

**DEVELOPMENT OF BIO-NANOCOMPOSITE FILMS
FROM POTATO STARCH FOR ACTIVE FOOD PACKAGING**

BY

**OMIWOLE, Kehinde Omisade
MEng/SIPET/2018/8053**

**A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL
UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF
ENGINEERING (MEng) IN CHEMICAL ENGINEERING**

OCTOBER, 2023

ABSTRACT

Continuous use of traditional synthetic polymers in food industries, due to affordable cost and desirable properties, imposes great sustainability issues with respect to plastic pollution of the environment and micro-plastic pollution of living cells. Wastes once accumulated in environment are now finding ways back into the human blood streams posing serious health concerns on human wellness and longevity. This has driven diverse innovations in bio-plastics productions. However, most bio-plastics typically have poor barrier, mechanical and thermal properties as compared to fossil fuel-based plastics, making it a challenging task to replace synthetic plastics. In this study, Potato starch nanocomposite films were prepared by solution casting method with cellulose nanofibers as reinforcement agent. Cellulose Nanofiber (CNF) of average diameter 43.23nm was isolated from Oil Palm Empty Fruit Bunches (OPEFBs) by grinding and ultrasonication processes. Poly Vinyl Acid (a synthetic bio-polymer) was added to strengthen the potato starch in 1:4 parts. 20 wt% of silver nanoparticles (AgNPs) was incorporated into the films as an antimicrobial agent while 20 wt% glycerol was added as plasticizer. The effects of the plasticizer, AgNPs and CNF contents on the barrier, mechanical, thermal and antimicrobial properties of the films were investigated. Increasing cellulose nanofibers content up to 7%, tensile strength of the films was improved by 46%. But then, this reduced the rate of transmission of water vapor by 15.6% and elongation at break by 12%. Incorporation of 20 wt% glycerol, however, decreased the tensile strength of the composite films by 14% but increased the film matrix's elongation at break and water vapor transfer rate by 17% and 24% respectively. Silver Nanoparticles as an antimicrobial agent were produced using an aqueous extract of fresh Aloe Vera leaves (varied from 1ml to 5ml concentrations). Higher concentrations of Aloe Vera leaf extract increases the quantity of bio-compounds required to reduce Ag^+ to Ag^0 . UV-Vis spectra confirmed the formation of AgNps whose antimicrobial activity in the films was investigated by the growth of inhibition zones of 8.0 ± 0.5 mm against E.coli and 7.0 ± 0.3 mm against Staphylococcus aureus. Control films without AgNps showed 0 mm zones and the antibiotic Ceftriaxone used as a reference was 14 ± 0.4 mm. Reduction of Ag^+ and capping of silver nanoparticles were caused by phenolic compounds present in Aloe Vera extract as characterized by the 1595 cm^{-1} wavenumber band in the FTIR spectra. The Thermogravimetric analysis of the nanocomposite films showed a gradual loss in weight between 80 - 120 °C thermal load while the weight loss was abrupt between 235 - 345°C thermal load. Abrupt weight loss was more pronounced in the films with increased quantity of CNF. Reduction in thermal stability of the nanocomposite films with increase in CNF quantities contradicts other findings and this is attributable to mechanical method (grinding and ultrasonication) employed to isolate CNF in this work.

TABLE OF CONTENTS

Content	Page
Cover Page	i
Title Page	ii
Declaration	iii
Certification	iv
Dedication	v
Acknowledgments	vi
Abstract	vii
Table of Contents	viii
List of Tables	ix
List of Figures	x
Nomenclature	xi
CHAPTER ONE	
1. INTRODUCTION	1
1.1 Background to the Study	1
1.2 Statement of Research Problem	3
1.3 Aim and Objectives of the Study	4
1.4 Justification of the Research	5
1.5 Scope of the Study	5

CHAPTER TWO

2.0	LITERATURE REVIEW	6
2.1	Food Packaging	6
2.2	Types of Packaging	8
2.3	Packaging Materials	8
2.3.1	Wood	8
2.3.2	Paper	8
2.3.3	Metals	8
2.3.4	Glass	9
2.3.5	Plastics	9
2.3.5.1	<i>Polyethylene (PE)</i>	9
2.3.5.2	<i>Polypropylene (PP)</i>	10
2.3.5.3	<i>Polyvinyl chloride</i>	10
2.3.5.4	<i>Polystyrene</i>	10
2.4	Bio-based Packaging	11
2.5	Edible Packaging	13
2.6	Bio-Based and Edible Packaging Challenges	14
2.7	Methods for Improving Properties of Biopolymer Based Packaging	15
2.7.1	Thermal treatment	16
2.7.2	Chemical modification	17
2.7.3	Irradiation	17
2.8	Nanotechnology in Food Packaging	18

2.9	Nanocomposites for Food Packaging	20
2.10	Bio-Based Reinforcement Agents for Bio-Nanocomposites	22
2.11	Biopolymer Structures	24
2.12	Isolation of Cellulose Nanoparticles	27
2.13	Starch Nanocrystals	29
2.14	Production and Characterization of Bio-Nanocomposites	31
2.15	Wet Processing	32
2.16	Dry Processing	33
2.17	Characterization Techniques	34
2.18	Bio-based Nanocomposite Active packaging	37
2.19	Antimicrobial Activities of Plant Extract	39
2.20	Smart Packaging	42
2.21	Health Impacts of Plastic Packaging	42
2.22	Food Packaging Hazards	43
2.22.1	Biocides	43
2.22.2	Bisphenol A (BPA)	43
2.22.3	Phthalates	44
2.22.4	Polyfluorinated	44
2.23	Ultrasonication	45
2.23.1	Parts of ultrasonicator	45
2.23.2	Working principle of ultrasonicator	45
2.23.3	Methods of sonication	47
2.23.3.1	<i>Direct ultrasonication method</i>	47
2.23.3.2	<i>Indirect ultrasonication method</i>	48

CHAPTER THREE

3.0	MATERIALS AND METHOD	49
3.1	Materials	49
3.2	List of Equipment Required	50
3.3	Preparation of Raw Materials	51
3.3.1	Delignification of OPEFBs	51
3.3.2	Ultrasonication of cellulose nanofibers from OPEFBs	52
3.3.3	Preparation of aloe vera extract	52
3.3.4	Preparation of silver nanoparticles	52
3.4	Preparation of Reinforced Active Nanocomposite Films	52
3.5	Characterizations	53
3.5.1	Characterization of cellulose nanofiber	53
3.5.2	UV-Vis spectrum of green synthesized silver nanoparticles	54
3.5.3.	X-Ray diffraction spectra of the bio-nanocomposite films	54
3.5.4	Infrared spectra (FTIR)	55
3.5.5	Determination of antimicrobial activity	55
3.5.6	Mechanical properties of the bio-nanocomposite films	55
3.5.7	Water barrier property	56
3.5.8	Thermogravimetric analysis	56

CHAPTER FOUR

4.0	RESULTS AND DISCUSSION	57
------------	-------------------------------	-----------

4.1	Scanning Electron Microscopy (SEM) of the Nanofibre	57
4.2	UV-Vis Spectrum of Green Synthesized Silver Nanoparticles (AgNP)	58
4.3	X-Ray Diffraction Spectra of the Bio-nanocomposite Films	60
4.4	Infrared Spectra (FTIR) Analysis of Potato Starch Nanocomposite Film	62
4.5	Determination of Antimicrobial Activity	65
4.6	Mechanical Tests	65
4.7	Water Vapor Transmission Rate (WVTR)	67
4.8	Thermogravimetric Analysis	67
 CHAPTER FIVE		
5.0	CONCLUSION AND RECOMMENDATIONS	69
5.1	Conclusion	69
5.2	Recommendations	70
5.3	Contribution to Knowledge	71
REFERENCES		72

LIST OF TABLES

Table		Page
2.1	Commonly Used Plastics and their Applications in Food Packaging	11
2.2	Items of Food Permitted for Irradiation Dose Under FDA Rules	18
2.3	Nanocomposites Types and Nanoclay Effects on Functional Properties of Biopolymer	22
3.1	Materials and Reagents Used	50
3.2	Required Equipment	51
4.1	Antimicrobial Investigation of Active BNC Films	64

LIST OF FIGURES

Figure		Page
2.1	Different Categories and Functionalities of Packaging Food Materials	7
2.2	Biopolymer Packaging Materials Classifications	12
2.3	Different Nanofillers in Biopolymers Reinforcement	20
2.4	Structural Composition of Lignocellulosic Biomass	25
2.5	Methods in the Production of Nonmaterial from Biomass	28
2.6	Cell Wall Structure of the Lignocellulosic Biomass	29
2.7	Active Scavenging and Releasing Systems in Food	39
2.8	Polystyrene Use in Food Packaging	44
2.9	Working Principle of Ultrasonication	46
2.10	Direct Ultrasonication Set Up	47
2.11	Indirect Ultrasonication Set Up	48
3.1	Oil Palm Empty Fruit Bunch	49
3.2	Process Diagram for Bio-nanocomposite Film Production	53
4.1	SEM Micrograph of Raw OPEBF	57
4.2	SEM Micrograph of Cellulose Nanofiber	58
4.3	AgNPs Green Synthesis (a) AgNO ₃ and (b) AgNPs	59
4.4	UV-Vis Spectra of Synthesized AgNPs at Different Concentrations	60
4.5:	Crystallinity Index Profile of PS/PVA Cellulose Nanocomposite Films	61
4.6	XRD Profiles of PS/PVA Bio-nanocomposite Films	62
4.7	FTIR Spectra of Bio-nanocomposite Films	63

4.8	The Incubation of E.coli , S.aureus with BNC Control and BNC/AgNPs	64
4.9	Tensile Strength of PS/PVA Nanocomposite Films	66
4.10	Elongation at Break of PS/PVA Nanocomposite Films	66
4.11	Water Vapour Transmission Rate of PS/PVA Nanocomposite Films	67
4.12	TGA Curves of Bio-nanocomposite Films with Varied CNF wt%	68

NOMENCLATURE

KGy	Irradiation dose
GPa	Giga Pascal
MPa	Mega Pascal
nm	Nanometer
CrI	Crystallinity index
I ₀₀₂	Biomass crystalline portion
WVTR	Water Vapour Transmission Rate
BNC	Bio-nanocomposite
CNF	Cellulose Nanofibre
OPEFB	Oil Palm Empty Fruit Bunches

CHAPTER ONE

1.0

INTRODUCTION

1.1 Background to the Study

Packaging protects the integrity of food and offers the consumer hygienic security (Wang *et al.*,2015; Tunc *et al.*, 2007). It facilitates handling, transportation, and storage of food materials by shielding them from physical, chemical, and biological harm (El-Wakil *et al.*, 2015). Additionally, it provides crucial details regarding the product's properties, nutritional value, and ingredient composition. Besides, packaging is known to be a global marketing tool because in 2019, the food packaging sector earned a total of about \$839 billion in revenue, and it is predicted that this industry will reach its peak between 2020 and 2027, when its worth would reach \$998 billion. Many decades ago, food industries use synthetic plastic materials due to their more advantageous properties, such as flexibility, safety, versatility, and low cost (Gil-López *et al.*,2019; Hassan *et al.*,2018). Global yearly production of plastics is projected to surpass 900 million tons by 2027 . This is equivalent to trillions of dollars when put in global economic indices. (Gil-López *et al.*,2019).

Over 40% of this is made up of packaging, which might take different shapes in terms packaging materials, composite items, casing material, sheets, bottling wares, cups, bags or scaffolds (Dehnad *et al.*,2014; Gil-López *et al.*,2019). But then, there are significant worries about the economic and environmental sustainability of these materials due to its extensive use in

food packaging. Food and beverage packaging makes up around 85% of the market in recent years, with pharmaceutical products coming in second at 10% (Ojogbo *et al.*,2019). Plastic packaging presently accounts for 6.8 million tons, with paper packaging accounting for 7.6 million tons (Demirtas *et al.*,. 2013). There has been increase in the demand for biodegradable packaging materials as an alternative to synthetic ones from consumers, food processors, environmentalists, and government bodies, particularly for use in short-term packaging and disposable applications (such as fast-food restaurants, disposable cutlery, drinking straws, cups, plates, and utensils) (Makaremi *et al.*,2017). When used, fossil-based polymers end up in landfills, incinerators, and bodies of water. While polymer world has supposedly been generated in water bodies all over the world, green house emissions are released when it is burned. When these synthetic polymers are consumed by aquatic life, they make their way back to human cells through seafood with negative health effects. These non-biodegradable food packaging materials thus pose a major threat to the health of everyone on the planet. In an effort to replace their non-degradable equivalents, numerous investigations on novel bio-materials being deployed in food packaging have been conducted recently. Due to the biodegradability of these materials, biopolymers have been one of the most promising alternatives to be explored and developed into eco-friendly food packaging materials (Fajardo *et al.*,2015). Using biopolymer-based food packaging materials, our environment becomes more sustainable because packaging made of biopolymers can be disposed into bio-waste collections for further composting, leaving behind recyclable organic byproducts like carbon dioxide and water.

Sadly, compared to typical non-biodegradable materials made from petroleum, the use of biopolymers as food packaging materials has disadvantages such as weaker physical, heat, and

water resistant properties. Sequel to this, diverse researches and investigations have been conducted to improve biopolymers' properties. Among these is the application of the bio-nanocomposite idea. According to these researches, bio-nanocomposite is a positive direction when it is desirable to improve mechanical and barrier properties of biopolymers. Biopolymers may have their mechanical and barrier properties improved by the addition of nanofillers including silicate, clay, and titanium dioxide (TiO₂), as well as additional uses as antimicrobial agent, sensing device and oxides scavenger in food packaging (Ojogbo *et al.*,2019).

Bio-nanocomposite films is useful in active food packaging because of its ability to interact with the contents in diverse ways. Beneficial agents like antimicrobials and antioxidants can be part of the composite, besides, removal undesirable components like oxygen or water is another benefits that come with bio-nanocomposite films. The bio-nanocomposite is also useful in smart packaging. This has the potential to identify the features of the contained food like microbes contamination or date of expiration and employ a process that records, transmits data and interpret data on the food's safety with its quality (Ojogbo *et al.*,2019).

1.2 Statement of the Research Problem

The pressing research problem addressed in this thesis revolves around the urgent need to develop and assess bio-nanocomposite films derived from potato starch as a sustainable alternative to conventional polymers for food packaging. The ubiquitous use of petroleum-based polymers in food packaging, while offering essential preservation and convenience benefits, has unleashed a plethora of environmental hazards (Jafari *et al.*,2018). The slow degradation of synthetic polymers after use has raised serious ecological concerns. This problem is being

compounded by rampant littering and inadequate disposal practices leading to contamination of ecosystems, soil and water bodies (Echegoyen *et al.*,2016). Beyond environmental concerns, the potential health risks associated with synthetic polymers have generated attentions. The leaching of chemical additives from packaging materials into food products raises apprehensions about the long-term health consequences of polymer exposure and this necessitates a thorough investigation into alternatives (Fernandes *et al.*, 2019). Additionally, as the global population burgeons, ensuring food security has emerged as a paramount challenge. Active food packaging materials that can extend the shelf life of food products, reduce food waste and enhance food safety are crucial (Jafari *et al.*,2018).

Hence, the central research problem of this thesis is to explore the viability of potato starch-based bio-nanocomposite films as a multifaceted solution. This investigation involves assessing the biopolymers' environmental sustainability, investigating their desirable properties and appraising their efficacy in active food packaging for enhancing food security. Addressing this problem holds the potential to revolutionize food packaging practices by introducing eco-friendly alternatives that minimize environmental hazards, mitigate health risks and contribute to global food security.

1.3 Aim and Objectives of the Study

The aim of this work is to develop biodegradable and reinforced nanocomposite films with desirable properties for effective and active food packaging using potato starch as the biopolymer matrix.

While the objectives of the research work are to:

1. Isolate and characterize Cellulose Nanofiber CNF from Oil Palm Empty Fruit Bunches as a nanofiller and reinforcement agent.
2. Synthesize Silver Nanoparticles as an antimicrobial agent in the film.
3. Formulate and characterize active bio-nanocomposite films from potato starch.
4. Measure the mechanical, thermal and barrier properties of the reinforced films.
5. Conduct bioassay of the anti-microbial activities of the films.

1.4 Justification of the Research

1. Bioplastics are derived from renewable agricultural resources and biomass feedstock which are eco-friendly and sustainable.
2. The bulk polymer matrix used (Potato) is locally available and it offers good film-forming and casting properties due to high amylose content (17% w/w). (Demirtas *et al.*, 2013).
3. Production of Cellulose Nanofiber is facile and OPEFBs as a precursor makes it economically viable.
4. Green synthesis process of AgNPs is eco-friendly, economically viable and sustainable

1.5 Scope of the Study

Active bio-nanocomposite films were produced from potato starch using solution casting method. Cellulose Nanofiber was isolated mechanically. Physical tests conducted were tensile strength, elongation at break, thermogravimetric analysis (TGA) and water barrier property tests. Characterizations conducted were SEM, UV-vis spectrum, XRD and FTIR while bioassays were done to analyze the antimicrobial property of the films.

