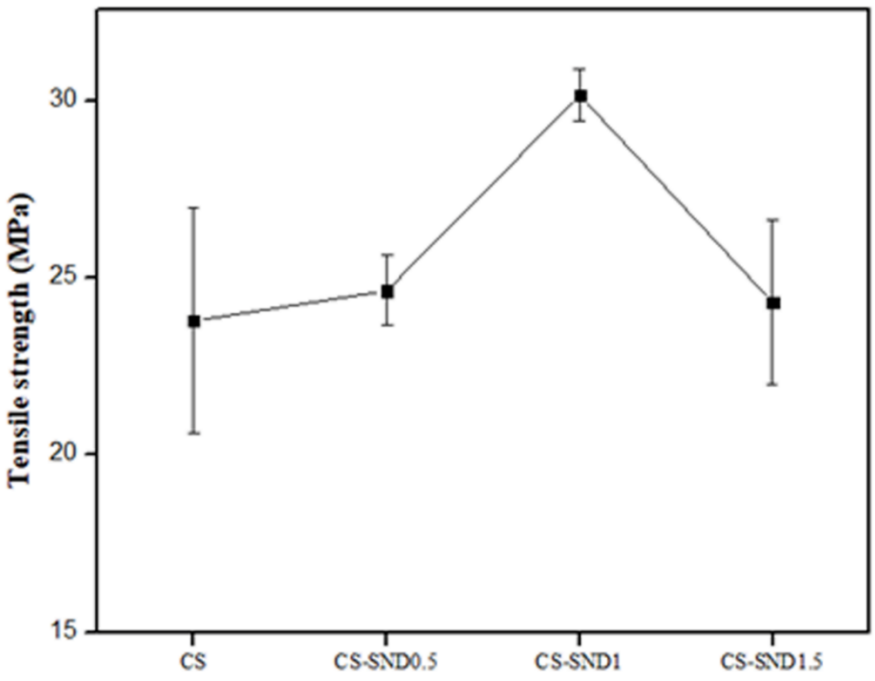


Figure 5.9 *Mechanical Properties of chitosan/silk nanodisc nanocomposite based edible coatings representing tensile strength.*

The surface properties of the fabricated edible coating materials are influenced by the incorporation of SND nanomaterials due to the interfacial interaction between nanofiller and base materials. As the surface wettability in terms of hydrophilicity/hydrophobicity of packaging materials is a crucial factor for proper food storage conditions, where contact angle measures of $\theta>65$ and $\theta<65$ signify hydrophobic and hydrophilic natures of polymer surfaces

(Ghosh, Bhasney, & Katiyar, 2020). Further, the surface properties of materials in terms of wettability and contact angle depend on several inherent factors such as surface chemistry, morphology, roughness, applied method of sample preparations and others (Ghosh, Bhasney, & Katiyar, 2020; Dhar et al., 2016). In this regards, the wettability of various materials is greatly affected by the introduction of different nanofiller materials. The hydrophobicity of packaging materials is desirable for reduced water absorbing properties. In this regard, the surface wettability and surface free energy of the fabricated edible coating has been evaluated at 27 °C using water (polar solvent) as represented in **Table 5.3** and **Figure 5.10**. The wettability analysis provides an insight into how the incorporation of SND particles affects the surface wettability of edible coating materials, where an average of the contact angles has been measured and represented. The wettability of CS materials is observed as 97.63 ± 2.6°. Additionally, the wettability of CS biocomposite materials has been increased upto ~108° showing an increase by 10° due to the application of SND nanofiller (1.5 wt%), which indicates the improved hydrophobicity of CS matrix suggesting suitable attributes for food packaging. Similarly, the surface free energy of the materials is reduced to 18.4 mN/m from 24.5 mN/m. In this regards, the surface properties of CS based packaging can be tailored-made according to the required packaging properties. The used nanofiller can be considered as an effective strategy in improving the hydrophobicity of the existing packaging materials for the safe storage life of perishable products.

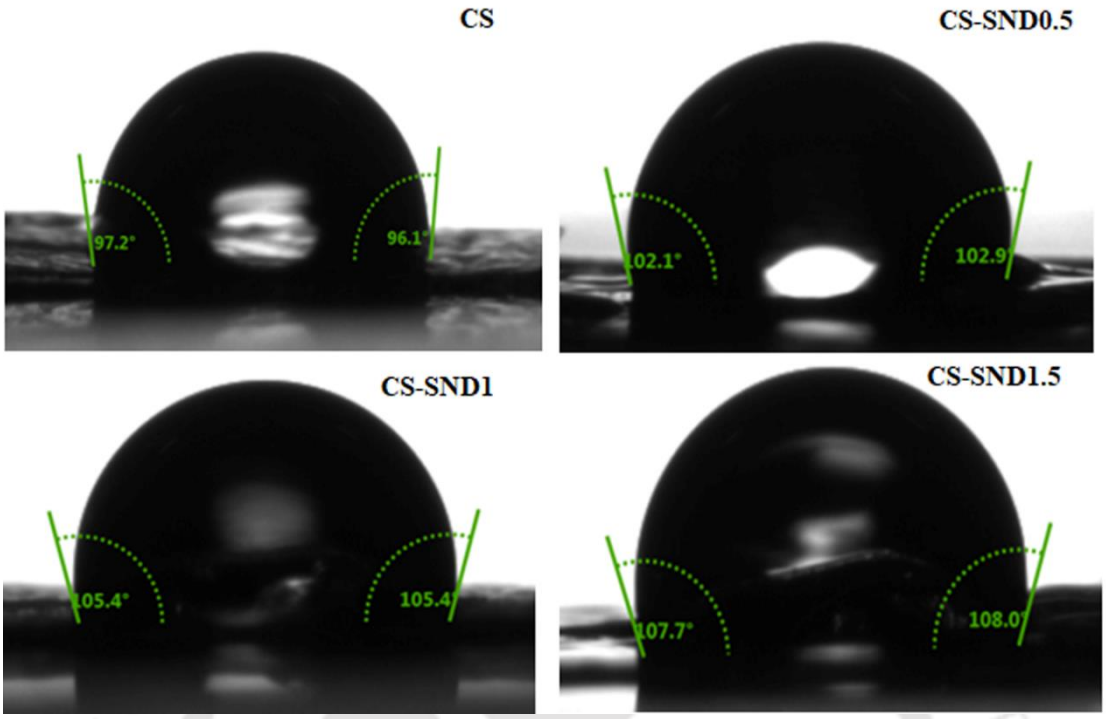


Figure 5.10 *Static contact angle measurement of fabricated edible coatings at 27 °C analyzed against water (polar solvent).*

Table 5.3 *Static contact angle and Surface free energy of CS/SND biocomposite edible coating materials.*

Testing Samples	Wettability (°)	Surface free energy (mN/m)
CS	97.63 ± 2.6	24.5
CS-SND0.5	102.25 ± 0.3	21.7
CS-SND1	105.72 ± 0.6	19.6
CS-SND1.5	107.79 ±0.9	18.4

The antimicrobial properties of CS and its biocomposites are carried out using disc diffusion technique against the two model food-borne microorganism such as *Staphylococcus aureus* ATCC 6538 (Gram positive bacteria) and *Escherichia coli* MTCC 723 as represented in **Table 5.4** and **Figure 5.11**. CS has more antimicrobial activity against *E. coli* (20.33 ±

0.47 mm) than *S. aureus* (18.67 ± 1.24 mm). However, the inclusion of SND in fabricating CS biocomposite reduce the antimicrobial activity of CS based biocomposites against both the foodborne microorganism, where SND addition may reduce the active sides of CS providing reduced antimicrobial property. The SF nanofibres have no antimicrobial property and are generally used to carry antimicrobial agents to provide antibacterial activity (Calamak et al., 2015). Thus, the biocomposites based on CS and SND based biocomposites are beneficial for providing antimicrobial property to be used as edible coating materials on food products.

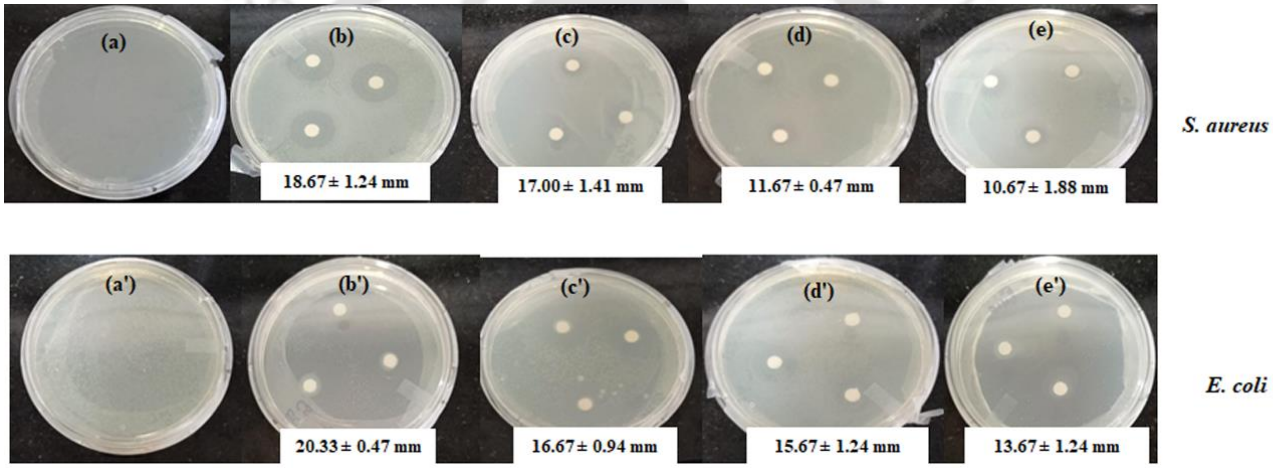


Figure 5.11 *Antimicrobial activity of CS and its biocomposites representing (a) CS; (b) CS-SND0.5, (c) CS-SND1, (d) CS-SND1.5 against S. aureus and (a') CS; (b') CS-SND0.5, (c') CS-SND1, (d') CS-SND1.5 against E. coli [CS: Chitosan; SND: Silk nanodisc].*

Table 5.4 *Zone of inhibition representing antimicrobial property of chitosan/silk nanodisc based edible coating agaisnt two food borne model bacteria.*

Sample Name	**Zone of Inhibition (mm)**	
	S. aureus	***E. coli***
CS	18.67 ± 1.24	20.33 ± 0.47
CS-SND0.5	17.00 ± 1.41	16.67 ± 0.94
CS-SND1	11.67 ± 0.47	15.67 ± 1.24
CS-SND1.5	10.67 ± 1.88	13.67 ± 1.24

As represented in **Figure 5.12**, the FTIR spectroscopy of CS and its spectroscopy is analyzed. The observed IR peaks of CS at 1650, 1554, 1419, and 1079 cm^{-1} are the characteristics peaks for amide II, amide I, alkane C-H stretching, and amide I, respectively. The IR peaks at 1156 and 891 cm^{-1} are observed for C-O stretching vibration. The characteristics FTIR peaks are observed in all the biocomposites of CS, which confirms the presence of base material in the biocomposites. However, there are observed some shift in the peaks due to the overlapping of SND and CS regions. Additionally, the peak intensity in CS nanocomposites has been found to decrease with increased SND content. The characteristics bands of SND has been superimposed with characteristics bands of CS or appear with low intensities due to reducing proportions of SND in developed SND dispersed CS biocomposites. Additionally, the peak at 2924 cm^{-1} in CS edible coating materials has shifted with changed intensities in the biocomposites due to the probable interactions between SND and CS materials.

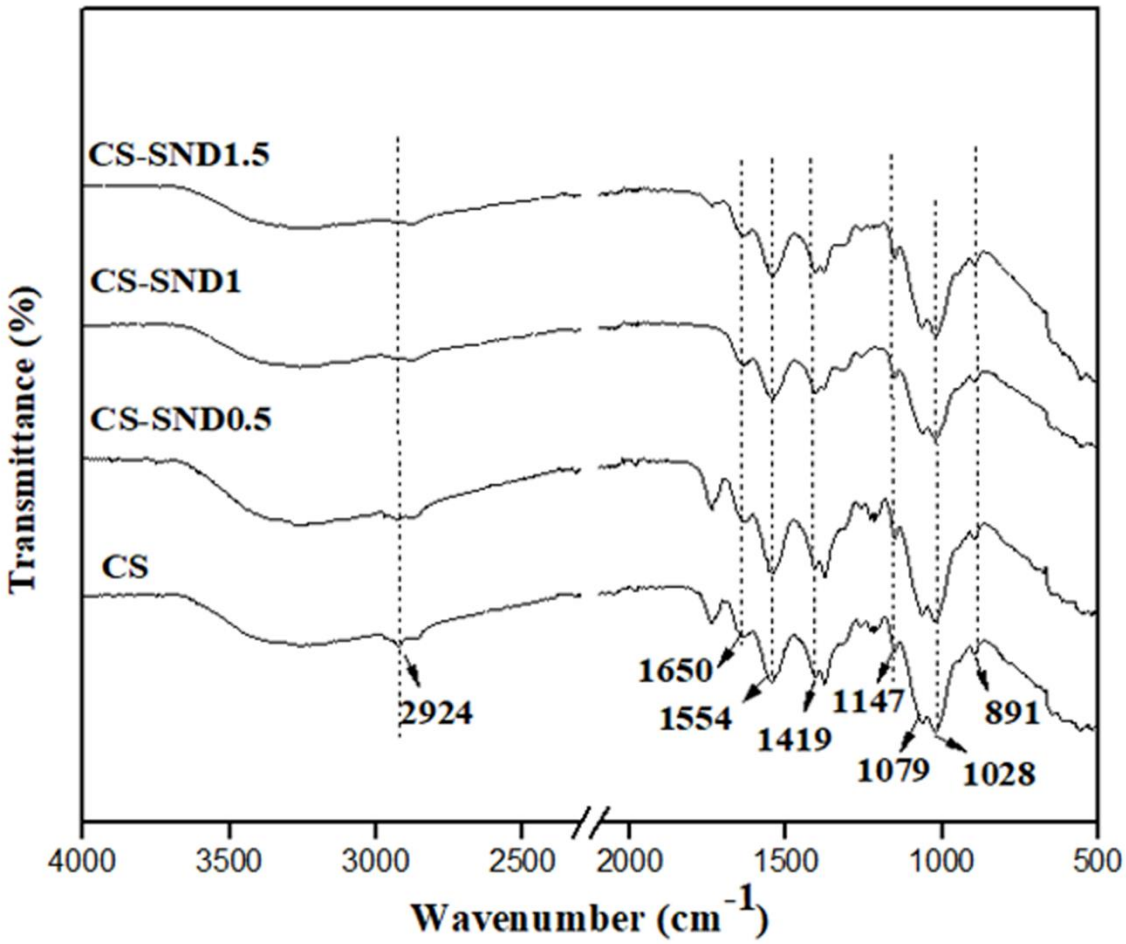
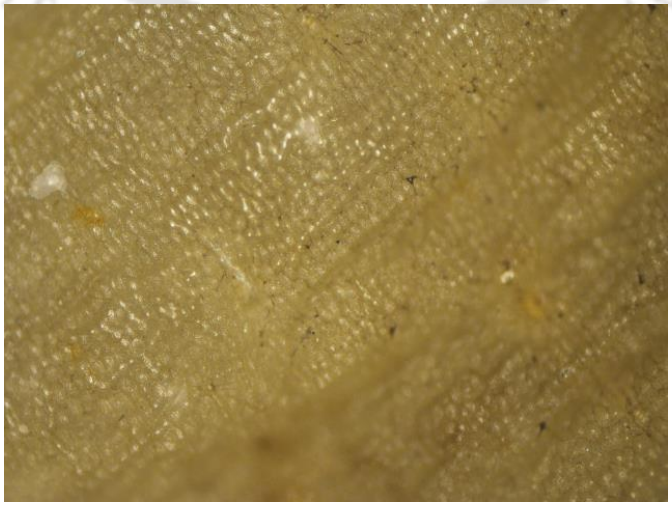


Figure 5.12 *FTIR spectroscopy of chitosan and silk nanodisc biocomposite based edible coating.*

5.2.3 Application of SND/CS Biocomposites Based Edible Coating on Banana Fruits

The CS-SND1.5 based edible coating provides most improved attributes in terms of wettability, thermal property, optical property, surface morphology, and mechanical property. However, the various selected proportion of the materials has been applied on the fresh banana fruits and a comparison has been made in regards to uncoated banana fruits. The study on EDX provides the various components that are present in the edible coating materials as mentioned earlier, and thus the absence of metal content directs the safe consumption of the materials. Further, to observe the appearance of the different coating materials on banana fruits, the coating materials are observed for their pictorial view with and without coating on banana fruits

as shown in **Figure 5.13(a)-(e)** using Nikon microscope connected with cross-polarizers and CCD camera. The schematic representation of developed edible coating on fresh banana fruits has been shown in **Figure 5.13**, with and without edible coating. Additionally, the edible coated banana has been taken for different quality measurements including weight loss, texture property, and color properties to observe the effectiveness of the noteworthy materials as edible packaging application. As mentioned in the earlier sections, the surface properties of CS/SND based nanocomposites has been found to modify in terms of surface wettability, surface energy and others. The observed surface wettability of SND dispersed CS nanocomposites has been enhanced by ~10° suggesting improved hydrophobicity. The increased hydrophobicity of the CS/SND nanocomposite may be the plausible reason for acting as a potential candidate in improved shelf life of banana. Further, FTIR results indicate the chemical interactions between SND and CS (Luangbudnark et al., 2012), which may deliver improved surface and thermal properties, which makes it a good candidate for edible nanocoating applications. Further, from the optical microscopy and FESEM analysis, the nanocomposite CS-SND1.5 has better intactness and uniformity in the matrix of chitosan, which make it a potential candidate to be used as edible coating materials.



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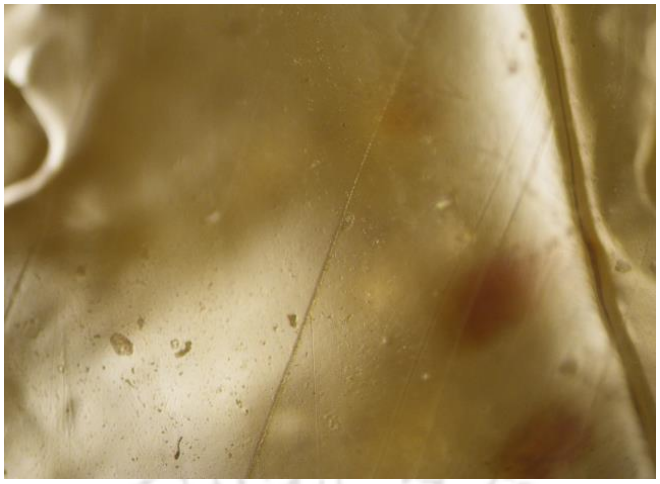

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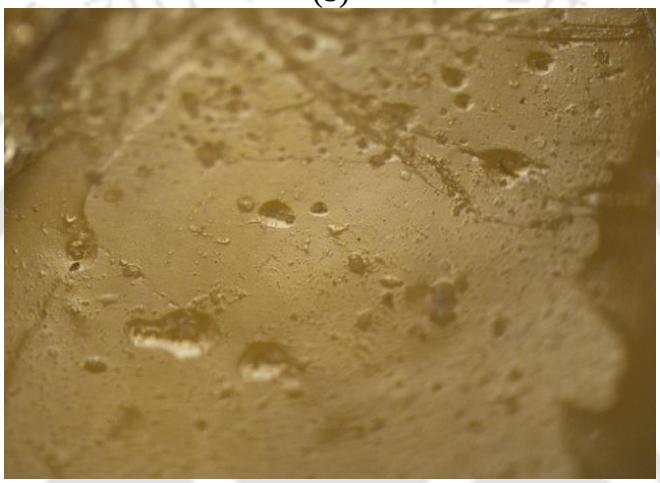

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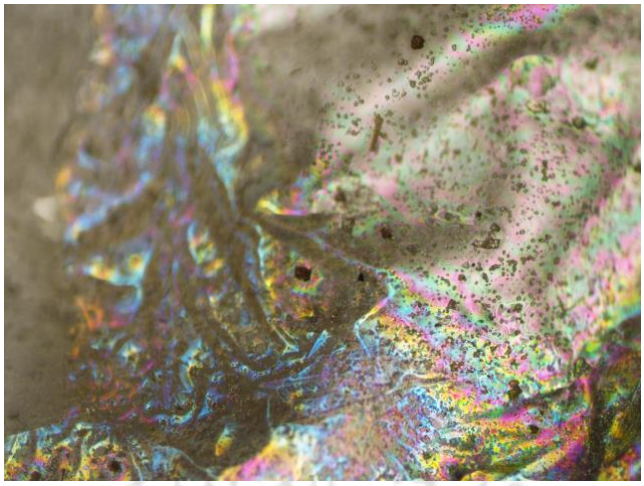


(e)

Figure 5.13 *Optical microscopy images of developed edible coating for various proportions of CS-SND representing Control (a), CS (b), CS-SND0.5 (c), CS-SND1 (d), CS-SND1.5 (e) observed using Nikon microscope connected with cross-polarizers and CCD camera*.

