Preparation and characterization of bioplastic based on *Gracilaria corticata* (J. Agardh) J. Agardh polysaccharides and reinforced with nanosilica

Dissertation submitted in partial fulfilment of the requirements

for the award of the degree of "**Master of Science**" in

BOTANY

By

SHARON THOMAS

Reg. No:AM20BOT015

DEPARTMENT OF BOTANY AND CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)

ERNAKULAM

2022

CERTIFICATE

This is to certify that the dissertation entitled **" Preparation and characterization of bioplastic based on *Gracilaria corticata* (J. Agardh) J. Agardh polysaccharides and reinforced with nanosilica"** is an authentic record of work carried out by **Sharon Thomas** under my supervision and guidance in the partial fulfilment of the requirement of the M. Sc. Degree of Mahatma Gandhi University, Kottayam. I, further certify that no part of the work embodied in this dissertation work has been submitted for the award of any other degree or diploma.

Ms. Anu Joy

Supervising Teacher

Department of Botany

St. Teresa's College (Autonomous)

Ernakulam.

Dr. Liza Jacob

Head, Department of Botany

St. Teresa's College (Autonomous)

Ernakulam

Place: Ernakulam

Date: 13/06/2022

Examiners

1. K. Madhusudhanan

2. Dr. Stephy Stephen 13/06/2022

ACKNOWLEDGEMENT

It is my privilege to express my deep sense of gratitude to Ms. Anu Joy, Faculty, Department of Botany, St. Teresa's College (Autonomous), Ernakulam, for her valuable guidance, help and encouragement at every stage of this work.

I express my sincere thanks to Dr. Liza Jacob, Head of the Department of Botany, St. Teresa's College (Autonomous), Ernakulam for her advices and constant support extended throughout the work.

I am highly indebted to Dr. Pressy P Prakasia, Assistant Professor, Department of Botany, All Saint's College, Trivandrum, for her vital guidance, which has promoted my efforts in all the stages of this project work.

I express my deep sense of gratitude to Dr. Jandas P. J., Post doctoral fellow, Department of Applied Chemistry, Cochin University of Science and Technology, Ernakulam, Dr. E. A. Siril, Professor and Head of the Department of Botany, Kariavattom Campus, Trivandrum, and Vishnu B, Research Scholar, Kariavattom Campus, Trivandrum, for their kind help and support in successful completion of my project.

I also acknowledge my sincere thanks to all the teaching and nonteaching staff of the Department of Botany for their suggestions, guidance and support during the course of this work.

Words fail to express my heartfelt thanks to my family and friends, for their cooperation, encouragement and support in my work and study.

I shall always remain grateful to the God Almighty without whose strength and blessing I would not have been able to complete the work successfully.

Place: Ernakulam SHARON THOMAS

Date:

CONTENTS

INTRODUCTION

Plastic has been a highly valued material on earth for its usefulness. Plastics contribute to our health, safety and peace of mind in our day to day life. Plastics are synthetic or semi-synthetic materials which are typically polymers of high molecular mass obtained from petroleum and natural gas. The phenomenal rise in the usage of plastics is due to their low cost and better properties which include flexibility, rigidness, brittleness and ability to be moulded into variety of shapes (Stevens, 2002). The history of plastics goes back more than 100 years. Life became much more convenient with the discovery of plastics because it has wide range of applications. In modern life, plastics are used in home appliances, electrical equipments, medical instruments, construction, automobiles and packaging (Rajendran *et.al.,* 2012).

The most important component of food packaging is that it aids in the preservation of food quality and ensures microbial safety (Abral *et.al.,*2021; Gaikwad *et.al.,*2019). Furthermore, packaging protects food products from physical, chemical, and microbiological harm by enabling the handling, transportation, and storage of various food products, resulting in improved quality preservation and shelf-life (Al-Tayyar *et*.al., 2020; Youssef *et.al.,*2019). However, petroleum-based polymeric materials or plastics, are the most employed in food packaging. Plastic packaging industry is thriving due to its unique characteristics, such as its capacity to be used as global marketing tool, low production costs, flexibility in usage, versatility in application and critical food product safety (Luijsterberg & Goossens, 2014*;* Padervand *et.al.,*2020). The food packaging sector generated US$ 839 Billion in revenue in 2015, with a 3.5 percent annual growth rate between 2015 and 2020, implying a value of about 1000 billion in 2020 (Qasim *et.al.,*2021; Gaikwad *et.al.,*2018). Furthermore, global plastics output grew by roughly 10 million metric tons per year, from 322 million metric tons in 2015 to 367 million metric tons in 2020 . This rise suggests that plastic production progresses while having a corresponding impact on the environment (Adrah *et.al.,*2020).

Plastic packaging has limitations in terms of biodegradability, recyclability, biocompatibility, and reusability. These restrictions on plastic packaging produce a massive amount of garbage, causing serious environmental difficulties such as soil contamination, marine pollution, air pollution, and global warming around the world .They have a poor recyclable potential and moreover, produce toxins in the recycling process. Furthermore, the plastic recycling process is quite challenging, as different plastics require different recycling

techniques. Only around 10% of the total plastic manufactured every year is recycled, with the rest dumped into the water bodies and landfills .The indigenous microorganisms do not have inherent potential to degrade these plastic wastes. As a result, both terrestrial and aquatic ecosystems are severely harmed. Discarded plastics have now contaminated the entire human habitat. (Sreenikethanam and Bajhaiya, 2021).

The ubiquity of plastic waste is essentially irreversible to be removed completely in the marine ecosystem as the pollution will always exceed this critical condition, making ocean as the ultimate grave of most mismanaged plastics. The ecological impacts occurring globally are ingestion and entanglement on tens of thousands of individual fishes, turtles, birds and mammals (Gall & Thompson, 2015). Besides that, the marine debris have also damaged the habitats and organisms including shorelines, coral reefs, shallow bays, estuaries, open ocean and deep sea. The marine plastics are frequently ingested by marine species and this may impact the food chain of fish and shellfish stocks, and their prey in diminishing their reproduction, growth and population level (Rochman *et.al.,* 2015). Eventually, these contaminated marine species maybe consumed by social communities as seafood and this indirectly affects the risk of human health. (Galloway *et.al.,* 2017).

Bioplastic made from renewable sources with similar qualities of fossil-based polymers is a viable alternative to overcome these major challenges. Bioplastics are defined as plastics that are made fully or partially from biomass or renewable sources, such as food crops, and have the identical function as the petroleum-based plastics . Bio-plastics can be made up of different materials which have different properties. With growing concern about the economic and environmental problems caused by the utilization off petroleum-based plastics, the demand for bioplastics has increased tremendously in recent years. The important characteristics of bioplastics include favourable mechanical and thermoforming properties, high gas and water vapour permeability, transparency and availability. They are biodegradable and environment friendly, which can lead to a more sustainable and circular economy. In addition, bioplastics can be entirely decomposed by soil microorganisms without producing any harmful by-productsby-products (Kadar *et.al.,* 2021).

A range of polymers utilised in bioplastic synthesis can be derived from key metabolites such as lipids, proteins, and carbohydrates. There are microbes that can utilise these polymers as a source of carbon and energy for their metabolism, and there are species that can produce exoenzymes to degrade them. Given the fact that bioplastics can be made

from a variety of renewable sources such as higher plants, bacteria, starch, and cellulose-based materials, algal plastics remain a high-demand research due to multiple advantages of algae as a feedstock. (Sreenikethanam and Bajhaiya, 2021).

There are numerous sources that can be used to manufacture bioplastics, mainly agricultural crop-based crops, such as corn, wheat, soy proteins, milk proteins, collagen and gelatin. The most extensively studied biopolymers, such as thermoplastic starch and poly (lactic) acid (PLA), are typically sourced from these terrestrial crops. However, this raises the concern on the sustainability of these feedstocks, such as the competition between land and water resources for human consumption (Lima, 2018). Furthermore, the process of extracting compounds, especially polymers from plants for the synthesis of bioplastics is difficult due to the presence of layered cell walls. In addition, the "green" plastics made from food crops, such as cassava, corn or sago, face the issues of poor water resistance and mechanical properties (Machmud *et.al.,* 2013).Therefore, algae have been emerging as a novel and potential biomass source to manufacture bioplastics since algae can be cultivated on non-arable lands and have short harvesting time (Chew *et.al.,* 2017).

Besides, algae do not compete with the food production for human consumption, are tolerant to harsh environmental conditions, can remediate wastewater and utilize carbon dioxide as a nutrient source for biomass production (Zhang *et.al.,*2019). During the manufacturing of algae-based plastic, the encapsulation of non-biodegradable polymer, for instance polyolefin, in the thermo-plastic algal blends can capture and store carbon dioxide in biomass form permanently. Consequently, carbon dioxide will not emit back into the atmosphere, thus alleviating the greenhouse effect. (Chia *et.al.*, 2020). Summing up, algal-based bioplastics serve as a promising and non-toxic alternative that can reduce the use of fossil fuels, enhance plastic quality and minimize negative environmental impacts brought by the excessive use of petroleum-based plastics (Beckstrom *et.al.,* 2020).

In the last few decades, the application of polysaccharide-based films and coatings in food packaging has increased dramatically. Noticeably, marine polysaccharides, from seaweeds, such as chitosan, alginate, agar, and carrageenan received significant attraction in the make-up of films and coatings in food packaging with the ability to protect from contamination and deterioration, thus improving the shelf-life and quality attributes. (Malhotra *et.al.,* 2015)

Agar is a high-molecular-weight polymer generated from the carbohydrate agarose, which serves as the supporting framework of certain algal species' cell walls. Agarophytes are red algae that belong to the group Rhodophytes (red algae). The primary commercial genera of agarophytes include *Gelidium, Pterocladiella, Gelidiella,* and *Gracilaria*. *Ahnfeltia, Acanthopeltis, Campylaephora, Ceramium, Gracilariopsis*, and *Phyllophora* are among the species that contain agar (Pereira *et.al.*, 2013).

Agar is a mixture of two polysaccharide components: agarose (gelling fraction) and agaropectin (non-gelling fraction). Agarose is a linear polysaccharide made up of repeating units of D-galactose and 3 6, anhydro-L-galactose linked by alternating a-(1.3) and ß-(1.4) glycosidic linkages, while agaropectin is branched and sulfated. Agarose makes up the majority of agar (50–90%) and is also responsible for agar's gelling properties (Nussinovitch & Gershon,1997). Different degrees of sulfation can be found in these polysaccharides. As a result, galactose, 3,6-anhydro-galactose, and inorganic sulfates linked to the carbohydrate are found in agar (Hand & Peat, 1938). However, some variance exists based on factors such as algae species and environment**.** Agar's remarkable properties as a thickening, stabilizing, and the gelling agent makes it an indispensable ingredient in the production of processed foods (Khalil *et.al.*,2017).

The genus *Gracilaria* is of considerable economic importance as an agarophyte, and it is the most abundant and promising resource of agar production. It has more than 150 species, distributed mainly in the temperate and subtropical zones (Radiah *et.al*., 2011). The value of *Gracilaria* has increased with demand because of the high production cost of the agar from the genus *Gelidium* and insufficient wild stock of this genus. Therefore, more than half of the world agarophyte tonnage consists of Gracilaria (McHugh, 1991). Different species of Gracilaria include *G. Edulis, G. Corticata, G. Millardetii, G. Debilis* (formally *G. Fergusonii*) and *G. Salicornia* have been reported to be potential sources of agar along the Indian Ocean water, including Tanzania (Jaasund, 1976; Kappanna and Rao 1963; Guiry and Guiry, 2021).