CHAPTER TWO

2.0

LITERATURE REVIEW

2.1 Food Packaging

Food quality and safety should be guaranteed through food packaging throughout the supply chain. Food packaging's primary purpose is to protect food from pathogenic microbes, chemical contamination, and unfavorable environmental conditions (such as insufficient oxygen gas, carbon (II) oxide, moistures, or photolysis) while in storage and transportation. The costs of energy and materials, recycling, sustainability, and disposability are other functions that are connected (Biji, *et al.*,2015). Therefore, it is important to choose packaging materials carefully in order to achieve the desired qualities like physio-mechanical, good thermal barriers, desirable optical barriers and antibacterial properties.

These are necessitated by the need to achieve desired food shelf-life, acceptable food qualities and to ensure safe standards (El-Wakil *et al.*,2015; Duncan, 2011). Petroleum-based plastics, such as polyethylene terephthalate (PET), polyvinylchloride (PVC), polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyamide, have been the most widely used among the packaging materials in use recently for food packaging. These materials include papers, boards, ceramics, glassware and metallic objects as shown in Fig 2.1. This is mostly because they are widely available, reasonably priced, and have excellent mechanical properties (tensile

strength), barrier to oxygen, carbon dioxide and aesthetic qualities (Denilson *et al.*,2003). By 2025, it is anticipated that yearly plastics output will have surpassed 300 million tons, representing trillions of dollars in worldwide economic gains. More than 40% of it is made up of packaging, such as films, bottle wares and different containers (Denilson *et al.*,2003). But then, the widespread use of the food packaging materials has been the source of significant worries about the sustainability of the economy and environment.

Short-term use of packaging and disposable materials (such as cutleries, drinking materials, plates and utensils etc), have the demand for biodegradable packaging materials in recent years from all major stakeholders in the food industries (Shen *et al.*,2014). The market for compostable and bio-plastic materials is anticipated to reach 900,000 tons globally by 2025 with annual increment of approximately 26% from 2012 to 2020 and approximately 21% from 2020 to 2025 (Moran *et al.*,2013).



Figure 2.1: Different Categories and Functionalities of Packaging Food Materials (Shen *et al.*,2014).

2.2 Types of Packaging

Packaging can be categorized into three groups: main, secondary, and tertiary. The main container for a product, like a processed food item, is called the primary container. Due to its direct interaction with food goods, the original place in which a product packaged and confined constitutes the most important aspect of packaging. The secondary packing assembles the initial boxes into one. Tertiary packing assembles every secondary package onto a single pallet (Quiferrada *et al.*,2016).

2.3 Packaging Materials

There are few packaging items that are ubiquitous today:

2.3.1 Wood

Primarily utilized for containers and pallets (heavy duty products). For cigars, presents, tea, cheese, and other items, some covered or hinged boxes are manufactured. Packaging made of bamboo is becoming more popular. Dell Inc. has created new bamboo-based packaging materials for laptops that are compostable (El-Wakil *et al.*,2015). When it comes to wine bottling, cork has a long history as a packaging material.

2.3.2 Paper

It is commonly used because it is inexpensive, retains its shape, and is simple to print. Most of the cellulose fiber used to make commercially accessible paper comes from pulped wood.

However, because it causes deforestation, its use has a negative impact on the ecosystem (El-Wakil *et al.*,2015).

2.3.3 Metals

The most often used metals in the packaging sector are tin plates and aluminum. Metals application as packaging material is becoming unpopular compared to synthetic plastics' applications in food industries, this can be attributed to their hefty weight and limited design possibilities (Dong *et al.*,2013).

2.3.4 Glass

Due to its inertness and good barrier properties, glass is a popular and practical packing material. Glass has a number of drawbacks, including its weight and fragility (Fakhouri *et al.*,2019).

2.3.5 Plastics

They are often moldable, synthetic, and frequently generated from petrochemicals. Plastics are utilized in an enormous and broadening variety of food goods due to their comparatively inexpensive cost, adaptability, and water resistance. A third of plastic is utilized in packaging in affluent nations. Packaging accounts for 42% of plastic consumption in India (El-Wakil *et al.*,2015). The following plastics are frequently found in packaging:

2.3.5.1 Polyethylene (PE)

It is the plastic that is used the most. About 80 million tons of PE are produced worldwide each year. Making bags, bottles, and films with it is the main application for packaging. PE is divided into a number of groups primarily depending on its molecular weight. These materials are

crucial for different packaging applications: Low Density Polyethylene (LDPE) has applications in tube, films and bottle wares. Reputable with low melting point and poor oxygen and moisture barrier property when formed into a film. Bottles and tubs are frequently made of high density PE. Its wide chemical resistance qualities and greater melting point are positives. For bottle seals, sachets, pouches, and bags, LDPE is mostly utilized as a packaging film over multiple laminating materials (Sharma *et al.*,2007)

2.3.5.2 Polypropylene (PP)

This thermoplastic material is employed in a wide range of tasks, including labeling and packaging. 45.1 million metric tons of polypropylene were sold on the world market in 2008. Bottles, jars, cartons, and trays can all be developed using this method (Goh *et al.*,2016).

2.3.5.3 Polyvinyl chloride

Following polyethylene and polypropylene, the third most common polymer manufactured. Bottles and other non-food packaging use it (Goh *et al.*,2016)..

2.3.5.4 Polystyrene

It is a synthetic aromatic polymer created using styrene as a monomer.

It can be used for disposable cutlery, containers, lids, bottles, trays, tumblers, and protective packaging (like packing peanuts). Table 2.1 summarizes these plastics properties *vis a vis* their applications (Goh *et al.*,2016).

Table2.1: Commonly used Plastics and their Applications in food Packaging

Polymer name	Properties	Applications
Low Density bread and Polythlene	Flexible, strong, easy to seal	Film applications like frozen food bags
High Density Polyethylene tubs	Resistant to chemical and moisture stiff, strong and easy to process	Bottles for milk, juices and water, margarines
Polypropylene microwave	Harder, denser, transparent, resistant to heat and chemicals	Yogurt containers, packaging material
Polyvinyl Chloride	Stiff, medium strong, transparent Material, resistant to chemicals	Bottles, packaging films and blister packs
Polystyrene containers, diposa-	Clear, hard and brittle material, Foaming, producing an opaque rigid, light material	Egg cartons, ble plastic silverware

Source: (Rodionova *et al.*,2012)

2.4 Bio-Based Packaging

It is feasible to make biodegradable materials from fossil-based raw materials or from a hybrid system of renewable and fossil-origin materials, bio-based packaging materials are produced using bio-polymers and -monomers derived from a complete renewable sources, which is expected to be compostable after use (Fakhouri, *et al.*,2019). The latter, however, is expected to be completely decomposed then returned to natural materials. Occasionally, but incorrectly, the terms "Bio-based" and "Biodegradable" are used alternatively (Homma *et al.*,2013). This research section will exclusively cover bio-based materials for packaging applications, this can be categorized in to one of these three groups (Category 1, 2, or 3) according to their place of origin and mode of production, as illustrated in Fig 2.2. Polymers that are solely or in combination derived from natural agricultural and marine precursors like as poly-saccharides and lipids, fall under Category 1. Polymerized materials made using traditional chemo-synthesis by employing sustainable bio-based monomeric materials, like Polylactic Acid-PLA, fall under category 2 (PLA is a bio-polyester polymerized from lactic acid monomers). Bacterial cellulose (BC) and poly (hydroxybutyrate) (PHB) are examples of polymers generated by microorganisms or genetically engineered bacteria in category 3 (Quiferrada *et al.*,2016; Jiang *et al.*,2015)

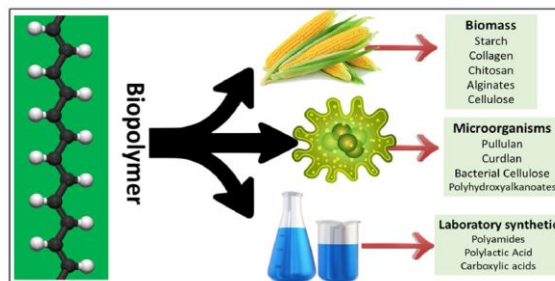


Figure 2.2: Biopolymer Packaging Materials Classifications (Hakalahti *et al.*,2015)

Regarding manufacture and renewability, few popular bio-based packaging materials in the industries include PLAs, PHA, starchy-plastics, cellulosic esters and plastics that are protein-based. Others include poly - (ethylene) monomers made through bio-processes like fermentation and pyrolysis or gasified biomass , polyethylene monomers made from the dehydration of bio-ethanol, polypropylene monomers made from the dehydration of bio-butanol, and biopolyurethane developed using hydrogenated vegetable oils. These types of trending technologies are anticipated to make impact in the nearest future (Saito *et al.*,2005).

Biodegradable packaging seems to be an alternative and substitute for plastics simply because it tends to successfully address the issues around sustainability and/or environmental risks related to creation, application and elimination of non-biodegradable food packaging materials because it has the right physical, mechanical, and barrier properties.

2.5 Edible Packaging

The definition of bio-based packaging that has been provided can include edible packaging. According to the law, all materials employed in this specific circumstance, both in their beginning (as ingredients in packaging) and final (as packaging) forms, must be edible. Coatings and films are typically used to create edible packaging. Coating as a super-thin layered substance that is placed onto a food materials and/or in between food stuffs (by immersive force, spraying, or shinning), later allowed to undergo drying process as applied to the surface of food producing a super-thin film with ability to serve the intended purpose. Even though thin coatings is removable, they may not be thrown away separated from foods. Layered coatings are typically viewed as a component of the finished product delivering additional benefits in terms of nutrition and quality, for instance by including bioactive ingredients therein. A film is the dried

film-forming solution that is applied on the food product as a self-standing material. The maximum thickness for a film to be evaluated is 254 μ m as investigated by Rodionova *et al.*(2012) and Homma *et al.*(2013). Simple or mixed ingredients, such as polysaccharides, proteins, lipids, and resins, can be used to create edible films and coatings in a variety of configurations, including single-layer, bilayer, and multilayer coatings. The limitations in their physicochemical qualities and biodegradability has been the reason they cannot function as a better replacement for synthetic packaging materials for prolonged food preservations . They can be extremely moisture-sensitive and have undesirable water vapour resistant property or they could be opaque in nature, with moderate rigidity and without stability despite having good water vapour barrier properties . There is still tendency for them to serve as replacement for few polymeric layers of the films when working with multilayer packaging systems, this functions as a supplement to raise residual food qualities, lengthen the food shelf life and boost the economic effectiveness of packaging items. (Torstensen *et al.*,2018).

2.6 Bio-Based and Edible Packaging Challenges

As compared to other conventional packaging materials, the use of biodegradable materials for in food industries is frequently constrained by a number of issues related to commercial application. The primary difficulties are attributable to the undesirable properties and performances exhibited (for example, relatively low tensile strength, poor processing properties and poor water inhibition property), varied raw material properties (for example, position and the harvesting time) and costs of productions (Dehnad *et al.*,2014). Additional issues relating to bio-based and edible materials should be taken into account - rates of degradation with different conditions, altered mechanical properties while the food is in storage, the capacity to develop the

needful microorganisms like amine groups, proteomics and protein-ligands conjugates), and possibility of interacting with other molecules in the contained food product (Arfat *et al.*,2015). In general, packaging films that comprises of proteins and polysaccharides have effective barrier against oxides (with relatively low RH) and desirable mechanical qualities. However, they also possess considerable permeable tendency for moistures primarily because of they have hydrophilic nature.

The inherent characteristics of bio-based polymers such as brittle nature, low viscous property, poor permeability and poor thermal property may limit their suitability for a variety of novel uses. Echegoyen *et al.*(2016) and Mackevica *et al.*(2016) are two researches investigating how to improve the packaging qualities of material made from bio-polymers; there are more research works also showing a huge enhancement of the packaging film properties. Jiang *et al.*(2015), for example worked on improving the physical and chemical properties of the films and the incorporated starch nanoparticles of cassava origin improved the barrier properties (water vapour at high RH of 90%). Nanotechnology has demonstrated distinct benefits when compared to all other researched options for improving the characteristics of bio-based packaging. Due to this, significant effort has been made over the past ten years to concentrate on improving bio-polymers and/or development of novel monomers and polymers by using nanoscience and nanotechnology.

2.7 Methods for Improving Properties of Biopolymer Based Packaging

Different techniques, both chemical and physical, have been suggested previously to enhance the mechanical strength and protective qualities of packaging made from biopolymers. These

techniques often involve thermal treatment, blending of multiple polymers to create composite films, altering the biopolymer chemically, using gamma irradiation, or adding various additives to the films.

2.7.1 Thermal treatment

Through thermal treatment, protein networks can readily establish strong covalent bonds between molecules, achieve close molecular packing, and decrease polymer mobility via cross-linking. However, many proteins undergo denaturation when exposed to high temperatures, exposing the amino acid groups of the protein to the solvent. This results in the formation of disulfide bonds, which cross-link the protein molecule. The degree of cross-linking is thought to be affected by variations in heating time and temperature. Numerous studies have examined the enhancement of film characteristics via heat-induced cross-linking. For instance, Shen et al.(2014) demonstrated that peanut protein films made from thermally treated solution (70°C for 30 min) had better tensile strength compared to untreated films. Previous studies have shown that heat treatment can enhance the Young's modulus of whey protein isolate films. Wang et al.(2015) have also shown that heat-treated wheat gluten films had significantly better tensile strength compared to untreated films. Additionally, blending different ratios of polysaccharides and/or proteins can lead to the creation of composite films with improved properties. For instance, composite films made from pectin and fish skin gelatin or soybean flour protein have demonstrated increased stiffness and strength, as well as reduced water solubility and water vapor transmission rate compared to pure pectin film. The tensile elongation of gellan/gelatin composite films also increased with an increasing proportion of gelatin. Wang et al.(2015)

demonstrated that combining whey protein isolate, gelatin, and sodium alginate led to improved mechanical and barrier properties of the resulting films.

2.7.2 Chemical modification

The functional qualities of films can be enhanced through the chemical modification of biomolecules. In the past, various methods for chemically treating biopolymers to create films have been investigated. For example, it was found that the percentage of elongation of soy protein-based film increased when it was mildly treated with alkali. In addition, the inclusion of cross-linkers such as glutaraldehyde and formaldehyde in a whey protein isolate solution resulted in an improvement in the tensile strength and a decrease in water solubility of the films formed. Furthermore, the cross-linking of casein with calcium reduced the water vapor and gas permeability of both sodium caseinate and calcium caseinate films (Vijaykumar et al.,2013).

2.7.3 Irradiation

Ionizing radiation is a technique used to process food by exposing it to gamma rays, X-rays, or electron beams. It is a cold process that can effectively preserve food, reduce the risk of food borne illness, prevent the spread of invasive pests, and delay or eliminate sprouting or ripening without significantly affecting its sensory and nutritional quality. This makes food irradiation one of the most extensively studied methods of food preservation. One of its significant advantages is its non-residual feature, which minimizes the use of chemicals applied to fruits and vegetables.

In 1980, a joint expert committee of the Food and Agriculture Organization, International Atomic Energy Agency, and World Health Organization concluded that the irradiation treatment of any food commodity up to an overall average dose of 10 kGy presents no radiological, microbiological, or toxicological hazard. Since then, this joint committee has approved the irradiation technology on the wholesomeness of food, and it is being commercially practiced in several countries and USA (Table 2.2). In India, only foods approved under the Food Safety and Standards Authority of India (FSSAI) rules can be irradiated and sold in the domestic market. Nowadays, using this technology to achieve similar results in fresh fruit products is one of the most challenging targets for processors (Khan et al.,2012).

Table 2.2: Items of Food Permitted for Irradiation Dose under FDA Rules

Name of Food	Dose of irradiation (kGy)	Purpose
Onio	0.03 – 0.09	Sprout inhibition
Potato	0.06 – 0.15	Sprout inhibition
Shallots	0.03- 0.15	Sprout inhibition
Rice, semolina, dried sea foods, whole wheat	0.25- 1.00	Insect disinfectant
Mango	0.25-0.75	Shelf life extension
Meat	2.50-4.00	Pathogen control
Fresh sea food	1.00-3.00	Shelf life extension

Source: (Khan *et al.*,2012).

2.8 Nanotechnology in Food Packaging

Nanotechnology is a concept that revolves around alteration of bulk material properties when the size is changed to nanoscale. This concept paves way for making novel materials with different properties from the existing ones using materials, electronics, or structures by manipulating their size and shape to a nanoscale which ranges from 1 to 100 nm. When particulate materials are decreased within these limits, the resulting material begins to show strange physicochemical that may differ dramatically from those at macro- or microscales, even when made of the same constituents (Makaremi *et al.*,2017). Since nanotechnology has the potential to revolutionize this industry by incorporating nano-enabled products, its use in food packaging has increased dramatically over the past ten years. These products offer a number of advantages, including strength, more flexibility and higher resistant packaging items, anti-microbes having better bioactivity with many biosensors with potentials to measure a range of variables. traces of bacteria, gases, or pollutants in packaged meals. In order to ensure the quality and safety of food products, this technique enables the development of the final packaging's functional performance, resistance, and physicochemical qualities (Dong *et al.*,2013).

Undoubtedly, the most active topic of food nanoscience research is the application of nanotechnology to food packaging. The market for nanotechnology-enabled food and drink packaging grew from \$ 5 billion in 2009 to \$ 9 billion in 2017, approximately 12% in rate of growth annually (Dong *et al.*,2013).The greater investment in this area is probably due to consumer acceptance surveys showing that consumers are more open to nanotechnology particularly when the applications are outside food industries as compared to when it is

employed in food industries directly (Hakalahti *et al.*,2015). One of the biggest problems for the food packaging business recently has been the development novel materials called bio-nanocomposites. Because of the combined effects of nanomaterials and their interference with bio-polymers, there is possibility for the enhancement of all the desirable physicochemical properties of bio-polymer matrixes (Hemmati *et al.*,2018; Tunc *et al.*,2007). Fig 2.3 shows different nano-fillers that have found their applications in food packaging industries.