The weight loss analysis is an important quality parameter, as weight loss phenomena are related to consumer acceptance of the product. Interestingly, the weight loss phenomena increase with time lapse phenomena as shown in **Figure 5.14**. The application of edible coating significantly reduces the weight loss phenomena of perishable fruit products (Ghosh et al., 2019). Further, silk fibroin has a significant application in weight loss management of perishable food products such as banana and strawberry fruits (Marelli et al., 2016). As shown in **Figure 5.14**, the weight loss phenomena of coated banana fruits are observed up to 7 days of storage at room temperature and the application of selected edible coating materials has a significant effect in comparison to the uncoated banana fruits. The edible coating materials *viz*. CS-SND1.5 is found to be the most effective in comparison to the other coating materials. The whole banana fruits can retain ~79% of the original weight at 7 days of storage, whereas, the uncoated control sample retain ~65% of original weight providing the effectivity of edible coating materials.

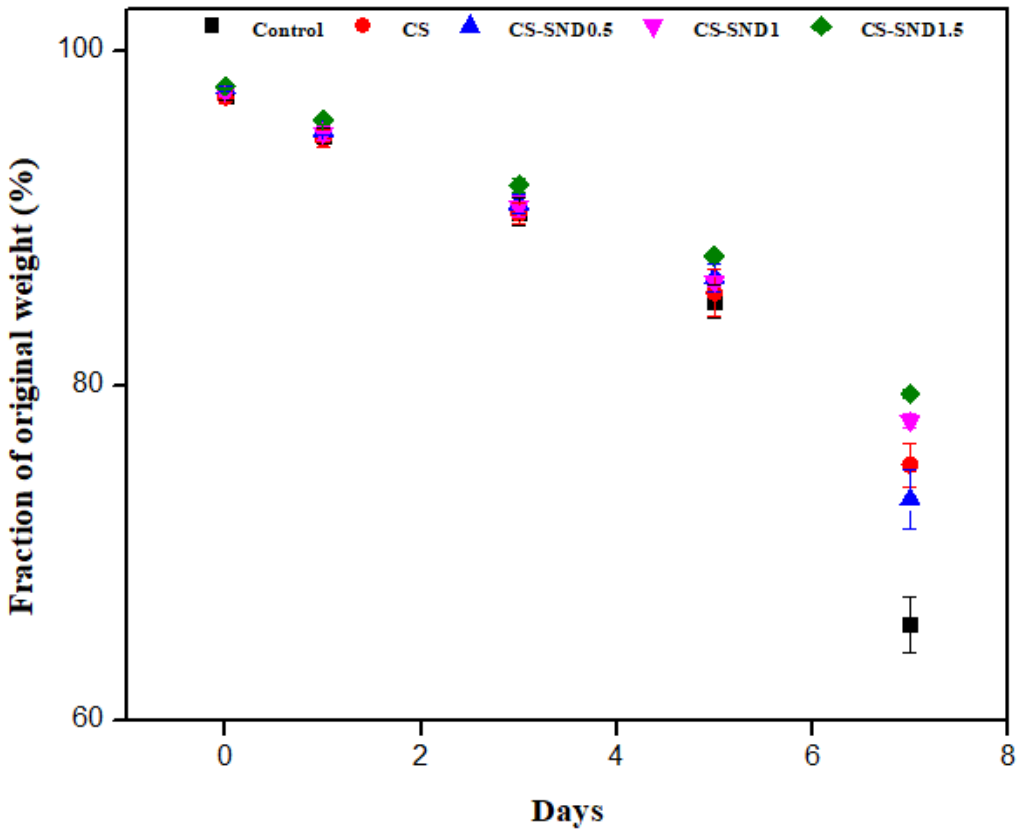


Figure 5.14 *Fraction of weight loss of edible coated banana using CS, CS-SND0.5, CS-SND1, and CS-SND1.5 at different time lapse stored at 25 °C.*

The texture loss is considered as one of the principal factors for reducing the storage life of perishable fruit products. The changes in the texture quality in terms of firmness of coated and uncoated banana fruits at room temperature have been represented in **Figure 5.15.** The firmness of the stored food products at initial storage time (at 0 days) is found to be at similar levels. However, as observed in **Figure 5.15**, the softening rate of uncoated banana fruits is the fastest in comparison to the coated fruit products. On 3rd day of storage, the firmness of the fresh banana fruits is better retained using CS-SND1.5 to ~39 N, whereas, the control testing sample has firmness of ~16 N. In this way, the developed edible coating materials can be a potential candidate for maintaining the firmness of fresh perishable food products. The reduced softening rate of control banana fruits at 6th day of storage is about ~ 8N. The loss in the

softening rate of fresh banana is affected by various environmental conditions, where the application of edible coating can act as a protective agent against degradation factors.

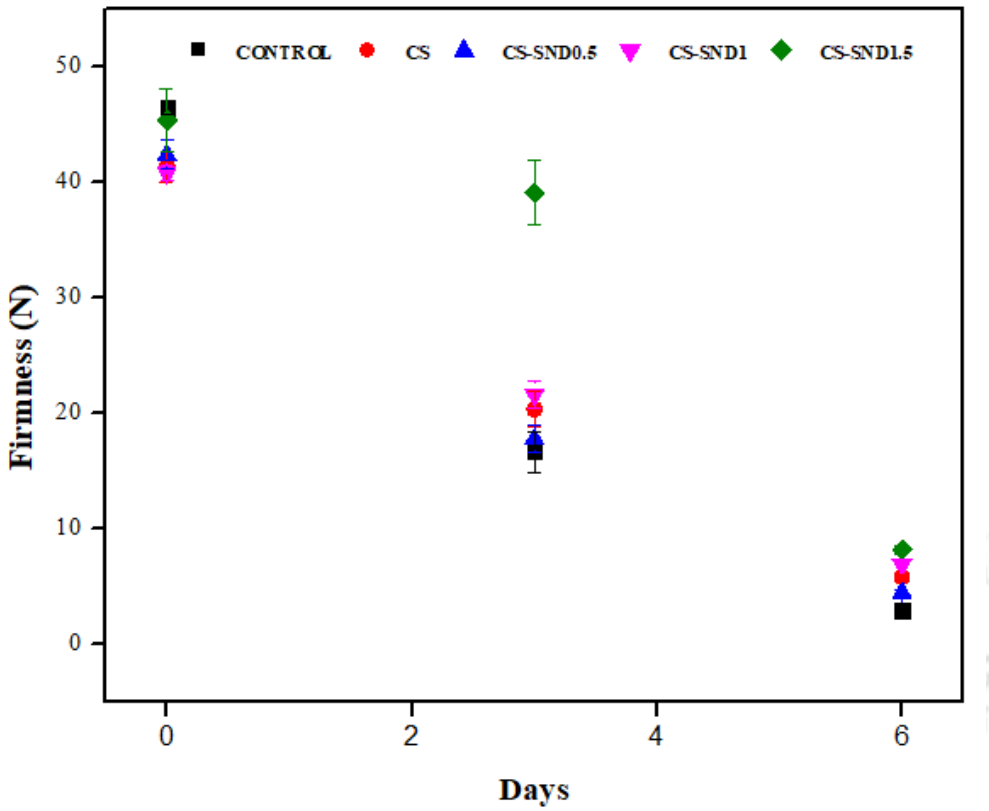
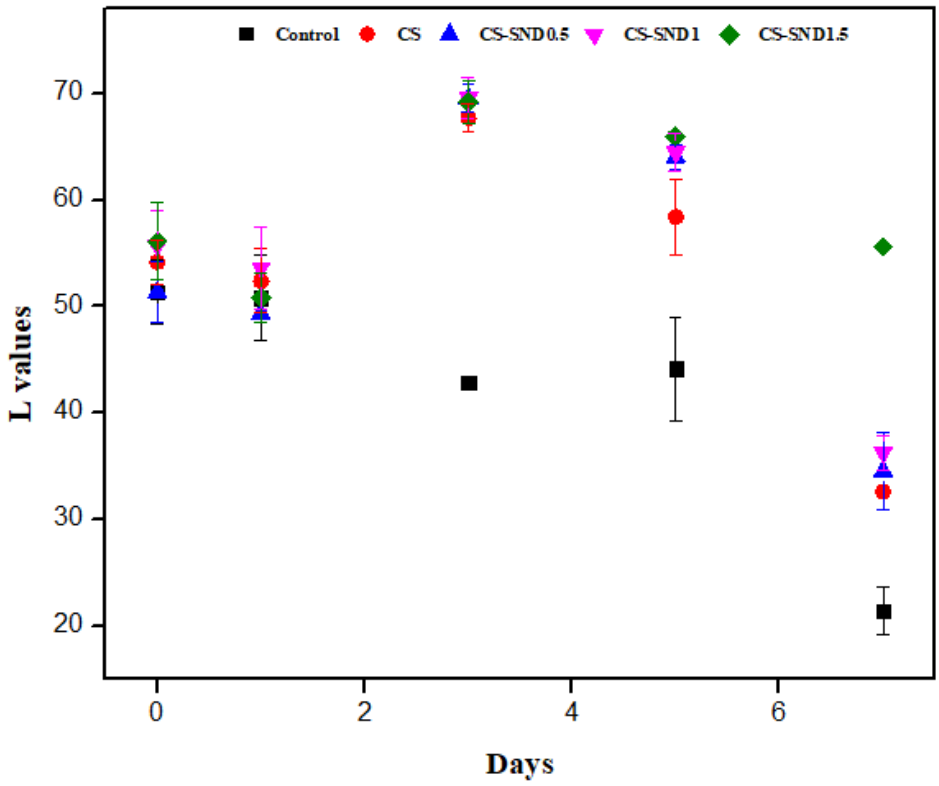


Figure 5.15 *Firmness of edible coated banana using CS, CS-SND0.5, CS-SND1, and CS-SND1.5 at different time lapse stored at 25 °C.*

The fresh banana fruits are dipped coated in different formulations of CS-SND solutions of 0.5, 1, 1.5% of SND nanomaterials and color parameters in terms of L, a*, b*, hue and chroma values have been investigated as represented in **Figure 5.16** to predict the end user impact on appearance of the products. The color properties can optimize the end user impact on edible coated product quality (Borkotoky et al., 2019). The application of CS-SND based biocomposite can significantly maintain the brightness of the stored banana at 25 °C significantly till 7 days of storage (**Figure 5.16a**). On 7th day of storage, L values of the stored banana are ~21, ~32, ~34, ~36, and ~55, for control, CS, CS-SND0.5, CS-SND1 and CS-

SND1.5, respectively. Thus, the enhancement of brightness is found to be increased by ~34 values. The brightness is a very crucial parameter for consumer acceptance of the fresh produces. Thus, the CS-SND1.5 is a very effective edible coating material for maintaining brightness of food products.

Additionally, as shown in **Figure 5.16b** and **c,** the redness factor and yellow color of the banana are preserved in an effective way using CS-SND1.5 based biocomposite material. Further, during storage of banana, the yellow color during storage may get fade away due to over ripening effect, which can be preserved using CS-SND1.5 based edible coating. The b* values of the edible coated banana fruits are found to be ~10, ~13, ~15, ~17 and ~39, respectively, for control, CS, CS-SND0.5, CS-SND1, and CS-SND1.5, respectively at 7^{th} days of storage. Thus, the application of edible coating materials is effective in maintaining brightness, yellow coloring effect, which suggest the noteworthy applicability of the synergistic effect of the biocomposite. The Chroma and hue values define the coloring effect and intensity, respectively, where, the color intensity is best preserved using the coating materials at several levels (**Figure 5.16d and e**). Further, the yellow coloration effect can be a predominant one during 7 days and maintained by using CS-SND1.5 edible coating materials.



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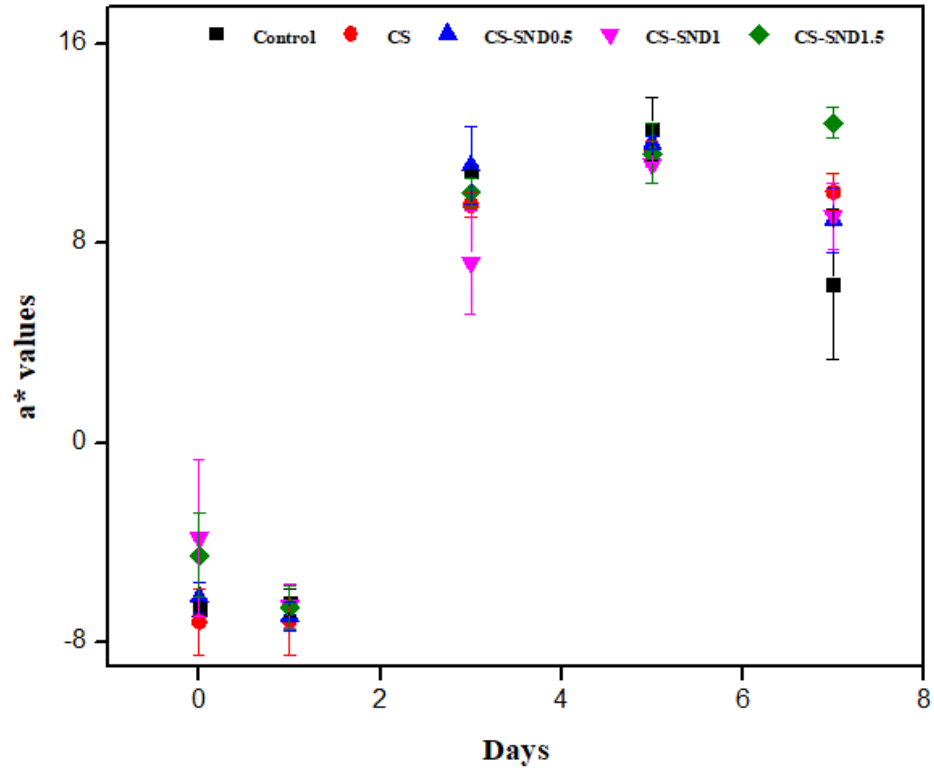

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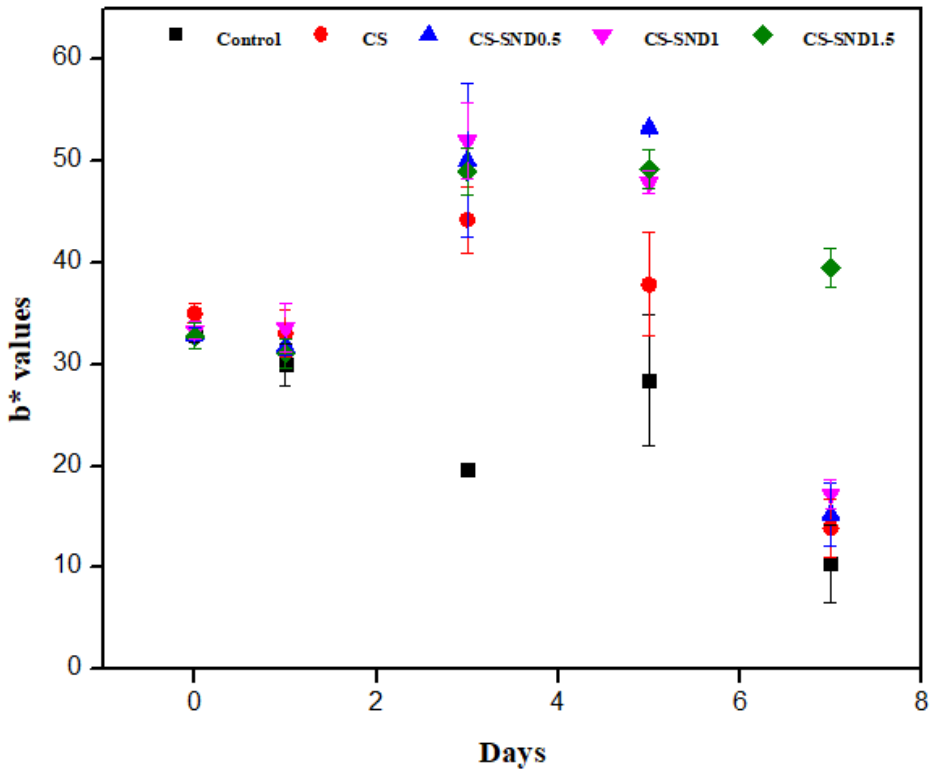

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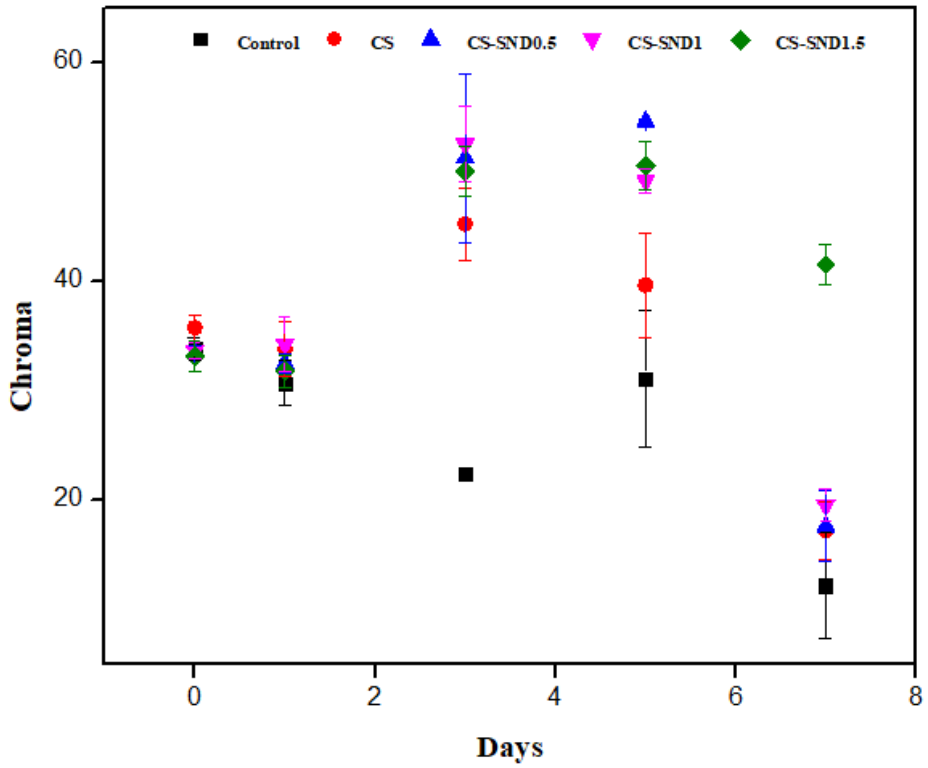

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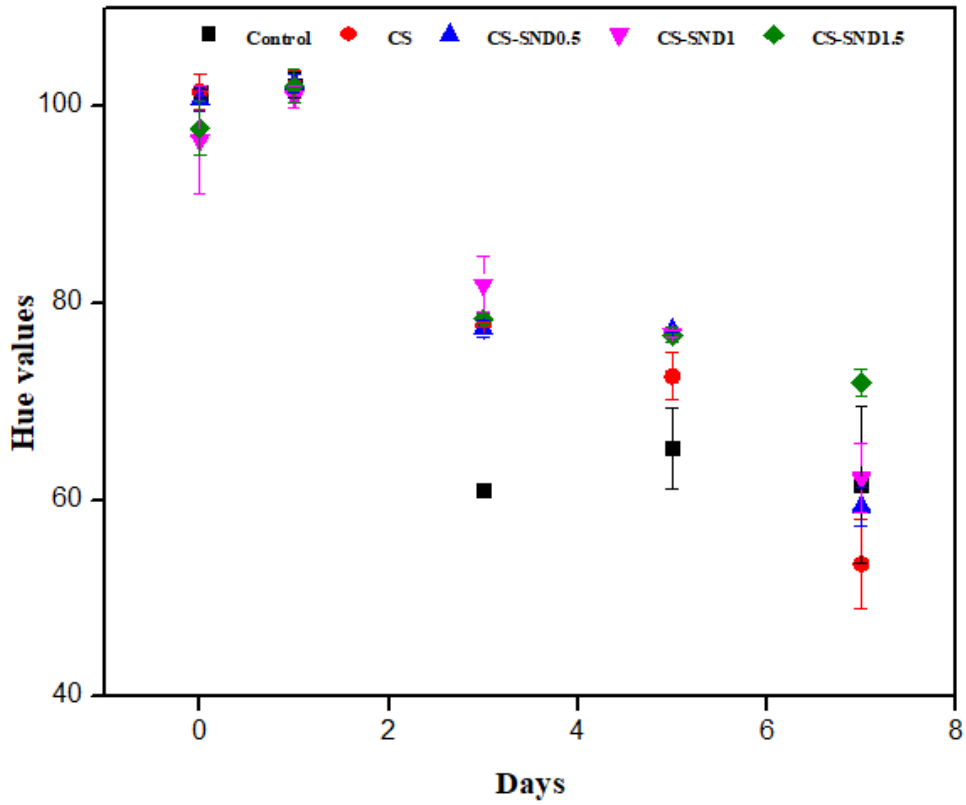


(e)

Figure 5.16 *Color properties of banana fruits during storage life representing L values (a); a* values (b); b* values (c); chroma values (d); and hue values (e).*

5.3 Conclusions

The noteworthy efficacy obtained for CS/silk nanodisc based edible coating materials by forming a uniform layer over banana fruits, wherein SND has a surface intactness in base materials as evident by FESEM micrographs and optical microscopy improving keeping quality of banana fruit in terms of original weight, firmness, and color attributes. The development of the biocomposite has been achieved following a simple stirring technique, where the coating solution form a uniform layer over the chosen fresh banana fruit products as evident by optical microscopy images. Additionally, the notable properties of silk nanostructures including structural and physical attributes increase its demand for improved shelf life of perishable fruit products. Thus, the developed coating is suitable for heat

dimensional treatment, mechanical damages of fruit products, light/moisture sensitive food products. Therefore, the present work delivers a new approach for decorating fresh food products with SND based coating materials for superior storage quality.