The water-soluble characteristic of agar has been very useful to produce films in a broad range of formulations .Nevertheless, elongation at break notably decreased as the amount of agar increased. In this sense, the flexibility of polymers and polysaccharide films can be improved by incorporation of plasticizers because plasticizers reduce brittleness and function as spacers between polymer chains, thereby decreasing intermolecular forces and

thus increasing the flexibility and extensibility of polysaccharide films. Plasticizers consist of polar and low-molecular-weight molecules that are capable of disrupting intermolecular bonds between polymer chains and forming new bonds with OH groups on the glucosidic units of polysaccharide chains. This facilitates chain movements and produces a more flexible material with a lower glass transition temperature (Tan *et.al.,* 2022).Typically, polyols such as glycerol, sorbitol, xylitol, sugar and mannitol are widely used plasticizers ,among which glycerol is the most commonly used, because the use of water and glycerol as a plasticizer improves the mechanical and optical properties of biodegradable and edible films (Madera-Santana *et.al*,2014) .

As compared with petroleum-based plastics, bioplastics present three main disadvantages: the processing window, the performance and the cost. The narrow processing window, poor gas and water barrier properties, unbalanced mechanical properties, low softening temperature and weak resistivity of plastics have collectively limited their broader applications. Through substantial research work, the above limitations could be overcome by the introduction of nanofillers. (Bhat *et.al.,* 2016; Al-Battashi *et.al.,* 2019)

Bioplastics reinforced with filler enhance the mechanical properties but reduce its hydrophilicity. In recent years, utilization of nano-sized fillers has bloomed in the fabrication of bioplastic due to their merits, such as low density, excellent mechanical properties, low abrasive nature and reactive surface for ease of modification. Numerous studies had reported that nano-sized fillers have a larger surface area than the conventional micro-sized fillers, thus enhancing the properties due to better interfacial interactions with the polymer matrix. Besides enhancing the mechanical and barrier properties, fillers are also capable of imparting specific functional properties to the bioplastics, e.g., electric conductivity or an antimicrobial character. Another added advantage of filler with nanoscale is in retaining the inherent transparency of the film, especially for the neat matrix.

Several popular types of nanofillers utilized in starch-based bioplastics are layered silicates (nanoclay, nanosilica and montmorillonites (MMT)), organic nanofillers (cellulose), inorganic nanofillers (metal or metal oxide) and carbonaceous nanofillers (nanotubes, graphene, graphene oxide) . (Tan *et.al.,* 2022).Nanosilica is a commonly used filler in polymer composites because of its contribution to thermal stability and mechanical properties (Yan *et.al.* 2007b; Battegazzore *et.al*. 2014). Silica can be obtained from rice husk, groundnut shell, bamboo leaves, and sugarcane bagasse (Vaibhav *et.al*. 2015).Battegazzore *et*

al.(2014) performed the reinforcement of silica extracted from rice husk and formulated it into the PLA matrix. It was found that the extracted silica from the rice husks enhanced the barrier properties and storage modulus of the PLA composites, as well as Young's modulus.

Active food packaging is a new method to prolong the shelf life of food products and to maintain of their safety, quality, and integrity. According to the European regulation (EC) No. 450/2009, active packaging consists of systems that interact with the food as they would absorb substances such as moisture, carbon dioxide, or odour from packaged food or release desired materials such as antimicrobial, antioxidant compounds or flavours into the packaged food (European Commission 2009) [5]. Despite the importance of active food packaging, there are limitations associated with the existing polymeric materials to serve as optimal active packaging and modifications are necessary. Such modifications involve addition of other additives such as antimicrobial and antioxidant agents which are required.

Oxidation processes are involved in most deterioration mechanisms present in nature, including in food products (López-de-Dicastillo et al. 2010). Active packages with antioxidant properties have received special attention because they are alternatives to traditional packaging, in which antioxidants are incorporated into or coated onto food packaging materials to reduce oxidation of the food, one of the main causes of food spoilage (López-de-Dicastillo et al. 2012).Currently, the tendency to reduce the use of synthetic additives in packaging, such as BHA or BHT, has focused interest on their substitution by natural antioxidants, such as tocopherol, plant extracts, and essential oils from herbs, that are safer and in most cases offer multiple health benefits (Li et al. 2014; López-de-Dicastillo et al. 2012).

Essential oils like those from cinnamon, clove, thyme, basil, lemon etc. Are excellent natural antioxidants. Cinnamon essential oil is obtained from the dried inner bark of the tropical perennial plant *Cinnamomum*, mainly the species *C. Zeylanicum* and *C. Cassia. Cinnamomum*, belonging to the family Lauraceae, is native to Sri Lanka and Malabar Coast of India. Phenolic compounds such as cinnamaldehyde, eugenol, carvacrol, cinnamic acetate and thymol are the main compounds found in cinnamon essential oil. Among these, cinnamaldehyde and eugenol act as the main bioactive antioxidant compounds because of their active functional groups. Thus the usage of cinnamon oil as additive can enhance the antioxidant property of the bioplastics for food packaging thus preventing food spoilage and improving shelf life of food products. (Weerasekara *et.al.,* 2021).

The present experimental study was aimed at developing a biodegradable food packaging film from the red algae, *Gracilaria corticata*. Nanosilica was incorporated as a filler to enhance mechanical properties and cinnamon essential oil added as antioxidant agent.

Objectives

- Extraction and characterization of polysaccharide from seaweed *Gracilaria corticata.*
- Extraction and characterization of silica nanoparticles from rice husk.
- Preparation of antioxidant biodegradable bioplastic film for food packaging based on extracted polysaccharide and reinforced with silica nanoparticles.

REVIEW OF LITERATURE

Biopolymers are obtained from different renewable natural sources such as plants, algae, animals, and microorganisms, or chemically synthesized from natural products, such as sugars, starch, natural fats, and oils. A biopolymer usually does not evoke an inflammatory/toxic response disproportionate to its beneficial effects. Biopolymers are eco-friendly with acceptable shelf-life, easy to be sterilized and biodegradable. Most of the plant-based polymers include pectin, starch, chitosan, xylan, galactoglucomannan, and lignin. These are usually derived from plant oil, cellulose, corn-starch, potato starch, sugarcane, weeds, and hemp etc. Algal phototrophs provide an excellent feedstock for biopolymer production owing to many advantages, such as high yield and ability to grow in diverse environments. The yield of cultivated algae is much greater (22 kg m2/year) than land plants(0.5e4.4 kg m2/year), a most favourable feature making them an ideal candidate for biopolymer production. Seaweeds are the excellent source of different biopolymers such as phycocolloids, alginate, agar, and carrageenan, which have different industrial applications in food, pharmaceutical, and biotechnology industries (Azeem et al., 2017).

Bio-packaging made from biodegradable materials is a current trend in the field of preservation of food products, however, it is still an important challenge for food technicians since the materials must meet specific requirements to protect food products. Chitosan-based edible films and coatings have successfully demonstrated their ability to fabricate different concepts of monolayer or composite films. The ability of chitosan(CS) to form edible films is mainly due to its polycationic nature that allows it to work in conjunction with other biopolymers and additives. This synergy causes strong physical interactions between all the components, which reflects the improvement of the physical, mechanical, and permeability properties. In addition, the bioactive potential of CS-edible films is originated by its intrinsic properties such as antimicrobial, antifungal and antioxidant capacities. These features make CS potentially interesting compared with other biomaterials (Díaz-Montes & Castro-Muñoz,2021).

The phylogenetic and molecular data from extant members of the green clade plant(Chlorobionta) were reviewed by Niklas et al. In 2017 and they showed that the capacity to synthesize the biopolymers cutin , suberin, lignin, and sporopollenin contributed significantly to the evolutionary success of the land plants by providing a number of

biological services, not the least of which is the conservation of water in a desiccating environment. It was concluded that the biosynthetic pathways of these biopolymers are extremely ancient, prefigured in a variety of unicellular and multicellular algal lineages (including the one that gave rise to the land plants), and evolutionarily derived by the co-option and evolutionary modification of very basic metabolic pathways, such as those that obtain the precursors to auxin, glucosinolates, tannins, and many other important secondary compounds.

Rhim and Ng(2007) studied recent advances in the preparation of natural biopolymer-based films and their nanocomposites, and their potential use in packaging applications. They remarked that the inherent shortcomings of natural polymer-based packaging materials such as low mechanical properties and low water resistance can be recovered by applying a nanocomposite technology. Polymer nanocomposites, especially natural biopolymer-layered silicate nanocomposites, exhibit markedly improved packaging properties due to their nanometre size dispersion. These improvements include increased modulus and strength, decreased gas permeability, and increased water resistance. Additionally, biologically active ingredients can be added to impart the desired functional properties to the resulting packaging materials. Consequently, natural biopolymer-based nanocomposite packaging materials with bio-functional properties have a huge potential for application in the active food packaging industry.

Furcellaran is a biopolymer produced from marine algae which has gained popularity in the recent years due to its abundance, water solubility and outstanding film forming properties. In 2020,Anjos et al. Reviewed the significance of furcellaran as a biopolymer, in which the innovative method of preparation, characterization and performance of furcellaran-based films as food packaging was highlighted.

Jhurry et al. (2005) reported on the extraction of biopolymers from *Hypnea, Eucheuma* and *Gracilaria* species collected around the coastal regions of Mauritius. Various extraction conditions were used and their effects on yield and structure of the corresponding biopolymers were investigated. The extracted polysaccharides were characterized by a combination of IR, NMR, SEC, viscometry and elemental analyses. These revealed that polysaccharide extracted from *Gracilaria* is a highly methylated agar and *Hypnea/Eucheuma* contain κ-carrageenan.

In 2008, Sousa et al. Extracted biopolymers from “Sargaço”, a name give to a mixture of dead seaweeds collected on Portuguese beaches. “Sargaço” is composed of algae containing biopolymers such as carrageenan, alginate and agar which are extensively used in food and pharmaceutical industries as gelling and stabilizing agents. A mixture of biopolymers was hot-extracted from alkali treated “Sargaço”. Films were made from an optimized extract, using the knife coating technique. Films’ physical properties were characterized and results showed that biodegradable films from “Sargaço” are more hygroscopic, less elastic, more deformable, and more permeable to water than films obtained from a commercial alginate and a domestic κ/ι-hybrid carrageenan. The applications of these biofilms as edible coatings for food were also studied and the preliminary tests showed that this material successfully preserves fruit textural properties.

Chen et al. (2016) successfully isolated nanocellulose from *Gelidium elegans* red algae marine biomass. The red algae fibre was treated in three stages namely alkalization, bleaching treatment and acid hydrolysis treatment. Morphological analysis was performed by field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM). TEM results revealed that the isolated nanocellulose had the average diameter and length of 21.8 ± 11.1 nm and of 547.3 ± 23.7 nm, respectively. Fourier transform infrared (FTIR) spectroscopy proved that the non-cellulosic polysaccharides components were progressively removed during the chemical treatment, and the final derived materials composed of cellulose parent molecular structure. X-ray diffraction (XRD) study showed that the crystallinity of yielded product had been improved after each successive treatments subjected to the treated fibre. The prepared nano-dimensional cellulose demonstrated a network-like structure with higher crystallinity (73%) than that of untreated fiber (33%), and possessed good thermal stability which is suitable for nanocomposite material.

In 2011,Wu et al. Observed that the green alga *Neochloris oleoabundans* was shown to be able to produce large quantities (up to 5 g/L) of high viscosity polymers with a weight-average molecular weight of 505 kDa from lactose under mixotrophic cultivation conditions. Aqueous solution of the polymers showed typical rheological behaviours of pseodoplastic fluids. The viscosity of the solutions was shown to be shear rate sensitive and temperature dependant. Adding 0.3–0.6 M NaCl into the aqueous solutions led to significant increase of viscosity. These properties suggest that the polymer could be utilize in a variety of fields including food, cosmetic, and oil industries.