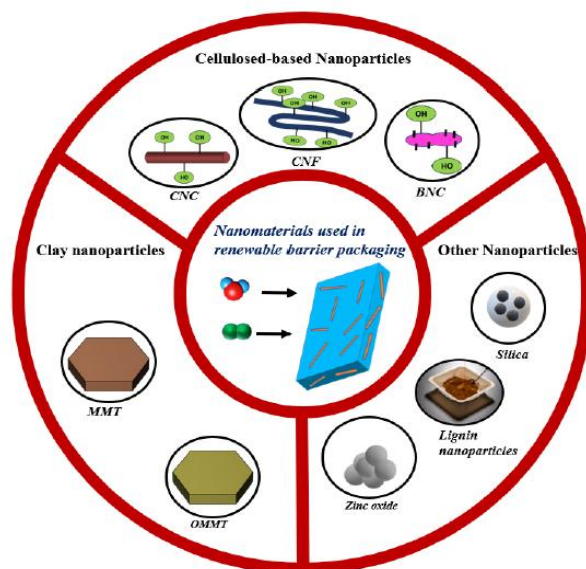


Figure 2.3: Different Nanofillers in Biopolymers Reinforcement(Mackevica *et al.*,2016)

2.9 Nanocomposites for Food Packaging

The success of utilizing inorganic nanostructures, such as Ag⁺ and Zn²⁺ in synthetic packaging has inspired further research into developing new nanocomposites from renewable sources for use in compostable packaging materials. These bio-nanocomposites are composed of multiple phases derived from renewable materials, including a continuous phase (matrix) and a

discontinuous nano-dimensional phase (nanostructure) that is obtained from fully renewable sources (El-Wakil et al.,2015). The nano-phase plays a major role in the structures of the biopolymer matrix acting as reinforcements to enhance its mechanical, barrier, and physicochemical characteristics by facilitating tension transference across the interface that has been produced between them. With the expectations that the existence of few reinforcing layered composite can delay the molecular journey and make the diffusive path more difficult, they are particularly promising in delivering superior barrier qualities (Fajardo *et al.*,2015). The addition of the reinforcement to bio-polymer architectures may create new opportunities for enhancing functional as well as physicochemical qualities (Hao *et al.*,2017). According to the number of dimensions that are nanoscale, the nano-dimensional phases may be split into 3 major categorical features: (1) Three-dimensional nanocrystals, nanospheres, or nanoparticles, and (2) nanowhiskers, nanofibrils, or nanotubes with few nanometers dimensionality that are different from one another *vis a vis* structural makeup (Jiang *et al.*,2015).

Data from the literature shows that nanoclay layered silicates have a significant impact on the properties of nanocomposite films due to their high aspect ratio, which may allow for greater energy transfer from one phase (the polymer) to another phase (the silicate layer) as well as the fact that, due to their nanoscale size, they interact with matter at the atomic, molecular, and macromolecular levels. Additionally, it was shown that adding nanoclay to polymer matrices caused a tortuous path in film leading to enhanced barrier properties of the nanocomposites (Table 2.3).

Table 2.3: Nanocomposite Types and Nanoclay effects on functional properties of biopolymer

Biopolymer	Nanofiller	Improvements
Starch	MMT	Tensile strength
Starch	CNT	Tensile strength
Starch	Na + MMT	Water vapour barrier
Agar	MMT	Tensile strength
Cellulose	Tourmaline	Water vapour barrier
Pectin	MMT	Water vapour barrier
Carrageenan	Mica	Tensile strength
Soy protein	MMT	Tensile strength
Whet protein	MMT	Water vapour barrier
Bovine gelatin	MMT	Tensile strength
Fish gelatin	MMT	Water vapour barrier
Whey protein	MMT	Antibacterial property

Source: (Hao *et al.*,2017).

2.10 Bio-Based Reinforcement Agents for Bio-Nanocomposites

The most popular bio-polymer based reinforcements agents for usage in continuous nanocomposites as applied to food materials safety and protection are presented in this section.

Nanocellulose whiskers is the most prevalent bio-polymer existing naturally, cellulose has significant and desirable properties. Given that it may be made from wastes and by-products of agricultural source like banana fibers or fermenting through *Glucanacetobacter* bacterium, this biopolymer is a practical material (Hayaka *et al.*,2009). These qualities make the nanosized material that are cellulosic in nature particularly appealing for application in reinforcements of e creation of biodegradable nanocomposites, in addition to their environmental friendliness. The two primary types of cellulose nano-reinforcements made from cellulose are nanofibrils and nanocrystals from cellulosic biomass, also known as cellulosic nano-whiskers (Mackevica *et al.*,2016). The molecules that make up cellulose nanofibrils are arranged in bundles that are lengthened and stabilized by hydrogen bonds (Quiferrada *et al.*,2016). These nanofibrils typically have a diameter of 2–20 nm and a length in the micrometre range. Both amorphous and crystalline regions make up these fibrils. The materials produced when the crystalline areas are segregated are referred to as nanowhiskers, nanorods or nanocrystals. Whiskers actually have a diameter of 8–20 nm and a length that spans from 500 nm to 1-2 m. Additionally, it was discovered that cellulosic nanocrystals may have a Young's modulus of roughly 160 GPa and a Tensile strength of roughly 12 GPa. These and some other good reasons constitute some extremely intriguing findings that might allow cellulose to take the place of single walled carbon nanotube in many applications.

The commonest technique for producing cellulosic nano-whiskers is acid hydrolysis, which preserves the crystalline areas while eliminating the amorphous regions (Bai *et al.*,2014). Due to the similar structures of the two bio-polymers, which enables the coupling of the physical and chemical features of chitosan with good physical capabilities of the cellulosic fibers that are

naturally existing, chitosan-cellulose blends are of great interest. According to Maestre *et al.*(2010), cellulose nanofibrils were successfully incorporated into chitosan films, yielding products with exceptional mechanical characteristics. Wang *et al.*(2015) demonstrated that the synthesis of PLA nanocomposite films using cellulosic nanoscaled crystals and lignins nanoparticle for reinforcing all the components proved successful., increasing the tensile strength and elastic modulus of their mechanical performance. Additionally, the two lignocellulosic nanosystems in combination with PLA nanocomposite films shown antibacterial action towards hazardous pathogens (i.e., *Pseudomonas syringae*) (Wang *et al.*,2015). In a PLA matrix of nanocomposites, Homma *et al.*, (2013) showed that the addition of cellulose nanowhiskers (concentration < 3% w/w) was well disseminated. This addition increased the water vapour and oxygen barriers of the nanocomposites by 82 and 90%, respectively, without affecting their thermal stability but did encourage the development of certain crystals (Homma *et al.*,2013).

2.11 Biopolymer Structures

Biopolymers are naturally occurring polymers that are typically characterized as having renewable resource origins. Both eco-friendly and biodegradable materials are referred to as biopolymers. The most alluring use of biopolymers among the several applications is food packaging (Tian *et al.*,2018). Table 2.3 lists several significant biopolymers' properties. Cellulose is the most successful and abundant bio-based polymer among the many biopolymers. The basic structure of cellulose is composed of millimeter-sized strings made of constantly small microfbers and microfbers that contain nanometer-sized microfbrils.. Cellulose is expected to be more widely employed in the packaging sector because it is non-toxic, less expensive, and has an incredible strength-to-weight ratio (Tian *et al.*,2018).

The manufacture of biodegradable food packaging using cellulosic materials produced from lignocellulosic biomass is depicted in Fig 2.4 as a viable and alluring solution to the problem of plastic food packaging. Lignocellulosic biomass includes non-cellulosic substances including pectin and waxes in addition to cellulose (crystalline region), hemicellulose, and lignin (amorphous region) (Ojogbo *et al.*,2019; Osman *et al.*,2019; Chen *et al.*,2019). In the 1980s, cellulose nanoparticles—a brand-new class of plant-based polymer that comprises nanoscale

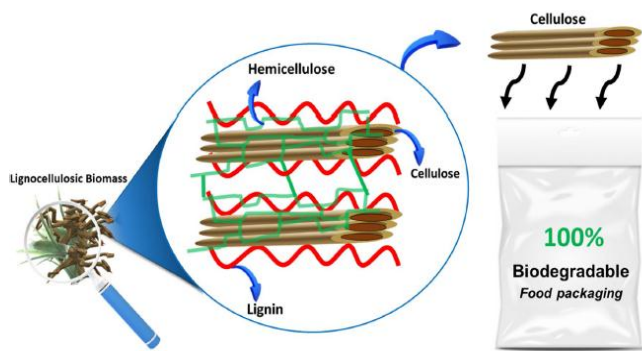


Figure 2.4: Structural Composition of Lignocellulosic Biomass (Maestre *et al.*,2010)

cellulose fibres and crystals—were discovered. The horizontal components range from 5 to 20 nm, whereas the longitudinal measurement ranges from a few tens of nanometers to a few microns (Yokohama and Welchons, 2007). Bacterial cellulose, cellulose nanofibers, and cellulose nanocrystals are three types of cellulose nanoparticles (Arfat *et al.*,2015). Acid hydrolysis produces cellulose nanocrystals with dimensions of 3 to 20 nm in width and 50 to 500 nm in length when applied to wood or any other material containing cellulose. To create linear or branching chains of cellulose nanofibers with widths and lengths ranging from 4 to 50 nm and 500 nm, respectively, physical treatments with or without chemical ones could be applied. Bacterial cellulose is manufactured by the bottom-up process, where bacteria produce cellulose in the form of nanofibrils, which then coalesce into a ribbon with a width of around 70–80 nm.

Bacterial cellulose is chemically comparable to plant cellulose (Raveendran *et al.*, 2014). The cost and utilization of chemical reagents for the removal of lignin and hemicellulose components can be reduced by synthesizing bacterial cellulose in its purest form, devoid of the noncellulosic components (Raveendran *et al.*,2014)

Despite the cellulose's above-mentioned qualities, its employment is still constrained because of its poor mechanical, thermal, and barrier properties as well as its limited capacity to absorb water. These drawbacks make cellulose less useful than traditional synthetic materials. The creation of composite materials using cellulose as a filler is most likely a solution to this issue. Multi-phase materials called nanocomposites have at least one dispersed phase constituent with a dimension of 100 nm or less (Brinchi and Cotana, 2013). Better stiffness is displayed by cellulose nanoparticles, which have a tensile modulus of 134 GPa and tensile strengths in the range of 0.8 to 10 GPa (Brinchi and Cotana, 2013; Wu *et al.*, 2021). Using the solvent casting approach, Kumar *et al.*(2003) created green nanocomposite films based on polylactic acid and cellulose nanofibers for use in packaging. Nanocellulose was recovered from agricultural waste made up of plum shells by Fakhouri *et al.*, (2019) and added to aliphatic polyester-based biodegradable films. Biodegradable scaffolds were created by Tang *et al.*(2017) for use in tissue engineering. Cellulose nanoparticles are a ground-breaking method for creating sustainable packaging with improved features in terms of design, an effectual design may include the product's (ecological packaging) quantitative and qualitative performance over the course of its full life cycle (Chuacharoen *et al.*,2016).

The design process for the isolation of cellulose nanoparticles will attest to the product's quality and the packaging's criteria, such as size, thickness, safety and stress levels, and ergonomics, as

well as the product's cost and quantitative life cycle assessment. As a result, the main function assigned to the design process is to define the potential, restrictions, and applicability of cellulose nanoparticles for the development of sustainable packaging (Jiang *et al.*, 2015). Given the innovative role that cellulose nanoparticles play in food packaging will provide the audience with a detailed look at the most recent studies relating to cellulose and cellulose nanoparticles. A detailed overview of pretreatments, cellulose nanoparticles characteristics, and processing methods for nanocomposites with an emphasis on food packaging applications has been studied. Finally, this research work discusses the concepts, significance, and difficulties of sustainable food packaging as well as the necessity of process designing (for cellulose nanoparticles).

2.12 Isolation of Cellulose Nanoparticles

International research community has its goal as the extraction of cellulose-enriched compounds with nanoscale (1-100 nm) dimensions from lignocellulosic biomass. A complex and compact hetero-matrix structure is formed by lignocellulosic biomass (Suvith and Philip. 2018). The cellulose is protected from isolation by lignin, hemicellulose, and their physical-chemical interactions (Herawan and Rivani, 2013; Zhong *et al.*, 2020). The two-step multistage process for isolating cellulose nanoparticles from biomass is acquired. In Fig 2.5, the first step is the fragmentation of lignocellulosic material to recover cellulose, and the second step is the treatment of cellulose to obtain its nanoscale dimension (Beigzadeh *et al.*,2016; Zhao. *et al.*, 2015). Nanofibrillated cellulose is produced by defibrillation using mechanical processes such as micro-fluidization, high-intensity ultrasonication, grinding, and high-pressure homogenization (Chen *et al.*, 2019). Chen *et al.*(2019) produced 18–20 nm-diameter nanofibrillated cellulose by mechanically grinding coir and then synthesizing it chemically. By refining and homogenizing at 50 Mpa, Stelte and Sanadi fibrillated hardwood and softwood pulps to obtain nanofibers. The

separation of cellulose nanoparticles by high-pressure homogenization was researched by Biji *et al.* (2015). The cellulose nanoparticles that were created had an average particle diameter of 5–20 nm (2018) stated that cellulose nanofibrils of 90–110 nm in diameter were created using balls of cerium-doped zirconia with a diameter of 0.4–0.6 mm.

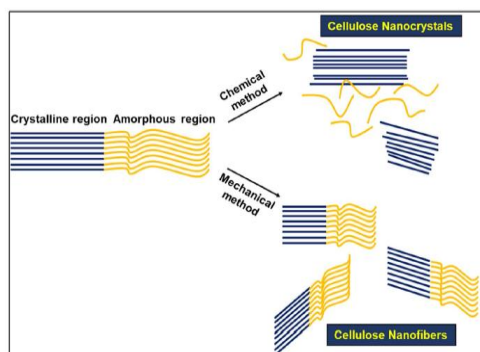


Figure 2.5: Methods in the Production of Nanomaterial from Biomass (Tian *et al.*,2018)

Several authors investigated wheat straw as a cellulose source. It was discovered that the crystals formed from the acid hydrolysis of wheat straw are little rods with an aspect ratio (length to diameter ratio) of 50 and lengths ranging from 150 to 300 nm. Hemmati *et al.*, (2018) created cellulose nanofibers using soy hulls and wheat straw. The researchers used chemomechanical techniques to create particles with a diameter of 10–80 nm and a length of several thousand nanometers (Isroi *et al.*,2016). Similar to this, Jafari *et al.* (2018) used the chemo-mechanical approach to extract cellulose nanofibrils with a diameter ranging from 5 to 10 nm from an oil palm empty fruit bunch and noticed a drop in the crystallinity. In order to extract cellulose nanoparticles from four different lignocellulosic fibres (wood, bamboo, wheat straw, and fax fibres), Chen *et al.*(2019) used a chemical ultrasonic approach. Wheat straw, wood, and bamboo were all used to successfully extract nanofibers with a diameter of 10–40 nm. In order to create cellulose nanocrystals from a lignocellulosic biomass (Fig 2.6), an acid hydrolysis procedure was

used. The resulting nanocrystals had an average length and diameter of 538.5 , 125.3 nm respectively and a diameter of 85.4 nm (Denilson *et al.*, 2003). Similar to this, fax fibres were acid hydrolyzed to create cellulose nanocrystals, and the resulting materials were thin rods with an average length of 327 .10 nm and a diameter of 21 .7 nm (Duran *et al.*, 2016).

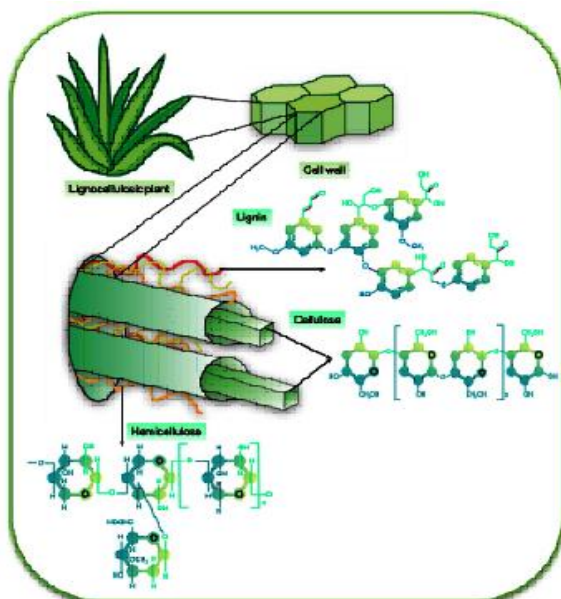


Figure 2.6: Cell wall structure of Lignocellulosic Biomass (Wang *et al.*,2015)

2.13 Starch Nanocrystals

Due to its biodegradability, availability and cheap cost, starch is a polymer derived from agricultural sources that has drawn a lot of interest. The 2-D-glucan constituents linear amyloses and heavily-branched-amylopectins make up the majority of starch structure (Sharma, *et al.*,2007). Despite the fact that starches derived from different sources such as potato, maize, pea and rice have different structural units, they possess similar physical properties. While the

structural units of wheat, cassava, waxy maize, and amylo maize are similar, their varying amyloses with amylopectins affect their physical and chemical characteristics, consequently, size distributions of their relative particulate constituents (Moran *et al.*,2013). Native granules can be macro to nanoscale (less than 100 nm), with changes in the amorphous region and crystalline casings (110-395 nm) with crystalline-amorphous lamella (6-8 nm) depending on the source of the starch (Makaremi *et al.*, 2017).