CHAPTER 6

Fabrication of Secondary Packaging for Coated Food Products Based on Blown Films of Poly Lactic Acid Biocomposites: An Industrially Viable Single Step Approach

Motivation

The increased food losses and wastes are mostly caused during product handling and transportation of food products due to undesirable conditions. The food losses during transportations are directly held due to the changes in the storage conditions, mechanical damages, and others. In this regards, the fabrication of secondary packaging for edible coated food products provides a solution to the safe delivery of products from the production section to the final delivery (end users). The three different levels of packaging (primary, secondary and tertiary) have their own functionality in terms of packaging criteria and the chapter focuses on the fabrication of green composites based on industrially viable secondary packaging for coated products to offer new prospects in maintaining food trade chains. It also focuses on tailoring the routes to improve the properties of chosen secondary sustainable packaging by using nanotechnology.

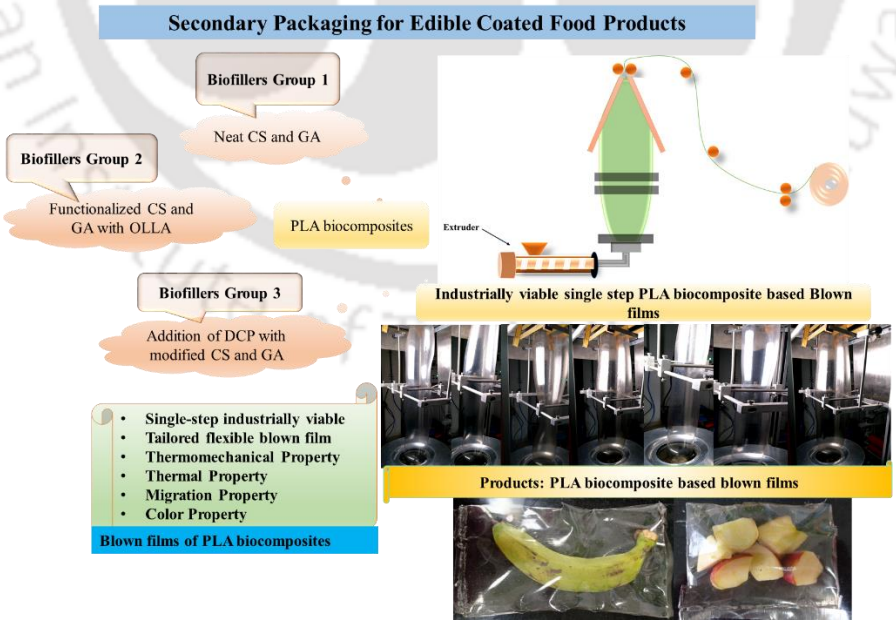
Parts of this research work has received for scientific recognition as follows:

Ghosh, T., Bhasney, S. M., & Katiyar, V. (2020). Blown films fabrication of poly lactic acid based biocomposites: Thermomechanical and migration studies. Materials Today Communications, 22, 100737.

Abstract

The present investigation demonstrates the development of a single-step industrially viable and flexible blown film based food packaging materials using biocomposites of Poly lactic acid (PLA) and functionalized biofillers. The modification of neat PLA (NPLA) based blown films is done using modified biofillers of chitosan (CS), gum Arabic (GA), with a cross-linking agent dicumyl peroxide for improved properties. The FTIR analysis gives the evidence of forming functional biofillers of CS and GA through grafting of oligomer lactic acid (OLLA) with CS and GA, giving a new peak at 1539 and 1755 cm^{-1}, respectively. The used biofillers help in tailoring PLA properties for developing blown films due to chain interactions, level of compatibility, loading percentage, and others. The formulated biocomposites provides improved properties over blow up ratio, mechanical, thermal, and thermomechanical properties of PLA. Moreover, the migration ratio of fillers in formulated biocomposites is within permissible limits confirming the safe use of packaging material.

Scheme of the Chapter



6.1 Introduction

Biodegradable polymers are a foremost requirement in food packaging industries due to the immense ability in reducing carbon footmark, and waste plastics, which in turn diminishes global warming (Shah et al., 2008; Siracusa et al., 2008; Narayan, 2011; Pawar, & Purwar, 2013). Generally, biodegradable polymers are derived from both renewable and non-renewable feedstock, and are commonly coined as "end of life", which can be recycled, biodegraded, or get composed into elemental components in nature, resulting in increased earth productivity (Yu, Dean, & Li, 2006; Gupta, Revagade, & Hilborn, 2007; Mohanty, Misra, & Hinrichsen, 2000; Soroudi, & Jakubowicz, 2013). Mostly used biodegradable polymers include poly lactic acid (PLA), poly glycolic acid (PGA), poly hydroxy alkanoates (PHA), polycaprolactone (PCL), and their fractions, which are having good packaging properties with the capability in substituting some of existing petrochemical-based packaging materials such as polyethylene (PE), polystyrene (PS), polypropylene (PP), polyethylene terephthalate (PET), etc. (Pal, & Katiyar, 2016; Dhar, et al., 2017). In this regard, petrochemical-based plastics such as PE, PS, PP, and PET are non-biodegradable which are made of fossil-based materials such as petroleum and natural gasses, which create several environmental problems. However, some of these plastic materials can be derived from biomass-based feedstock and can be regarded as biobased (bio-PE) or partly biobased plastic (bio-PET). In this regard, PET is generally developed using the monomers terephthalic acid and ethylene glycol, where both the monomers are obtained from non-biodegradable materials. On the otherhand, bio-PET is developed from mono ethylene glycol (a biodegradable source) and terephthalic acid. However, biodegradable polymers convey some limitations over some packaging properties in comparison with conventional polymers. In this regards, the properties of the biodegradable polymers in terms of crystallinity, barrier, mechanical, and thermal properties can be tuned by reinforcing biofillers (biocomposites) or blending with other polymers (polymer blends) (Dhar, et al.,

2017). However, most of the biopolymers being hydrophilic in nature need to be chemically modified for making them suitable fillers to be well dispersed in hydrophobic polymer matrixes (Pal, & Katiyar, 2016; Wu, & Wu, 2006).

The biodegradable polyester PLA is a chemically derived polymer (Phattarateera, & Pattamaprom, 2019), having comparable mechanical and thermal properties against some of the fossil based polymers such as PS and PET (Herrera et al., 2016), which gains an extreme enthrallment in the field of food packaging industry, biomedical application for preparing scaffolds for bones in tissue engineering, etc. (Pal, & Katiyar, 2016). From very early days, PET and PS are widely used conventional food packaging materials. Thus, the use of PLA can act as a good alternative for the existing packaging materials. Further, the high transparency of PLA is similar to PS make it a good candidate for packaging application. However, the poor crystallization property of PLA makes it a poor candidate for packaging application, which can be modified using different biofillers and cross linking agents. PLA is primarily synthesized from lactic acid (2-hydroxy propionic acid) monomer, which is achieving marketable importance day by day due to its renewable carbohydrate based feedstock such as wheat, corn, sweet potato, tapioca starch, agricultural wastes, etc. (Pal, & Katiyar, 2016). Furthermore, PLA is a nontoxic, biocompatible, natural, and commercially accepted polymer with comparable properties against some of the conventional polymers, which makes it a favourable candidate to be used in the field of food packaging and beverage industries (Nampoothiri, Nair, & John, 2010; Colomines et al., 2008; Jin, & Zhang, 2008). However, PLA has some existing shortcomings which may limit its use in the field of food packaging applications such as inherent brittleness, low impact resistance, poor crystallization behavior, poor toughness, etc. (Soroudi, & Jakubowicz, 2013; Phattarateera, & Pattamaprom, 2019; Jin, & Zhang, 2008). In this regard, the development of biocomposites and blends can help to obtain tailor-made properties of PLA based packaging.

Chitosan (CS), a class of polysaccharides isolated from a renewable resource, is a biopolymer abundantly available in nature (Abd El-Fattah et al., 2016) and a cationic polysaccharide which is the result of deacetylation of chitin (Islam, Khan, & Alam, 2016). It is extensively used in foodstuffs packaging due to its excessive functional properties and can be extracted from crab, lobster, shrimp, prawns, muga silkworm, etc. Moreover, it is nontoxic, biocompatible, edible, hydrophilic in nature, and has a wide variety of applications to be used in the form of fillers and matrix materials (Elsabee, & Abdou, 2013; Costa da Silva et al., 2016; Reyes-Avalos et al., 2016). The gaining interest in CS is due to its involvement in the active food packaging, its blends with other polymers improve the packaging properties such as antimicrobial, antioxidant, antifungal properties (Lozano-Navarro et al., 2017; Zou et al., 2016; Gomes, Paschoalin, & Del Aguila, 2017; Sigroha, & Khatkar, 2017). On the other hand, gum Arabic (GA) is a kind of edible, antimicrobial, antioxidant biopolymer, which can be obtained from exudates of stems of Acacia tree (Kaddam et al., 2017; Murmu, & Mishra, 2017; Tabarsa, et al., 2017; Saha et al., 2017). GA is a highly branched macromolecule having a galactose unit connected with β-1,3-glycoside. Moreover, many reports have suggested that both CS and GA has the capability of reducing bacterial, yeast, and fungal growth in food products, so migration of these materials in food surfaces will not cause any harmful effect to the food products. In adverse, CS and GA have a common limitation of aggregation due to their hydrophilicity, when acting as a filler material in PLA films. Therefore, the elimination of such limitations may possible by grafting CS and GA with lactic acid. It can provide a better filler material for PLA films, where grafted compound can be easily dispersed in PLA polymers developing perfect materials for food packaging (Pal, & Katiyar, 2016; Tripathi, & Katiyar, 2016). Additionally, the use of cross-linking agents into polymer composites can provide better packaging materials with improved interactions of molecules, which in turn can offer many beneficial impacts to the current packaging material.

The film blowing is a plastic processing technique consisting of a single screw extruder in connection with a blown film die to develop tubular films or blown films. The extruder is used to prepare the polymer melt, which is further passed into a blown film die or tubular die, where the air is blown to obtain the blown film. This method of plastic processing is extensively utilized to prepare packaging films for food products. Moreover, blown films of PLA blends with other polymers and biofillers have been reported with enhanced properties in comparison with neat PLA. Moreover, the incorporation of biofillers in PLA outburst the idea of providing green composites based on aliphatic polyester providing tuned properties. In this regards, modification of PLA can be achieved by blending with other polymers or reinforcing fillers into it. Some of the studies of formulating PLA composite film includes silk fibroin in PLA (Tesfaye et al., 2016), clay in PLA (Katiyar et al., 2011), magnetic cellulose nanocrystals in PLA (Dhar, Kumar, & Katiyar, 2016), CS in PLA by solvent evaporation (Pal, & Katiyar, 2016), functionalized GA in PLA (Tripathi, & Katiyar, 2016), etc. However, development of PLA blown films is a technique to produce packaging films, where problems due to brittle nature, poor melt strength, poor elongation capability, and higher unwrapping properties of the formed films occur, which make the material unacceptable for acting as a packaging material. In this context, biocomposites of bioplastics with modified biopolymers are promising candidates for performing as perfect packaging in food and beverage industries.

To the best of our literature survey, no study has been reported detailing development of blown films of PLA with unmodified and modified biofillers with the cross-linking agent. Based on this discussion, the main aim of the current investigation is to study the packaging properties and effects of the filler materials on PLA based blown films. The development of films include preparation of the blown film of PLA incorporating neat biofiller (CS, GA), functionalized CS (MCS) and functionalized GA (MGA), DCP linked MCS (DMCS) and MGA (DMGA). Further, the developed films are analyzed for their blown, mechanical, thermal,

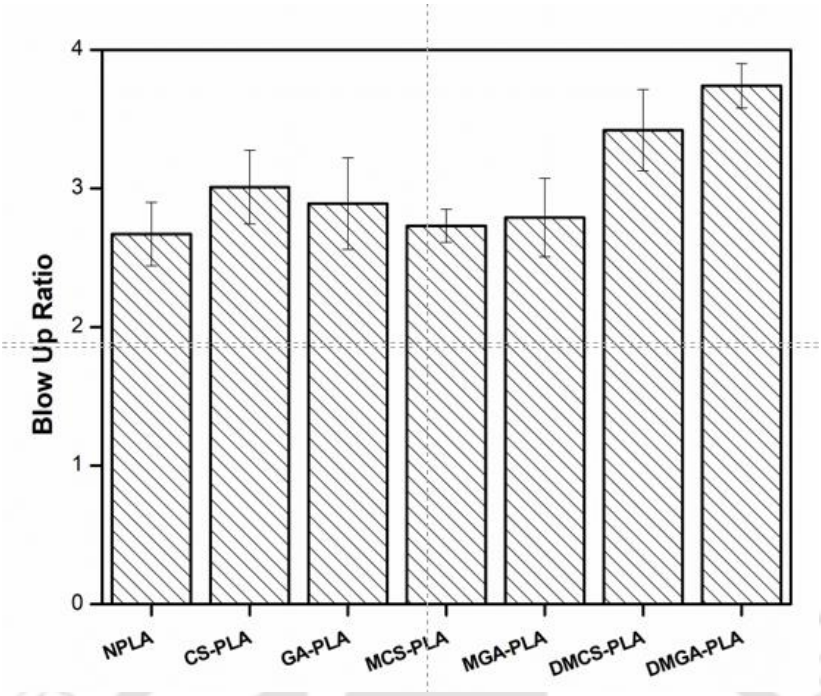
color and migration properties. The surface morphology and dispersion of biofillers in developed films are also studied using various characterization techniques.

6.2 Results and Discussions

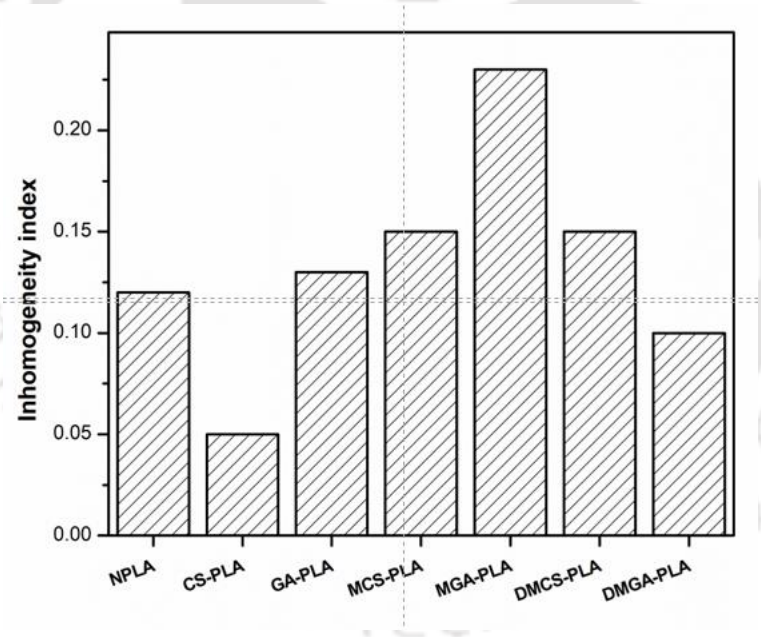
6.2.1 Fabrication and Characterization of PLA Biocomposite based Blown Films: Structural and Functional Property

The preparation of PLA composite based blown films has been carried out using modified filler materials. The various properties of PLA blown films are tuned by modifying the biofillers with the aid of DCP (cross-linking agent). The different fillers used in the study are classified into three groups based on their modifications and aided a cross-linking agent such as (i) neat CS and GA, which develop uniform but thick blown film, (ii) Grafted CS and GA with OLLA targeted to form a blown film with thin but light brown colored films, and (iii) Addition of DCP with modified CS and GA, this reactive blown film helps in increasing the width of blown films. Further, DCP having a half-life of ~180 days is used as cross-linking agents, generates peroxide radicals at high temperature and shear rates during the preparation of the blown films (Dhar et al., 2017). The addition of neat CS and GA enhance the BUR by ~11% and ~7 %, respectively in comparison to NPLA films. There is noticed to form stable blown formation with the addition of neat fillers. However, various filler materials provide various levels of interactions with PLA due to various active and inactive sites, which efficiently change the blowing properties of the developed films in both machine direction and transverse directions which in turn affects the BUR of films. The addition of MCS and MGA enhance the BUR ratio by ~ 2 and ~4% in comparison to NPLA. Interestingly, BUR provides a wide idea about the alignment of polymer molecules thus alters the proficiency of fillers onto composite film. It is suggested that having greater BUR indicates that the developed films are being elongated in the transverse direction to obtain the ultimate width of the formed bubble in

the blown films. In this regard, DMCS-PLA and DMGA-PLA based composite improve the BUR ratio by ~21% and ~28% in comparison to NPLA, respectively. Moreover, the films are stretched in the machine direction due to the need of adjusting it into the roller for balancing its uniform application of force. In this way, the stretching action in both machine and transverse direction provide bubbles in the films with a greater diameter. It is noticeable that the mechanism provides a tunable property from both the directions resulting in balanced shrink properties in both the directions. In this regards, the cross-linking property of DCP has an ability to increase the bubble diameter, so DMCS-PLA and DMGA-PLA have greater bubble diameter in comparison to other filler materials as shown in **Figure 6.1(a)**. In adverse, having low BUR indicates less orientation in transverse direction resulting in less shrinkage and tear strength. The noticeable thing is that higher the BUR less is the thickness of the developed films. Further, defects, like dancing bubbles and draw resonance can highly affect bubble formations during blown film fabrication. In this case, unmodified biopolymers i.e. CS and GA provide uniform and stable blown formations than others. Moreover, studies on inhomogeneity index suggested that variation in thickness is found higher in the case of MGA-PLA followed by DMCS-PLA (**Figure 6.1**). In adverse, the inclusion of biofillers i.e. CS and modified GA with DCP provide stable thickness throughout the developed films as compared to others. It is noticed that grafted chitosan with and without DCP provide an almost similar inhomogeneity index. By this, according to the necessity of stored food products, specific biofillers can be utilized for obtaining the targeted microstructural properties in PLA based blown films.