Madera-Santana et al. (2011) conducted experimental studies in which agar obtained from the red alga *Hydropuntia cornea* was blended with polyvinyl alcohol (PVOH) in order to produce biodegradable films. They compared the properties of biopolymeric films formulated with agars extracted from *H. Cornea* collected at different seasons (rainy and dry) in the Gulf of Mexico coast and PVOH as synthetic matrix. The films were prepared at different agar contents (0%, 25%, 50%, 75%, and 100%) and their optical, mechanical, thermal, and morphological properties analysed. The tensile strength of PVOH–agar films increased when agar content was augmented. The formulation with 50% agar from rainy season (RS) had a significant higher tensile strength when compared to those from dry season (DS; $p<0.05$). Tensile modulus also displayed an increasing trend and likewise, for 50% and75% agar blends from RS showed higher values than those from DS ($p<0.05$). In contrast, elongation at break decreased as the agar content increased, independently of the season. Based on the above mentioned results, blends of PVOH and 75% agar from *H. Cornea* collected in rainy season showed good properties for applications in the biodegradable packaging industry.

In 2014, le Guerrero et al. Determined the chemical structure of the agar obtained from *Gelidium sesquipedale* (Rhodophyta) by 13C nuclear magnetic resonance (13C NMR) and Fourier transform infrared spectroscopy (FTIR). Agar (AG) films with different amounts of soy protein isolate (SPI) were prepared using a thermo-moulding method, and transparent and hydrophobic films were obtained and characterized. This is a novel approach to the characterization of agar-based films and provides knowledge about the compatibility of agar and soy protein for further investigation of the functional properties of biodegradable films based on these biopolymers

The utilization of red algae waste as raw material for the production of high quality cellulose nanocrystals (CNC) and it's application in polymer nanocomposites were investigated by El Achaby et al. In 2017.Red algae waste was chemically treated via alkali, bleaching and acid hydrolysis treatments, in order to obtain pure cellulose microfibers and CNC. Needle-shaped CNC was successfully isolated, having a diameter of 5-9 nm and length of 289-315nm. The as-extracted CNC were used as nanofillers for the production of polyvinyl alcohol (PVA)-based nanocomposite films with improved thermal and tensile properties, as well as optical transparency. It is shown that the addition of 8 wt % CNC into the PVA matrix increased the Young's modulus by 215 %, the tensile strength by 150 %, and the toughness by 45 %.

In 2014,Cian et al. Studied the physicochemical and antioxidant properties of phycobiliproteins-phycocolloids-based films, obtained from mixtures of two aqueous fractions extracted from *Porphyra columbina* red seaweed, one enriched in phycocolloids (PcF) and the other in phycobiliproteins (PF). Films with different ratios of PF:PcF (0, 25, 50, 75, 100% [w/w]) and without plasticizer addition were prepared by casting. PcF films had excellent mechanical properties (tensile strength ~50 Mpa, elongation at break ~3% and an elastic modulus ~17.5 Mpa). The addition of PF to formulations exerted a plasticizing effect on the PcF matrix, which was manifested in moisture content, water solubility and mechanical properties of the resulting films but not in its water vapour permeability. The antioxidant capacity (TEAC) of the PcF films was significantly increased by the addition of PF and a direct relationship between TEAC and the total phenolic compounds (r2R-phycoeryt = 0.9998) and R-phycoerythrin (r2=0.9942) was observed.

Sousa *et.al.* in 2010 produced biodegradable agar films from *Gracilaria vermiculophylla,* collected in Ria de Aveiro, Portugal, and the conducted a study on the effect of glycerol, an hydrophilic plasticizer, on the properties of the films and on subsequent application in edible coating of fresh fruits and vegetables. The plasticizer addition revealed an increase in elongation and decrease in tensile strength. The films were transparent and optically clear, showing good properties similar to the commercial agar films. Results of the coating application tests showed that coatings made with *Gracilaria* extracted agar/glycerol solutions were effective in extending cherry tomatoes shelf life in terms of weight loss and firmness although during the second half of the test period this difference tended to vanish. Visual inspection of the fruits revealed that the control fruits lost their gloss whereas the coated fruits kept a light gloss up to the end of the test.

Microwave-assisted extraction (MAE) of agar from *Gracilaria vermiculophylla,* produced in an integrated multitrophic aquaculture (IMTA) system, from Ria de Aveiro (north-western Portugal), was tested and optimized using response surface methodology by Sousa et al. In 2010.The quality of the extracted agar compared favourably with the attained using traditional extraction (2 h at 85°C) while reducing drastically the extraction time, solvent consumption and waste disposal requirements.

Experimental studies conducted by Kadam et al. In 2015 demonstrated that brown seaweed *Ascophyllum nodosum* extract can be incorporated in gelatin and casein films as an active ingredient for the development of bio functional films. The total phenolic content and

antioxidant activity of the film increased at higher levels of seaweed extract incorporation. An increase in hydrophilicity and glass transition temperature of films was also observed.

Castro-Enríquez et al. Conducted a study in 2020 in which a protein hydrolysate obtained from alga *Fucus spiralis* was included in a gelatine-based biofilm and used as packaging system in European hake slices during refrigerated storage. Hake samples packed in alga -gelatine condition showed a partial inhibitory effect on microbial activity development, lipid oxidation and hydrolysis in comparison with control fish samples.

García-Soto et al. (2015) applied a polylactic acid (PLA) biodegradable film including lyophilised alga *Fucus spiralis* and sorbic acid during megrim *(Lepidorhombus whiffiagonis)* refrigeration and evaluated its effect on fish quality loss .Thus, sensory assessment showed that samples wrapped up with PLA film including 8% alga and 1% sorbic acid were still acceptable on day 11, while control fish specimens (kept under polyethylene film) were rejected at that time. Under such biodegradable film condition, a preservative effect was also implied according to chemical indices assessment related to microbial activity and lipid oxidation development; additionally, lower mean numbers for different microbiological groups were detected. This result provides a promising replacement strategy to enhance refrigerated fish quality and reduce the waste material content.

Ku et al. In 2007 prepared *Gelidium corneum* films using cinnamaldehyde as a cross-linking agent and their physical properties were determined. Tensile strength (TS) value of the film containing 0.01% cinnamaldehyde was higher than the control by 831 Mpa. However, increasing cinnamaldehyde from 0.01% to 0.1% significantly decreased TS from 9.54 Mpa to 0, 03 Mpa, and no film was formed at 1% cinnamaldehyde. On the contrary, when cinnamaldehyde content was increased from 0.01% to 0.1%,% elongation was increased from 1.44% to 2.75%. Water vapour permeability (WVP) of the film containing 0% to 0.1% cinnamaldehyde were 1.64 ng m/m^2 sPa and 1.42 ng m/m^2 sPa respectively. These results suggested that 1.5% *Gelidium corneum* treated with 0.01% cinnamaldehyde should be most suitable condition for film formation.

A study was conducted to improve the life-span of the biofilm produced from algae by evaluating the decomposition rate with the effect of cinnamon extraction oil (CEO) by Othman et al. In 2018.The biofilm was fabricated using the solution casting technique. The soil burying analysis demonstrated low moisture absorption of the biofilm, thus decelerating the degradation due to low swelling rate and micro-organism activity, prolonging the shelf-

life of the biofilm. Hence, the addition of CEO also affects the strength properties of the biofilm. The maximum tensile strength was achieved with the addition of 5% CEO, which indicated a good intermolecular interaction between the biopolymer(algae) and cinnamon molecules.

MATERIALS AND METHODS

Study Site

Kerala has a coastline of about 580 km, which is extended in 9 districts of the state from Poovar, Thiruvananthapuram district in south to Thalapady, Kasaragod district in north. The coast of Kerala supports a large number of marine flora and fauna, owing to its variety of habitats such as beaches, back waters, estuaries, cliffs, lagoons, mangroves and coral reefs. Thus it forms an integral part of the marine biodiversity of India. Gracilaria corticata, the sample for our study was collected from coast of Thikkodi beach in Kozhikode district. The beach has latitude of 11° 28' 20.8" N and a longitude of 75° 37' 04.5" E. In this area rocks of different kinds and granite stones are found in the intertidal and subtidal region with luxuriant growth of various green, brown and red algae of few seaweed vegetation is found on them.

Collection of Sample, Preservation and Processing

The seaweed sample was collected randomly from the intertidal regions, during the low tides by hand picking and use of knife. The collected sample was washed thoroughly in seawater and stored in polythene bags along with seawater for delaying degradation during the transport from the collection site to the laboratory. The sample was identified and confirmed as *Gracilaria corticata* by reference of literature (Srinivasan, 1973; Desikachary et al., 1998; Jha et al., 2009) (Fig. 1). In the laboratory, the sample was washed under running water, all the surface debris as well as other smaller organisms was removed. For preparing the dried samples, the specimen was kept under shade for 6-7 days and finally dried in hot air oven. It was then powdered and used for further processes.

Extraction of polysaccharides from Gracilaria corticata

100 g of the milled G. Corticata was treated with 1L isopropyl alcohol (80% w/v) under constant mechanical stirring overnight at room temperature to remove pigments, lipids,

some phenols, and low molecular weight compounds. To separate the sediment from the solvent (isopropyl alcohol), a refrigerated centrifuge with the controlled temperature at 10°C; 8,000 rpm for 10 minutes was applied, and then the supernatant was discarded. The residual was rewashed with isopropyl alcohol (80% w/v), rinsed with acetone, and centrifuged at 10,000 rpm (10°C) for 10 minutes again, and dried at room temperature in a fume hood overnight. To extract the polysaccharides, 20 g of de-pigmented powder was added to 500 mL distilled water and the extraction was carried out at 65 °C with a stirrer for 2 hours. The supernatant was collected after centrifugation at 10°C and 10,000 rpm for 10 minutes, and the extraction was conducted twice. The supernatant was concentrated using the rotary evaporator under reduced pressure at 60°C. The concentrated extract was frozen at −20°C prior to lyophilisation to obtain the dried form of polysaccharides (Fig. 2).

Characterization of the extracted polysaccharide

Determination of Physical properties

Solubility

The solubility of the isolated polysaccharides was tested according to the method of Wheet (2011). About 1 mg of pure compound was added into 5 small test tubes. Different solvents such as DMSO, water and ethanol added separately. The solubility of the compound in each of these solvents was observed and recorded.

Fourier Transform Infra-Red spectrophotometry (FT-IR)

Fourier-transform infrared (FT-IR) spectra were recorded using a Thermoscientific Nicolet iS50 FT-IR spectrometer, catalogue number 912A0760. It has got a spectral range of 15 to 27,000 cm-1 and has an automated beam splitter exchanger (ABX) which can easily detect varies bond formation.

X-ray diffraction (XRD) analysis

XRD was performed with an Aeris Benchtop X-ray Diffractometer Malvern PANalytical to investigate the phase and crystallinity of the extracted polysaccharide powder. The XRD

patterns were recorded in the region of 2θ from 0° -80° (Liang and Wang, 2017). The crystallinity of the molecules was calculated using the following equation.

Crystallinity percentage = (Total area of crystalline peak÷ Total area of all peaks) ×100

In vitro antioxidant activity

To different volume of extract, 0.5 ml of 1 mM ethanolic solution of DPPH was added and made up to 2.0 ml using ethanol. The mixture was allowed to react at room temperature for 30 minutes. Ethanol served as the blank and a tube without the extracts served as the positive control. After 30 minutes of incubation, the discoloration of the purple colour was measured at 518 nm in a spectrophotometer. The assay was calculated as:

Radical scavenging activity = [(Control- Test) ÷ Control] ×100

Nanosilica synthesis from Rice husk

Nanosilica is synthesized by sol-gel method from rice husk. The chemical reaction for the precipitation of silica involved:

$SiO2 + 2NaOH \rightarrow Na2SiO3 + H2O$

$Na2SiO3 + 2HCl \rightarrow SiO2 + 2NaCl + H2O$

Washing and drying: Rice husk was washed thoroughly with water to remove the soluble particles, dust, and other contaminants. It was then dried in an air oven at about 110°C for 24 hours.