By carefully regulating the temperatures, acidic concentrations, starch contents, time of hydrolysis, starch-based nano-crystals produced by acidic hydrolysis of native granular starches (typically using sulfuric acid) can be used as reinforcement material when creating nanocomposites (Makaremi *et al.*,2017). The semicrystalline nature and three-dimensional architecture of these reinforcement agents may be disturbed when used in hot water, causing a phase transition from the ordered granular structure into a disordered state in water. This may limit their use when creating plasticized starch bio-nanocomposites (Dehnad *et al.*,2014).

Fajardo *et al.*(2015) demonstrated that native cassava starch films reinforced with catechin and starch nanocrystals (obtained by acidic hydrolysis from waxy maize starch granules) improved the mechanical performance and thermal stability of such edible films. In order to create nanocomposite films, Hayaka *et al.*(2009) used a continuous-phase of yam starch with glycerol as a plasticizer and waxy maize starch grains that had undergone starch plasticized with glycerol as the continuous phase and waxy maize starch grains that had undergone acid hydrolysis to produce starch nanocrystals to be the reinforcing medium. There was a demonstration of 360% increment of shear modulus (at 60 °C) and also a 50% reduction for water-vapour permeability

with reinforced films. The nanoparticles were evenly distributed throughout the cassava starch matrix, which was related to the permeability decreasing (Hayaka *et al.*,2009). According to Jiang *et al.* (2015), compared to unreinforced peers, film wax of maize starch strengthened with a modest proportion (2.6% w/w) of nanoscale starch crystals increased their storage modulus by 470% at 50°C.

2.14 Production and Characterization of Bio-Nanocomposites

A minimum of one polymer capable of forming a continuous matrix must be used in the formulation of a packaging substance. However, other materials may be incorporated, to result in the formation of composites so as to obtain desirable properties for food packaging. Although the majority of these materials require inclusion of plasticizing agents to enhance their processability, elasticity, and thermogravimetric properties (among other things),the final product may not be necessarily regarded as composites. In order to lower the glass transition temperature and increase flexibility and toughness, the plasticizer will be introduced either during the process of polymerization or rather when the compounds are being mixed (Mhd Haniffa *et al.*,2017).

There has been a thorough evaluation of the plasticizers utilized in biodegradable packaging (Mhd Haniffa *et al.*,2017). Wet process and dry process are two techniques for making packaging products. The "wet process" entails dispersing the polymer or solubilizing it in a solvent that will eventually evaporate, enabling a film to form on a substrate. In laboratory procedures and whenever Category 1 biopolymers are used, this technique is frequently applied. Dry process; which relies on the components' thermoplastic properties, entails mixing and

smelting the bio-polymer that is formed into a mold and laminates via the created film. But additional approaches, including in situ polymerization and bi-layer technologies, have also been proposed as remedies (Quiferrada *et al.*,2016; Jang *et al.*,2016; Sharma *et al.*,2007). It is possible to create bio-nanocomposite materials by incorporating nanostructures into biopolymer matrices. These materials will have improved packaging properties and rely on effective homogenous dispersion and/or deposition. The concluding characteristics usually have to do with how the nanostructure will organize itself in the matrix and interact with the biopolymer. There are numerous studies that describe how silicates and clays will organize and how that organization will impact the general properties of packaging materials. Intercalation, flocculation, or exfoliation processes can be used in the polymer-matrix under used, depending on the processing technique; the latter is the most preferable shape because it demonstrates the greatest affinity in-between nanostructures and biopolymers (Fernandes *et al.*,2019). Because of this, it is obvious that the methods used to prepare and produce packaging materials have a significant impact on the final characteristics of those materials.

2.15 Wet processing

Solution casting and evaporative drying of the liquid in which the bio-polymer shows to be soluble are the foundations of moist processing. In contrast to synthetic materials, where dry processing is more prevalent, this technique popularly called solution casting approach find extensive application in the creation of bio-polymer films. In as much the films forming solutions can be applied to a surface (primarily food surfaces) to form a covering which is able to dry and make film - layer surface, the wet method is the one that is most frequently used to create the biodegradable nanocomposites being used in layer coating. Water is the most common

solvent, but ethanol and other chemical solvents can also be used. With or without temperature rise (the use of temperature is required for some proteins and polysaccharides), the biopolymer is distributed in the solution till the bio-polymer totally dissolved in the solvent. The film-forming solution can then undergo some modifications like changes in pH or/and inclusion of plasticizing agents, surfactants and additives. The inclusion of the nanostructured materials is carried out immediately in the solutions of the film for the creation of nanocomposites, or a prior mixture is prepared in the same or a separate solvent, then combined together. The preparation used during the mixing phase has a significant impact on how well the nanostructures disperse in the film matrix. Occasionally, ultrasonication, ultrasound, or high-pressure homogenization are required to ensure effective dispersion of the nanoscale materials. However, since they can alter the structure of the biopolymer, applications of these methods in the creation of bio-nano nanocomposites should be cautiously considered.

The final step involves applying the nanocomposite forming solution to a firm surface (like Petri dishes or glass platters covered with teflon) and allowing it to dry. When drying, the containers used, whether they have forced air flow or not, with the temperatures profile, RH, total time spent on solvent evaporation, are crucial elements to consider (Fernandes *et al.*,2019).

2.16 Dry Processing

The method that makes it possible to produce packing materials at the lowest cost without a question is dry processing. Due to the lack of organic solvents, this technique is not only inexpensive but also ecologically beneficial. Processing methods used to create manufactured packaging materials, such as sheet rolling, extrusion, or injection molding, are being modified to

create bio-based packaging materials. Industrial size processing and production are used to create materials like PLA, PHA, and PHB. The bio-polymer with the nanoparticles are combined and sub-heated over the polymer's soft point and this can occur while static or compressed, to create bio-nanocomposites. When mixing the biopolymer in this instance, ultrasounds have also been used to enhance the spread of nanostructures (Ghaderi *et al.*,2014). The majority of biopolymers, including protein and polysaccharide groups, are harmed by heat processing, showing a little instances of narrow spectrum of stable process, and having a limited use in typical extrusion or moulding machinery. However, some authors have demonstrated that it can be feasible to apply the most economical method like this to develop these types of materials (Maestre *et al.*,2010), with gelatinous starch nanocomposites (Hakalahti *et al.*,2015), soy- proteins (Jiang *et al.*,2015) and chitosan-starch composites (Denilson *et al.*,2003). There is still a dearth of knowledge regarding the dry processing of bio-nanocomposites, and more research into particular processing variables, chemicals, or formulas is required.

2.17 Characterization Techniques

To ensure their effective application and to determine whether they are suitable for the planned food uses, packaging materials should be characterized. The primary qualities and traits of packing materials can be affected by a wide range of variables. Several academic and industrial scientists are currently attempting to link those structures of the creation of bio-polymers and bio-based composites and their final features. This makes it possible for packing materials' property to be tailored to the requirements of the food product, guaranteeing the purity and safety of the latter. Biopolymer qualities, such as chemical structures, crystalline nature, polarities, densities, directions and molecular weights, will also have an impact on the qualities of

packaging films. but also by the qualities of the nanoscale materials that make up the mixture and affect the materials (Denilson *et al.*,2003; Mackevica *et al.*,2016). The finished properties of packaging materials will also be influenced by the technique used to prepare and handle the film in relation to those variables. The primary disadvantage of using bio-based materials for container manufacturing is their inferior physicochemical characteristics in comparison with those of fossil-based polymers. The primary benefits of nanostructures' applications are their exceptional mechanical and thermal qualities, which have an impact on the characteristics of biopolymers and enhance their thermal resilience and stiffness. Additionally, the creation of bio-nanocomposites with improved barrier and optical characteristics will result from the nanoscale impact and potential chemical interactions created between materials. However, nanostructures can be added to packing materials to functionalize them with the goal of creating smart packaging, their impact on all needed properties like physical, heat, and transit characteristics should also be taken into account.

Tensile strength profiling and elongation at break are among the most researched properties ; however, other mechanical tests, such as modulus of elasticity, compressive strength, and piercing strength are used as well. When it comes to heat load properties, the glass transition (T_g) and melting point temperature—which can be obtained by differential scanning calorimetry (DSC) are the most researched because they are crucial when the "dry process" is used to create packaging materials. Thermogravimetric analysis (TGA), which measures weight loss in response to temperature rise and is associated with the deterioration of composite materials, is another method used to study the thermal stability of materials. The ability of packing materials to create a boundary between food products and the outside world is their key characteristic.

Some vapours and flammable substances, including water vapor, oxygen, carbon dioxide, and ethylene, are prevented from permeating by this physical barrier. Three key factors—diffusion, sorption, and permeation—all affect the rate at which molecules diffuse through packaging materials to transfer mass: (1) sorption, which is influenced by the molecular solubility of the starch matrix and (2) permeation, which is the rate at which molecules diffuse through a packaging matrix (Goh *et al.*,2016). In this instance, adding a nanostructure will also have an impact on the end characteristics, which is primarily described by the material's increased tortuosity (Saito *et al.*,2005). Techniques like Fourier transform infrared spectroscopy (which provides details about the materials chemical fingerprints), X-ray diffraction (which gives knowledge about the crystalline nature), can be used to evaluate the chemical with changes in morphology on bio -packaging materials. These methods can be very helpful for understanding how the characteristics of packing materials are affected by nanostructures when combined with measurements of thermal, mechanical, and transport properties. The use of nanomaterials raises a number of questions about the security of tiny elements. Packaging should ensure compliance with regulations.

This being the case, it is sometimes necessary to conduct migration experiments to guarantee direct application of the composites in food application and edible items packaging. When it comes to plastic-based items and items permitted to make contact with food, the European Commission is very clear, and the restrictions and recommended food simulants are well explained (European Commission, 2009). Several works have detailed the migration characteristics of compounds for food simulants in addition to the rules, and some approaches have been suggested (Arfat *et al.*,2015).

The biodegradability as well as sustainability of these materials as packing components is one of the primary assertions of bio-nanocomposites. However, it is crucial to assess their biodegradability to make sure the created bio-nanocomposite degrades in accordance with global standards (e.g., ISO14855). The ultimate packing system (packaging required to store food items) should be the centre of a life cycle assessment (LCA), which should be carried out and be decided in accordance with international standards, such as ISO14040. Several instances demonstrated that some materials, even those drawn from bio-based sources, are not biodegradable and viable. On the other hand, it is possible for materials to be biodegradable even if they were produced using petroleum. For instance, PLA made from gasoline degrades while biopolyethylene developed using ethanol as raw material obtained from sugarcanes does not (El-Wakil *et al.*,2015; Tian *et al.*,2018).

2.18 Bio-based Nanocomposite Active Packaging

The use of antimicrobials in bio-based packaging has drawn a lot of attention from the food business recently in an effort to improve the safe consumption of food items. The interests that can only be primarily ascribed to customers' increasing demand for food items that are more natural, lightly processed, and handy. As a consequence, research into natural antimicrobial substances that can function as an option to synthetic preservatives has received more focus (Tian *et al.*, 2018). Antimicrobial packaging works in conjunction with the packed food or the headspace of the container to lessen, delay, or even prevent the development of pathogenic/spoiled microbes.

The bioactive substance may be directly incorporated into the polymer matrix for antimicrobial active packaging, coated on the packing surface, or immobilized in sachets, among other options. Once food spoilage microbes come into touch with the food surface, this form of container may make it easier to regulate their development. Furthermore, when applied directly to food by dipping or sprinkling, antimicrobial packaging can activate the bioactive compounds in the food, preventing effectiveness loss or fast migration of compounds within the food mass (Sharma *et al.*,2007).

By limiting microbial development and pollution, as well as by eradicating and destroying microorganism communities in food items, the use of nanostructures with antibacterial qualities may enhance food safety and quality. The greater surface area of nanostructures compared to micro- or macrostructures enhances the antibacterial effectiveness of the container made of nanocomposite materials (Torstensen *et al.*,2018). Numerous antibacterial substances used in the creation of nanocomposites can be either biopolymers (such as chitosan), naturally occurring antimicrobial substances (such as enzymes and nisin), or organic or inorganic substances (such as MMT clay, silver, ZnO, and TiO₂) (Torstensen *et al.*,2018; Hassan *et al.*,2018). One of the most popular nanostructures for antimicrobial nanocomposite packing uses is metal and metal oxide nanoparticles. Numerous experiments using metallic antimicrobial nanoparticles in bio-nanocomposite films are being carried out, and the results have shown encouraging antimicrobial action against both Gram-positive and Gram-negative microorganisms. Some of these instances include cellulose-based antibacterial nanocomposites with alginate, gelatin, and metal nanoparticles (Rodionova *et al.*,2012). Toxic non organic substances that are found in food containers that ultimately move into food items and are consumed could harm human cells. As a

result, it's crucial to investigate naturally food-graded antimicrobials like essential oils to integrate into biodegradable packing and guarantee their impacts on human health after consumption. Nonmetal or nonmetal-oxide nanoparticles have been the subject of some research to provide antimicrobial function when included in biobased food containers. Consider the antimicrobial properties of bio-nanocomposite containers composed of biologically modified nanoclay. Two chemically adjusted MMT clays (Cloisite 20A and Cloisite 30B) with an unaltered MMT (Cloisite Na⁺) were combined to create agar/clay nanocomposite sheets by El-Wakil *et al.* (2015) to depict active scavenging and releasing systems (Fig 2.7) . While Cloisite 20A and Cloisite Na⁺ films lacked antibacterial activity. This is best narrated by the existence of quaternary- ammonium ions in organically transformed Cloisite 30B nanoclay, which serve as an antibacterial agent (El-Wakil *et al.*,2015).

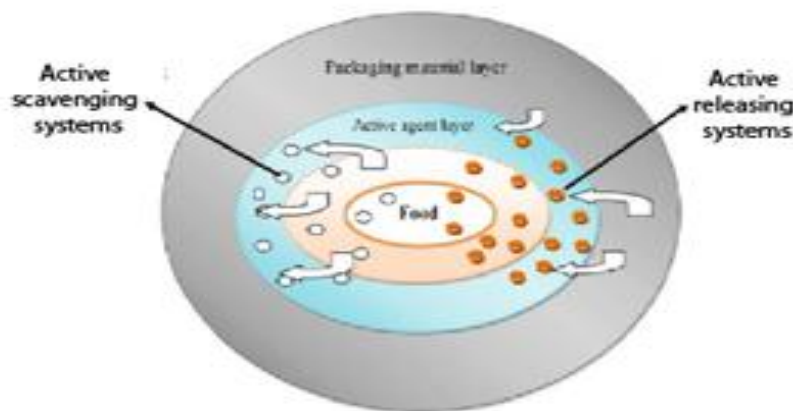


Figure 2.7: Active Scavenging and Releasing Systems in food (Shen *et al.*,2014)

2.19 Antimicrobial Activities of Plant Extract

The food industry has been prompted to consider natural alternatives by consumers' preference for natural food additives and their concern about the cancer-causing potential of synthetic food

preservatives like nitrates, benzoates, sulphites, sorbates, and formaldehyde (Khan *et al.*,2012). Natural plant extracts can help preserve the color, odor, and flavour characteristics of food while keeping its look, flavor, and quality. Numerous substances also have potent antimicrobial qualities in addition to their antioxidant properties (Osman *et al.*,2019). The primary effects of herbal plant extracts and essential oil actions are on cellular membrane and structure. It has been demonstrated that bioactive substances present in infusions affect the porosity of the cell septum or barrier bringing the intracellular boundaries outside of the cell, which may be pursued by the disruption of the cell's electron transfer system, enzymatic activity, and capacity to absorb nutrients (Fernandes *et al.*,2019). Since the dawn of the human species, pathogenic microorganisms have posed a threat to man as a primary source of morbidity and death. According to the type of microorganism, the bioactive substances exhibit various modes of action (Bai *et al.*,2014). Simple phenols, quinones, tannins, coumarins, flavones, terpenoids, and alkaloids are phenolic substances that have antibacterial properties (Sathishkumar *et al.*,2012). By changing the porosity of microbial cells, they have the ability to cause the loss of proteins from inside cells. Because of their interactions with membrane proteins, phenols disrupt the shape and function of cell membranes. Functions of the membranes that are impacted include electron transfer, nutrient absorption, protein and nucleic acid synthesis, and enzyme activity (Fernandes *et al.*,2019)..