(a)



(b)

Figure 6.1 *(a) Blow up ratio (BUR) and (b) Inhomogeneity index of blown films of PLA and its differently aided reinforcements representing effects on microstructural properties.*

PLA Biocomposite based Blown Films: Gel Permeation Chromatography

With the aid of GPC, the molecular weight calculation in terms of weight average (M_w) and number average molecular weight (M_n) of developed blown films are represented in **Table 6.1**. The analysis depicts the difference in molecular weights due to the variation in the filler, their compatibility and mode of interactions with the matrix materials. However, in the case of both MCS and MGA, the molecular weights are decreased which may be due to the presence of low molecular weight lactic acid within the composites (Pal, & Katiyar, 2016; Tripathi, & Katiyar, 2016). Further, the incorporation of DCP helps in improving the cross-linking between filler and matrix materials, thus improving the molecular weights. So, the investigation provides that inclusion of DCP which is a radical initiator improved the dispersibility of modified fillers making more interactions between filler and matrix material.

Table 6.1 *Weight average and number average molecular weights and polydispersity index of developed blown films.*

Sample	M_w	M_n	PDI
NPLA	129200	59400	2.2
CS-PLA	132800	65700	2.0
GA-PLA	131400	66400	2.0
MCS-PLA	126200	62800	2.0
MGA-PLA	112100	59000	1.9
DMCS-PLA	131500	50100	2.6
DMGA-PLA	129400	53900	2.4

PLA Biocomposite based Blown films: FTIR spectroscopy

FTIR spectroscopy provides an outgrowth of knowledge about the functional groups present in the developed films and their grafting mechanism through the development of new bonds as shown in **Figure 6.2**. The FTIR spectroscopy study of neat PLA having various functional groups C=O stretching vibrations (~ 1749 cm^{-1}), asymmetric C-O-C stretching (1180 cm^{-1}), symmetric and asymmetric -CH_3 vibrations (1128 and 1042 cm^{-1}), C-OH side groups vibrations (1078 cm^{-1}), –CH stretching (954 cm^{-1}) and C-C vibration (868 cm^{-1}), -CH- bending (754 and 1383 cm^{-1}) (Pal, & Katiyar, 2016; Dhar et al., 2017)**.** The peak at 872 cm^{-1} observed in all samples which is due to C-C stretching and featured for amorphous properties of films. Further, the presence of a peak at 1539 cm^{-1} confirms the formation of amide ester bond (-OCONH-) in case of MCS-PLA films. In the case of MGA-PLA, the presence of oligomer molecules is witnessed by the presence of a peak at 1755 cm^{-1}, which indicates the presence of -C=O stretching vibration (Pal, & Katiyar, 2016; Dhar et al., 2017). However, CS-PLA and GA-PLA having very low intensity in comparison to others are unable to provide visible peaks. In this way, he FTIR spectroscopy confirms effectiveness of the used biofillers, modified biofillers and cross linking agent in the matrix of PLA. Additionally, as discussed in the earlier section, the use of different materials in the PLA matrix significantly affects the molecular weights at different levels.

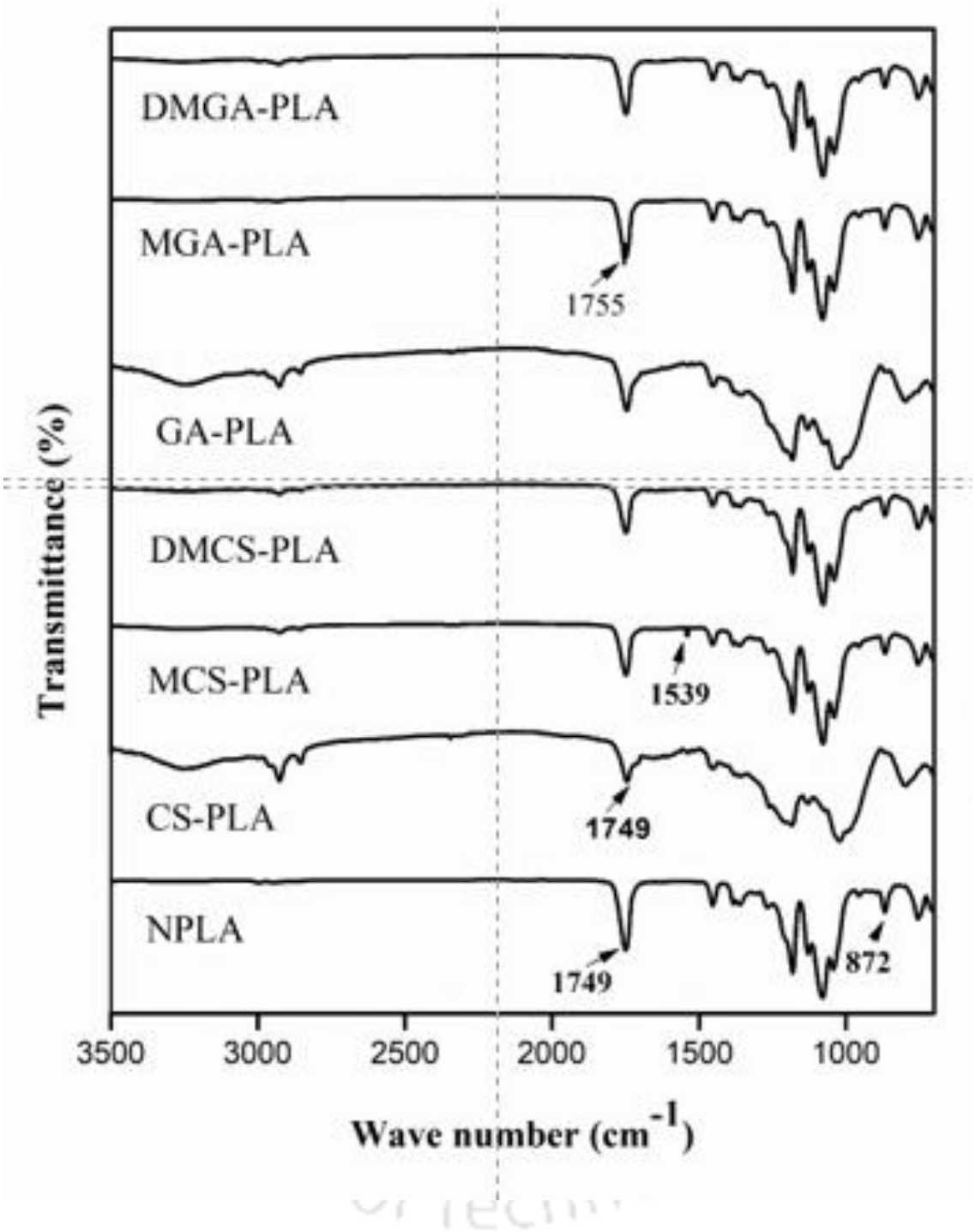


Figure 6.2 *FTIR spectrogram of PLA and its differently incorporated reinforcements presenting functional bonds in the range of 3500-200 cm^{-1}.*

6.2.2 PLA Biocomposite based Blown films: Wettability/Contact Angle Test

The wettability of polymer surface is an indispensable factor to be considered in terms of hydrophobicity and hydrophilicity for proper food packaging application (Pal, & Katiyar, 2017). The surface property of hydrophobicity and hydrophilicity is determined by measuring contact angles, where the hydrophobic and hydrophilic angles signify by ɵ >65 and ɵ <65°, respectively (Arrieta et al., 2014a; Arrieta et al., 2014b). The wettability of polymer films is greatly affected by the surface property such as morphology, chemical property, roughness, etc. Further, wettability/contact angle measurement provides an idea about a better understanding of polar and non-polar liquid adhesion property towards the composite film surface. Interestingly, hydrophilic property of films is an undesirable property, so hydrophobic property is required to ignore the water attraction property. In all cases for wettability measurement with water and glycerol, the polymer surface has obtained an angle of more than 65°, which provide a great field for use as polymeric material for food packaging application. Further, the addition of CS and GA tends to decrease the contact angle due to hydrophilic property of addressed biofiller. In adverse, functionalized CS and GA has a property of improving hydrophobicity of PLA material, providing a breakthrough to be utilized in the field of food packaging. The enhancement of hydrophobicity due to incorporating MCS and MGA may be due to creating a strong interaction through intermolecular hydrogen bonding with PLA molecule. From **Figure 6.3**, it can be concluded that MCS-PLA with water has attained improved properties in terms of acting as a packaging film material. Interestingly, with glycerol, the contact angle decreases, however it has improved for DMCS-PLA film (**Figure 6.3**). For the non-polar solvent, GA-PLA has achieved highest wettability among all the selected films. Further, the wettability of MCS-PLA and DMCS-PLA had almost similar values with diiodomethane. However, wettability of DMGA-PLA with diiodomethane has increased in comparison to MGA-PLA.

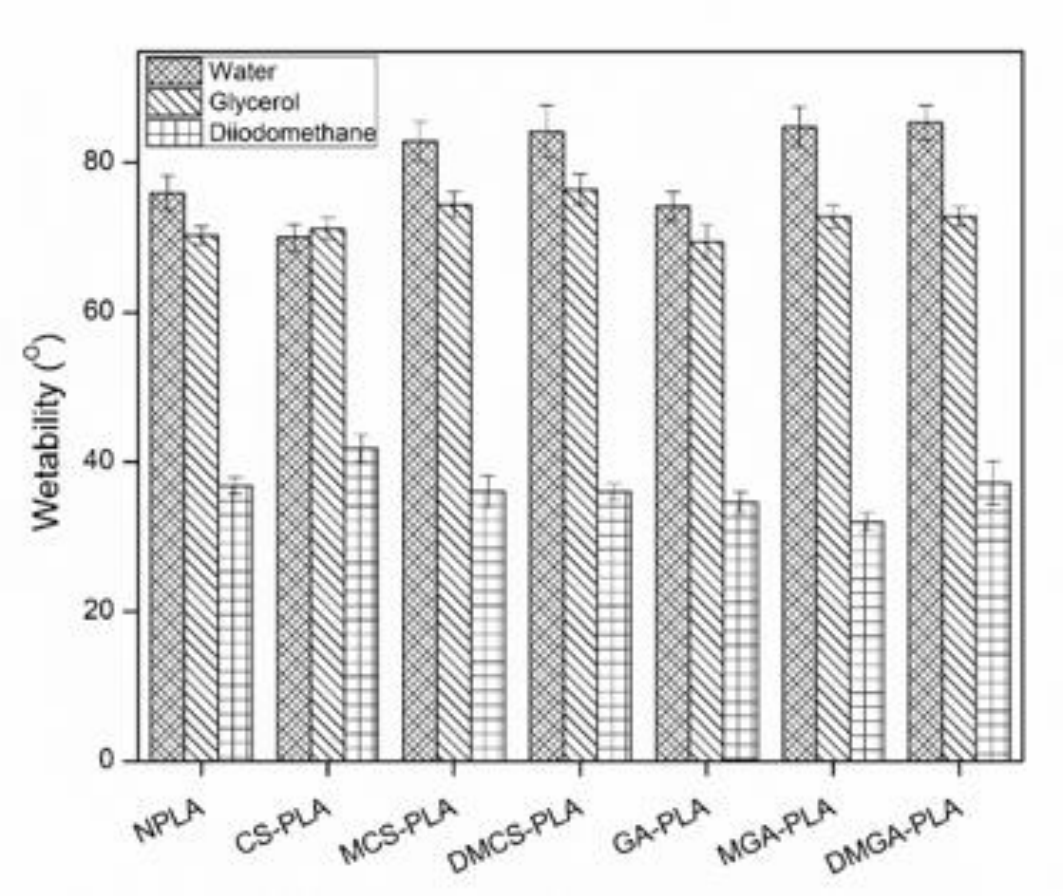


Figure 6.3 *Wettability of PLA based composite films with water, glycerol and diiodomethane.*

PLA Biocomposite based Blown films: Optical Polarimetry

The specific rotation and optical purity of developed blown films are presented in **Table 6.2**, moreover, as mentioned earlier % of L-lactic acid present in the sample is 98.6%, and the observed value for specific rotation for NPLA (-158.40) is approximately equal as reported earlier (Dhar, Kumar, & Katiyar, 2016). The specific rotation of a product depends upon how monochromatic plane polarized light rotates when passed through a sample. If the passed light rotates clockwise then denotes dextrorotary giving positive values. In adverse, in case of anticlockwise rotation, the sample provides negative values representing presence of levorotary. In this regards, the PLA and its biocomposites contain levorotary compounds. Further, the specific rotation of compounds is found to be changed with incorporation of filler materials. It is noticed for CS-PLA and GA-PLA that there is a change in specific rotation by

~16 and ~13 °, respectively due to the effect of filler onto PLA materials in comparison to PLA films. Interestingly, the variation in specific rotation for MCS and MGA reinforced PLA is changed by ~5 and ~14 ° in comparison to PLA. The change in specific rotation may be due to the grafting of OLLA chains onto the CS and GA molecules which reduce the specific rotation (Pal, & Katiyar, 2017). However, there is not a significant change in specific rotation is found when incorporated with the cross-linking and modified fillers together as shown in **Table 6.2**.

Table 6.2 *Optical polarimetry of blown films of PLA and its biocomposites representing its specific rotation (°) and optical purity (%).*

Sample	Specific Rotation (°)	Optical Purity (%)
NPLA	-158.40	100.00
CS-PLA	-142.06	89.68
GA-PLA	-145.45	91.82
MCS-PLA	-153.25	96.75
MGA-PLA	-144.90	91.48
DMCS-PLA	-158.37	99.98
DMGA-PLA	-157.03	99.14

PLA Biocomposite based Blown Films: UV-Vis Spectroscopy

Transparency of blown films of PLA films with various fillers and cross-linking agents are an important parameter according to the view point of consumer acceptance as food packaging materials as shown in **Figure 6.4**. The observed transparency of NPLA is found to be ~ 91 %, which gives the evidence that compared to other processes such as twin screw extrusion (Dhar et al., 2017) and solution casting (Dhar et al., 2015). Further, the application of various fillers noticeably affects transparency of developed films as it is a function of thickness, processability, loading percentage dispersion property of fillers etc. Considering

these parameters, transparency for CS-PLA and GA-PLA is found to be ~ 55% and ~ 75%, respectively. The reason for such reduction may be due to particle size of both the unmodified fillers and their agglomeration property with PLA during film formation, which makes PLA less transparent. Further, MCS-PLA has got transparency of ~ 89% which is closer to that of NPLA. Further, decrease in transparency of MGA-PLA (~83%) is found, where plausible reason may be the intense brown coloration effects on PLA films. On the other hand, with the aid of DCP to functionalized filler materials shows alternative effects. The transparency of DMCS-PLA is reduced to ~83% from ~89% (MCS-PLA), in adverse, the transparency of DMGA-PLA is increased to ~86% from ~83% (MGA-PLA). The plausible reason for this may be improved dispersion in case of functionalized GA, carbon content and thickness parameters. The BUR of DMGA-PLA is more than DMCS-PLA, which indicates the better transparency of DMGA-PLA in comparison with DMCS-PLA. The transparency of the films is measured according to the method followed by Molinaro *et al*. 2013 (Molinaro *et al*. 2013). So, it can be concluded that transparency can be well influenced by the use of filler materials, processing techniques, and dispensability.

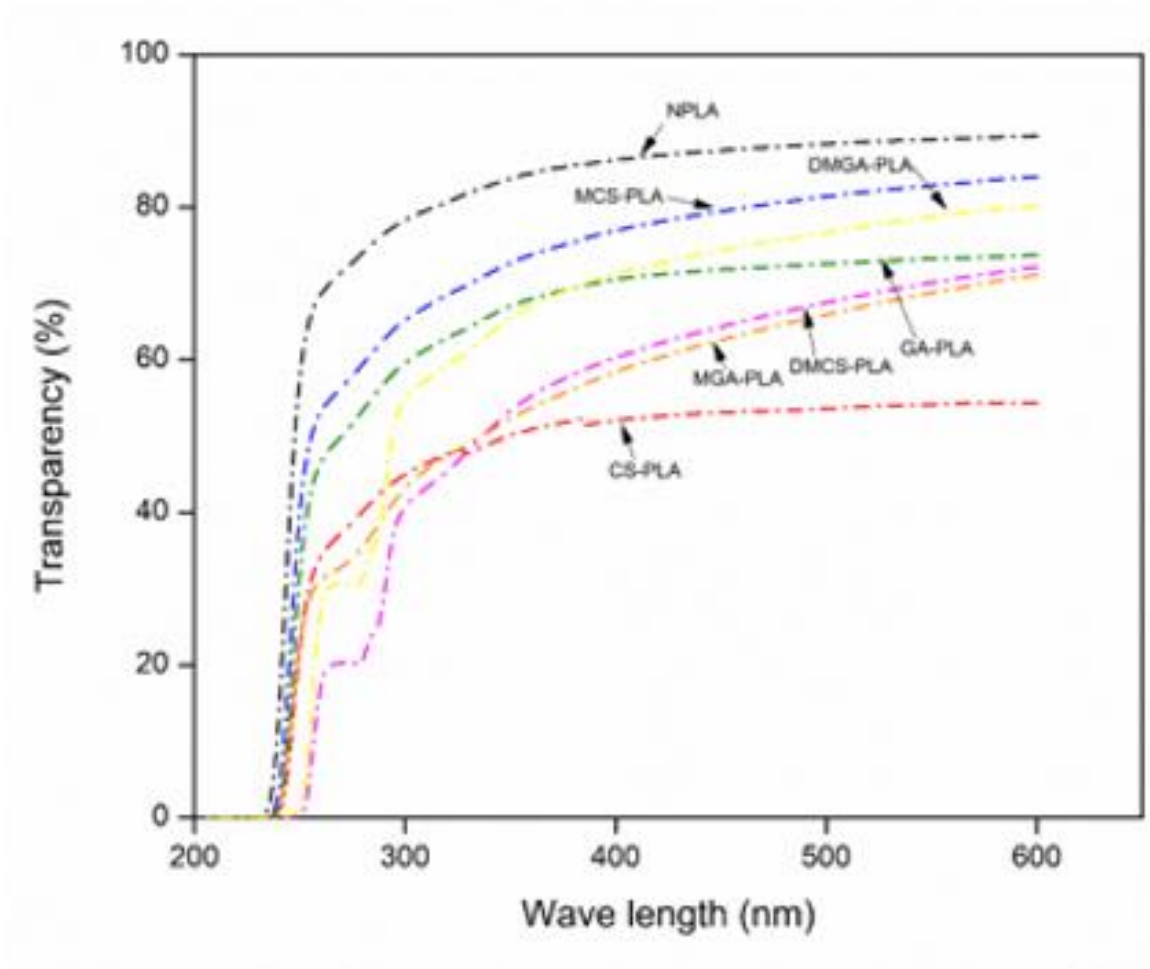
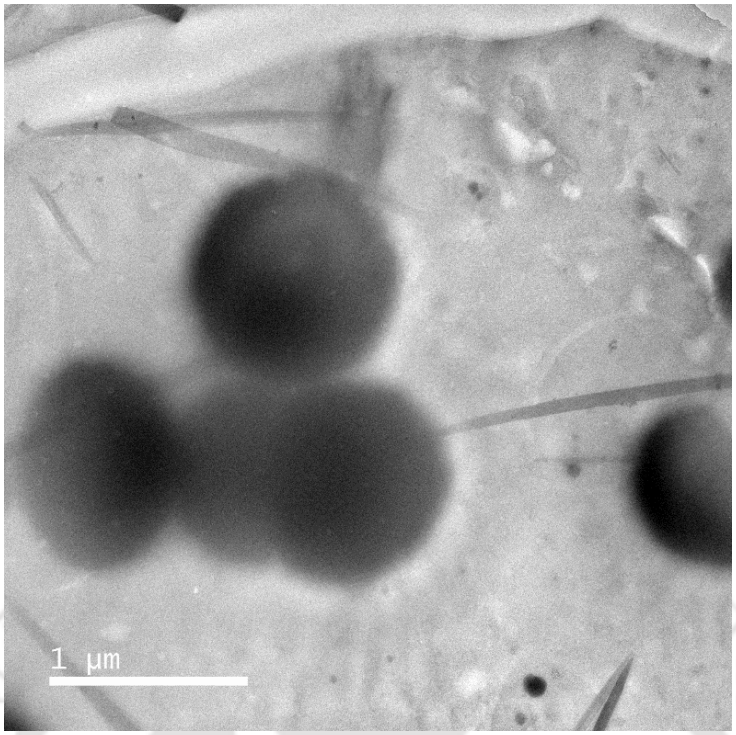


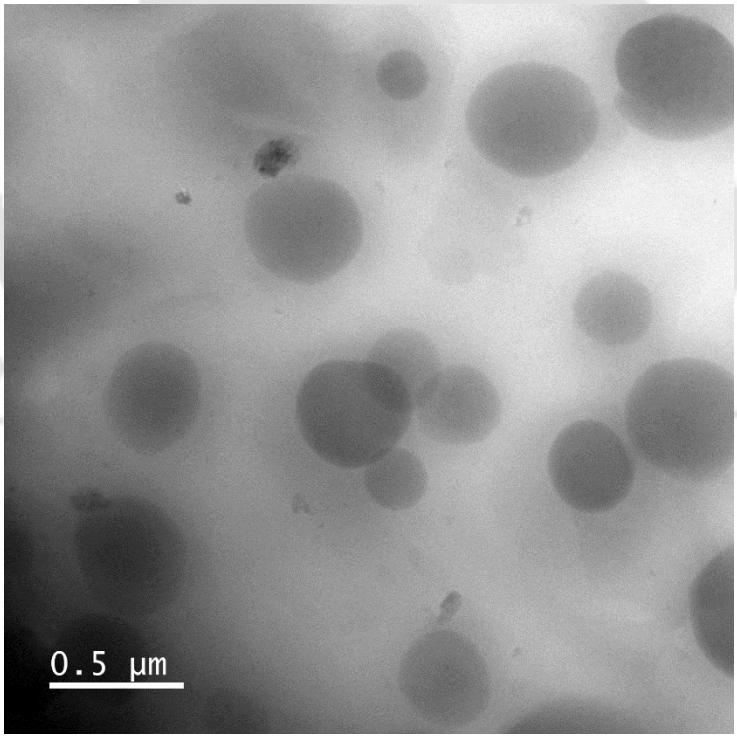
Figure 6.4 *UV-Vis spectroscopy of developed PLA and its biocomposite films.*

6.2.3 PLA Biocomposite based Blown films: Morphological Analysis

FETEM analysis of formulated MGA-PLA and MCS-PLA films provide spherical size nanoparticles dispersion of MGA (a) and MCS (b) throughout PLA matrix, which proves the presence of nanofiller materials in PLA matrix (**Figure 6.5**). The dimension of MGA and MCS particles are found to be in the range of 50 to 900 nm and 150 to 500 nm, respectively. The surface morphology gives the evidence that there is no agglomeration of functionalized fillers on to the PLA matrix and different particles are scattered on the matrix, where it proves that modified fillers are compatible with the matrix material. However, the addition of DCP reduces the particle size with better interaction and decrease in particle size, where DMGA-PLA has a particle size of 20-50 nm, and DMCS-PLA has a size range of 40-110 nm.