Thermal treatment: The washed rice husk was weighed and subjected to heat treatment to obtain the ash. Sample was burned inside a programmable furnace at 700°C for 6 hours.

Acid treatment: Acid washing step was done to remove the small quantities of minerals prior to silica extraction from rice husk ash (RHA). Ten grams of RHA sample were dispersed in 60 ml of I N HCl for 5 minutes. It was then washed thoroughly.

Silica extraction: A sample of 2.5 g RHA was stirred in a 250 ml of 0.5N sodium hydroxide solution. The solution was heated in a covered beaker by stirring constantly, allowed to stand at room temperature and then filtered.

Nanosilica preparation: HCl was added until neutralized. The precipitate silica was washed repeatedly with warm, deionized water and then centrifuged at 5000 rpm for 10minutes. It was repeated for 3 times to obtain a neutral pH. The product was dried at 110°C for 24 hours in the oven and crystallization in a programmable furnace at 450°C for1 hour. The obtained silica was crushed and preserved until further experiments.

Characterization of Silica nanoparticles

X-ray diffraction (XRD) analysis

XRD was performed with an Aeris Benchtop X-ray Diffractometer Malvern PANalytical to investigate the phase and crystallinity of the Nano-silica particles, of which the XRD patterns were recorded in the region of 2θ from 0° -80° (Liang and Wang, 2017). The crystallinity of the molecules were calculated using the following equation

Crystallinity percentage = Total area of crystalline peaksTotal area of all peaks ×100

Preparation of Biodegradable bioplastic for food packaging

The bioplastic preparation was carried out by trail and error method by fixing the concentration of extracted polysaccharide powder at 1.5% (w/v) and varying the concentration of silica nanoparticles and glycerol from 1% to 7% (w/w of Polysaccharide powder) and 5% to 15% (w/w of Polysaccharide powder) respectively. Commercially bought cinnamon essential oil was added to incorporate antioxidant property to the bioplastic at a concentration of 20% of w/w of silica nanoparticles. The three components in appropriate concentrations along with the essential oil were mixed and stirred for 30 minutes at 90°C. Sufficient amount of the above mixture was poured in a clean oiled petri dish and kept in hot air oven at 60°C to 80°C for 3 hours for setting. The film was removed after proper drying and evaluated for various properties.

Characterization of developed bioplastic film

Solubility ratio:

The water solubility (WS) ratio was determined as per basic standard method reported earlier (Wang & Rhim, 2015; Arham et al., 2016; Sanyang et al., 2016). The sample was oven dried at 220 ºF for 24 hours and weighed (W1) properly. The dried pieces were then immersed in centrifuge tubes containing 30 ml distilled water and kept in water bath at 25ºC with slow shaking for overnight. The solutions were filtered and the remnants on filter paper were dried at 220 °F for two hours and reweighed (W2). The undissolved dry matter was calculated by using the formula given below.

Solubility (%) = [(w1-w2)÷w1]×100

Rate of biodegradability

The developed bioplastic film pre-weighed (B1) and buried for a month in pots containing conditioned garden soil. The final weight (B2) of the film was recorded and the difference in the weight of the films was calculated by using the following equation (Hii et al., 2016).

Biodegradability (%) = [(B1 – B2)÷B1]×100

In vitro antioxidant activity

To different volume of extract 0.5 ml of 1 mM ethanolic solution of DPPH was added and made up to 2ml using ethanol. The mixture was allowed to react at room temperature for 30 minutes. Ethanol served as the blank and a tube without the extracts served as the positive control. After 30 minutes of incubation, the discoloration of the purple colour was measured at 518 nm in a spectrophotometer. The assay was calculated as:

Radical scavenging activity = [(Control- Test) ÷Control]×100

(a) Habit

(b) Single filam

Figure 1. ***Gracilaria corticata***

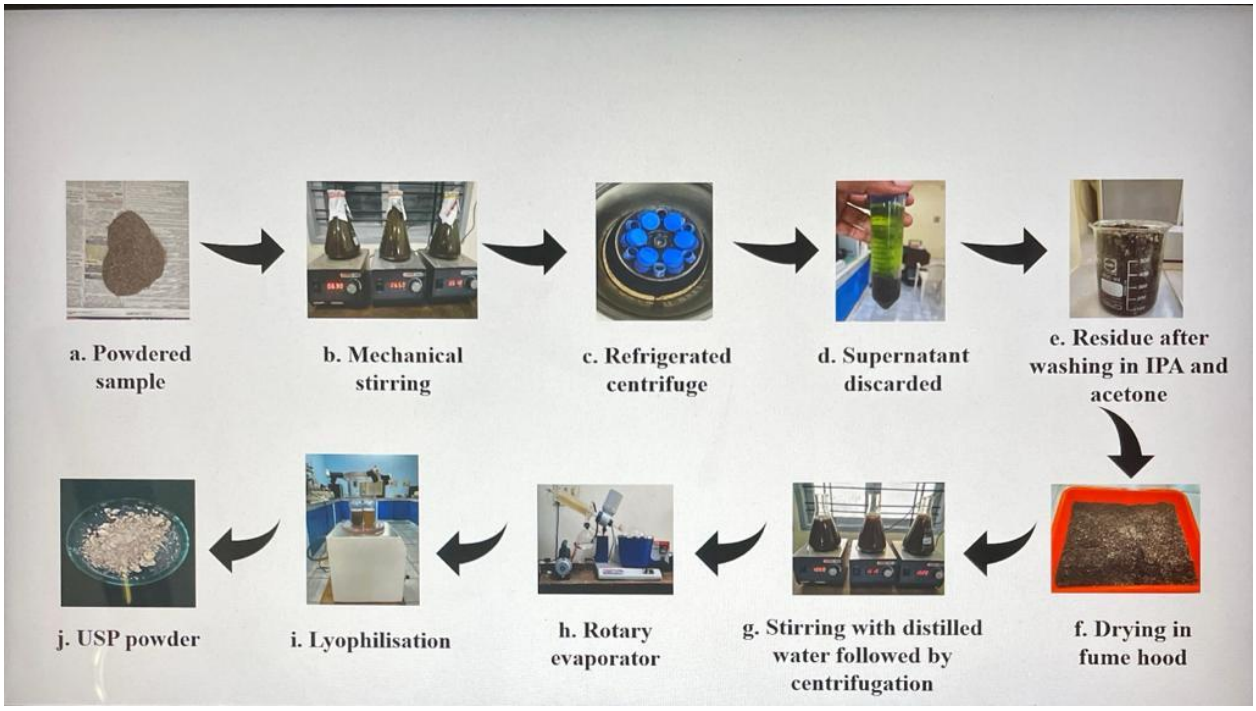

Figure 2. Extraction of Polysaccharide.

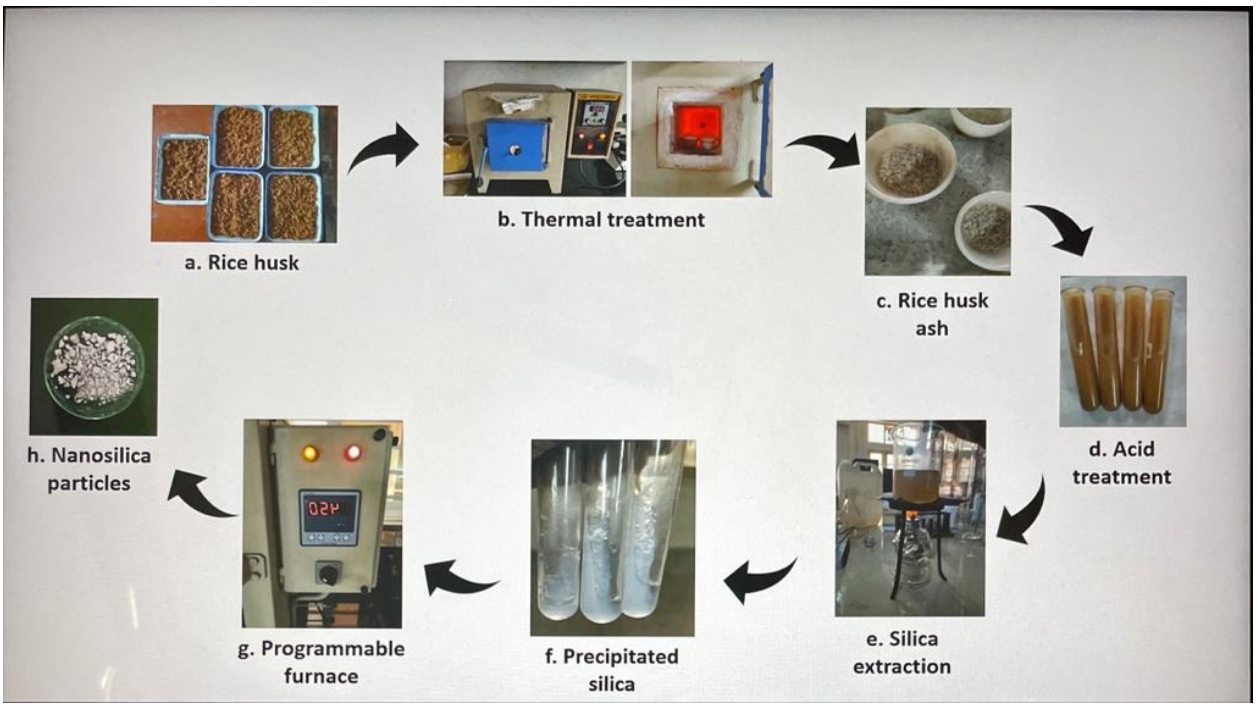

Figure 3. Extraction of nanosilica

Polysaccharide powder

+

Nanosilica particles

+

Glycerol

+

Cinnamon bark essential oil

Constant stirring at 90°C

Poured into an appropriate mould

Bioplastic film

Figure 4. Preparation of bioplastic film

RESULTS

Collection and processing of fresh specimen

Seaweed (*Gracilaria corticata*) for the experimental study was collected from Thikkodi beach in Kozhikode district of Kerala. Around 7 kg of fresh specimen was collected from which 420 grams of dried powdered specimen was obtained.

Extraction of polysaccharide from *Gracilaria corticata*

Using the cold extraction procedure, the yield of polysaccharide obtained was 19.99 grams per 50 grams of powdered algal specimen (39.98%).

Characterization of polysaccharide

Determination of physical properties

Solubility

The polysaccharide extracted was found to show solubility in the solvents water, ethanol and DMSO (Dimethyl sulphoxide).

Fourier Transform Infra-Red Spectroscopy

The FT-IR spectra of polysaccharide is shown in figure. 5. The graph showed vibrational peaks at 3362.42 cm-1 which indicates the presence of O-H stretching and the corresponding broad peak is around 3400cm-1 due the hydroxyl groups of polysaccharides and hydrogen bonding. In 1000cm-1 there is CH bending (Figure). It is also observed that the presence of band at 989.94 cm-1 1040.27 cm-1 and 1094.56 cm-1 indicates the presence of anhydrous groups.