The effect of the antimicrobial activity that plant-derived chemicals generate against microorganisms relies on their structural variety skeletal arrangement (Suvith and Philip, 2018). Since the hydroxyl (-OH) groups in phenolic substances engage with the bacterial cell membrane to damage the structures of membranes and cause leakage of cellular components, it is believed

that these groups have inhibitory effects. Thymol and carvacrol share a similar structural makeup, but when evaluated in an agar medium, it was found that they had different antimicrobial potencies against Gram-positive and Gram-negative microorganisms. This discrepancy has been ascribed to the different amount of double bonds between the -OH group in the ortho position in carvacrol and the meta position in thymol. Citronellol was found to be less effective than geraniol and nerol because it only has one double bond, whereas geraniol and nerol both have two double bonds and exhibit greater antimicrobial activity against the yeast and bacteria (*Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus*) that were tested (*Candida albicans*). According to some accounts, flavonoids play a significant antibacterial role in plant life. Plants have an inherent immunity that includes multiple levels of defensive mechanisms to stop the spread of pathogens, some of which include the biosynthesis of flavonoids (Ghaderi *et al.*,2014). Many different types of vegetation contain phenolic acids, which are primarily found in seeds, berries, peels, and foliage. According to reports, caffeic acid has antiviral, antibacterial, and antifungal properties, whereas eugenol has been categorized as bacteriostatic . The passage of the non-dissociated acid across the membrane, which results in the acidification of the cytoplasm and, on rare occasions, cell death, is the mode of action for low-molecular-weight phenolic acids. It should be emphasized that a variety of elements, including pH, ring replacements, and side chain saturation, have a significant impact on the action of cinnamic acids. Tannins have long been known to have antimicrobial properties, which include inhibiting extracellular microbial enzymes, depriving microbes of the substrates they need to grow, directly affecting their metabolism by inhibiting oxidative phosphorylation, depriving microbes of metal ions, or forming complexes with their cell membranes, which alter the morphology of the cell wall and increase permeability of the membrane (Fukuzumi *et al.*,2013).

2.20 Smart Packaging

Nanomaterials are used in smart/intelligent packing to sense biological or microbial modifications to the food, such as identifying particular bacteria growing in the food or particular gases from food spoilage. Nanoparticles can be used as reactive particles in packing materials to provide information about the condition of the packed product in smart packaging. In order to connect, educate, and recognize the product with the goal of ensuring its quality and safety, so-called nanosensors are able to react to changes in external stimuli. Recent advancements in polymer nanoparticles for smart food packing include the ability to identify products, track their history, and detect oxygen and spoilage (Goh *et al.*, 2016).

2.21 Health Impacts of Plastic Packaging

Globally, the production of food contact materials (FCMs) involves the use of about 12,000 substances. FCMs are substances and items, such as kitchenware, industrial machinery, storage receptacles, and food packaging, that come into touch with human food (Fernandes *et al.*, 2019).

Many of these substances are added to plastic containers as fillers, colorants, or additives to increase elasticity and longevity in hot or sunny environments. Along with inks and colors, paper, fibers, and mixed-material food containers are all made using a variety of substances. Plastic containers may contain more than 4,000 substances. 906 of these chemicals have been found as potentially present in plastic packaging, 68 of which are especially dangerous to the ecosystem and 63 of which are especially dangerous to human health (Fernandes *et al.*, 2019)..

These substances have the potential to harm the immune, hormonal, and neurological systems. Numerous substances that are allowed to be used in food contact uses, such as food packaging, can lead to cancer and other diseases or impede growth and fertility. Many more chemicals that come into touch with food could be dangerous, but their toxicity has not yet been sufficiently evaluated (Duran *et al.*,2016).

Packaging contains chemicals that may leak into our food and drinks. It is well known that materials like that of glass, metallic alloys, and ceramic are more solid and less likely to enable chemical migration into their innards. Contrarily, non-inert materials like papers, plastics, and cardboard allow chemicals to more easily migrate from the substance to the meal. The way food is presently distributed and packaged poses a long-term threat to both human health and the ecosystem (Dehnad *et al.*,2014).

2.22 Food Packaging Hazards

By offering physical security and fostering the ideal physicochemical conditions, food packing materials aim to extend the shelf life of goods . The market for food packing is currently worth \$250,000,000 and is anticipated to hit \$300,000,000 by 2030 (Jeeva *et al.*,2014a).. The migration of chemicals from the container into the food, however, can make traditional food packaging products a source of chemical food contaminants. In the United States and Europe, the manufacturing of food contact packaging is lawful for 176 chemicals with known hazardous characteristics, according to a recent research by the Food Packaging Forum. These hazardous substances are frequently used in food packaging (Jeeva *et al.*,2014a).

2.22.1 Biocides - Many biocides used to clean surfaces, such as propanol and glutaraldehyde, are pollutants and sensitizers that affect the skin, eyes, and nasal membranes. Asthma and sensitive contact rashes can result from them (Hao *et al.*,2017).

2.22.2 Bisphenol A (BPA) It is the most prevalent type of polycarbonate plastic and a crucial component in the manufacturing of epoxy resins. BPA had an impact on female fecundity and the start of adolescence. Additionally, it negatively impacts the male reproductive system and is toxic (Hao *et al.*,2017)..

2.22.3 Phthalates - used primarily as plasticizers (substances added to plastics to increase their flexibility, transparency, durability, and longevity). It has a negative impact on health and is linked to obesity and lessened masculinization in infant males (Hao *et al.*,2017)..

2.22.4 Polyfluorinated – Substances like Fluorosurfactants are frequently found in containers as polyfluorinated substances. These substances significantly lower the sheets' surface tension. However, these substances are damaging to the skin and eyes and, at greater doses, can cause liver, renal, and testicular cancer. The adding of possibly toxic substances to food packaging is being restricted by a number of government organizations, including the Food and Drug Administration. These preventative measures will lessen the danger to human health, but they cannot fully eliminate it. The creation of food containers made of biopolymers is an alternative strategy. Due to the lack of dangerous compounds or toxins and the fact that they are biodegradable, bioplastics are totally safe because they decompose into harmless substances. Fig 2.8 depicts the hazards that accomplish continuous use of polystyrene in food packaging (Kelly *et al.*,2003).

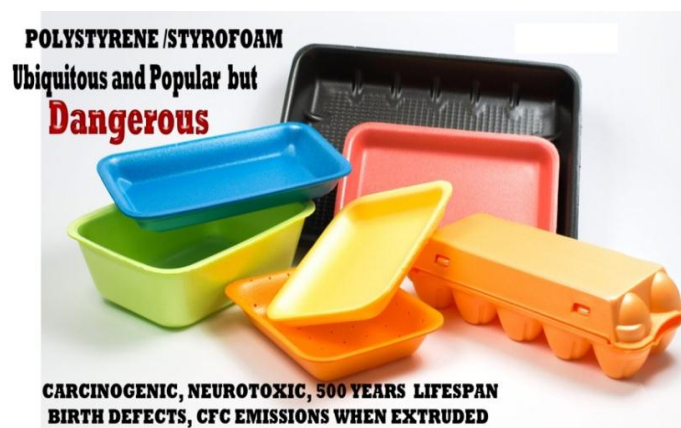


Fig 2.8: Polystyrene's Application in food Packaging(Osman *et al*,2019)

2.23 Ultrasonication

The act of agitating the particles in solutions using sound vibrations is known as ultrasonication. These disturbances are used to combine the solutions, quicken the rate at which a solid dissolves in a liquid, and remove dissolved gases from the liquids (Jafari *et al.*,2018).

2.23.1 Parts of ultrasonicator

The equipment used for sonication is known as a sonicator. The following are the three parts of the sonicator (Jafari *et al.*,2018):

- A generator
- A transducer
- A probe .

The generator is employed to convert the electrical power supplied as input into an electrical signal that powers the sensor. In order to transform an electronic input into vibration, a transducer is used. By increasing this vibration into a longitudinal vibration that creates a cavity in the sample, the vibration is used in the probe point. The cavitation produced by the ultrasonic energy disturbs the sample and makes it simple to break down the larger particles into smaller ones (Rodionova *et al*,2012).

2.23.2 Working principle of ultrasonicator

Ultrasonic sound pulses are used during the sonication procedure. Due to the pressure being applied, thousands of tiny vacuum bubbles are produced in the fluid during the procedure. The process of cavitation causes the created bubbles to burst into the fluid. In the cavitation field, bubbles collapse, creating ripples in the process that releases a tremendous amount of energy. The molecular connections between the water molecules are broken down as a consequence. The chemical bonds decrease, which causes the particles to start separating and allowing the mixing process to happen. The sound waves discharge energy, causing friction in the solution as a consequence. To keep the sample from burning up during and after the sonication procedure, ice blocks are used (Tian *et al*,2018).

Cavitation causes the liquids to disperse, homogenize, disintegrate, form emulsions, separate, and have sonochemical effects during the ultrasonication process. Regions of high pressure (known as compression) and low pressure are created in the liquid as a result of the introduction of high power ultrasound (known as rarefaction). The pace of ultrasound application determines how quickly these areas are formed (Fig 2.9). Small vacuum bubbles are formed in the liquid when low pressure is applied because this causes high-intensity ultrasonic vibrations to be generated. In the high-pressure cycle, balloons disintegrate as soon as they hit their saturation point. Cavitation is the name of this procedure. The liquid droplets during cavitation can discharge at speeds of up to 280 m/s (Tian *et al*,2018). The illustration below demonstrates the sound wave's propagation in the liquid resulting in the formation of bubbles and their collapse .

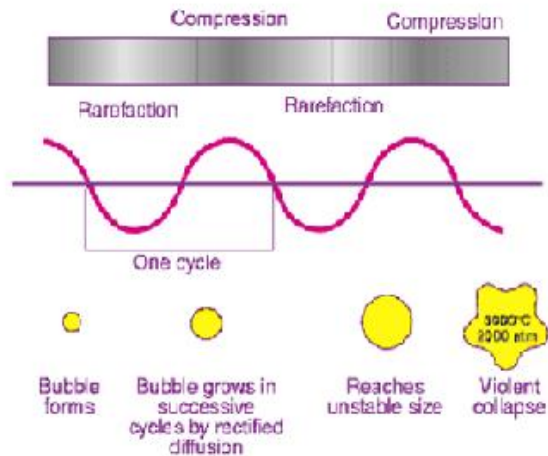


Figure 2.9: Working Principle of Ultrasonication (Dehnad *et al*,2014)

2.23.3 Methods of sonication

There are two sonication methods and they are:

- Direct sonication method
- Indirect sonication method

2.23.3.1 Direct ultrasonication method

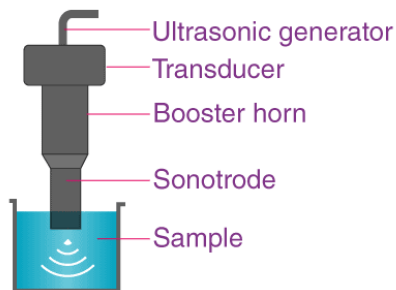


Figure 2.10: Direct Ultrasonication Set Up (Dehnad *et al*,2014).

The most popular sonication technique is direct sonication, which involves inserting the instrument right into the material (Fig 2.10). With this technique, the energy is delivered straight

from the probe to the material. The sample processing happens rapidly because this is a high-intensity procedure. The amount of the substance to be processed is calculated by measuring the diameter of the probe's point. These ends are either fixed titanium pieces or replaceable ones. The strength of sonication increases with decreasing tip width because the energy is concentrated in smaller spaces. Although the working capacity grows as the tip width does as well, the intensity decreases. The output of the large-diameter probes can be increased by using boosters and high gain horns (Isroi *et al*,2016).

2.23.3.2 Indirect ultrasonication method

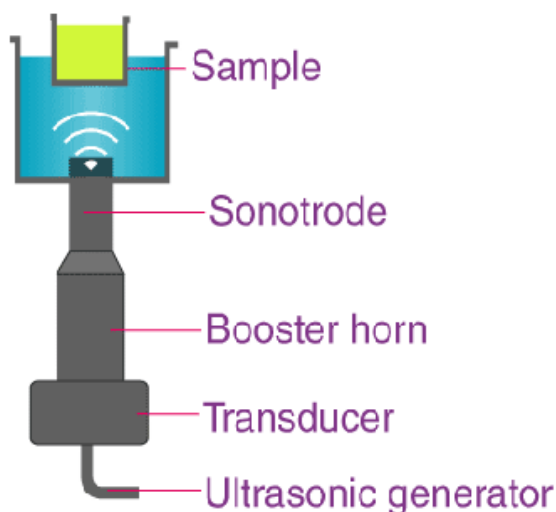


Figure 2.11: Indirect Ultrasonication Set Up (Dehnad *et al*,2014)

The sample and probe make no touch when using the indirect sonication technique. The high-intensity ultrasonic water is another name for this technique. Through the water, the trumpet transmits ultrasonic energy to various vessels holding the sample. Since it minimizes foaming and sample loss, this technique is favoured when the sample amount is small (Hassan *et al*,2018). Because it avoids sample contamination, the indirect sonication technique is used with

pathogenic or clean materials. The two indirect sonicators thought to be best for volume uses are cup horns and microplate horns (Figure 2.11).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Materials

Materials needed for this project includes raw material for the isolation of cellulose nanofibers as a reinforcement agent in the bio-nanocomposite which is a product of oil palm. The Oil Palm Empty Fruit Bunches OPEFB biomass was considered because of its relative availability as a byproduct of oil production from palm trees (Fig 3.1). The polymer matrix for the composite was starch sourced from sweet potato and Aloe Vera extract as the reducing and capping agent for Silver Nitrate and Silver Nanoparticles respectively. Silver Nanoparticles were incorporated as antimicrobial agent to transform the film to an active packaging film. Glycerol was used as the plasticizer and other materials needed are the chemicals required for bleaching and biomass pretreatments as tabulated in Table 3.1



Figure 3.1 : Oil Palm Empty Fruit Bunch

Table 3.1: Materials and Reagents Used

No	Materials/reagents	Manufacturer	Source
1	OPEFB		Palm farm
2	Potato Starch		Minna Market
3	Glycerol	Burgodine & Co.,India	Panlac stores Minna
4	Polyvinyl Alcohol	Kermel (UN No. 2265)	Chemistry lab Minna
5	Oven	Kregol Ltd, England	Chemistry lab Minna
6	NaOH	BDH Chemicals England	Panlac stores Minna
7	Aloe vera extract		Farm
8	AgNO ₃	Kermel (UN No. 2265)	Panlac stores Minna
9	Sodium hypochlorite	BDH Chemicals England	Panlac stores Minna

3.2 List of Equipment Required

The equipment used in this study were categorized into two types; production and analysis tools.

All equipment used for production were operated at standard temperature and pressure to

guarantee reliable results with high accuracy and precision. Table 3.2 shows the list of equipment used in this research work.

Table 3.2: Required Equipment

No	Equipment	Model	Source
1	Weighing Scale	Adventure –AR223CN	Chemical Engineering lab FUT
2	Ultrafine Blender	Gallenkamp(OEF/021/10)	Step B laboratory, FUT Minna
3	Magnetic Stirrer	PEC medical (USA 85-2)	Step B laboratory, FUT Minna
4	Ultrasonicator	Quantachrone Novawin	Step B laboratory, FUT Minna
5	Sieves	BSS (ASTM-IS460)	Step B laboratory, FUT Minna
6	SEM-EDS	PerkinElmer (634-BSS21)	Allschoolab scientific, Lagos
7	FTIR	PerkinElmer	Allschoolab scientific, Lagos
8	Water Bath Shaker	PEC medical (USA 85-2)	
9	TGA Analyzer	AlabTech (LSB-0455)	Allschoolab scientific, Lagos

3.3 Preparation of Raw Materials

3.3.1 Delignification of OPEFBs

To eliminate impurities, dry OPEFBs were sliced to a length of about 1 centimeter and boiled in water for 1 hour at 100° C. After being submerged in a 1000 ml solution of 6% NaOH for 11 hours at room temperature, 50 g of sliced OPEFBs were rinsed to a pH neutral state. OPEFBs were bleached by immersing them in 12% hypochlorite solution at ambient temperature for 5 hours. Before creating cellulose nanofibers, the acquired cellulose pulp was neutral pH rinsed and placed in a refrigerator.

3.3.2 Ultrasonication of cellulose nanofibers from OPEFBs

Using an electric mixer, the cellulose material was mixed with water. The concentration of the pulp slurry was then modified to between 1 and 2 percent by introducing water. The mixture was then run 30 times at 1500 revolutions per minute through an ultrafine mill. After that, the suspension was processed using ultrasonication for 1 to 3 hours at 40 Amplitude.

3.3.3 Preparation of aloe vera extract

Leaves of Aloe vera were gathered freshly from a neighboring yard. They were cleaned with distilled water and 10g portion was combined with 100ml of distilled water and heated for 10 minutes. The extract was then gathered, put in a container, and sifted through Whatman filter paper.

3.3.4 Preparation of silver nanoparticles

Aqueous solution (0.01M) of silver nitrate (AgNO_3) with different concentrations of Aloe Vera extract ranging from 1.0 ml to 5.0 ml were made in separate parts. To every concentration of the leaf extract, 10 ml of 0.01M AgNO_3 was added. After 25 minutes, the solution changed

color from light brown to darkish brown, this indicated that Silver Nanoparticles are formed. The final solutions of silver nanoparticles was later characterized with UV-Vis spectrophotometer.