(a)



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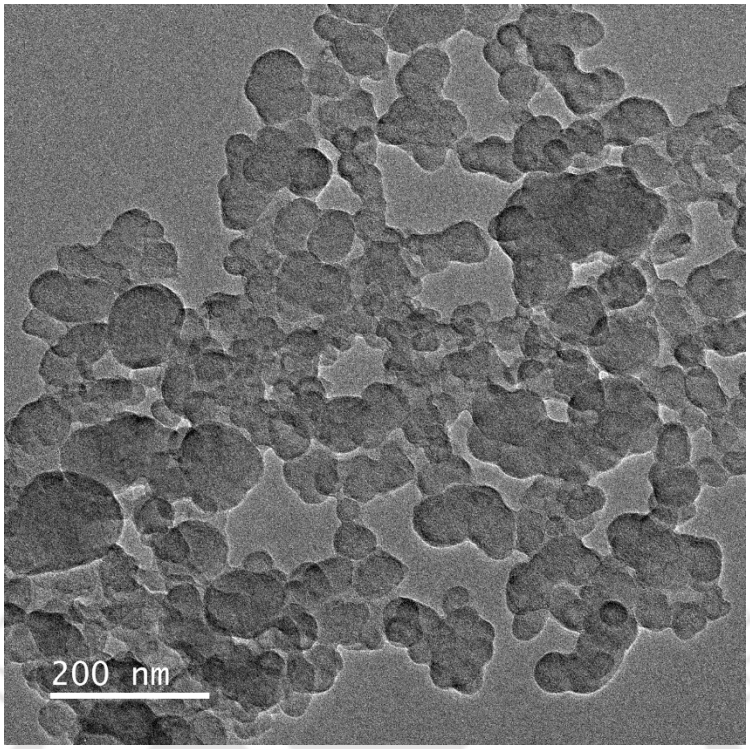

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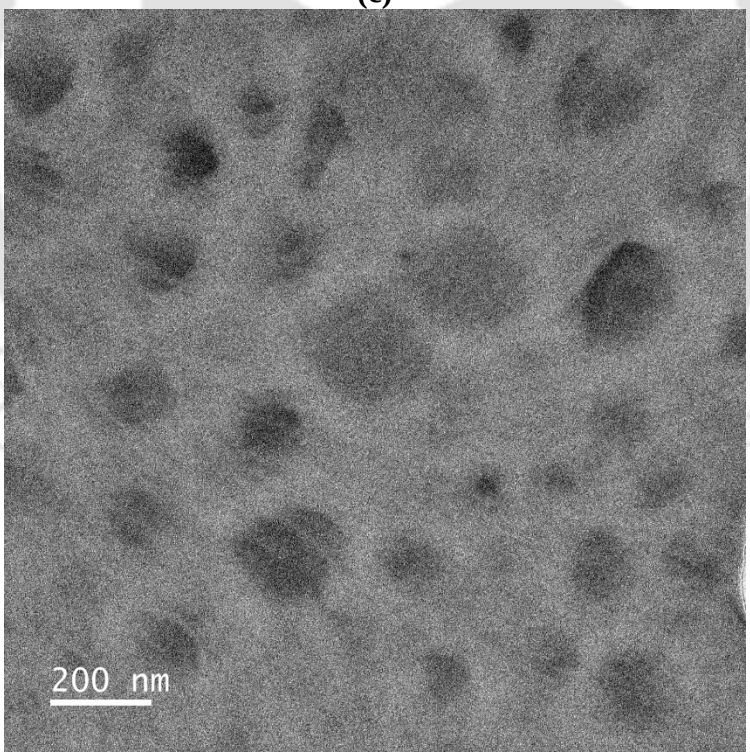

(d)

Figure 6.5 *Representative FETEM micrographs of (a) MGA-PLA, (b) MCS-PLA, (c)DMGA-PLA, and (d)DMCS-PLA*

PLA Biocomposite based Blown Films: XRD Study

The XRD diffractogram of developed biocomposite films is represented in **Figure 6.6**, where an intense peak at 2θ=16.3° and 18.8° are observed indicating the presence of α-crystals of PLA for the planes (010) and (110/200). In case of blown films of composites of PLA, the sharp peaks are observed at 16.3° in all cases, showing no significant change in peak positions, confirming the presence of characteristics diffraction pattern. Further, the crystallinity index values of PLA based blown films are determined where the involvement of DCP increase the crystallinity index by many folds.

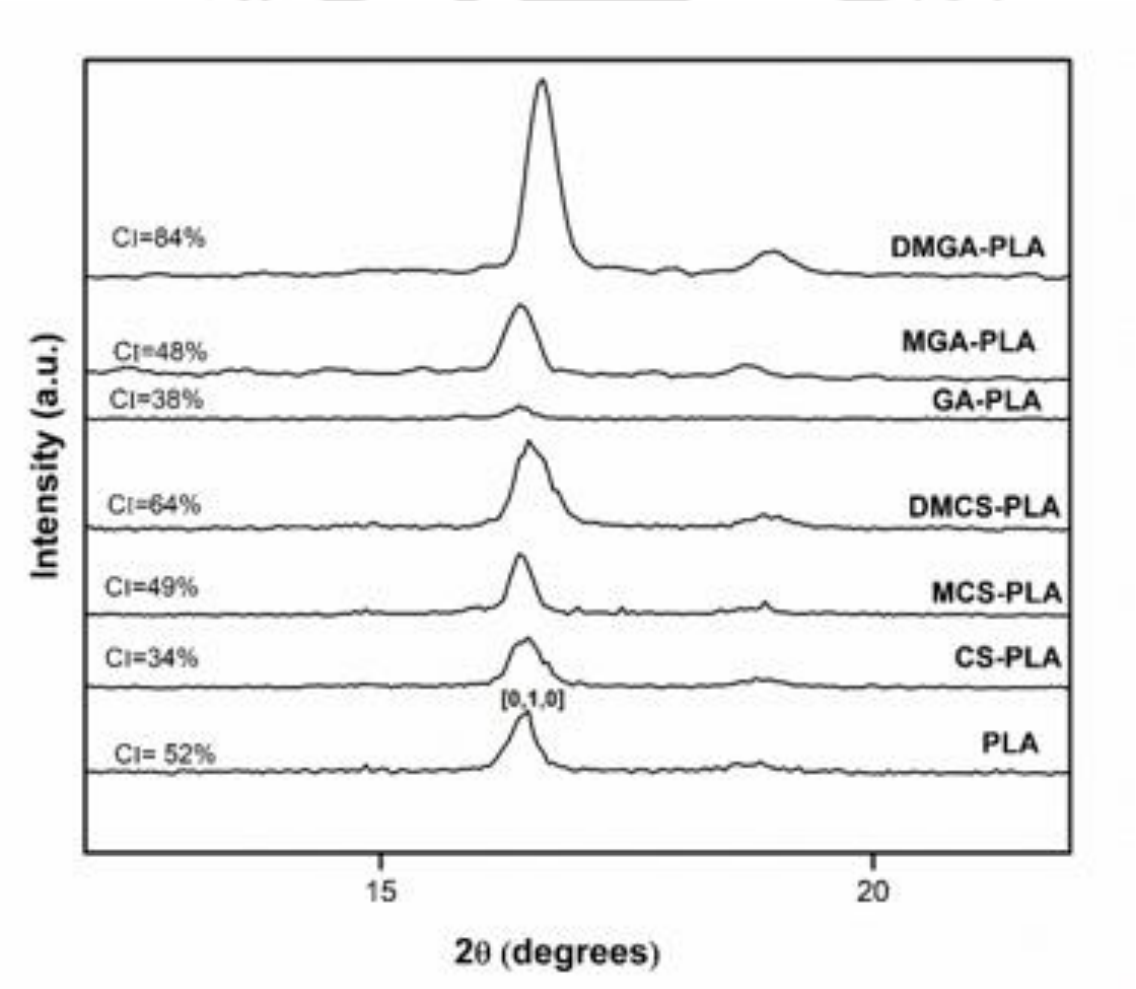

Figure 6.6 *XRD patterns of PLA based biocomposite blown films*

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6.2.4 PLA Biocomposite based Blown films: Thermal Properties

DSC analysis of PLA and its composites are carried out to understand the crystallization and melting strategy of developed blown film samples considering two heating and cooling cycles as mentioned earlier. The first heating cycle is conducted to remove bound water from the films and the second heating cycle is considered for the present study as shown in **Figure 6.7**. The investigation shows almost no significant in T_g values, however, crystallization temperature and crystallinity index are influenced by the incorporation of filler and DCP. The T_{cc} values are reduced to ~106 and ~105 °C for DMCS-PLA and DMGA-PLA from ~110 °C (NPLA), respectively as shown in **Table 6.3**. However, incorporation of unmodified filler materials increases the T_{cc} values to ~115 and ~112 °C for CS-PLA and GA-PLA, respectively. Moreover, the % crystallinity values ($\%X_c$) is found to improve for DMCS-PLA and DMGA-PLA for improving crystallinity effect due to DCP, which are found to be in the same pattern as that of XRD results.

Table 6.3 *Thermal properties of PLA based composites for blown film application*.

Sample name	T_g	T_{cc}	T_m	ΔH_{cc}	ΔH_m	Xc%
NPLA	61.3	110.6	169.3	33.29	42.4	9.73
CS-PLA	61.9	115.1	171.0	42.37	46.68	4.60
MCS-PLA	61.2	110.8	170.7	29.67	37.97	8.86
DMCS-PLA	61.6	106.2	168.7	37.39	47.36	10.68
GA-PLA	61.0	112.9	170.7	48	53.34	5.80
MGA-PLA	60.9	110.9	170.7	59.34	67.23	8.42
DMGA-PLA	61.4	105.5	168.8	6.303	40.64	36.68

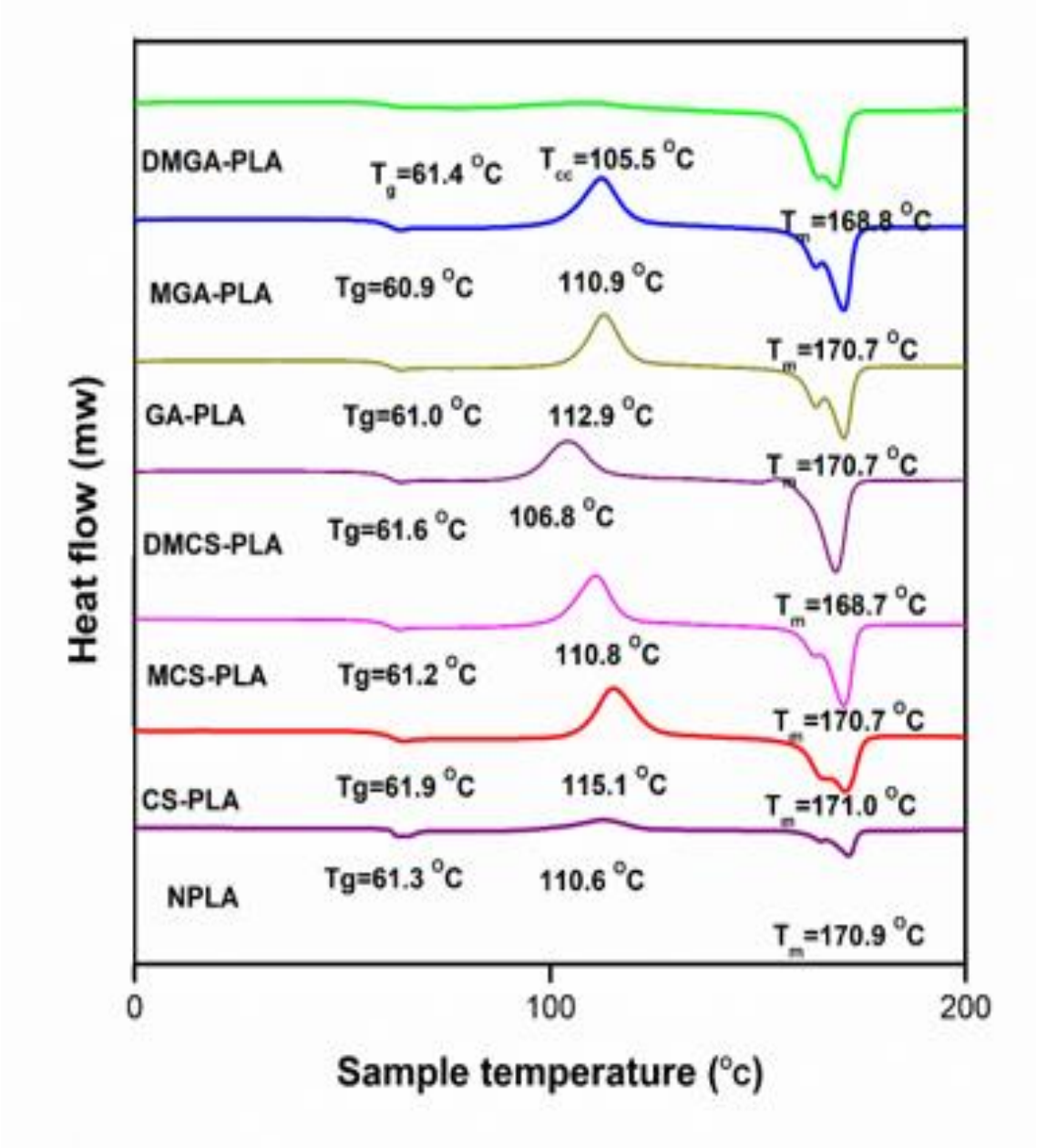


Figure 6.7 *DSC thermograms of PLA based biocomposites*

PLA Biocomposite based Blown films: Thermogravimetric Analysis

The thermal study of PLA and its composites show that the T_{on} degradation temperature of CS-PLA, MCS-PLA, DMCS-PLA, GA-PLA, and MGA-PLA are found to be increased in comparison with NPLA as shown in **Table 6.4**. On the other hand, T_{on} of DMGA-PLA is found to be decreased, which may be due to the availability of cross-linking agent in MGA-PLA filmmaking the polymer chains more flexible. Further, T_{off} of CS-PLA, MCS-PLA, DMCS-

PLA, GA-PLA, MGA-PLA, and DMGA-PLA are increased which shows their better thermal stability than NPLA. Another term i.e. peak temperature is also found to be improved by incorporating biofillers except for DMGA-PLA (~368 °C). But, the thermal cycle of MGA-PLA is different from the others, where two degradation paths are easily noticeable. The residual weights are 0.82, 0.58, 0.58, 0.36, 0.29, 0.70 and 0.52% for NPLA, CS-PLA, MCS-PLA, DMCS-PLA, GA-PLA, MGA-PLA, and DMGA-PLA, respectively.

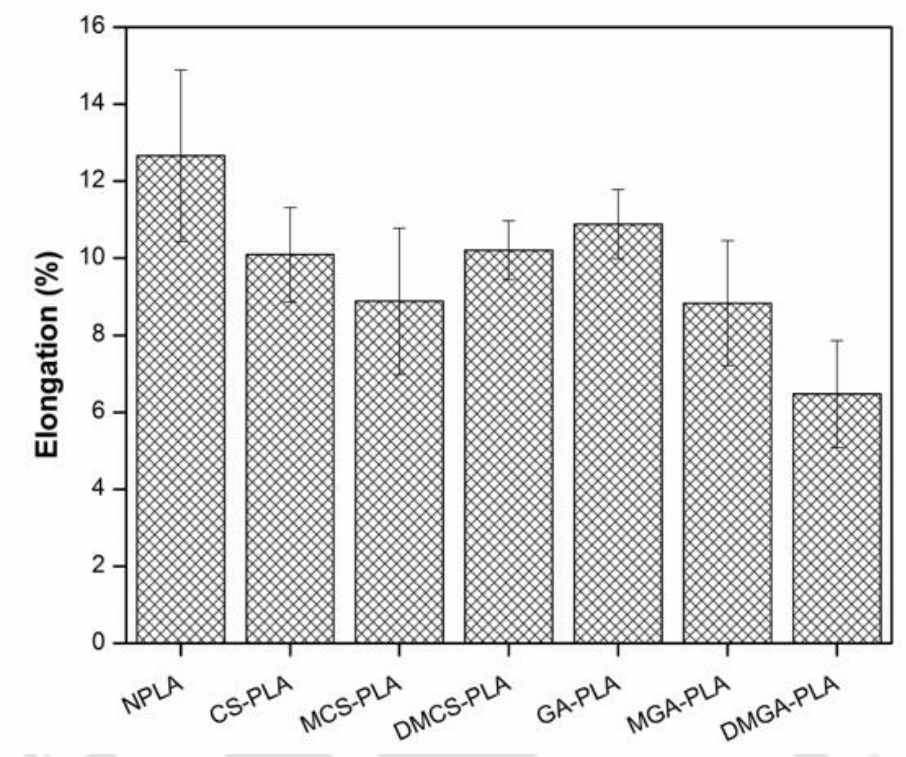
Table 6.4 *Thermal stability of blown films of PLA and its biocomposites.*

Sample	Onset temperature (T_{on} °C)	Peak temperature (T_p °C)	Offset temperature (T_{off} °C)
PLA	336	371	389
CS-PLA	348	379	395
MCS-PLA	337	373	390
DMCS-PLA	340	377	393
GA-PLA	337	374	392
MGA-PLA	341	377	394
DMGA-PLA	328	368	388

6.2.5 PLA Biocomposite based Blown films: Mechanical properties

The mechanical properties of the developed films are investigated, where % elongation, peak load (N), stress (N), UTS (N/mm^2) and break load (N) are measured and presented in **Figure 6.8**. It is worthy to mention that mechanical properties of polymer composites are mainly influenced by processing methods, processing temperature, filler type, dispersion of

fillers, polymer interactions, intermolecular forces, etc. Interestingly, the stress required to break the polymer samples under the tensile mode of CS-PLA, MCS-PLS, DMCS-PLA, GA-PLA, MGA-PLA, and DMGA-PLA are reduced by 19%, 44%, 43%, 24%, 11% and 5% respectively. The observed % elongation of NPLA is found to be 12.66%, in comparison to this, a variation is found in % elongation for developed PLA composites due to the influence of filler materials. The results show that % elongation of CS-PLA, MCS-PLA, DMCS-PLA, GA-PLA, MGA-PLA, and DMGA-PLA is lessened by 20%, 29%, 19%, 14%, 30%, and 48%, respectively, where CS-PLA, DMCS-PLA have almost similar % elongation. The variation may be due to the various interaction of filler materials in the long chain of PLA matrix, decreasing its intermolecular forces of attraction. Further, alternate effects of DCP has been found with the functionalized fillers MCS and MGA. As the incorporation of DCP, it reduces the value of % elongation of MCS-PLA, and in the adverse incorporation of DCP increases the % elongation of MGA-PLA. This may be due to the better interaction of DCP with MGA molecules, making the MGA molecules more flexible to move around in matrix molecule. Considering the tensile strength of developed films, it is noticed that UTS for all films are found to be decreased in all cases in comparison with NPLA. The UTS of CS-PLA, MCS-PLA, DMCS-PLA, GA-PLA, MGA-PLA, and DMGA-PLA are reduced by 25%, 49%, 38%, 25%, 11%, and 15%, respectively. However, similar effects of tensile strength for CS and GA fillers on PLA matrix have been found. MGA-PLA has found to have better tensile strength than MCS-PLA. Further, in consideration, the addition of DCP decreases the tensile strength of both the modified filler incorporated PLA films. The observed peak load shows that GA-PLA has the highest peak load followed by CS-PLA, DMGA-PLA, NPLA, MGA-PLA, MCS-PLA, and DMCS-PLA. The results show that the peak load of CS-PLA, GA-PLA, DMGA-PLA have been improved by 79%, 135%, and 71%, respectively. Whereas, the peak load of MCS-PLA, DMCS-PLA, MGA-PLA is decreased by 29%, 37%, and 26%, respectively.