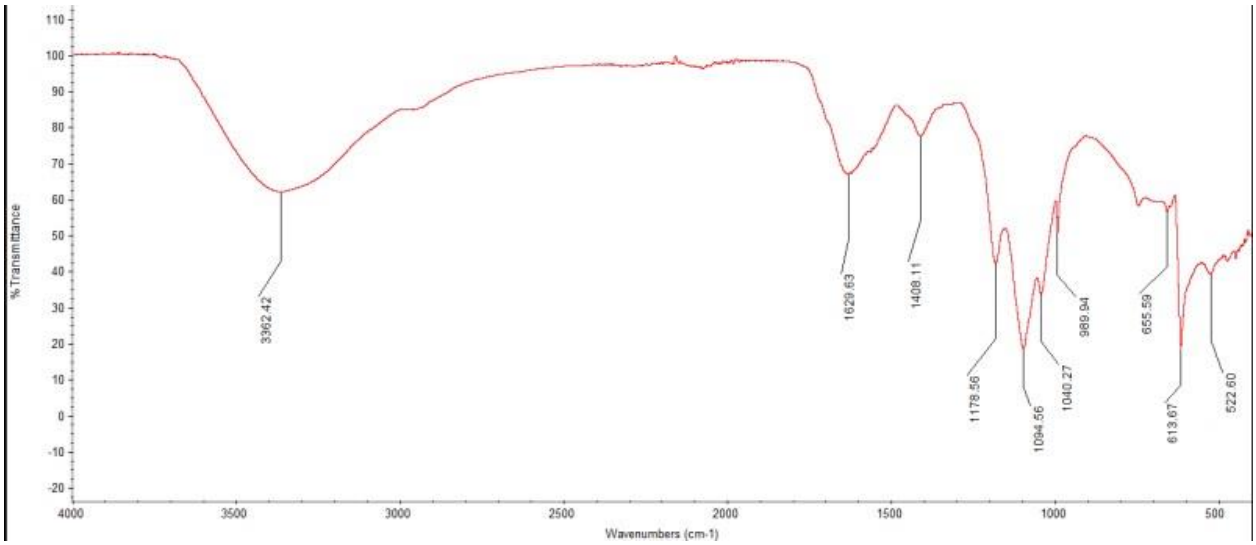

Figure 5

X-ray diffraction (XRD) analysis

The XRD spectra of polysaccharide powder is shown figure 6 and figure 7. In the graph, 4 major peaks were identified using the software OriginLab and the material was found to exhibit 55.44% crystallinity.

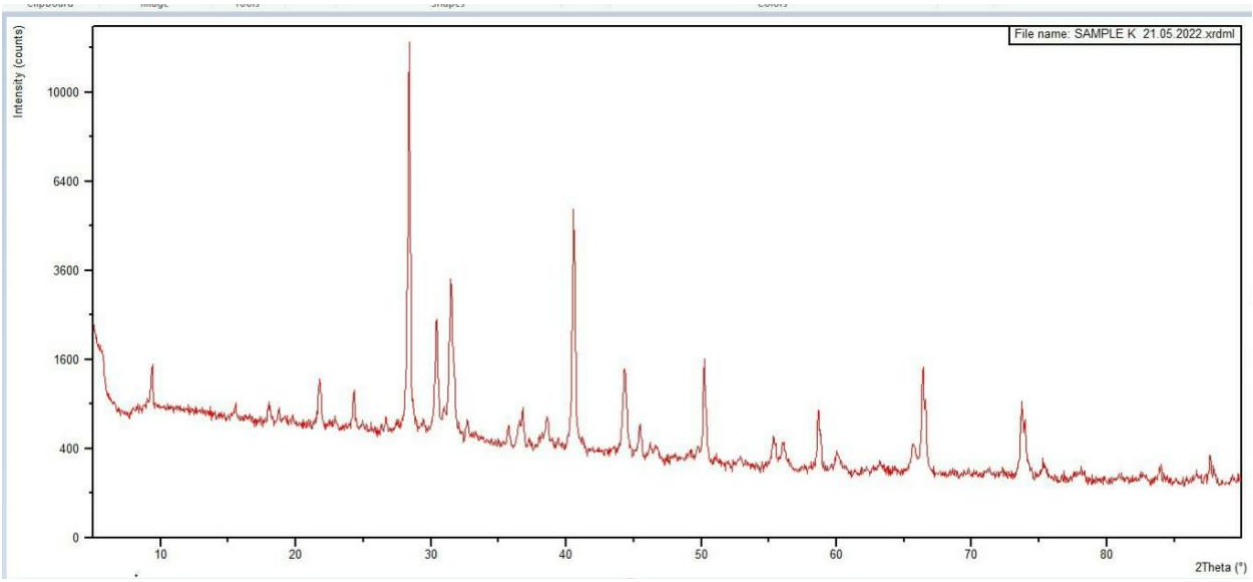

Figure 6

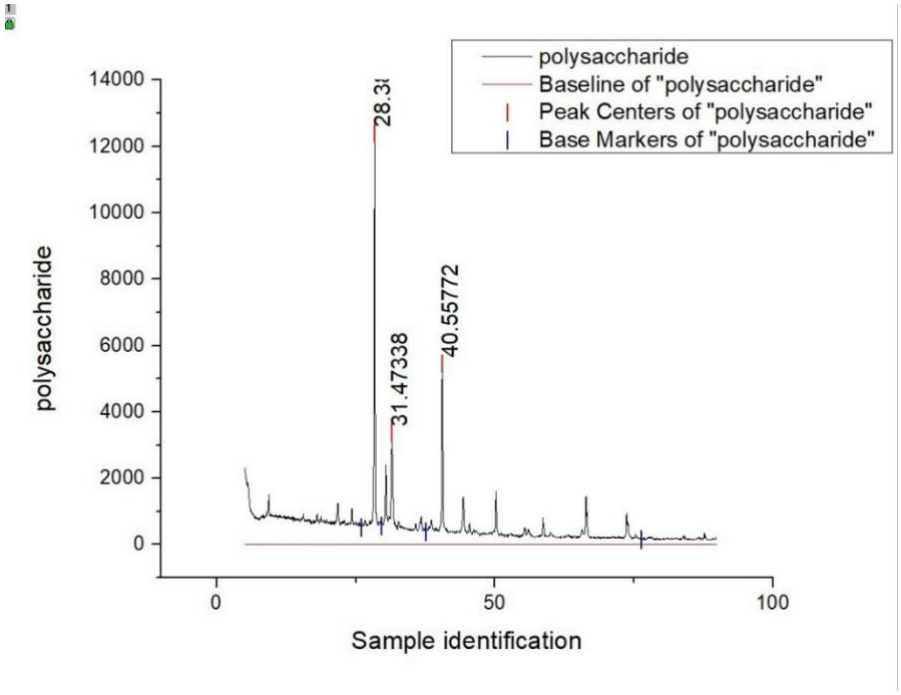

Figure 7

In vitro antioxidant activity

In vitro antioxidant activity antioxidant activity of the sample polysaccharides powder is shown in the table 1. The strongest antioxidant activity was at a concentration of 300µL with an inhibition rate of 10.7%.

Volume of sample (µl)	OD at 518 nm	% of Inhibition
100	0.418	4.12
200	0.402	7.79
300	0.389	10.7

Table 1- Invitro antioxidant activity of polysaccharide.

Nanosilica synthesis from rice husk

Nanosilica synthesis was carried out using 120g of rice husk as starting material and and an yield of 16.88g (14.06%) of nanosilica was obtained.

Characterization of nanosilica

X-ray diffraction (XRD) analysis

The XRD pattern of nanosilica is depicted In figure 8 and figure 9. From the graph, 4 major peaks were identified using the software OriginLab and the material was found to be 98.43% crystalline. A broad peak at 2θ angle of about 22°C indicates amorphous nature of nanosilica and the sharp peaks indicate crystalline nature.

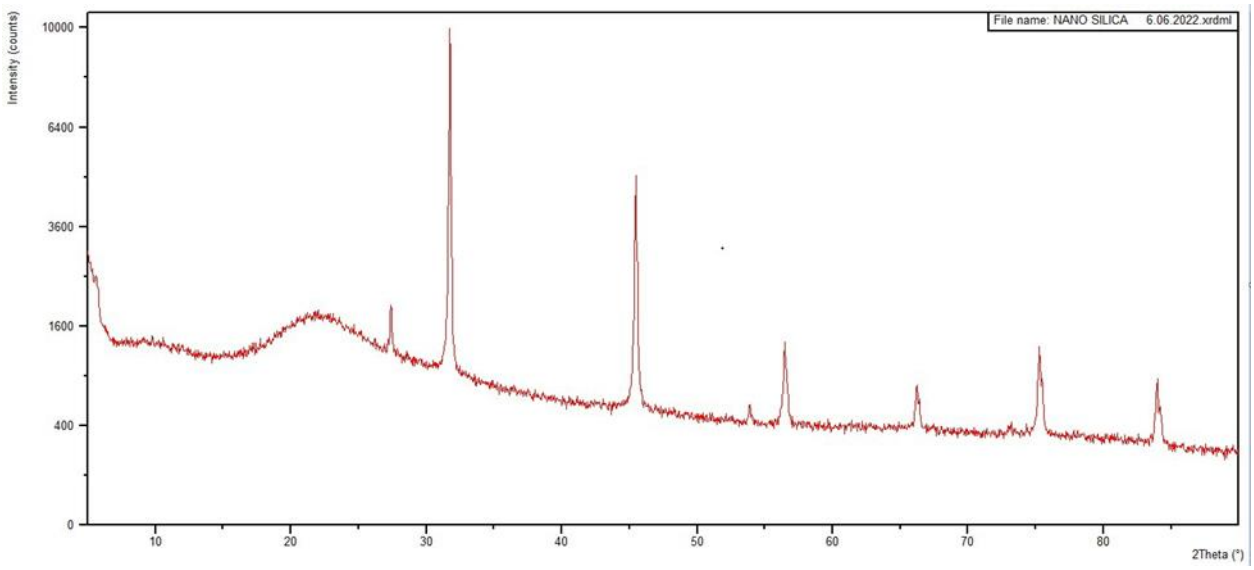

Figure 8

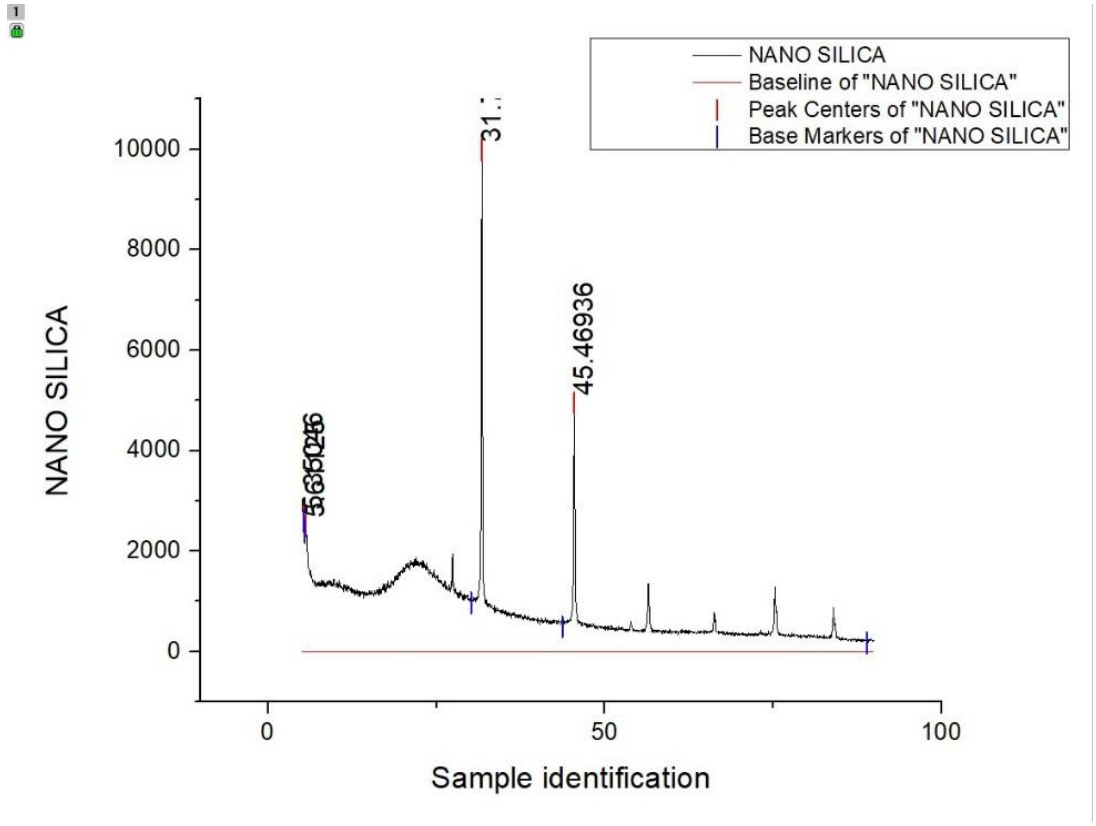

Figure 9

Preparation of biodegradable bioplastic film for food packaging

The bioplastic preparation was carried out by trial and error method by fixing the concentration of extracted polysaccharide powder at 1.5% (w/v). A concentration of 5% (w/w of polysaccharide) of extracted Nanosilica particles and 11% (w/w of polysaccharide) of Glycerol were selected as the ideal concentrations for the preparation of mechanically stable, non-brittle, biodegradable bioplastic film with uniform thickness

Characterization of bioplastic

Solubility ratio

The initial weight (W1) of bioplastic sample was 0.0206g and final weight (W2) after immersion in water was 0.0175g. Using the standard equation, the solubility percentagr of bioplastic was calculated as 15.05%.