3.4 Preparation of Reinforced Active Bio-nanocomposite Films

By using the solution casting technique, reinforced composite films with the inclusion of cellulose nanofibers were produced. In a ratio of 4:1, potato starch and PVA were combined and diluted in water. For 10 minutes, the mixture was heated to 90°C while being stirred. Granular flour underwent gelatinization in this stage. Glycerol to plasticize (0 and 20 wt%), 20 wt% AgNPs solution, and cellulose nanofibers (0, 2, 4, and 6 wt%) were added to the mixture, which was then agitated for 18 minutes. The substance was then moulded in a Teflon mould and dried in an electric oven for 48 hours at 45°C as illustrated in Fig 3.2. SPG-aNF-b is the designation for a set of PS/PVA cellulose nanocomposite materials. In this notation, S stands Starch, P represents PVA, G is glycerol, 'a' represents percentage of glycerol, NF stands for nanofiber content while 'b' stands for the nanofibers weight % in each sample.

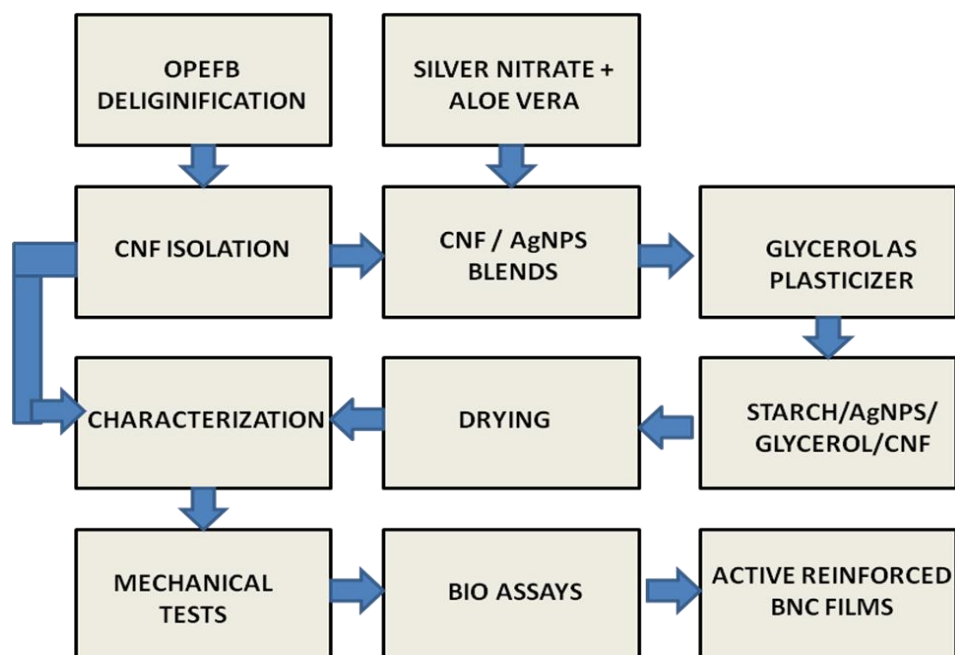


Figure 3.2: Process Diagram for Bio-nanocomposite Film Production

3.5 Characterizations

3.5.1 Characterization of cellulose nanofiber

Analysis using Scanning Electron Imaging (SEM). A field-emission scanning electron microscope (FE-SEM) (Sirion 200, FEI, Eindhoven, Netherlands) with an accelerating voltage of 5 kV was used to examine the morphology of the Cellulose Nanofiber (CNF). Prior to FE-SEM imaging, the freeze-dried CNF were covered with platinum using a vacuum sputter coater.

3.5.2 UV-vis spectrum of green synthesized silver nanoparticles (AgNPs)

Aloe vera extracts ranging in concentration from 1 to 5 milliliter and an aqueous solution of silver nitrate (0.01M) were made independently. 10 milliliter of the made 0.01M AgNO₃ solution was added to each sample of the leaf extract. The solution's colour shifted from light yellow to dark brown after 20 minutes, signifying the creation of Silver Nanoparticles (AgNPs).

A UV-Vis spectrophotometer was used to analyze the silver colloidal fluid that resulted. The method used to verify the formation of metal nanoparticles was UV-Vis spectroscopy. Using a UV-Vis spectrophotometer Shimadzu-UV 1800 and distilled water as a standard, the absorbance spectrum of the colloidal material was measured in the 200-800 nm region.

3.5.3 X-Ray diffraction spectra of bio-nanocomposite films

Utilizing a Philips PW3040/00 X'Pert MPD instrument, an X-ray diffractometer (XRD) was used to examine the crystallinity index of CNF (Eindhoven, Netherlands). The crystallinity index, CrI, was determined using equation (3.1) after the X-ray diffractogram was obtained in the 2θ range of 5 to 50°

$$\text{CrI} = \frac{I_{002} - I_{\text{am}}}{I_{002}} \times 100\% \quad (3.1)$$

whereas I_{002} is equal to $2\theta = 23^\circ$ and it refers to the peak of the crystalline portion of biomass (i.e., cellulose), and I_{am} refers to the peak at about $2\theta = 18^\circ$, which correlates to the amorphous region (Hemmati *et al.*, 2018).

3.5.4 Infrared spectra (FTIR)

Film with Starch, Polyvinylalcohol, glycerol 25% ,Cellulose Nanofibre 6wt% (SPG25NF6) and SPG0NF0 Control films' were analyzed with FTIR spectra using a Perkin Elmer FTIR/NIR Frontier model (Waltham, MA, USA) with a precision of 4 cm^{-1} between 4500 and 500 cm^{-1} .

3.5.5 Determination of antibacterial activity

Using disc diffusion technique, the antibacterial effects of the SPG20NF6 and reference films were evaluated against *Staphylococcus aureus* and *Escherichia coli*. In Mueller-Hinton agar dishes that were incubated at 37°C for 24 hours, a 6 mm film disc was utilized. By quantifying the zone of inhibition around the circumference of the disc (millimeter) after incubation, the antibacterial efficacy of the films was ascertained. Ceftriaxone as an antibiotic with known mechanism of action was used in the antimicrobial disc as a control.

3.5.6 Mechanical properties of bio-nanocomposite films

Using a micrometer, the thickness of films was determined at eight distinct places (Pittsburg, USA). Using the Instron Universal Testing 3367, the films' tensile strength (TS) and percent elongation at break (E%) were calculated. Each film was divided into 15 mm x 100 mm strips and stored at 25 °C and 50% Relative Humidity (RH) until analysis. The strip was placed between the device's jaws and drawn at a rate of 200 mm/s. The Bluehill 2 software (Norwood, MA, USA) was used to calculate the strain at fracture as well as the maximum force applied to the specimen at the moment of fracture.

3.5.7 Water barrier property

The different films' water permeability was tested in line with ASTM E96/E96M-16. Barrier cups with an exposed surface of 8 cm² were used to quantify water vapour transfer rates (WVT). The barrier containers were filled with distilled water (RH=100%) and then covered with securely sealed films of 0 wt% and 20 wt% glycerol as well as various CNF wt% 0, 2, 4, and 6. After that, the containers were put inside a desiccator box set to 23°C and 30% relative humidity (RH). Each film's weight changes as a consequence of water permeability were monitored over

the course of several days. The following algorithm was used to calculate WVT numbers (g/m².h):

$$WVTR = \Delta m / \Delta t \times 1/A \quad (3.2)$$

Where WVTR represents water vapor transmission rate (g/m²/hour), Δm is the difference in mass of the containers used (mg), Δt is the differences in time, and A is area (m²).

3.5.8 Thermogravimetric analysis

On a TA Q500 TGA, thermogravimetric analyses were carried out. The heating rate from ambient temperature to 650 °C was fixed at 5 °C per minute, and the nitrogen purge rate was 10 ml per minute.

CHAPTER FOUR

4.0

RESULTS AND DISCUSSION

4.1 Scanning Electron Microscopy (SEM) of Cellulose Nanofibre (CNF)

Isolation of CNF was done mechanically using ultrasonication method. CNF produced after 4 hours was characterized with SEM so as to examine ultrasonication's impact on raw OPEFB (Figure 4.1). The average diameter of the CNF produced was determined using Image J software. The Scanning Electron Micrographs in Fig 4.2 shows that the OPEFB cellulose size was reduced after about 3.5 hours of ultrasonication to fibrillated cellulose. Besides, the SEM images

showed raw OPEFB to be in lower magnification of micrometer range (X1,000) when compared to that of the CNF magnification (X10,000) of nanometer range. This result has indicated that the hydrogen-bonding (HB) linking the CNF was reduced due to ultrasonication; the smallest diameters of CNF is obtainable after 4 hours of ultrasonication. This was sufficient to prove that ultrasonication has the ability to break the weak forces of attraction among cellulosic fibers bonded together by hydrogen bonds. (Ojogbo *et al.*,2019).

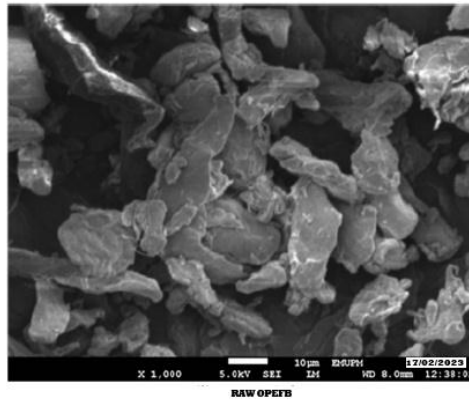


Figure
of Raw OPEBF

4.1: SEM Micrograph

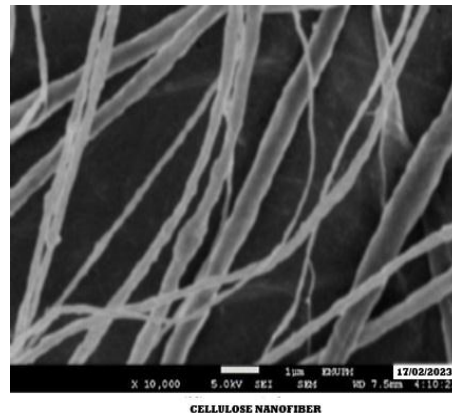


Figure 4.2: SEM Micrograph of Cellulose Nanofiber

4.2 UV-Vis spectrum of Green Synthesized Silver Nanoparticles (AgNPs)

AgNO₃ solution before and after adding extract of Aloe Vera leaf as the reducing and capping agent is shown in Fig 4.3. The UV–vis absorptions spectra of silver nanoparticles using varied concentrations of Aloe Vera (1, 2, 3, 4 and 5 ml) are recorded and depicted in Fig 5 with absorbance intensities of 0.6, 0.8, 1.15, 1.3 and 1.75 respectively. Production of silver nanoparticles by reduction reaction was depicted via solution change of color from light brown to darkish color and this can be noticed with visual inspections (Figure 4.3). Metallic nanoparticulate materials possess electrons that are free, the free electrons can produce surface plasmon resonance (SPR) absorption band (the peaks), because of the mutual vibration of the free electrons of metallic nanoparticles in resonance with light wave. The peaks sighted in Fig 4.4 show the characteristics of surface plasmon resonance of silver nanoparticles. The absorption spectra exhibit a gradual decrease of the absorbance, accompanied by a shift in the wavelength from 435 to 423 nm. The UV–Vis spectrum indicates the significant roles of AgNO₃ with the phytochemicals present in the leaves for the formation of silver nanoparticles.

The absorbance intensity is increased when the concentrations of the leaf extracts is increased. It is noticed that the surface plasmon peaks occurs at 422 nm and gradually tilted in the direction of low wavelengths when the concentrations are high. The shifts depend on the sizes of the particles as well as shapes (Kelly *et al.*, 2003). In accordance with Wu *et al.*(2021) findings, these bands correspond to the absorption effected by the colloids of silver nanoparticles in the sub-region (410–460 nm) because of the excited surface plasmons. To this effect, the samples with higher concentrations were used for more analytic researches. As the concentrations of Aloe Vera extract increase, there is increase in the quantity of the bio-active compounds needed to reduce Ag⁺ to Ag⁰



Figure 4.3:AgNPs Green Synthesis (a) AgNO₃ and (b) AgNPs

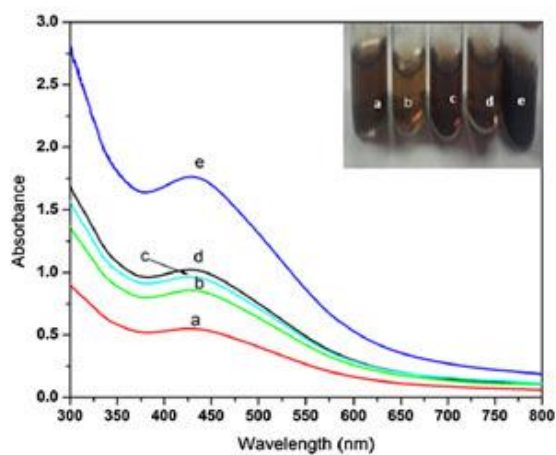


Figure 4.4 :UV-Vis Spectra of Synthesized AgNPs at Different Concentrations (a.u)

4.3. X-Ray Diffraction Spectra of Bio-nanocomposite films

The Crystallinity of nanocomposite films and X-ray diffraction profiles can be seen in Fig 4.5 and Figure 4.6 respectively. Film with 0% glycerol shows a higher crystallinity index in comparison with the films incorporated with glycerol. Chen *et al.*(2019) gave the report of polyvinyl acid (PVA) diffraction peaks at 18.5° . Figs 4.6 (a) and 4.6 (b), shows that every peak in normal starch was not depicted while all pattern of diffractions look to be within range of PVA's; despite the fact that in bio-nanocomposite films, starch content there is about 4 times. This is attributable to the degradation of starch granules crystallinity orchestrated by gelatinization while mixing the entire system using PVA at 92°C for 12 minutes. Besides, it is glaring that ultrasonication immensely affected the crystallinity of cellulose nanofiber having decreased it in a significant measure. This has indicated that the CNF structures can be disrupted by ultrasonication process leading to reduction in the films' crystallinity indices. This claim was corroborated by Isroi *et al.*(2016) and it indicated that ultrasonication has the capacity to disintegrate the H-bond of cellulosic fibers which is the cause of damages to the crySTALLINE portion of the cellulose nanofibers. Both Figs 4.5(a) and 4.5(b) show that increase in CNF lead to gradual increase in the differential peak at 22.5° . But then, incorporation of glycerol has been traced to the reason for the change in the diffraction pattern of the bio-nanocomposites. This entails that the crystallinity of the bio-nanocomposites was influenced by PVA present, glycerol molecule and CNF. Due to the presence of glycerol, the crystalline index of starchy film is reduced due to molecular interaction within the starch chains. As CNF increases, the crystalline index of the nanocomposites is increased. Krishnaswamy *et al.*(2014) investigated that the inherent crystallinity in CNF is helpful in improving the crystalline index of the bio-

nanocomposites. As the percentage of crystallinity of the nanocomposites increases, the tensile strength is equally improved due to the compact structures in a well-ordered alignment .

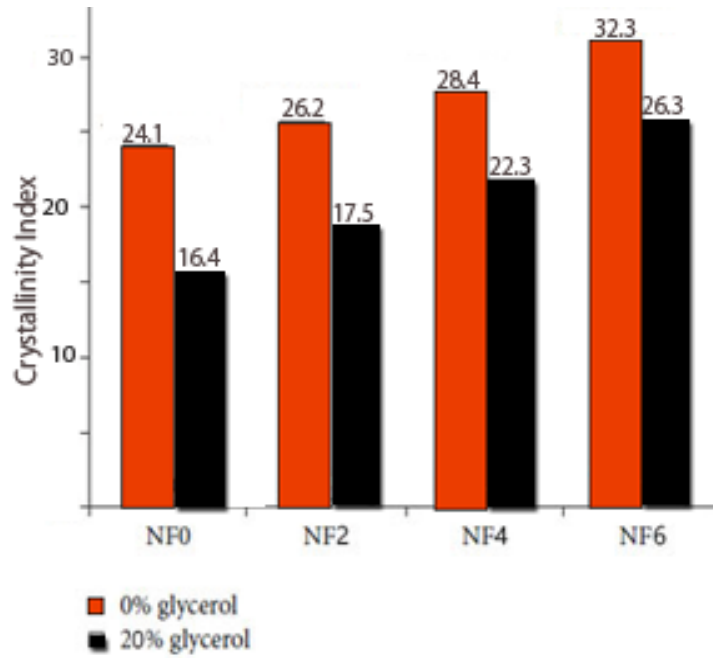


Figure 4.5: Crystallinity Index Profile of PS/PVA Cellulose Nanocomposite Films

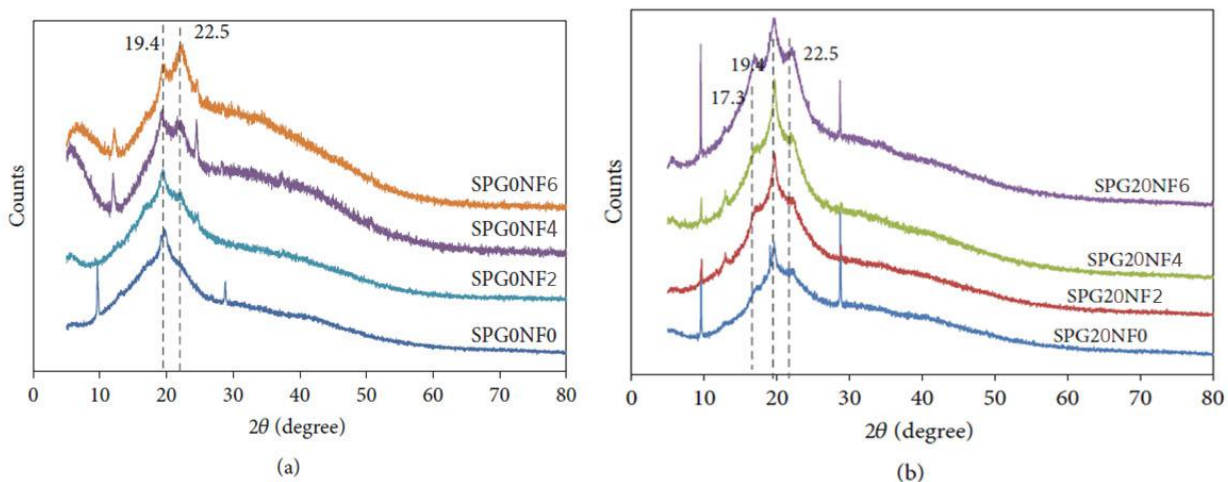


Figure 4.6: XRD Profiles of SP/PVA Bio-nanocomposite Films (a) without glycerol; (b) with glycerol.(Vertical Scale in Arbitrary Unit-a.u.)