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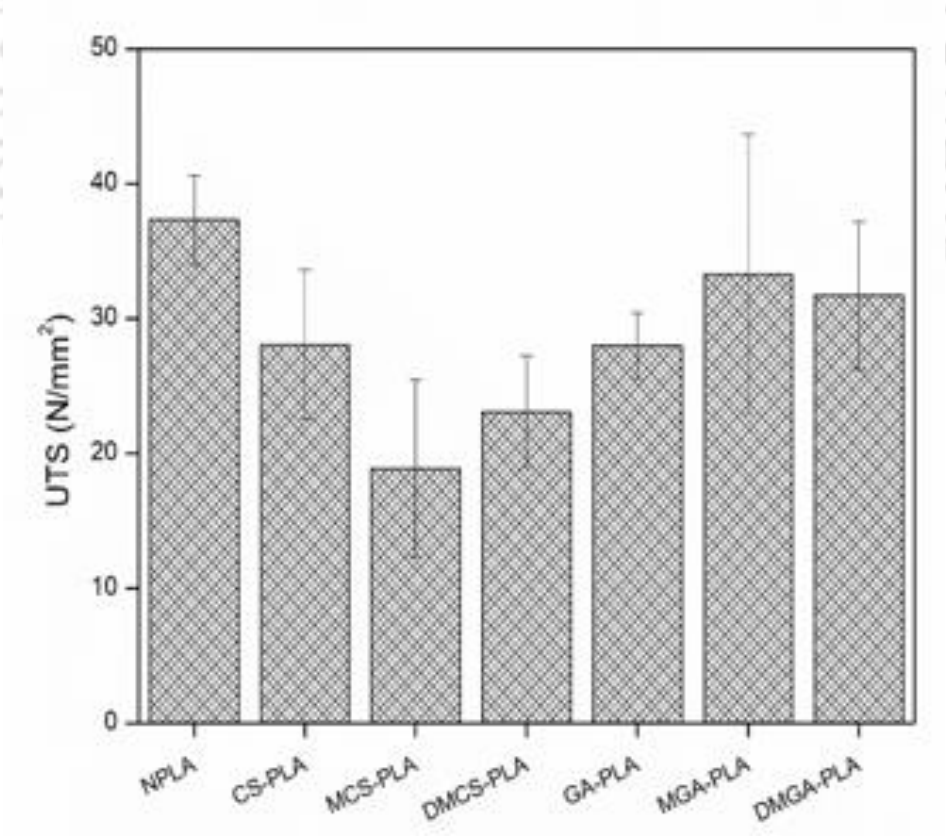


(b)

Figure 6.8 *Mechanical properties of PLA based blown films with different compitibilizers and cross linking agent representing (a) % Elongation, (b) UTS (N/mm²).*

6.2.6 PLA Biocomposite based Blown Films: Thermomechanical Properties

The effect of storage modulus due to the effect of used biofillers are studied over a temperature region of 25-120 °C for 3.0 K/min under tension (5×0.166×5). The E' is found to be increased and greatly influenced by biofillers due to the transfer of load, and formation of cross-links through the use of DCP, which provides better interfacial properties between the polymer matrix and biofillers as shown in **Figure 6.9**. The E' of CS-PLA, MCS-PLA, DMCS-PLA, GA-PLA, MGA-PLA, and DMGA-PLA are improved by ~56 %, 17 %, 43%, 63 %, 25 %, and 26 %, respectively. The loadings of various classes of biofillers in PLA matrix has improved the storage modulus by various magnitudes. Further, significant improvement in storage modulus is observed in case of DMCS-PLA followed by MGA-PLA. Interestingly, alternate effects of DCP on fillers have been observed. Involvement of DCP has found to improve the storage modulus of MCS-PLA, wherein, it provides the inverse effect to DMGA-PLA reducing its storage modulus in comparison to modified one. For a better understanding of the effect of biofillers on thermomechanical properties of PLA, another parameter i.e. filler effectiveness coefficients are determined using the equation (6.1) as described below. It is reported that higher value of effectiveness coefficient 'C', lesser is the effect of biofillers on the PLA matrix and vice versa.

$$C_{FE=} \left(\frac{E_g'}{E_r'}\right)_{Composite} \div \left(\frac{E_g'}{E_r'}\right)_{Matrix} \quad \text{.....6.1}$$

Where E'_g and E'_r are the storage modulus for PLA at their glass transition state (46 °C) and rubbery state (85 °C) for a frequency of 1 Hz. The observed result shows that C-factor of CS-PLA, MCS-PLA, DMCS-PLA, GA-PLA, MGA-PLA, and DMGA-PLA are 1.6, 1.1, 1.9, 1.8, 1.9, and 1.8 respectively, which proves the comparable effectiveness of functionalized CS property over other biofiller materials. Therefore, from this can be concluded that effect of fillers on storage modulus can be modified with the proper use of filler materials.

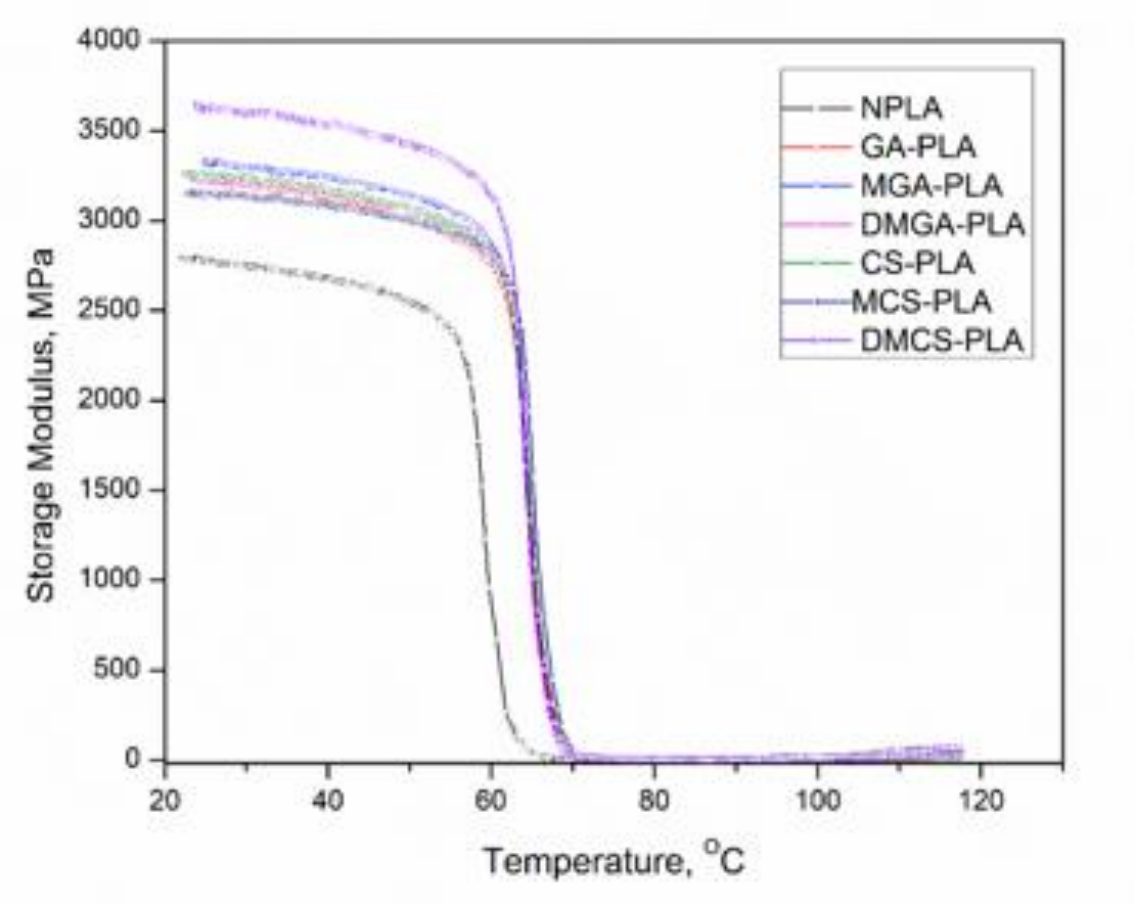
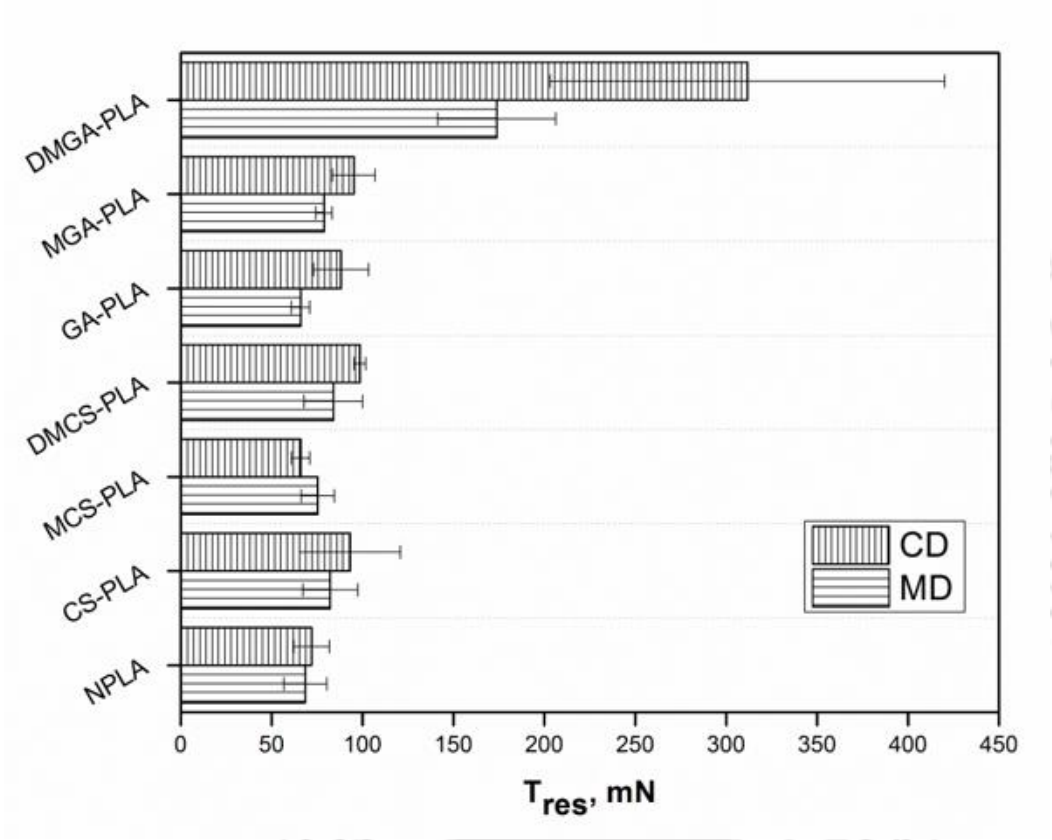


Figure 6.9 *Thermomechanical behavior of blown films of PLA and its biocomposites at constant frequency of 1 Hz over a temperature range of 25-120 °C for 3.0 K/min.*

The tear testing of developed blown films shows the improvement of tear resistance of biocomposites of PLA blown films in comparison to NPLA films. The result shows that the tear resistance (towards MD) of CS-PLA, MCS-PLA, DMCS-PLA, MGA-PLA, and DMGA-PLA are improved by ~16.7 %, 9.1 %, 18.4%, 13.1 %, and 60.6 %, respectively. In adverse, the tear resistance (in the machine direction) of GA-PLA is observed to be reduced by ~3.95 %, showing the opposite effect on PLA matrix. Further, towards CD, the tear resistance is

improved by ~22.7, 27, 18.4, 24.4, and 76.9% for CS-PLA, DMCS-PLA, GA-PLA, MGA-PLA, and DMGA-PLA, respectively. For, MCS-PLS, the tear resistance is decreased by ~9.1% in the CD. Moreover, the effect of tear factor and tear index is represented in **Figure 6.10**. Therefore, a good statement can be made that the materials capability to susceptible tearing resistance is well improved through the incorporation of used biofillers.



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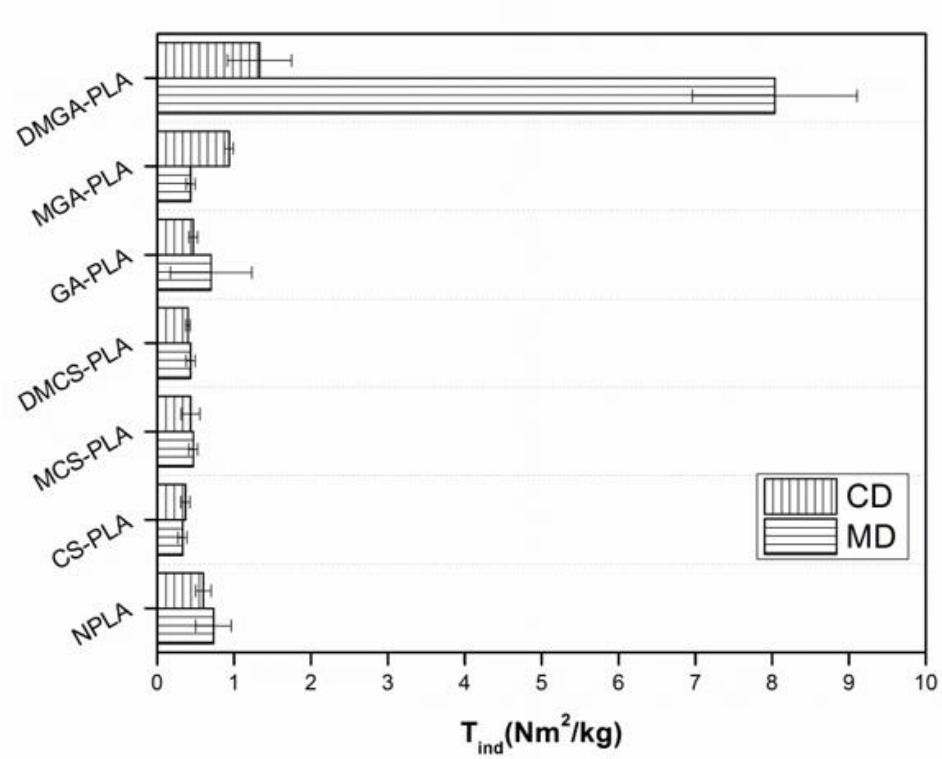

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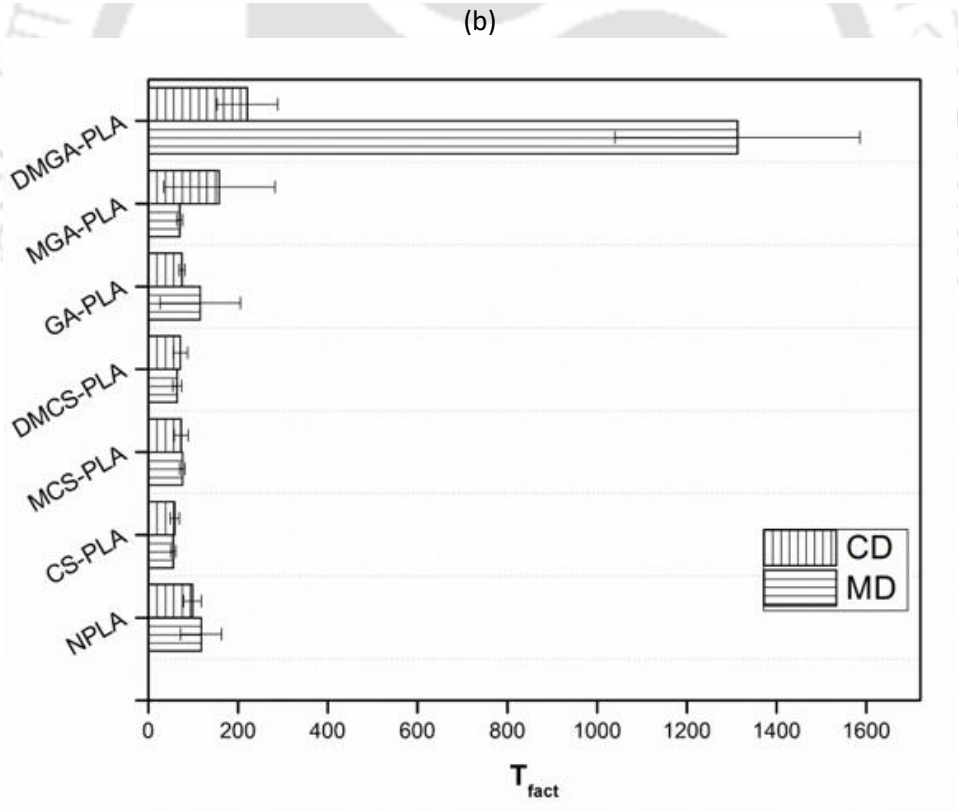