Rate of biodegradability

The initial weight (B1) of bioplastic sample was 0.0389g and the final weight (B2) after burial in soil for a week was 0.0379g. Using the standard equation, the biodegradability rate of bioplastic sample was calculated as 10.54%.

In vitro antioxidant activity

In vitro antioxidant activity of the sample bioplastic film is shown in the table 2.The strongest antioxidant activity was at a concentration of 300µL with an inhibition rate of 85.4%

Volume of sample (µl)	OD at 518 nm	% of Inhibition
100	0.168	75.4
200	0.132	80.7
300	0.10	85.4

Table 2 – Invitro antioxidant activity of nanosilica.

Figure 10. Polysaccharide powder

Figure 11. Nanosilica particle.

Figure 12 . Bioplastic film

Figure 13. Solubility test.

Figure 14. Biodegradability test

DISCUSSION

Lack of degradability and the closing of landfill sites, as well as growing water and land pollution problems, have led to concern about plastics (Maheswari & Ahilandeswari, 2011). Bioplastic materials, biodegradable and from renewable resources, are the most promising substitutes of non-biodegradable, petroleum-based plastics, toward green and sustainable packaging solutions (Tran *et.al.*,2020). Seaweeds are important autotrophic and photosynthetic plants, which are now often explored frequently due to the presence of some vital polysaccharides such as alginate, agar, floridean starch and carrageenan (Rajendran, *et.al*.,2012). Agar, that is present intracellularly within the cell walls of red seaweeds (Martins *et.al*., 2012)possess good gelling & emulsifying abilities and is a promising agent for bioplastic synthesis (Wu *et.al.*, 2009; Hii et al.,2016; Tabassum, 2016). In the present study, a biodegradable bioplastic film for food packaging was prepared utilizing the polysaccharide extracted from red algae, *Gracilaria corticata* with glycerol as plasticizer. The bioplastic film was reinforced with silica nanoparticles to improve the mechanical properties and cinnamon essential oil to impart antioxidant activities.

The specimen, *Gracilaria corticata* was collected from the coast of Thikkodi beach in Kozhikode district of Kerala. The collection process was carried out in the month of January when luxuriant growth of red algae is usually observed and a considerable quantity of fresh specimen was obtained.

The polysaccharide powder was extracted from *Gracilaria corticata* using the cold extraction method which was developed experimentally to suit the specimen under study. Cold extraction methods are advantageous in that they prevent the decomposition or alteration of natural compounds. On the other hand, hot extraction methods are carried out under high temperatures and may cause solvents to be lost, leading to extracts of undesirable impurities and decomposition of thermolabile components. In the current study, a significant yield of the polysaccharide obtained was obtained, i.e., 39.98%. This yield is much better compared to the maximum agar yields achieved in earlier findings by Villanueva *et al*., 2010 (33% by *G. vermiculophylla*) and Wang *et.al*., 2017 (23% by *G. tenuistipitata*). The impact of cold weather and techniques for the agar production is known to influence agar yield among seaweeds and may vary from species to species (Soriano & Bourret, 2003; Nil *et.al.*,

2016) . Thus the differences in agar polysaccharide yield may be attributed to seasonal variations and differences in the extraction procedure.

The extracted polysaccharide was characterized for the determination of its properties. The polysaccharide was obtained as an ivory coloured powder and was observed to be soluble in the solvents- water, ethanol and DMSO. Similar results have been reported by Wu *et.al.* in 2017 and Castro et.al. in 2018 who extracted water soluble polysaccharides from *Gracilaria lemaneiformis* and *Gracilaria intermedia* respectively. The XRD pattern of polysaccharides showed four major peaks and the compound was revealed to be semi crystalline in nature. This result aligns with the typical XRD pattern of a semi crystalline polymer. It was found to be similar to reports by Ghribi *et.al.*(2015) and Kolsa *et.al.* (2017) where the XRD patterns of chickpea water soluble polysaccharide and *Sargassum vulgare* polysaccharide indicate semi crystalline nature.

In recent years, silica nanoparticles (Si-NPs) have attracted the attention of researchers due to their unique characteristics such as high surface area, high pore volume, tunable pore size, excellent biocompatibility, ability to encapsulate hydrophilic as well as hydrophobic materials, and scalable synthetic availability. The possibility to specifically manage the particle size, shape, porosity, and crystallinity, and the existence of nanosilica in different forms like solid silica, mesoporous silica,hollow or core−shell structures, virus-form silica, rod shaped particles, and silica gels have garnered them attention in diverse applications (Flikkema *et.al.*,2003; Rahman *et.al.*,2009). The surface modification of silica can also be done easily using different molecular or polymeric moieties making them highly compatible with biological environments. Nanosilica particles have have wide range of biomedical applications ranging from targeted drug delivery, cancer therapy to bioimaging, and bio sensing. Recently the silica-based drug Cornell Dots (C' dots) has been approved by the U.S. Food and Drug Administration (FDA) for molecular imaging of cancer for first-in-human clinical trials (Benezra *et.al.*, 2011).

Different approaches like sol−gel process, chemical precipitation method,24 microemulsion processing, plasma synthesis, chemical vapor deposition (CVD), combustion in a diffusion flame and pressurized carbonation are employed for preparing Si-NPs. Among these, the sol−gel process also known as "Stöber method", developed in 1968, is an excellent procedure to follow for synthesizing better-quality of spherical NPs with narrow size distribution (Stöber *et.al.* 1968; Tang *et.al.*, 2012). In the present study, silica nanoparticles

were extracted from rice husk by the sol-gel method and an yield of 14.06% was obtained. This is a better result compared to the nanosilica yields of 9.26% and 9.03% obtained by Setyawan and Yuliani in 2021 (from rice husk) and Kaulder and Yadav in 2018(from paddy straw). These variations in yield may be due to difference in the material used and the extraction procedure adopted.

The XRD analysis of the extracted nanosilica showed sharp peaks indicating the crystalline nature of the compound. This can be attributed to the thermal treatment of rice husk at high temperature (700°C) when the silica cristolabite form in rice husk will become crystalline form (Rozainee *et.al.,* 2008).

Nanosilica is a commonly used filler in polymer composites because of it's contribution to thermal stability and mechanical properties (Yan *et.al.,* 2007). Therefore the incorporation of nanosilica into bioplastic, in the current study, has been significant in improving the mechanical strength and thermal resistance. Similarly De Azêvedo *et.al.* in 2021 incorporated silica powder, from sugarcane waste ash, into corn and potato starch bioplastics doped with sodium silicate solution bioplastics which improved the properties of elongation at break and increase the thermal resistance of the bioplastics.

The bioplastic food packaging film was prepared using the polysaccharide, extracted from *Gracilaria corticata,* as backbone and glycerol as plasticizer. The film was also reinforced with silica nanoparticles and cinnamon essential oil as antioxidant agent. Clean petri dishes coated with vegetable oil were used as moulds for film preparation. The bioplastic film thus prepared was nearly transparent with a brownish tinge and was of uniform thickness. It was observed that the film showed considerable flexibility, non brittleness and mechanical strength. The flexibility and non brittle nature can be attributed to the addition of glycerol as plasticizer and improved mechanical properties to the nanosilica incorporation. The obtained bioplastic film was characterized for its solubility and biodegradability rates as well as antioxidant activity.

The rate of solubility of bioplastics is an important feature reflecting the ability of bioplastic being disintegrated in the presence of moisture, post-consumption, when utilized commercially (Arham *et.al.,* 2016). Bioplastics having low grade solubility are considered to be the best as they resist moisture for a longer period of time and helps to increase the shelf life of a products; whereas, some edible bioplastics used in the packaging of food material mostly degrade rapidly (Sanyang *et.al*., 2015; Arham *et.al*., 2016; Melanei *et.al*., 2017). In

the present study, the solubility rate was found to be comparatively lower, indicating that it is less susceptible to water degradation and thus it could be used as a food packaging material for improving the shelf life of food products. Soil burial test (Biodegradability test) was performed to analyze the level of deterioration caused to the bioplastics via microbial growth when discharged into the soil. As the base material , plasticizer and filler used in this study, agar, glycerol and nanosilica are known to have hydrophilic properties (Vieira *et.al*., 2011; Hii *et.al*., 2016) the bioplastic sample showed weight loss. This indicates that the bioplastic film prepared is biodegradable and can be an eco-friendly alternative for food packaging.

Foodborne illnesses are resulting from consumption of food contaminated with pathogenic bacteria while the oxidation of lipid in food is also a big concern of public health because it causes a large number of illnesses and economic damage (Alam *et.al.,* 2013; Jovin *et.al.,* 2007).As a solution of foodborne problems, food industries use today more than 2500 synthetic additives to avoid food spoilage, increase food shelf-life and protect consumers from poisoning. Nevertheless, many countries has limited several of these chemical molecules because of the accumulation of residues in food and feed chain, the emergence of microbial resistance, their bad effects on people's health, such us carcinogenicity, liver damage, respiratory, dermatological, gastrointestinal diseases (Ait-Ouazzou *et.al*.,2012).In this scenario, active packages with antioxidant properties have received special attention because they are alternatives to traditional packaging, in which antioxidants are incorporated into or coated onto food packaging materials to reduce oxidation of the food, one of the main causes of food spoilage (López-de-Dicastillo *et.al.* 2012).Currently, the tendency to reduce the use of synthetic additives in packaging, such as BHA or BHT, has focused interest on their substitution by natural antioxidants, such as tocopherol, plant extracts, and essential oils from herbs, that are safer and in most cases offer multiple health benefits.

Cinnamon essential oil is obtained from the dried inner bark of the tropical perennial plant *Cinnamomum*, mainly the species *C. zeylanicum* and *C. cassia*. Cinnamon bark essential oil (CO) contains a great variety of chemical compounds (mainly cinnamaldehyde, cinnamic acid, coumaric acid, cinnamyl alcohol, and eugenol) that present antioxidant and antimicrobial activities in an individual and collective manner . In the present study, the bioplastic film was incorporated with cinnamon essential oil. The DPPH assay results of the bioplastic samples indicate high free radical scavenging activity or antioxidant activity. This activity can be attributed to the presence of the main CO antioxidant, which is eugenol; however, it could also be due to the presence of cinnamaldehyde, which has been reported to

have a lower activity than eugenol, or to a synergetic effect among chitosan, eugenol, and cinnamaldehyde. Similarly Ganeson *et.al.* in 2022 has developed a cinnamon oil emulsion based gelatin edible film with improved antioxidant activity. Therefore the biodegradable bioplastic film incorporated with cinnamon oil could be developed as a sustainable alternative to replace plastic packaging in the future.