4.4 FTIR Analysis of Potato Starch Nanocomposite Film

FTIR analysis for SPG0NF0 (control) and SPG20NF6 nanocomposites are given in Figure 4.7. Methylcellulose structures are revealed by its characteristics bands in all films. Bands at 1470 cm^{-1} , 1367 cm^{-1} , 1335 cm^{-1} , and 957 cm^{-1} are major features of C-H bond of C-H₂ and C-H₃. Bands at 1060 cm^{-1} depicts characteristics of COC bonds for ethers existence in methylcellulose. The set of bands recorded at 2910 cm^{-1} can be attributed to the CH bonds and also stretch in the OH bonds at the hydroxyl functional groups available at the methylcellulose structure (Oliveira *et al.*,2015). Presence of phenols sourced from Aloe Vera extracts incorporated into the SPG25NF6 film was discovered with the observed band at 1596 cm^{-1} , this characterizes C=C-C bonds peculiar to benzene groups (the aromatic rings). Moreover, discovery of a minute band at 1411 cm^{-1} has served as support to the previous claim and this has confirmed presence of bio-active materials in the methylcellulose matrix.

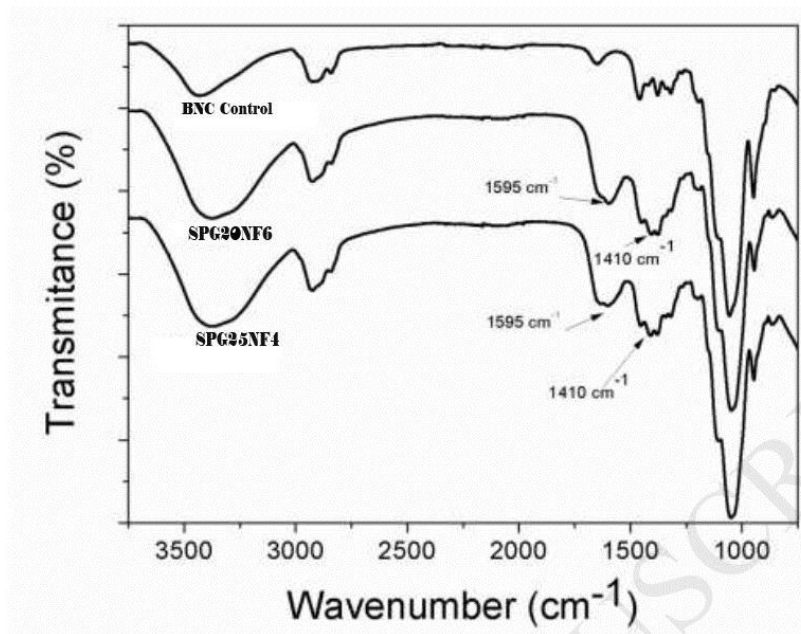


Figure 4.7: FTIR Spectra of Bio-nanocomposite Films

4.5 Determination of Antibacterial Activity

The films' antimicrobial activities were examined using inhibition zone method for halo of gram-positive bacteria *S. aureus* and gram-negative bacteria *E. coli*. The results are depicted in Table 4.1. The observation showed that the BNC Control sample had no active effect. Other films incorporated with Aloe Vera extracts were unable to show any significant inhibition to the microbes to a large extent under the conditions tested; because the bioactive compound presence was in a low concentrations. (Nazarenko *et al.*, 2007). SPG25NF6 film incorporated with AgNPs showed an improved antimicrobial properties on both microbes. An inhibition zone was discovered for the gram-negative bacteria, *E. coli*, 8.0 ± 0.5 mm. For the gram-positive bacteria, *S. aureus*, a halo of 7.0 ± 0.3 mm was observed as depicted in Fig 4.8. An antibiotics Ceftriaxone was part of the experimental set-up as a reference point, this indicated an inhibitory halo of 14 ± 0.3 mm

for *E. coli* and 14 ± 0.4 mm for *S. aureus*. These results agree with the outcomes of Hemmati *et al.*, (2018). The differences noticed in the halo is attributable to the fact that the gram-negative bacteria, *E. coli*, has a thinner cell wall due to a smaller layer of peptidoglycan. Several AgNPs mechanisms against bacterial growth are described in the literature (Khan *et al.*,2012). Mechanisms include the accumulation of AgNPs on the bacterial membrane, blocking the exchanges with the external environment and decreasing its integrity. Size of the nanomaterials has great impact on this mechanism .

Table 4.1: Antimicrobial Investigation of Active BNC Films

Sample	<i>Escherichia</i>	<i>Staphylococcus aureus</i>
BNC Control	_____	_____
Aloe Vera Extract	3.5 ± 0.3mm	4.0 ± 0.3mm
Active BNC	8.0 ± 0.5mm	7.0 ± 0.3mm
Ceftriaxone	14.0 ± 0.3mm	14.0 ± 0.4mm

Without inhibition: _____

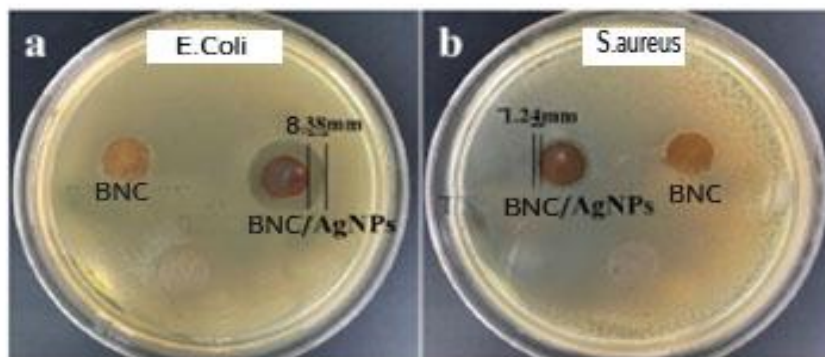


Figure 4.8: The Incubation of *E. coli* , *S.aureus* with BNC Control and BNC/AgNPs

4.6 Mechanical Tests

Tensile strength of Potato Starch Bio-nanocomposite films is depicted in Fig 4.9. While the tensile strength of unalloyed bio-nanocomposite film was 47.4 MPa. Obviously, the addition of cellulose nanofibers improved tensile strength of the film. With increasing cellulose nanofibers content up to 7%, tensile strength of PS/PVA films without glycerol improved 46% from 47.30 MPa to 60.76 MPa. Both matrix PS/PVA and cellulose nanofibers as filler have identical chemical nature, that is, hydroxyl groups. Therefore, a good affinity between them could happen, causing the formation of strong hydrogen bonded percolation cellulose nanofibers network at their interfaces and giving reinforcement effect. The increase of tensile strength due to the addition of cellulose nanofibers indicates that interfacial adhesion between PS/PVA as polymer matrix and cellulose nanofibers occurred.

Figure 4.9 exhibits that there is a difference in the tensile strength between nanocomposite films with glycerol and the films without glycerol. Glycerol reduces the inter-molecular and intra-molecular forces of the polysaccharide chain so that the film structure becomes more flexible (Hassan *et al.*,2018). The addition of glycerol caused tensile strength to decrease significantly. Fig 4.10 indicates that increase in cellulose nanofibers content decreased the elongation at break. The films with 0% glycerol displays lower elongation values as compared with the films with glycerol added. Incorporation of the plasticizer in Potato Starch has the tendency to disrupt the double helices formed through amyloses in conjunction with amylopectin branches; then there is reduction in the affinity between the molecular structures of amyloses and amylopectins; thereby increasing the film's flexibility (Dong *et al.*,2013).

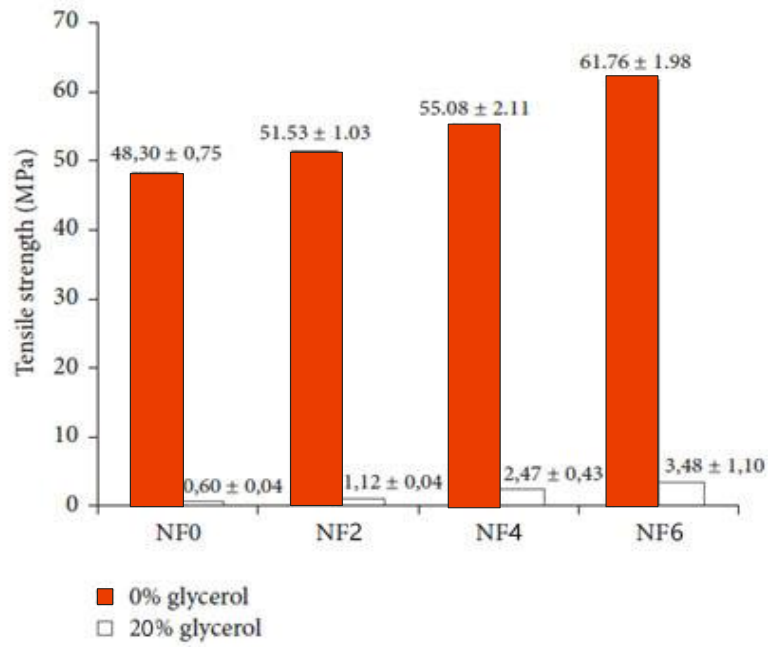


Figure 4.9: Tensile Strength of PS/PVA Nanocomposite Films

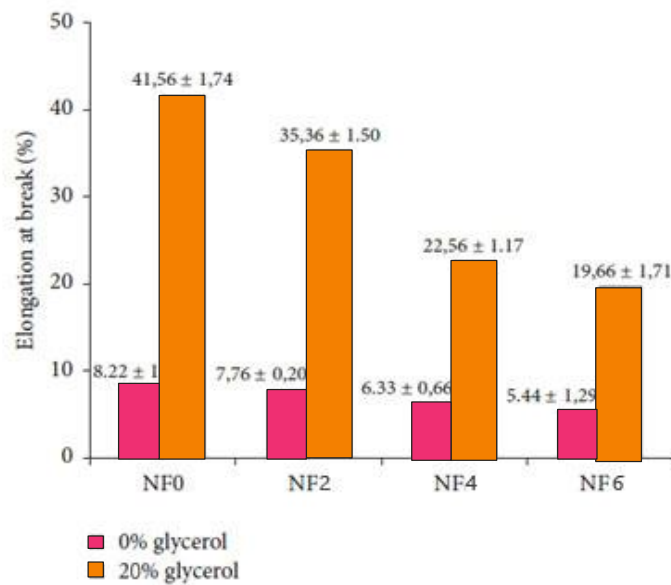


Figure 4.10: Elongation at Break of PS/PVA Nanocomposite Films

4.7 Water Vapor Transmission Rate (WVTR)

The WVTR of PS/PVA bio-nanocomposite films is shown in Fig 4.11. Incorporation of CNF reduces the WVTR. The cause is likely to be because the cellulose nanofibers that renders the direct diffusion of the water molecules into the composites matrix tortuous; thereby blocking the water vapor passage through the films (Ghaderi *et al.*,2014). The films void of glycerol indicates that the WVTR is lower when compared to the films with glycerol added. Glycerol is known to be hydrophilic, this can improve the hygroscopic properties giving it the ability to absorb more water from the ambience air thereby increasing the WVTR of the material.

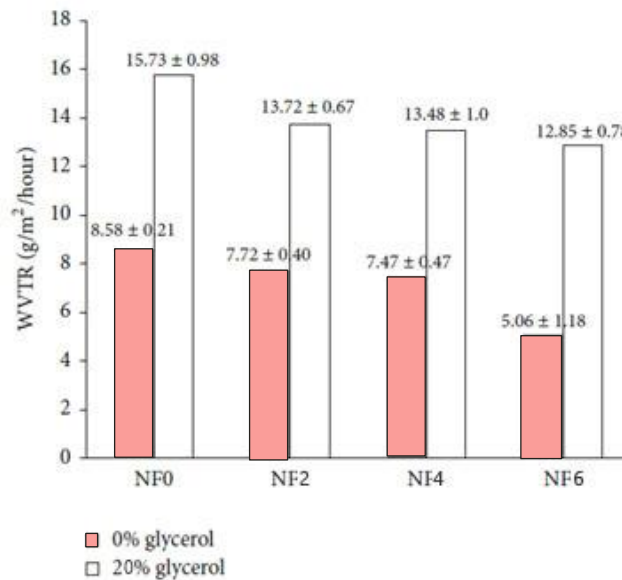


Figure 4.11: Water Vapour Transmission Rate of PS/PVA Nanocomposite Films

4.8 Thermogravimetric Analysis

Thermogravimetric analysis was used to determine the effect of loading CNF to the thermal stability of the bio-nanocomposite films. Fig 4.12 shows the TGA curve of the bio-nanocomposite film for each treatment. The gradual loss in weight at around 80–120°C is attributed to the vaporization of bound water. The abrupt loss in weight starting around 250°C

and ending at approximately 350°C is due to the pyrolysis of thermoplastic starch and cellulose. This stage corresponds to the major weight loss of the film. Further loss in weight beyond 350°C could be ascribed to the decomposition of the remaining carbonaceous material (Suvith and Philip, 2018). The temperature at maximum rate of degradation, T_{max} , of the films decreased by the addition of CNF. This indicates that the thermal stability of the CNF-reinforced films decreased relative to Temperature. This result is contrary to those reported in literature that addition of crystalline cellulose could enhance thermal stability of the films. The lowering of T_{max} could be attributed to the lower thermal stability of ultrasonicated CNF. The ultrasonication of the isolated CNF in this study may have been extensive due to longer exposure of the fibres.

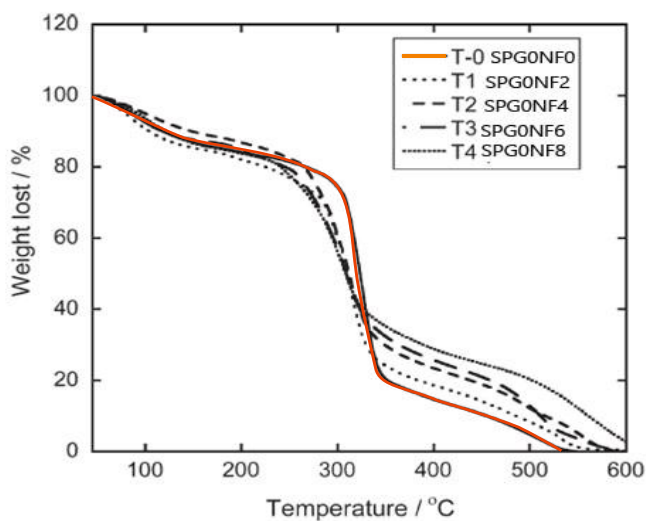


Figure 4.12: TGA Curves of Bio-nanocomposite Films with Varied CNF wt%

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

This research proposed replacement of petroleum-based packaging films in food industries with Bio-nanocomposite films produced from Potato Starch as the polymer matrix , glycerol as the plasticizer and reinforced with Cellulose Nanofibers derived from OPEFB. The following are the conclusions:

5.1 Conclusion

1. Isolation of Cellulose Nanofiber from Oil Palm Empty Fruit Bunches was accomplished mechanically using grinding and ultrasonication processes yielding nano-sized fibers of diameter 40-100 nm according to the SEM images, cellulose nanofiber showed to be a good candidate for reinforcements in the bio-nanocomposite films.
2. Bio-reduction of AgNO_3 using Aloe Vera extract as the reducing and capping agent was successfully accomplished as confirmed by the UV-Vis spectrum of the green synthesized AgNPs with characteristic antimicrobial properties in the composite film.
3. Reinforced and Active Bio-nanocomposite films were formulated at an optimum condition of 45 deg Celsius within 48 h using wet processing (solution casting) with up to 46% improvement in tensile strength, 17% modification in elasticity and antibacterial bioactivity properties.
4. The research has also demonstrated that addition of the cellulose nanofibers lowered the water vapour transmission rate while glycerol incorporation increased it. Also, the thermal stability of the films decreased as the thermal load increases, but then, contrary to

common findings, increase in the quantity of CNF in the films did not correspond to higher thermal stability.

5. Films without glycerol exhibited better tensile strength, however, there was reduction in elongation at break yielding a relatively brittle film. Addition of CNF also resulted into the reduction of the film elongation at break while increase in the quantity of CNF increased the film crystallinity. Glycerol incorporation also lowered the film's crystallinity when compared to the films without glycerol.
6. The presence of the phenolic compounds (as shown in the characterization analysis) from the Aloe Vera extract is responsible for the reduction of AgNO₃, capping of AgNPs and antimicrobial activity of AgNPs in the film. However, due to a larger surface area ratio of metal nanoparticles, AgNPs showed a better antimicrobial action than Aloe vera extract.