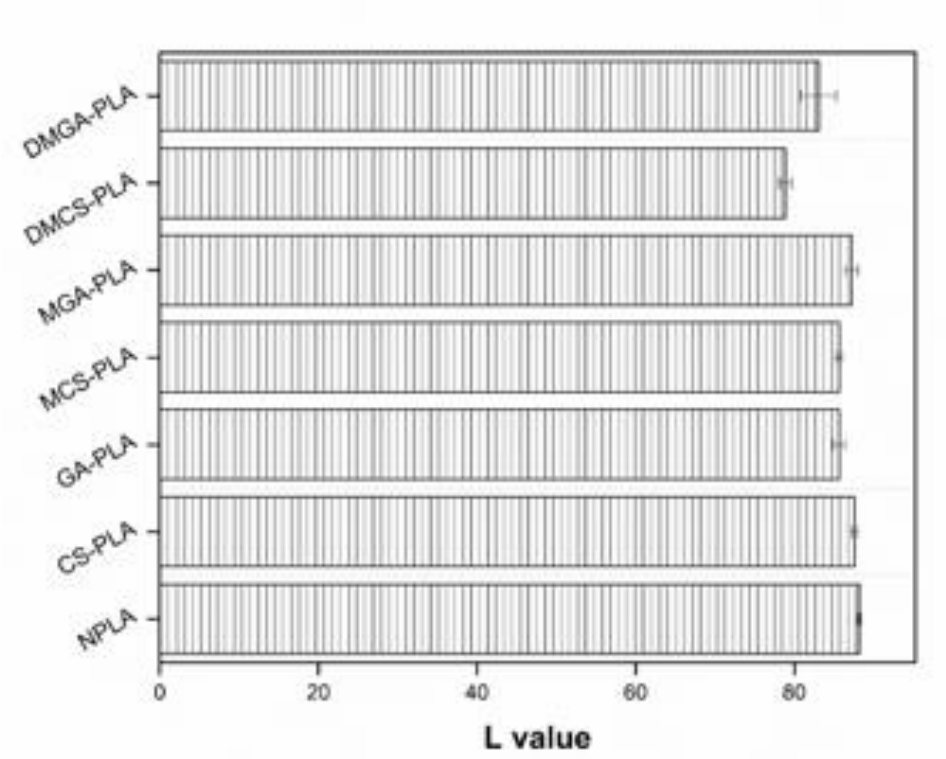
(c)

Figure 6.10 *Tearing strength testing of PLA and its biocomposites representing (a) Tear resistance, (b) Tear index, and (c) Tear factor.*

PLA Biocomposite based Blown films: Color determination

The color scale factors (L, a*, b*, hue angle, and Chroma) of packaging films is an indispensable fact used for better understanding the coloring effects of reinforcements on matrix material (PLA) in preparing polymer composites. In addition, optical properties are a significant aspect to be recognized by end users (Ahmadzadeh et al., 2016), which can be better explained by color factors. In this regards, a study on coloring effects of filler materials on developed blown films has been carried out, where the parameters L, a*, b* are calculated as shown in **Figure 6.11**. The term 'L' defines the brightness (50-100) and darkness (50-100) of the targeted sample, where NPLA has better brightness as compared to other packaging materials followed by CS-PLA, GA-PLA, MCS-PLA, DMCS-PLA, DMGA-PLA, and MGA-PLA. The decrease in L value in DMGA-PLA (0.5% MGA) and DMCS-PLA (0.5% MCS) in comparison to MGA-PLA (1% MGA) and MCS-PLA (1% MCS) may be due to the greater crosslinking effect for DCP in the polymer matrix with filler material making dark coloring effect in polymers reducing whiteness. Further, the term a* indicates about the red (+a*) vs. green (-a*) color, where the observed values for a* shows that all the sample has red colorants effects. The experimented values depict that MGA-PLA has highest red coloring effects followed by DMGA-PLA, MCS-PLA, DMCS-PLA, NPLA, GA-PLA, and CS-PLA. As noticed, both MGA and MCS increase a* value due to their dark brown color which imparts red coloring effect to the developed packaging material. In adverse, the term b* indicates the existence of yellow and blue colorants in the sample, where positive values indicate its yellow color and negative values indicate its blue color effects. The observed values show that all samples have yellow color effects and among all MGA-PLA has more yellow color followed by DMGA-PLA, MCS-PLA, DMCS-PLA, CS-PLA, GA-PLA, and NPLA, which may be related to their dispersibility and instrument temperature during the analysis. Moreover, the detection of more yellow coloration in composites of MGA-PLA and MCS-PLA may be due

to the uniform and more filler loading, filler temperature, etc. (Ahmadzadeh et al., 2016). Moreover, hue, chroma and % reflectance (520-570 nm) have been determined as shown in **Table 6.5**. Further, hue angle is an additional factor that defined a comparable effect from other colorants present (red, green, blue and yellow). Different hue angle specifies different coloring effects such as hue angle of 0°, 90°, 120°, and 240° defines the coloring effects of red, yellow, green and blue, respectively. In respect to this, the developed blown films have yellow coloring effects. Further, chroma value defines the color intensity of developed blown films, considering that the color intensity of MGA-PLA is highest followed by DMGA-PLA, DMCS-PLA, MCS-PLA, CS-PLA, GA-PLA, and NPLA films. Another term, % reflectance displays the effect of fillers how it affects the light reflection at the wavelength 520-570 nm range when falls on film. The % reflectance of MGA-PLA is found to be the lowest, where NPLA had the highest % reflectance. Therefore, it can be concluded that filler materials, their modifications, and addition of cross-linking agent significantly affect reflectance, which can be a considerable tool for tuning the polymer materials. Interestingly, incorporation of fillers to the PLA had a great effectiveness towards color parameters 'L', 'a*, and 'b*', which in turn greatly influences the consumer acceptance for acting as a proper packaging material.



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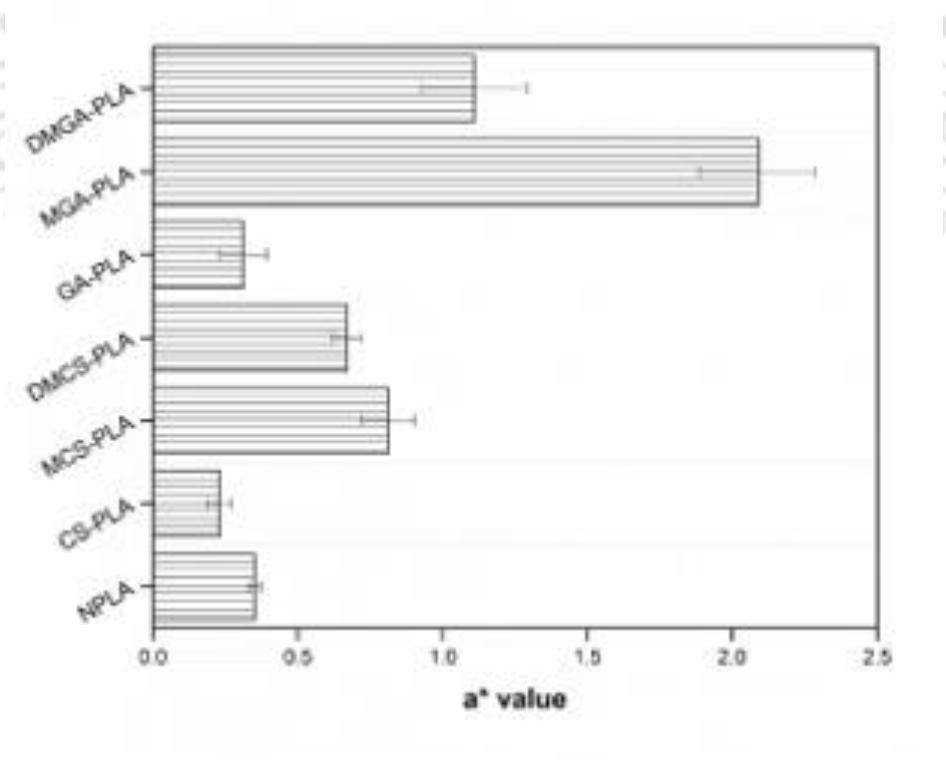

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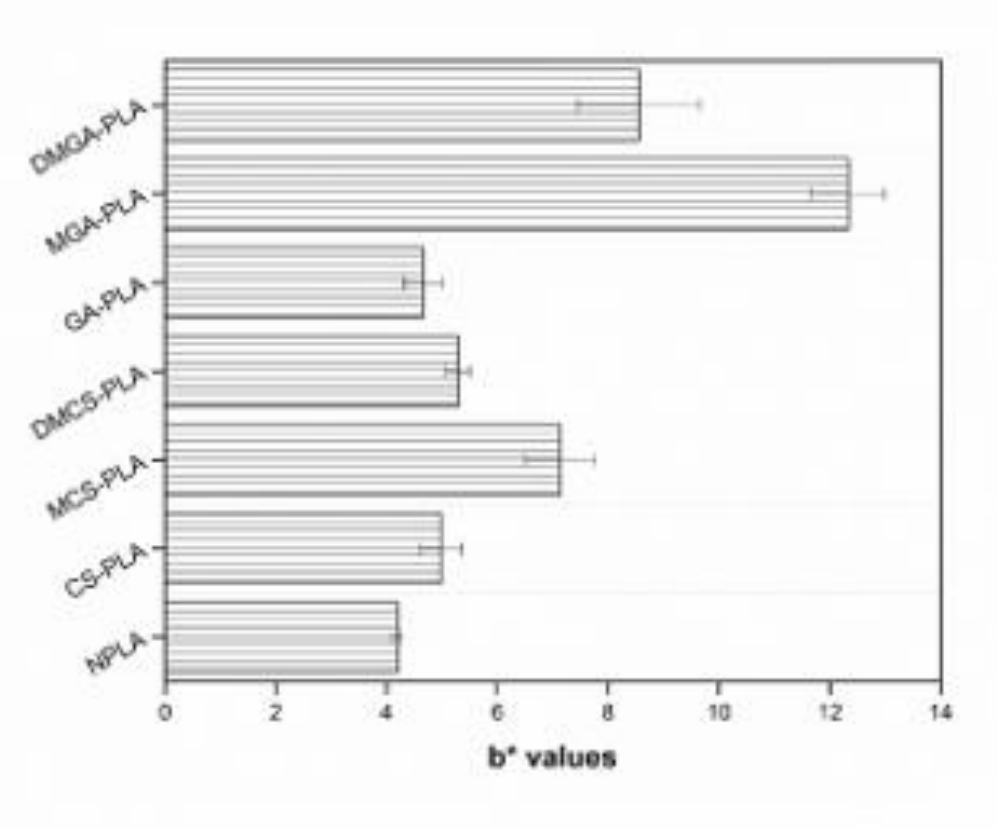

(c)

Figure 6.11 *Color parameters L, a*, b* of PLA and its biocomposites*

Table 6.5 *Hue, Chroma and % reflectance (520-570 nm) of PLA and its biocomposites.*

Sample	Hue (^{O})	Chroma	%Reflectance (520-570 nm)
NPLA	85.27±0.22	4.20±0.08	72.25±0.22
CS-PLA	87.31±0.66	4.98±0.36	70.32±0.59
GA-PLA	86.18±0.76	4.66±0.35	67.89±0.50
MCS-PLA	83.52±0.21	7.18±0.64	67.03±0.80
MGA-PLA	80.38±0.41	12.50±0.70	51.21±1.16
DMCS-PLA	82.78±0.33	7.92±0.37	66.01±0.60
DMGA-PLA	82.60±0.41	8.62±1.11	64.71±0.81

The haziness of particular packaging films is mainly related to the reflecting surface of packaging films caused by irregular surface and affected by various factors such as dispersibility, filler type and compatibility of materials (Sothornvit, Rhim, & Hong, 2009). The haze value of developed blown films shows that application of filler materials has increased the haziness, whereas functionalized fillers are shown to have reduced haziness in comparison to the other non-functionalized and DCP incorporated filler materials (**Figure 6.12**). On the basis of this discussion, the functionalized CS and GA has a better capacity to formulate a uniform surface in comparison with other filler material. Interestingly, NPLA has better haziness property due to better regularities in comparison to other film materials.

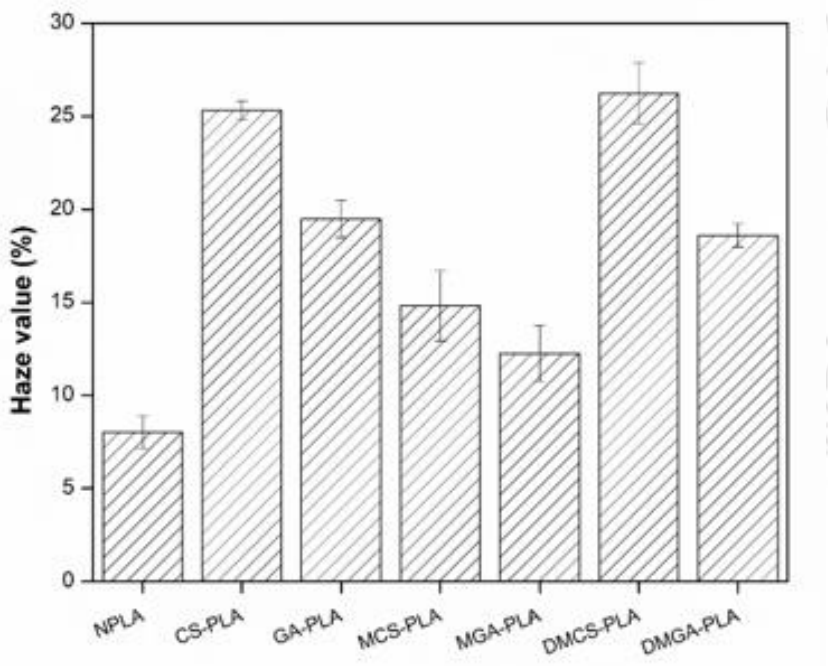

Figure 6.12 *Haze value of developed PLA blown films and its composites.*

6.2.7 PLA Biocomposite based Blown films: Migration Property

The overall migration analysis is carried out for developed PLA based biocomposites using three simulants 50 % ethanol (v/v), 3% acetic acid (v/v), and distilled water for incubation temperature of 40 °C for 10 days in the need of measuring proportions of fabricated composites

that may migrate into the food simulants are represented in **Table 6.6**. It is obvious that various filler materials will show a various rate of migration with PLA film due to different loading and interactions. The overall migration value for NPLA is found to be the lowest among all the blown films. Further, the incorporation of filler in PLA films affects migration property and the migration limit for all the biocomposites of PLA is found to be within the acceptable limit (10 mg/dm^2) according to the legislation European Commission 2007 (Souza, & Fernando, 2016; Schmidt et al., 2009). Interestingly, CS-PLA films show the highest migration in the selected simulants in comparison to others. The functionalized CS and GA had less migration due to their better compatibility in comparison to non-functionalized filler material, as fabrication of modified GA and CS provide hydrophobicity in comparison to neat filler one, so provide less migration compared with others. Among all fillers used, the non-functionalized filler (CS and GA) have more overall migration in all the three simulants. This gives the confirmation that making fillers compatibility with matrix material could reduce migration value. Therefore, the present investigation for developing blown films from biobased polymers taking PLA polymer reinforcing modified (MCS, MGA) and unmodified biofillers (CS, GA) with permissible limit of cross-linking agent (dicumyl peroxide) provides a packaging material with low migration ratio, which will not harm food material packed within it with higher rate of migration rate. Thus the experiment successfully provides an industrial approach for developing packaging film with permissible migration value.

Table 6.6 *Overall Migration of PLA and its biocomposites in three various food simulants: 50 % ethanol (v/v), 3% acetic acid (v/v), and distilled water.*

Sample	Total migration for 50 % ethanol (v/v) (mg dm^{-2})	Total migration for 3% acetic acid (v/v) (mg dm^{-2})	Total migration for distilled water (mg dm^{-2})
NPLA	2.3±0.3	2.5±0.1	2.2±0.4
CS-PLA	7.3±0.7	8.2±0.3	7.0±0.3
GA-PLA	6.2±0.7	6.5±0.5	5.8±0.5
MCS-PLA	4.5±0.1	4.8±0.7	3.3±0.7
MGA-PLA	4.3±0.7	5.1±0.2	3.9±0.8
DMCS-PLA	5.5±1.2	5.8±0.6	4.9±0.5
DMGA-PLA	6.3±0.9	7.3±0.5	5.4±0.8

6.3 Conclusions

PLA and its various biocomposites are developed using film blowing procedure, where it is found to have a remarkable effect of filler onto the properties of PLA. The adopted process could help in preparing packaging materials for apposite storage of food products for improving shelf life. Further, film blowing of PLA and its biocomposite can be commercialized in terms of transporting goods to avoid mechanical damage and thermal damage. Further, the biodegradable packages provide limited migration property, transparency and improved color properties which helps in consumer acceptance. In this regards, the developed film blowing materials have an ability to replace fossil-based polymer materials for storing perishable food commodities. In this regard, the developed blown film materials can be used to replace the existing conventional packaging materials, where the biodegradability of plastic can be

improved under optimized composting conditions, and further, the thermophilic composting condition may fasten the biodegradation process. The efficient biodegradation of PLA can also be obtained *via* selecting the proper PLA-degrading microorganisms. The use of some biofiller materials may also stimulate the enzyme productions which are responsible for the biodegradation of PLA based composites.

CHAPTER 7

Conclusions and Future Prospects

This chapter summarizes the key findings of the current research work and further provides the future perspectives of the edible nanocoating studies along with other packaging systems.

7.1 Conclusions

This chapter provides findings of the research work and highlights the fundamental issues alongwith future scope of the current work. The main objective of the work is to develop functionalized biopolymeric nanocomposite as a potential candidate to develop edible nanocoating on food products and its sustainable secondary packaging. The innovations and applications of developed functionalized biopolymeric nanocomposite based edible nanocoating in the current thesis work include: (i) fabrication of curcumin doped iron functionalized cellulose nanofiber dispersed edible chitosan based nanocoating on cut pineapple and whole kiwifruits; (ii) fabrication of nanochitosan modified starch and guar gum based functionalized nanocomposite as a candidate of edible nanocoating on cut apple fruits; (iii) fabrication of silk nanodisc dispersed edible chitosan coating on banana for improved product life; and (iv) finally, fabrication of poly lactic acid biocomposite based secondary packaging for coated food products. In this regards, the thesis has addressed the use of emerging nanomaterials as a component in modifying the inherent characteristics features of existing biopolymers for edible coating applications. The developed edible nanocoating materials are noteworthy in respect to improved storage life of food products. The main outcomes in this thesis work are: Firstly, iron fortified ready to eat cut pineapple with anticancer activity and further improved shelf life the tested products, where, the system provides

significant medicinal properties with improved shelf life; Secondly, a simple and easy processable edible nanocoating system consisting starch, guar gum and nanochitosan having hydrophobic, highly thermal stable, antimicrobial active has been done; Thirdly, protein resources (from waste muga cocoons) based edible coating with a strategy to convert waste to wealth has been done; Fourthly, development of sustainable secondary packaging for coated food products for safe delivery of food products has been done. In this context, the conclusions drawn from the thesis work are summarized in the next section as follows.

- The fabrication of mgCNF following a single step co-precipitation technique is a way to adsorb iron particles on CNF and is used as a reinforcement in chitosan (CS) biocomposites. Additionally, the formulation of curcumin (Cur) loaded mgCNF/CS nanocomposite as edible coating on cut pineapple delivers functionalized nanocomposite with anti-cancerous and iron-fortified ready to eat pineapple fruit products. The edible nanocoatings are applied on kiwifruits and are analysed during the storage period (10 days and 10 °C).
- A simple edible coating system for perishable fruit products. NCS is another polysaccharide nanostructure fabricated following ionic gelation method and are used in modifying starch (ST) and guar gum (GG) based edible coatings. The inclusion of NCS (a food based nanomodifier) in ST-GG biocomposites improve the surface, thermal, optical, antimicrobial, mechanical, and other properties. The development of edible nanocoating on cut apple using NCS modified ST-GG biocomposites significantly improved the food properties during storage life.
- The formulation of silk nanodisc (SND) dispersed edible CS coating for bananas acts as a candidate with superior thermal, hydrophobic, optical, mechanical and food properties. It is noteworthy to mention that SND dispersed CS edible coatings provide an improved texture of banana fruits. Thus, the current thesis work addresses the use of

functionalized biopolymeric nanocomposite as an edible coating to improve the storage of perishable fruits.

- In line with this, the development of industrially viable secondary packaging for edible coated foods providing an in-depth understanding of modified PLA blown films from the aspects of thermomechanical, crystallinity, thermal stability, migration, wettability and others.

7.2 Future Directives of the Research

The current thesis work can be moved further with the listed future prospective for improved shelf life of food products:

- The scale up production of developed functionalized biopolymeric nanocomposite to be used as edible coatings on food products. The optimization of the processing parameters for large scale development with the life cycle assessment study of the food products.
- Application of edible coating on food products applying different other edible coating method for large production such as brushing, spray coating and others. The study on the cost effectiveness with their shelf life prediction study.
- Application of developed edible coating on various food products including vegetables, dried foods, fried foods and others to check the effectivity of the coating materials
- The in-detail shelf life study of coated food products packed in secondary packaging materials. The study includes the changes in physicochemical properties, weight loss, firmness and other properties.
- Application of different packaging technology such as modified atmospheric packaging, controlled atmospheric packaging, smart packaging and others for coated food products packed in secondary packaging materials.
- The study on effectiveness of stored food products during transportation with primary and secondary packaging such as mechanical damages due to vibration and others.
- Development of tertiary packaging system for exportation of coated food products.