SUMMARY AND CONCLUSION

Environmental concerns associated with petroleum based plastics has led to increasing importance of bioplastics derived from renewable biological sources and are eco-friendly. The current study was aimed at developing a biodegradable, bioplastic film for food packaging based on polysaccharides extracted from red algae, *Gracilaria corticata*. Glycerol was used as plasticizer and the bioplastic film was reinforced with silica nanoparticles and essential oil from cinnamon to improve mechanical properties and antioxidant activity respectively.

The algal specimen for the study, *Gracilaria corticata* was collected from Thikkodi beach in Kozhikode district of Kerala , in the month of January when luxuriant algal growth is observed in the area. The algal specimen was cleaned, dried and powdered for further processes. The powdered specimen was used in the extraction of polysaccharide using cold extraction procedure and considerable yield was obtained which was better than earlier documented studies. The extracted polysaccharide polysaccharide powder was characterized for its physical properties and antioxidant activity. The polysaccharide was found to be soluble in water, ethanol and DMSO. The FTIR spectra of the compound showed various various peaks indicative of typical bonds in polysaccharide and XRD results revealed its crystalline nature. A small antioxidant activity was also shown by the polysaccharide in DPPH assay.

Nanosilica particles to be incorporated in the film was extracted from rice husk by sol gel method which is comparatively better than other methods of extraction. A significant amount of nanosilica was obtained, the yield being better than some previous studies. The dried nanosilica was powdered and XRD analysis was conducted which revealed the crystalline nature of the obtained compound.

The bioplastic film was finally prepared using the polysaccharide extracted from *Gracilaria corticata* with appropriate concentrations of glycerol, nanosilica and essential oil from cinnamon bark. The film was nearly transparent with a brownish tinge and was non-brittle and flexible with excellent mechanical and thermal properties. The addition of glycerol resulted in the flexibility of the film and improved mechanical properties may be attributed to nanosilica reinforcement. The solubility test of the film has shown that it is not easily soluble

in water and is less susceptible to degradation by water. The biodegradability test led to the inference that the film is considerably biodegradable. DPPH assay of bioplastic sample showed that it has high antioxidant activity. This activity can be attributed to the presence of the main cinnamon oil antioxidant, which is eugenol; however, it could also be due to the presence of cinnamaldehyde, which has been reported to have a lower activity than eugenol, or to a synergetic effect among chitosan, eugenol, and cinnamaldehyde.

Bioplastics developed from sustainable biological resources ,with properties similar to plastics is the the most viable solution to overcome the challenges associated with petroleum derived plastics.

Bioplastic materials, biodegradable and from renewable resources, are the most promising substitutes of non-biodegradable, petroleum-based plastics, toward green and sustainable packaging solutions (Tran *et.al.*,2020). Polysaccharides from seaweeds are renewable biomass resources and are polymers made from sugars which contain carbon, they could be used to create biodegradable and high quality bioplastics. Agar polysaccharide extracted from *Gracilaria corticata* has shown excellent gelling and emulsifying properties and its properties can be further improved by the addition of plasticizers and antioxidant agents. In the present study the addition of glycerol improved the plasticity of the bioplastic film and reinforcement with silica improved the tensile strength. The addition of cinnamon bark essential oil has imparted antioxidant activity which improves the shelf life of food particles by preventing oxidative degradation of food. It is possible to further improve the properties and health of the bioplastic by the addition of bioactive compounds during film preparation. Thus with appropriate research, the obtained bioplastic film can be developed into a safe and sustainable to plastic food packaging in the future.

REFERENCES

1. Abral, H., Pratama, A.B., Handayani, D., Mahardika, M., Aminah, I., Sandrawati, N., Sugiarti, E., Muslimin, A.N., Sapuan, S.M. and Ilyas, R.A. (2021). Antimicrobial edible film prepared from bacterial cellulose nanofibers/starch/chitosan for a food packaging alternative. *International Journal of Polymer Science feelbpeacs* , 2021.
2. Adrah, K., Ananey-Obiri, D., & Tahergorabi, R. (2020). Development of Bio-Based and Biodegradable Plastics: Novelty, Advent, and Alternative Technology. *Handbook of Nanomaterials and Nanocomposites for Energy and Environmental Applications*, 1-25.
3. Ait-Ouazzou, A., Lorán, S., Arakrak, A., Laglaoui, A., Rota, C., Herrera, A., Pagán, R. and Conchello, P. (2012). Evaluation of the chemical composition and antimicrobial activity of *Mentha pulegium, Juniperus phoenicea*, and *Cyperus longus* essential oils from Morocco. *Food Research International, 45*(1),313-319.
4. Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi pharmaceutical journal, 21*(2), 143-152.
5. Al-Battashi, H., Annamalai, N., Al-Kindi, S., Nair, A. S., Al-Bahry, S., Verma, J. P., & Sivakumar, N. (2019). Production of bioplastic (poly-3-hydroxybutyrate) using waste paper as a feedstock: Optimization of enzymatic hydrolysis and fermentation employing *Burkholderia sacchari*. *Journal of cleaner production, 214*, 236-247.
6. Al-Tayyar, N. A., Youssef, A. M., & Al-Hindi, R. R. (2020). Antimicrobial packaging efficiency of ZnO-SiO2 nanocomposites infused into PVA/CS film for enhancing the shelf life of food products. *Food Packaging and Shelf Life, 25*, 100523.
7. Arham, R., Mulyati, M. T., Metusalach, M., & Salengke, S. (2016). Physical and mechanical properties of agar based edible film with glycerol plasticizer. *International Food Research Journal, 23*(4), 1669.
8. Azeem, M., Batool, F., & Iqbal, N. (2017). Algal-based biopolymers. In *Algae Based Polymers, Blends, and Composites* (pp. 1-31). Elsevier.
9. Bastos Lima, M. G. (2018). Toward multipurpose agriculture: food, fuels, flex crops, and prospects for a bioeconomy. *Global Environmental Politics, 18*(2), 143-150.

10. Battegazzore, D., Bocchini, S., Alongi, J., & Frache, A. (2014). Rice husk as bio-source of silica: preparation and characterization of PLA–silica bio-composites. *RSC Advances, 4*(97), 54703-54712.
11. Beckstrom, B. D., Wilson, M. H., Crocker, M., & Quinn, J. C. (2020). Bioplastic feedstock production from microalgae with fuel co-products: A techno-economic and life cycle impact assessment. *Algal Research, 46*, 101769.
12. Benezra, M., Penate-Medina, O., Zanzonico, P.B., Schaer, D., Ow, H., Burns, A., DeStanchina, E., Longo, V., Herz, E., Iyer, S. and Wolchok, J. (2011).Multimodal silica nanoparticles are effective cancer-targeted probes in a model of human melanoma. *The Journal of clinical investigation*, 121(7), 2768-2780.
13. Bhat, A. H., Khan, I., Amil Usmani, M., & Rather, J. A. (2016). Bioplastics and Bionanocomposites Based on Nanoclays and Other Nanofillers. In *Nanoclay Reinforced Polymer Composites* (pp. 115-139). Springer, Singapore.
14. Bozin, B., Mimica-Dukic, N., Samojlik, I., & Jovin, E. (2007). Antimicrobial and antioxidant properties of rosemary and sage (Rosmarinus officinalis L. and Salvia officinalis L., Lamiaceae) essential oils. *Journal of agricultural and food chemistry, 55*(19), 7879-7885.
15. Castro, J. P. L. D., Costa, L. E. C., Pinheiro, M. P., Francisco, T. D. S., Vasconcelos, P. H. M. D., Funari, L. M., … & Freitas, A. L. P. (2018). Polysaccharides of red alga *Gracilaria intermedia*: structure, antioxidant activity and rheological behaviour. *Polímeros*, 28, 178-186.
16. Castro-Enríquez, D. D., Miranda, J. M., Barros-Velázquez, J., & Aubourg, S. P. (2020). Quality increase of refrigerated fish by employment of a gelatine biofilm including a protein hydrolysate obtained from alga *Fucus spiralis*. *Bulg. Chem. Com, 52*.
17. Chen, Y. W., Lee, H. V., Juan, J. C., & Phang, S. M. (2016). Production of new cellulose nanomaterial from red algae marine biomass *Gelidium elegans*. *Carbohydrate polymers, 151*, 1210-1219.
18. Chew, K.W., Yap, J.Y., Show, P.L., Suan, N.H., Juan, J.C., Ling, T.C., Lee, D.J. and Chang, J.S. (2017). Microalgae biorefinery: high value products perspectives. *Bioresource technology, 229*, 53-62.
19. Chia, W. Y., Tang, D. Y. Y., Khoo, K. S., Lup, A. N. K., & Chew, K. W. (2020). Nature's fight against plastic pollution: algae for plastic biodegradation and bioplastics production. *Environmental Science and Ecotechnology*, *4*, 100065.

20. Cian, R. E., Salgado, P. R., Drago, S. R., González, R. J., & Mauri, A. N. (2014). Development of naturally activated edible films with antioxidant properties prepared from red seaweed *Porphyra columbina* biopolymers. *Food chemistry*, *146*, 6-14.
21. De Azêvedo, L.C., Rovani, S., Santos, J.J., Dias, D.B., Nascimento, S.S., Oliveira, F.F., Silva, L.G. and Fungaro, D.A. (2021). Study of renewable silica powder influence in the preparation of bioplastics from corn and potato starch. *Journal of Polymers and the Environment*, 29(3),707-720.
22. Desikachary, T.V., V. Krishnamurthy and M.S. Balakrishnan. (1998). Rhodophyta Vol. II. Part-II (B). Madras Science Foundation, Chennai, 359.
23. Díaz-Montes, E., & Castro-Muñoz, R. (2021). Trends in chitosan as a primary biopolymer for functional films and coatings manufacture for food and natural products. *Polymers*, *13*(5), 767.
24. Ding, Y., Wu, E.Q., Liang, C., Chen, J., Tran, M.N., Hong, C.H., Jang, Y., Park, K.L., Bae, K., Kim, Y.H. and Kang, J.S. (2011). Discrimination of cinnamon bark and cinnamon twig samples sourced from various countries using HPLC-based fingerprint analysis. *Food chemistry*, *127*(2),755-760.
25. El Achaby, M., Kassab, Z., Aboulkas, A., Gaillard, C., & Barakat, A. (2018). Reuse of red algae waste for the production of cellulose nanocrystals and its application in polymer nanocomposites. *International journal of biological macromolecules*, *106*, 681-691.
26. Frech, C. B. (2002). Green Plastics: An Introduction to the New Science of Biodegradable Plastics (Stevens, ES).
27. Gaikwad, K. K., Singh, S., & Ajji, A. (2019). Moisture absorbers for food packaging applications. *Environmental Chemistry Letters*, 17(2), 609-628.
28. Gaikwad, K. K., Singh, S., & Lee, Y. S. (2018). Oxygen scavenging films in food packaging. *Environmental Chemistry Letters*, *16(2)*, 523-538.
29. Galanakis, C. M. (2012). Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. *Trends in Food Science & Technology*, *26*(2), 68-87.
30. Gall, S. C., & Thompson, R. C. (2015). The impact of debris on marine life. *Marine pollution bulletin*, *92*(1-2), 170-179.
31. Galloway, T. S., Cole, M., & Lewis, C. (2017). Interactions of microplastic debris throughout the marine ecosystem. *Nature ecology & evolution*, *1*(5), 1-8.