5.2 Recommendations

This current work opens the following research pathways for more work to expand the boundaries of knowledge in the use of bio-nanocomposites in food packaging materials.

1. More in-depth researches are needed in ascertaining the potential risks of about 10,000 chemicals approved to be used in Food Contact Materials (FCM) whose toxicity are not fully investigated yet.
2. Safe use of nanomaterials in food packaging as well as migration potentials of chemicals are not fully understood by research community. Hence, more toxicity

studies are needed before these materials can be incorporated as reinforcement agents in food packaging materials.

3. Further works are needed to be done in finding cost effective and eco-friendly raw materials and/or processes in the production of nanocellulose crystals and fibres for the reinforcement of biopolymers. This will reduce the cost of biopolymer packaging materials and make it acceptable to food industries .

5.3 Contribution to Knowledge

Active Bio-nanocomposite films from potato starch for food packaging were developed in this research work. Incorporation of 7 wt % Cellulose Nanofiber (CNF), 20 wt % glycerol and 20 wt % silver nanoparticles respectively improved the films' tensile strength by 46%, increased the films' matrix elongation at break by 17% and indicated antimicrobial property of the films within 8.0 to 7.0 mm of inhibition zones against gram positive and gram negative bacterial. Due to the ultrasonification method used in the CNF isolation, the films' thermal stability decreased with increase in CNF quantities in contradiction to other findings; abrupt weight loss between 235 °C - 345°C thermal load was more pronounced in the films with higher concentration of CNF (Fig 4.12).

REFERENCES

- Arfat, Y.A., Benjakul, S., Prodpran, T., Sumpavapol, P., Songtipya, P., (2015). Physico-mechanical characterization and antimicrobial properties of fish protein isolate/fish skin gelatin—zinc oxide (ZnO) nanocomposite films. *Food Bioprocess Tech.* 9, 101–112.
- Bai, H., C. Huang, H. Xiu, Q. Zhang, H. Deng, K. Wang, F. Chen, Q. Fu, (2014). Significantly Improving Oxygen Barrier Properties of Polylactide via Constructing Parallel-Aligned Shish-Kebab-Like Crystals with Well-Interlocked Boundaries, *Biomacromolecules*. 15 1507–1514. <https://doi.org/10.1021/BM500167U>.
- Beigzadeh G, S., Esmaili, M., Almasi, H., (2016). Characterization of chitosan–nanoclay bio-nanocomposite active films containing milk thistle extract. *Int. J. Bio. Macromol.* 613–621.
- Biji, K.B., Ravishankar, C.N., Mohan, C.O., Srinivasa Gopal, T.K., (2015). Smart packaging systems for food applications: a review. *J. Food Sci. Technol.* 52, 6125– 6135.
- Brinchi, L. & Cotana, F., (2013). Production of nanocrystal cellulose from lignocellulosic biomass: Technology and applications. *Carbohydrate Polymer.* ; 94: 154-169
- Chen, M.J., Cerqueira, M.A., Ruiz, H.A., Fougnes, C., Richel, A., Vicente, A.A., Teixeira, J.A., Aguedo, M., (2019). Use of wheat bran arabinoxylans in chitosan-based films: effect on physicochemical properties. *Ind. Crops Prod.* 66, 305–311.
- Chuacharoen, T., Sabliov, C.M., Smith, T., (2016). Development of gluten/ titanium dioxide nanocomposites for active food packaging. *Carbohydr. Polym.* 124, 337–346.
- Dehnad, D., Mirzaei, H., Emam-Djomeh, Z., Jafari, S. M., & Dadashi, S (2014). Thermal and antimicrobial properties of chitosan-nanocellulose films for extending shelf life of ground meat. *Carbohydrate Polymers*, 109, 148-154.
- Demirtas, I., Erenler, R., Elmastas, M., & Goktasoglu, A (2013). Studies on the antioxidant potential of flavones of *Allium vineale* isolated from its water-soluble fraction. *Food Chemistry*, 136(1), 34-40.
- Denilson, S. P., Suzelei, M., & Michel, R. V (2003). TEMPO-Mediated Oxidation of Cellulose III. *Biomacromolecules*, 4, 1417-1425.
- Duncan, T.V., (2011). Applications of nanotechnology in food packaging and food safety: barrier materials, antimicrobials and sensors. *J. Colloid Interface Sci.* 363, 1–24.
- Dong, H., Snyder, J. F., Williams, K. S., & Andzelm, J. W (2013). Cation-Induced Hydrogels of Cellulose Nanofibrils with Tunable Moduli. *Biomacromolecules*, 14(9), 3338-3345.

- Duran, N., Duran, M., DeJesus, M. B., Seabra, A. B., Favaro, W. J., & Nakazato, G (2016). Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity. *Nanomedicine*, 12(3), 789-799.
- Echegoyen, Y., Rodríguez, S., Nerín, C., (2016). Nanoclay migration from food packaging materials. *Food Addit. Contam.* 33, 530–539.
- El-Wakil, N.A., Hassan, E.A., Abou-Zeid, R.E., Dufresne, A., (2015). Development of wheat gluten/nanocellulose/ titanium dioxide nanocomposites for active food packaging. *Carbohydr. Polym.* 124, 337–346.
- European Commission, (2009). Commission Regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food. *Off. J. Eur. Union*, pp. 3–11. European Parliament and Council, 2011. Regulation (EU) No 10/2011 of the European Parliament and of the Council of 14 January 2011 on plastic materials and articles intended to come into contact with food. *Off. J. Eur. Union* pp. 1–89.
- Fajardo, A.R., Pereira, A.G.B., Muniz, E.C., (2015). Hydrogels nanocomposites based on crystals, whiskers and fibrils derived from biopolymers. In: Thakur, K.V., Thakur, K.M. (Eds.), *Eco-friendly Polymer Nanocomposites: Chemistry and Applications*. Springer India, New Delhi.
- Fakhouri, F.M., Costa, D., Yamashita, F., Martelli, S.M., Jesus, R.C., Alganer, K., Collares-Queiroz, F.P., Innocentini-Mei, L.H., (2019). Comparative study of processing methods for starch/gelatin films. *Carbohydr. Polym.* 95, 681–689.
- Fernandes, S.C.M., Oliveira, L., Freire, C.S.R., Silvestre, A.J.D., Neto, C.P., Gandini, A., Desbrieres, J., (2019). Novel transparent nanocomposite films based on chitosan and bacterial cellulose. *Green Chem.* 11, 2013–2019.
- Fukuzumi, H., Fujisawa, S., Saito, T., & Isogai, A (2013). Selective Permeation of Hydrogen Gas Using Cellulose Nanofibril Film. *Biomacromolecules*, 14(5), 1705-1709.
- Ghaderi, M., Mousavi, M., Yousefi, H., and Labbafi, M (2014). “All-cellulose nanocomposite film made from bagasse cellulose nanofibers for food packaging application,” *Carbohydrate Polymers* 104, 59-65. DOI: 10.1016/j.carbpol.2014.01.013
- Gil-López, D. L., Lois-Correa, J. A., Sánchez-Pardo, M. E., Domínguez-Crespo, M. A., Torres-Huerta, A. M., Rodríguez-Salazar, A. E., and Orta-Guzmán, V. N (2019). “Production of dietary fibers from sugarcane bagasse and sugarcane tops using microwave-assisted

- Goh, K., Heising, J. K., Yuan, Y., Karahan, H. E., Wei, L., Zhai, S., Chen, Y (2016). Sandwich-Architected Poly(lactic acid)–Graphene Composite Food Packaging Films. *ACS Applied Materials & Interfaces*, 8(15), 9994-10004.
- Hakalahti, M., Salminen, A., Seppala, J., Tammelin, T., & Hanninen, T (2015). Effect of interfibrillar PVA bridging on water stability and mechanical properties of TEMPO/NaClO₂ oxidized cellulosic nanofibril films. *Carbohydr Polym*, 126, 78-82.
- Hao, J., Xu, S., Xu, N., Li, D., Linhardt, R. J., & Zhang, Z (2017). Impact of degree of oxidation on the physicochemical properties of microcrystalline cellulose. *Carbohydrate Polymers*, 155, 483-490.
- Hassan, E. A., Fadel, S. M., & Hassan, M. L (2018). Influence of TEMPO-oxidized NFC on the mechanical, barrier properties and nisin release of hydroxypropyl methylcellulose bioactive films. *International Journal of Biological Macromolecules*, 113, 616-622.
- Hayaka Fukuzumi, Tsuguyuki Saito, Tadahisa Iwata, Yoshiaki Kumamoto, & Isogai, A (2009). Transparent and High Gas Barrier Films of Cellulose Nanofibers Prepared by TEMPO-Mediated Oxidation. *Biomacromolecules*, 10(1), 162-165.
- Hemmati, F., Jafari, S. M., Kashaninejad, M., & Barani Motlagh, M (2018). Synthesis and characterization of cellulose nanocrystals derived from walnut shell agricultural residues. *International Journal of Biological Macromolecules*, 120(Pt A), 1216-1224.
- Herawan T and Rivani M. (2013) Sandwich-Architected Poly(lactic acid)–Graphene Composite Food Packaging Films. *ACS Applied Materials & Interfaces*, 8(15), 9994-10004
- Homma, I., Fukuzumi, H., Saito, T., & Isogai, A (2013). Effects of carboxyl-group counter-ions on biodegradation behaviors of TEMPO-oxidized cellulose fibers and nanofibril films. *Cellulose*, 20(5), 2505-2515.
- Isroi, Y., Cifriadi A;Panji T. Bioplastic production from oil palm empty fruit bunch. *International Conference on Biomass 2016: 10-11th October 2016, Bogor, Indonesia.*
- Jafari, S. M., Bahrami, I., Dehnad, D., & Shahidi, S. A (2018). The influence of nanocellulose coating on saffron quality during storage. *Carbohydrate Polymers*, 181, 536-542.
- Jeeva K, Thiyagarajan M, Elangovan V, Geetha N, Venkatachalam P (2014a) *Caesalpinia coriaria* leaf extracts mediated biosynthesis of metallic silver nanoparticles and their antibacterial activity
- Jiang, C., Oporto, G. S., Zhong, T., & Jaczynski, J (2015). TEMPO nanofibrillated cellulose as template for controlled release of antimicrobial copper from PVA films. *Cellulose*, 23(1), 713-722.

- Kelly L, Coronado E, Zhao LL, Schatz GC (2003). The optical properties of metal nanoparticles: the influence of size, shape and dielectric environment. *J Phy Chem B* 107:668–677
- Khan Z, Hussain JI, Hashimi AA (2012) Shape-directing role of cetyltrimethylammonium bromide in the green synthesis of Agnanoparticles using neem (*Azadirachta indica*) leaf extract. *Coll Surf B* 95:229–234
- Krishnaswamy, K., Vali, H., & Orsat, V (2014). Value-adding to grape waste: Green synthesis of gold nanoparticles. *Journal of Food Engineering*, 142, 210-220.
- Mackevica, A., Olsson, M. E., & Hansen, S. F (2016). Silver nanoparticle release from commercially available plastic food containers into food simulants. *Journal of Nanoparticle Research*, 18(1).
- Maestre, R., Micol, V., Funes, L., & Medina, I (2010). Incorporation and Interaction of Grape Seed Extract in Membranes and Relation with Efficacy in Muscle Foods. *Journal of Agricultural and Food Chemistry*, 58(14), 8365-8374.
- Makaremi, M., Pasbakhsh, P., Cavallaro, G., Lazzara, G., Aw, Y. K., Lee, S. M., & Milioto, S (2017). Effect of Morphology and Size of Halloysite Nanotubes on Functional Pectin Bio-nanocomposites for Food Packaging Applications. *ACS Applied Materials & Interfaces*, 9(20), 17476-17488.
- Mhd Haniffa, M. A. C., Ching, Y. C., Chuah, C. H., Yong Ching, K., Nazri, N., Abdullah, L. C., & Nai-Shang, L (2017). Effect of TEMPO-oxidization and rapid cooling on thermo-structural properties of nanocellulose. *Carbohydrate Polymers*, 173, 91-99.
- Moran JI; Alvarez V A; Cyras V P; Vzquez A. (2013). Extraction of cellulose and preparation of nanocellulose from sisal fibers. *Cellulose*. 15:149-159.
- Nazarenko, S., P. Meneghetti, P. Julmon, B.G. Olson, S. Qutubuddin,(2007). Gas barrier of polystyrene montmorillonite clay nanocomposites: Effect of mineral layer aggregation, *J. Polym. Sci. Part B Polym. Phys.* 1733–1753. <https://doi.org/10.1002/POLB.21181>.
- Ojogbo, E., E.O. Ogunsona, T.H. Mekonnen,(2019). Chemical and physical modifications of starch for renewable polymeric materials, *Mater. Today Sustain.* 7–8 (2020) 100028. <https://doi.org/10.1016/j.mtsust.2019.100028>.
- Osman, T.,; Alvarez V A; Cyras V P; Vzquez A. (2019). Extraction of cellulose and preparation of nanocellulose from sisal fibers. *Cellulose*. 2019; 15:149-159.
- Quiferrada, P., Choy, Y. Y., Calvert, C. C., Waterhouse, A. L., & Lamuela-Raventos, R. M (2016). Use of metabolomics and lipidomics to evaluate the hypocholesterolemic effect

- of Proanthocyanidins from grape seed in a pig model. *Molecular Nutrition & Food Research*, 60(10), 2219-2227.
- Raveendran P, Fu J, Wallen SL (2003) Completely green synthesis and stabilization of metal nanoparticles. *J Am Chem Soc* 125:13940–13941 Reddy NJ, Vali DN, Rani M, Rani SS (2014)
- Rodionova, G., Eriksen, Y., & Gregersen, P (2012). TEMPO-oxidized cellulose nanofiber films: effect of surface morphology on water resistance. *Cellulose*, 19(4), 1115-1123
- Saito, T., Shibata, I., Isogai, A., Suguri, N., & Sumikawa, N (2005). Distribution of carboxylate groups introduced into cotton linters by the TEMPO-mediated oxidation. *Carbohydrate Polymers*, 61(4), 414-419.
- Sathishkumar G, Gobinath C, Karpagam K, Hemamalini V, Premkumar K, Sivaramakrishnan S (2012). Phyto-synthesis of silver nano scale particles using *Morinda citrifolia* L. and its inhibitory activity against human pathogens. *Coll Surf B* 95:235–240
- Sharma, S. D., Meeran, S. M., & Katiyar, S. K (2007). Dietary grape seed proanthocyanidins inhibit UVB-induced oxidative stress and activation of mitogen-activated protein kinases and nuclear factor-kappaB signaling in in vivo SKH-1 hairless mice. *Molecular cancer therapeutics*, 6(3), 995-1005.
- Shen, Z., Luo, Y., Wang, Q., Wang, X., & Sun, R (2014). High-value utilization of lignin to synthesize Ag nanoparticles with detection capacity for Hg(2)(+). *ACS Applied Materials Interfaces*, 6(18), 16147-16155.
- Suvith, O. P., & Philip, D. Y. (2018) Catalytic degradation of methylene blue using biosynthesized gold and silver nanoparticles. *Spectrochim Acta A Mol Biomol Spectrosc* 118:526–532
- Tian, Y., Pukanen, A., Alakomi, H. L., Uusitupa, A., Saarela, M., & Yang, B (2018). Antioxidative and antibacterial activities of aqueous ethanol extracts of berries, leaves, and branches of berry plants. *Food Research International*, 106, 291-303.
- Torstensen, J. O., Liu, M., Jin, S. A., Deng, L., Hawari, A. I., Syverud, K., . . . Gregersen, O. W (2018). Swelling and Free-Volume Characteristics of TEMPO-Oxidized Cellulose Nanofibril Films. *Biomacromolecules*, 19(3), 1016-1025.
- Tunc, S., Angellier, H., Cahyana, Y., Chalier, P., Gontard, N., & Gastaldi, E (2007). Functional properties of wheat gluten/montmorillonite nanocomposite films processed by casting. *Journal of Membrane Science*, 289(1-2), 159-168.

- Vijaykumar M, Priya K, Nancy FT, Noorlidaha A, Ahmed ABA (2013) Biosynthesis, characterization and anti-bacterial effect of plant-mediated silver nanoparticles using *Artemisia nilagirica*. *Ind Crops Prod* 41:235–240
- Wang, Z., Xia, T., & Liu, S (2015). Mechanisms of nanosilver-induced toxicological effects: more attention should be paid to its sublethal effects. *Nanoscale*, 7(17), 7470-7481.
- Wu, F., Misra, M., A.K. Mohanty, (2021). Challenges and new opportunities on barrier performance of biodegradable polymers for sustainable packaging, *Prog. Polym. Sci.* 117 (2021) 101395. <https://doi.org/10.1016/j.progpolymsci.2021.101395>.
- Yokohama K, Welchons DR (2007) The conjugation of amyloid beta protein on the gold colloidal nanoparticles surfaces. *Nanotechnology* 18:105101–105107
- Zhao. X., E. van der Heide, T. Zhang, D. LiuX. Zhao, E. v. d. H., T. Zhang and D. Liu, Delignification of Sugarcane Bagasse with Alkali and Peracetic Acid and Charecterization of the Pulp. *Bio-resources* (2015), 5 (3), 1565-1580