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Research Outputs

Patents:

1. Vimal Katiyar and Tabli Ghosh, "Method for producing starch and guar gum biocomposite based edible packaging" (*In process*).

Publications

From Thesis:

1. **Ghosh, T.,** Teramoto, Y., & Katiyar, V. (2019). Influence of Non-Toxic Magnetic Cellulose Nanofibers on Chitosan based Edible Nanocoating: A Candidate for Improved Mechanical, Thermal, Optical, and Texture Properties. ACS Journal of agricultural and food chemistry, 67(15), 4289-4299. (**IF: 4.192**)
2. **Ghosh, T**., Bhasney, S. M., & Katiyar, V. (2020). Blown films fabrication of poly lactic acid based biocomposites: Thermomechanical and migration studies. Materials Today Communications, 22, 100737. (**IF: 2.678)**
3. **Ghosh, T.,** Nakano, K., Mulchandani, N., & Katiyar, V. (2021). Curcumin loaded iron functionalized biopolymeric nanofibre reinforced edible nanocoatings for improved shelf life of cut pineapples. Food Packaging and Shelf life (Accpeted). (**IF: 4.244**)
4. **Ghosh, T.,** Nakano, K., & Katiyar, V. (2021). Curcumin doped Functionalized Cellulose Nanofibers based Edible Chitosan Coating on Kiwifruits. Carbohydrate Polymers (Under revision).
5. **Ghosh, T.,** Mondal, K., Giri, B. S., & Katiyar, V. (2021). Silk Nanodisc Based Edible Chitosan Nanocomposite Coating for Fresh Produces: A Candidate with Superior Thermal, Hydrophobic, Optical, Mechanical and Food Properties. Food Chemistry (Under Review).
6. **Ghosh, T.,** Mondal, K., Giri, B. S., & Katiyar, V. (2021). Nanochitosan Incorporated Starch/Guar gum Biocomposites Based Edible Coating: A Potential Candidate with Hydrophobic, Transparent, Thermally stable, Ductile Materials for Fresh cut Apple. (Ready for submission)

Other:

1. Borkotoky, S. S., **Ghosh, T.,** Bhagabati, P., & Katiyar, V. (2019). Poly (lactic acid)/modified gum arabic (MG) based microcellular composite foam: Effect of MG on foam properties, thermal and crystallization behavior. International journal of

biological macromolecules, 125, 159-170. (**IF: 5.162**)

2. Borkotoky, S. S., **Ghosh, T.,** Patwa, R., & Katiyar, V. (2019). Silk Nanocrystal (SNC) Reinforced Poly (lactic acid) based Microcellular Foam: Impact on Porous Structure, Crystallinity, Thermomechanical and Surface Property. Materials Today Communications. (Accepted).
3. Borkotoky, S. S., **Ghosh, T.,** and Katiyar, V. *"Hydrolytic and UV irradiated Degradation of Modified Gum Arabic (MG) Reinforced Poly lactic acid Based Microcellular Nanocomposite Foams."* (Ready for submission)
4. Borkotoky, S. S., **Ghosh, T.,** and Katiyar, V. *"Degradation studies of PLA and PLA/SNC based microcellular nanocomposite foams."* (Ready for submission)

Books

1. Vimal Katiyar, Raghvendra Gupta, **Tabli Ghosh** (2019) "Advances in Sustainable Polymers: Processing and Application", in the series Materials Horizons: From Nature to Nanomaterials by Springer Nature, ISBN: 978-981-329-804-0. (Edited Book)
2. Vimal Katiyar, and **Tabli Ghosh** (2020) "Nanotechnology in Edible Food Packaging: Food Preservation Practices for a Sustainable Future" in the series Materials Horizons: From Nature to Nanomaterials by Springer Nature, ISBN 978-981-336-169-0. (Authored book)

Book Chapters

1. **Ghosh, T.,** & Katiyar, V. 2019 book chapter titled as 'Chitosan Based Edible Coating: A Customize Practice for Food Protection' in the book titled as "Advances in Sustainable Polymers: Processing and Application" under in the series Materials Horizons: From Nature to Nanomaterials by Springer Nature, ISBN: 978-981-329-804-0.
2. **Ghosh, T.,** Borkotoky S. S., & Katiyar, V. 2019 book chapter titled as 'Green Composites Based on Aliphatic and Aromatic Polyester: Opportunities and Application' in the book titled as "Advances in Sustainable Polymers: Processing and Application" under in the series Materials Horizons: From Nature to Nanomaterials by Springer Nature.
3. **Ghosh**, T., Dhar, P., & Katiyar, V. (2020) book chapter entitled "Nanocellulose: Extraction and Fabrication Methodologies" in book entitled "Cellulose Nanocrystals:

An Emerging Nanocellulose for Numerous Chemical Processes" by Walter de Gruyter GmbH. ISBN: 978-3110644524

4. **Ghosh**, T., Dhar, P., & Katiyar, V. (2020) book chapter entitled “Nanocellulose for Food Packaging Applications” in book entitled "Cellulose Nanocrystals: An Emerging Nanocellulose for Numerous Chemical Processes" by Walter de Gruyter GmbH. ISBN: 978-3110644524
5. **Ghosh**, T., Monika, Katiyar, V., (2020) Emerging Sustainable Nanostructured Materials Facilitated by Herbal Bioactive Agents for Edible Food Packaging. In *Nano-food Engineering* (pp. 259-285). Springer, Cham.
6. **Ghosh, T.,** Mondal K., & Katiyar, V. (2020) Current Prospects of Bio-Based Nanostructured Materials in Food Safety and Preservation. In book *Food Product Optimization for Quality and Safety Control: Process, Monitoring, and Standards*, 111.
7. **Ghosh, T.,** & Katiyar, V. (2021) book chapter entitled “Edible Food Packaging: An Introduction” in book entitled "Nanotechnology in Edible Food Packaging" (Under production).
8. **Ghosh, T.,** Das, D., & Katiyar, V. (2021) book chapter entitled “Edible Food Packaging: Targeted Biomaterials and Synthesis Strategies” in book entitled "Nanotechnology in Edible Food Packaging"
9. **Ghosh**, T., Hazarika, D., & Katiyar, V. (2021) book chapter entitled “Cellulose based Nanostructured Materials in Edible Food Packaging” in book entitled "Nanotechnology in Edible Food Packaging" (Under production)
10. **Ghosh, T.,** Mondal K., & Katiyar, V. (2021) book chapter entitled “Chitosan based Nanostructured Materials in Edible Food Packaging” in book entitled "Nanotechnology in Edible Food Packaging" (Under production)
11. **Ghosh, T.,** Das, M., Katiyar, V., (2021) book chapter entitled “Starch based Nanostructured Materials in Edible Food Packaging” in book entitled "Nanotechnology in Edible Food Packaging" (Under production)

Awards/Recognization:

1. **DST INSPIRE Fellowship**, 2016 for perusing Doctoral degree in the Department of Chemical Engineering, Indian Institute of Technology Guwahati.
2. Tabli Ghosh has received **Best Poster Award** based on the title “Development of Non-toxic Magnetic Cellulose Nanofiber Dispersed Chitosan Based Edible Nanocoating”

during POLY-CHAR 2019 (Polymers for Sustainable Global Development), May 19-23, 2019 in Kathmandu, Nepal.

3. Tabli Ghosh has received **Best Poster Award** based on the title "Tailor-made Functionalized Chitosan Dispersed Poly Lactic Acid based Blown Films: An Industrially Viable, Biodegradable and Flexible Food Packaging Material" during 27th Indian Convention of Food Scientists and Technologists (ICFoST), January 30 to February 1st, 2020.
4. A **special research student** at the **United Graduate School of Agricultural Science, Gifu University, Japan** from 1st October, 2017 to 31st March, 2018 in Gifu University, Gifu, Japan.
5. A **special research student** at the **United Graduate School of Agricultural Science, Gifu University, Japan** from 1st October, 2018 to 31st March, 2019 in Gifu University, Gifu, Japan.

Conference Presentations

1. Attended a seminar on "special lecture on agricultural II" from 15th November, 2017 to 17th November, 2017 at the United Graduate School of Agricultural Science, Gifu University, Japan.
2. Attended a seminar on research activity and convention on biological diversity and its protocols from 24th January 2018 to 25th January 2018 at the United Graduate School of Agricultural Science, Gifu University, Japan.
3. **Tabli Ghosh**, Yoshikuni Teramoto, Kohei Nakano and Vimal Katiyar, "Chitosan/Magnetic Cellulose Nanofiber Based Edible Nano-Coating Facilitated By Bioactive Compounds for Kiwi fruit products", International Symposium on Sustainable Polymers & National Symposium on Chemistry Education for Sustainable Engineering, IIT Guwahati, India [2019]
4. **Tabli Ghosh**, Yoshikuni Teramoto, and Vimal Katiyar, "Development of Non-toxic Magnetic Cellulose Nanofiber Dispersed Chitosan Based Edible Nanocoating", POLY-CHAR 2019 (Polymers for Sustainable Global Development), Kathmandu, Nepal [2019]
5. **Tabli Ghosh**, Akhilesh Kumar Pal, Siddharth M. Bhasney, Vimal Katiyar, "Blown films of Poly lactic acid: Characterization and property", Abstract no. FPC-123, SPSI-Macro-2018 [2018]

6. **Tabli Ghosh**, Akhilesh Kumar Pal, Siddharth Mohan Bhasney, Vimal Katiyar, "A Development of blown films of Poly lactic acid and its biocomposites: Its influence on color and migration properties", Fourth International Symposium on Advances in Sustainable Polymers, ASP-17 [2018]
7. **Tabli Ghosh** and Vimal Katiyar, Curcumin aided Functionalized Cellulose Nanofibre Based Bioactive Edible coating, Japan Rubber Association's 31st Elastomer Debate, 2020.

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Article

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Influence of Nontoxic Magnetic Cellulose Nanofibers on Chitosan Based Edible Nanocoating: A Candidate for Improved Mechanical, Thermal, Optical, and Texture Properties

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Supporting Information

ABSTRACT: The present work demonstrates the formulation of cellulose nanofiber (CNF) or magnetic cellulose nanofiber (mgCNF) dispersed chitosan-based edible nanocoating with superior mechanical, thermal, optical, and texture properties. The fabrication of mgCNF is successfully achieved through a single-step coprecipitation route, where iron particles get adsorbed onto CNF. The thermal stability of mgCNF is improved considerably, where ∼17% reduction in weight is observed, whereas CNF degrades completely under identical conditions. TGA analysis shows that there is an improvement in thermal stability for both CNF- and mgCNF-reinforced CS nanocoatings, where mgCNF provides more heat dimensional stability than CNF-dispersed CS nanocoatings. Further, the edible nanocoatings are stable even at the temperature of heat treatment such as food sterilization. The mechanical property of the mgCNF-dispersed chitosan (CS) shows a remarkable improvement in tensile strength (57.86 ± 14 MPa) and Young's modulus (2348.52 ± 276 MPa) in comparison to neat CS (6.27 ± 0.7 and 462.36 ± 64 MPa, respectively). To determine the developed materials to be safe for food, the quantification of iron is made by using ICP-MS technique. It is worth mentioning that mgCNF-coated CS helps in improving the texture of cut pineapples in comparison with uncoated pineapple slices under ambient conditions.

KEYWORDS: *cellulose nanofiber, magnetic cellulose nanofibers, chitosan, edible nanocoating, packaging property*

INTRODUCTION

Cellulose nanofibers (CNFs) have been systematically investigated biomaterials due to their unique features of higher aspect ratio, better mechanical properties and surface properties, etc. in various fields of life including biomedicine and food packaging.[1,2] Interestingly, hydroxyl groups on the surface of cellulose act as reducing and nucleating agents for metal nanoparticles (NPs) such as Fe, Ag, Co, Cu, Ni, etc.[3] Generally, CNF-adsorbed metal particles provide improved properties in terms of mechanical, thermal, and medicinal properties with various health-promoting factors for treating diseases. Among the available metal particles, magnetite acts as a medicinal herb and is also used in the preparation of Chinese medicative diets,[4] providing immense health-promoting factors. Further, in China, an Erlong Zuoci decoction (ELZCD) consisting of magnetite as one of the herbs is used for the treatment of age-related hearing loss.[5] Iron NPs having low toxicity and low cost can be coated with polyethylene glycol and perindopril for treating hypertension.[6,7] Iron oxide nanoparticles with *Argemone mexicana* L. leaf extract provide better antibacterial activity and further improve the properties for drug delivery.[8] Additionally, iron oxide based nanomagnets have attracted interest due to the internal properties, conjugation ability with bioactive molecules, colloidal stability, etc.[9] Magnetite has various medicinal properties such as (1) magnetite with white quartz and rice soup are used for treating asthenia and (2) wine with powdered magnetite is used for treating impotence. Magnetic NPs with the required modifications can be used in the treatment of hyperthermia, drug delivery, tissue repair, cell repair, etc. In addition, the use of metallic nanoparticles and polysaccharide nanoparticles together impart a property of enhancing the therapeutic index of drugs with enhanced drug efficacy.[10] On the basis of this discussion, magnetic CNF with tunable properties can be utilized for preparing edible nanocoatings on perishable food products to improve product life with the added advantages of suppressing human diseases.

Further, cellulose and its various derivatives, CNF, cellulose nanocrystals, and cellulose whiskers, are widely utilized with other materials to formulate edible coating, targeting perishable food products with improved mechanical, thermal, optical, and texture properties.[11−16] CNF-based edible films such as mango puree reinforced with CNF provide enhanced properties including mechanical, thermal, and barrier for an improved shelf life of food products.[17] Further, CNFs have a beneficial property of forming a network structure due to the presence of functional groups when they are used with chitosan. However, CNFs do not have antimicrobial properties and are prone to microbial degradation. Thus, CNF-based composites with chitosan promise to be beneficial packaging materials, as chitosan has antibacterial and antimicrobial properties which help to reduce the microbial degradation of CNF.[18] Additionally, as discussed, magnetic CNF (mgCNF) has the ability to provide additional medicinal properties and can be chosen to be

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Blown films fabrication of poly lactic acid based biocomposites: Thermomechanical and migration studies

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ABSTRACT

The article addresses the modification of Poly lactic acid (PLA) based blown films using modified biofillers of chitosan (CS), gum Arabic (GA), with a cross-linking agent dicumyl peroxide for improved properties. The FTIR analysis gave the evidence of forming functional biofillers of CS and GA through grafting of oligomer lactic acid (OLLA) with CS and GA, giving a new peak at 1539 and 1755 cm^{-1}, respectively. The used biofillers helped in tailoring PLA properties for developing blown films due to chain interactions, level of compatibility, loading percentage, and others. The formulated biocomposites showed improved properties over blow up ratio, mechanical, thermal, and thermomechanical properties of PLA. Moreover, the migration ratio of fillers in formulated biocomposites was within permissible limit confirming the safe use of packaging material. In this regards, the used biofillers can overcome the shortcomings of PLA, for providing a single step method of developing a flexible food packaging material.

1. Introduction

Biodegradable polymers are a foremost requirement in food packaging industries due to its immense ability in reducing carbon footmark, and waste plastics, which in turn diminishes global warming [1–4]. Generally, biodegradable polymers are derived from both renewable and non-renewable feedstock, and are commonly coined as “end of life”, which can be recycled, biodegraded or get composed into elemental components in nature, resulting in increased earth productivity [5–8]. Mostly used biodegradable polymers include poly lactic acid (PLA), poly glycolic acid (PGA), poly hydroxyl alkanoates (PHA), polycaprolactone (PCL), and their fractions, which are having good packaging properties with the capability in substituting some of existing petrochemical-based packaging materials such as polyethylene (PE), polystyrene (PS), polypropylene (PP), polyethylene terephthalate (PET), etc. [9,10]. In this regard, petrochemical-based plastics such as PE, PS, PP, PET are non-biodegradable which are made of fossil-based materials such as petroleum and natural gasses, which create several environmental problems. However, some of these plastic materials can be derived from biomass-based feedstock and can be categorised as biobased (bio-PE) or partly biobased plastic (bio-PET). In this regard, PET is generally developed using the monomers terephthalic acid and ethylene glycol, where both the monomers are obtained from non-biodegradable materials. On the otherhand, bio-PET is developed from mono ethylene glycol (a biodegradable source) and terephthalic acid. Though biodegradable plastic materials provide many beneficial traits over using conventional plastics, however, these class of plastic materials convey some limitations over some packaging properties in comparison with conventional polymers. In this regards, the properties of the biodegradable polymers in terms of crystallinity, barrier, mechanical, and thermal properties can be tuned by reinforcing biofillers (biocomposites) or blending with other polymers (polymer blends) [10]. However, most of the biopolymers being hydrophilic in nature need to be chemically modified for making them suitable fillers to be well dispersed in hydrophobic polymer matrixes [9,11].

The biodegradable polyester PLA is a chemically derived polymer [12], having comparable mechanical and thermal properties against some of the fossil based polymers such as PS and PET, which gains an extreme enthrallment in the field of food packaging industry, biomedical application for preparing scaffolds for bones in tissue engineering, etc. [9]. PLA is primarily synthesized from lactic acid (2-hydroxy propionic acid) monomer, which is achieving marketable importance day by day due to its renewable carbohydrate based feedstock such as wheat, corn, sweet potato, tapioca starch, agricultural wastes, etc. [9]. Furthermore, PLA is nontoxic, biocompatible, natural, commercially accepted polymer with comparable properties against some of the conventional polymers, which makes it a favorable candidate to be used in the field of food packaging and beverage industries [13–15].

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Curcumin loaded iron functionalized biopolymeric nanofibre reinforced edible nanocoatings for improved shelf life of cut pineapples

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Abstract

The current research investigates the development of curcumin (Cur) loaded chitosan (CS)/iron functionalized cellulose nanofibers (mgCNF) based edible coating for perishable cut fruit, which is a non-toxic eatable packaging with improved anti-cancer, physicochemical, thermal, and food properties. The modification in cellulose nanofibres (CNF) through fabricating mgCNF improved the surface properties of Cur loaded CS materials (suggested by FESEM). Further, the weight percentage of various elements such as carbon, oxygen, iron, and nitrogen was found to be as 54.4, 38.4, 5.2, and 1.9 % (w/w), respectively, in the developed Cur loaded CS/mgCNF (1.5 % (w/w)) based materials (obtained by EDX analysis). Interestingly, the work provides value addition to the perishable cut pineapple fruit in form of eatable packaging materials with the iron content of ~3.6 ppm, which is within permissible limit according to WHO guidelines. It is worth mentioning that the presence of Cur with CS based biocomposites exhibited anti-cancer activity by disrupting the cell membrane of HeLa (cervical cancer) cells with cell viability of ~17 % and ~98 % for CS/mgCNF (1.5 % (w/w)) with and without Cur, respectively. The thermal properties in terms of onset degradation temperatures (10 % weight reduction) for Cur loaded CS, CS/CNF (1 % (w/w)) and CS/mgCNF (1 % (w/w)) were 78.6, 90.8, and 82.3 °C, respectively, which provides an additional approach to use these materials for heat unstable food items. Therefore, the present investigation provides a novel approach towards supplementing the iron functionalized food products with anti-cancer activity and improved performance to reduce food waste.

Keywords: Cellulose nanofibers; Magnetic cellulose nanofibers; Chitosan; Curcumin; Edible coating; Packaging

1 Introduction

In the recent past, fresh-cut fruit products have gained considerable attention for being convenient and high quality ready-to-eat food products (González-Aguilar, Ruiz-Cruz, Cruz-Valenzuela, Rodrıguez-Félix, & Wang, 2004; Issa-Zacharia, Kamitani, Miwa, Muhimbula, & Iwasaki, 2011). Among the available fresh fruit products, pineapple being a type of non-citrus fruit product is widely eaten as fresh-cut forms. The flesh of cut pineapple has a sweet texture and is a rich source of polyphenolic compounds and ascorbic acids (Mantilla, Castell-Perez, Gomes, & Moreira, 2013). However, the quality deterioration in fresh-cut pineapple products is mainly caused by the microbial attack and environmental agents during storage. Specifically, the food spoilage for fresh-cut fruit products takes account of surface discoloration (browning), change in firmness, texture loss, increased respiration rate, which results in a decreased shelf