32. Ganeson, K., Razifah, M. R., Mubarak, A., Kam, A., Vigneswari, S., & Ramakrishna, S. (2022). Improved functionality of cinnamon oil emulsion-based gelatin films as potential edible packaging film for wax apple. *Food Bioscience*, *47*, 101638.
33. García-Soto, B., Miranda, J. M., Rodríguez-Bernaldo de Quirós, A., Sendón, R., Rodríguez-Martínez, A. V., Barros-Velázquez, J., & Aubourg, S. P. (2015). Effect of biodegradable film (lyophilised alga *Fucus spiralis* and sorbic acid) on quality properties of refrigerated megrim (L epidorhombus whiffiagonis). *International Journal of Food Science & Technology*, *50*(8), 1891-1900.
34. Ghribi, A.M., Sila, A., Gafsi, I.M., Blecker, C., Danthine, S., Attia, H., Bougatef, A. and Besbes, S. (2015). Structural, functional, and ACE inhibitory properties of water-soluble polysaccharides from chickpea flours. *International Journal of Biological Macromolecules*, 75,276-282.
35. Guerrero, P., Etxabide, A., Leceta, I., Peñalba, M., & De la Caba, K. (2014). Extraction of agar from *Gelidium sesquipedale* (Rhodophyta) and surface characterization of agar based films. *Carbohydrate polymers*, *99*, 491-498.
36. Hands, S., & Peat, S. (1938). Isolation of an anhydro l-galactose derivative from agar. *Nature*, *142*(3600), 797-797
37. Hii, S. L., Lim, J. Y., Ong, W. T., & Wong, C. L. (2016). Agar from Malaysian red seaweed as potential material for synthesis of bioplastic film. *Journal of Engineering Science and Technology*, 7, 1-15.
38. Jha, B., Reddy, C. R. K., Thakur, M. C., & Rao, M. U. (2009). Seaweeds of India: the diversity and distribution of seaweeds of Gujarat coast (Vol. 3). CSMCRI, Bhavnagar, Vol. 3, 116-124.
39. Jhurry, D., Bhaw-Luximon, A., Mardamootoo, T., & Ramanjooloo, A. (2005, December). Biopolymers from the Mauritian marine environment. In *Macromolecular symposia* (Vol. 231, No. 1, pp. 16-27). Weinheim: WILEY-VCH Verlag.
40. Júnior, L. M., Vieira, R. P., Jamróz, E., & Anjos, C. A. R. (2020). Furcellaran: An innovative biopolymer in the production of films and coatings. *Carbohydrate Polymers*, 117221.
41. Kadam, S. U., Pankaj, S. K., Tiwari, B. K., Cullen, P. J., & O'Donnell, C. P. (2015). Development of biopolymer-based gelatin and casein films incorporating brown seaweed *Ascophyllum nodosum* extract. *Food Packaging and Shelf Life*, *6*, 68-74.

42. Kadar, N. A. H. A., Rahim, N. S., Yusof, R., Nasir, N. A. H. A., & Hamid, H. A. (2021). A review on potential of algae in producing biodegradable plastic. *International Journal of Engineering Advanced Research, 3*(1), 13-26.
43. Kauldhar, B. S., & Yadav, S. K. (2018). Turning waste to wealth: A direct process for recovery of nano-silica and lignin from paddy straw agro-waste. *Journal of Cleaner Production, 194*, 158-166.
44. Khalil, H.A., Saurabh, C.K., Tye, Y.Y., Lai, T.K., Easa, A.M., Rosamah, E., Fazita, M.R.N., Syakir, M.I., Adnan, A.S., Fizree, H.M. and Aprilia, N.A.S. (2017). Seaweed based sustainable films and composites for food and pharmaceutical applications: A review. *Renewable and sustainable energy reviews, 77*,353-362.
45. Kolsi, R.B.A., Salah, H.B., Jardak, N., Chaaben, R., Jribi, I., El Feki, A., Rebai, T., Jamoussi, K., Allouche, N., Blecker, C. and Belghith, H. (2017). Sulphated polysaccharide isolated from *Sargassum vulgare*: Characterization and hypolipidemic effects. *Carbohydrate polymers,* 170,148-159.
46. Ku, K. J., Seo, Y. B., & Song, K. B. (2007). Physical Properties of *Gelidium corneum* Films Treated with Cinnamaldehyde-Research Note. *Preventive Nutrition and Food Scienc*e, *12*(2), 122-125.
47. Li, J. H., Miao, J., Wu, J. L., Chen, S. F., & Zhang, Q. Q. (2014). Preparation and characterization of active gelatin-based films incorporated with natural antioxidants. *Food Hydrocolloids*, *37*, 166-173.
48. Liang, T., & Wang, L. (2017). Thermal treatment of poplar hemicelluloses at 180 to 220 C under nitrogen atmosphere. *BioResources*, 12(1), 1128-1135.
49. Lopez-de-Dicastillo, C., Alonso, J. M., Catala, R., Gavara, R., & Hernandez-Munoz, P. (2010). Improving the antioxidant protection of packaged food by incorporating natural flavonoids into ethylene− vinyl alcohol copolymer (EVOH) films. *Journal of agricultural and food chemistry*, *58*(20), 10958-10964.
50. López-de-Dicastillo, C., Gómez-Estaca, J., Catalá, R., Gavara, R., & Hernández-Muñoz, P. (2012). Active antioxidant packaging films: Development and effect on lipid stability of brined sardines. *Food Chemistry, 131*(4), 1376-1384.
51. Luijsterburg, B., & Goossens, H. (2014). Assessment of plastic packaging waste: Material origin, methods, properties. *Resources, Conservation and Recycling*, 85, 88-97.
52. Machmud, M. N., Fahmi, R., Abdullah, R., & Kokarkin, C. (2013). Characteristics of red algae bioplastics/latex blends under tension. *International Journal of Science and Engineering, 5*(2), 81-88.

53. Madera-Santana, T. J., Freile-Pelegrín, Y., & Azamar-Barrios, *J. A.* (2014). Physicochemical and morphological properties of plasticized poly (vinyl alcohol)–agar biodegradable films. *International journal of biological macromolecules*, *69*, 176-184.
54. Madera-Santana, T. J., Robledo, D., & Freile-Pelegrín, Y. (2011). Physicochemical properties of biodegradable polyvinyl alcohol–agar films from the red algae *Hydropuntia cornea*. *Marine biotechnology*, *13*(4), 793-800.
55. Maheswari, N. U., & Ahilandeswari, K. (2011). Production of bioplastic using *Spirulina platensis* and comparison with commercial plastic. *Res. Environ. Life Sci*, 4(3), 133-136.
56. Mahmud, N., Islam, J., & Tahergorabi, R. (2021). Marine Biopolymers: Applications in Food Packaging. *Processes*, *9*(12), 2245
57. Malhotra, B. H. A. N. U., Keshwani, A., & Kharkwal, H. A. R. S. H. A. (2015). Natural polymer based cling films for food packaging. *Int. J. Pharm. Pharm. Sci*, *7*(4), 10-18.
58. Martins, J. T., Cerqueira, M. A., Bourbon, A. I., Pinheiro, A. C., Souza, B. W., & Vicente, A. A. (2012). Synergistic effects between κ-carrageenan and locust bean gum on physicochemical properties of edible films made thereof. *Food Hydrocolloids*, 29(2), 280-289.
59. Mathew, S., & Abraham, T. E. (2006). Studies on the antioxidant activities of cinnamon (Cinnamomum verum) bark extracts, through various in vitro models. *Food chemistry*, *94*(4), 520-528.
60. Melanie, S., D. Fransiska, M. Darmawan, and H. E. Irianto. "Barrier and physical properties of Arrowroot starch-carrageenan based biofilms." *Journal of Bio-Science 25* (2017): 45-56.
61. Niklas, K. J., Cobb, E. D., & Matas, A. J. (2017). The evolution of hydrophobic cell wall biopolymers: from algae to angiosperms. *Journal of Experimental Botany*, *68*(19), 5261-5269.
62. Nussinovitch, A., & Gershon, Z. (1997). Physical characteristics of agar—yeast sponges. *Food hydrocolloids*, *11*(2), 231-237.
63. Othman, M., Rashid, H., Jamal, N. A., Shaharuddin, S. I. S., Sulaiman, S., Hairil, H. S., … & Zakaria, M. N. (2019). Effect of Cinnamon Extraction Oil (CEO) for Algae Biofilm Shelf-Life Prolongation. *Polymers*, *11*(1), 4.
64. Padervand, M., Lichtfouse, E., Robert, D., & Wang, C. (2020). Removal of microplastics from the environment. A review. *Environmental Chemistry Letters*, 18(3), 807-828.

65. Pereira, L., Gheda, S. F., & Ribeiro-Claro, P. J. (2013). Analysis by vibrational spectroscopy of seaweed polysaccharides with potential use in food, pharmaceutical, and cosmetic industries. *International Journal of Carbohydrate Chemistry*, 2013.
66. Qasim, U., Osman, A. I., Al-Muhtaseb, A. A. H., Farrell, C., Al-Abri, M., Ali, M., … & Rooney, D. W. (2021). Renewable cellulosic nanocomposites for food packaging to avoid fossil fuel plastic pollution: a review. *Environmental Chemistry Letters*, 19(1), 613-641.
67. Rahman, I. A., Vejayakumaran, P., Sipaut, C. S., Ismail, J., & Chee, C. K. (2009). Size-dependent physicochemical and optical properties of silica nanoparticles. *Materials Chemistry and Physics*, 114(1), 328-332.
68. Rajendran, N., Puppala, S., Sneha Raj, M., Ruth Angeeleena, B., & Rajam, C. (2012). Seaweeds can be a new source for bioplastics. *J. Pharm. Res*, 5(3), 1476-1479.
69. Rhim, J. W., & Ng, P. K. (2007). Natural biopolymer-based nanocomposite films for packaging applications. *Critical reviews in food science and nutrition*, *47*(4), 411-433
70. Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D.V., Lam, R., Miller, J.T., The, F.C., Werorilangi, S. and The, S.J. (2015). Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. *Scientific reports*, *5*(1), 1-10.
71. Rozainee, M., Ngo, S. P., Salema, A. A., Tan, K. G., Ariffin, M., & Zainura, Z. N. (2008). Effect of fluidising velocity on the combustion of rice husk in a bench-scale fluidised bed combustor for the production of amorphous rice husk ash. *Bioresource Technology*, 99(4), 703-713.
72. Sanyang, M. L., Sapuan, S. M., Jawaid, M., Ishak, M. R., & Sahari, J. (2015). Effect of plasticizer type and concentration on tensile, thermal and barrier properties of biodegradable films based on sugar palm (*Arenga pinnata*) starch. *Polymers*, 7(6), 1106-1124.
73. Sanyang, M. L., Sapuan, S. M., Jawaid, M., Ishak, M. R., & Sahari, J. (2015). Effect of plasticizer type and concentration on tensile, thermal and barrier properties of biodegradable films based on sugar palm (*Arenga pinnata*) starch. *Polymers*, *7*(6), 1106-1124.
74. Setyawan, N., & Yuliani, S. (2021, April). Synthesis of silica from rice husk by sol-gel method. In IOP *Conference Series: Earth and Environmental Science* (Vol. 733, No. 1, p. 012149). IOP Publishing.
75. Shankar, S., & Rhim, J. W. (2018). Bionanocomposite films for food packaging applications. *Reference module in food science*, *1*, 1-10.

76. Sousa, A. M., Alves, V. D., Morais, S., Delerue-Matos, C., & Gonçalves, M. P. (2010). Agar extraction from integrated multitrophic aqua cultured *Gracilaria vermiculophylla*: evaluation of a microwave-assisted process using response surface methodology. *Bioresource technology*, *101*(9), 3258-3267.
77. Sousa, A. M., Martins, S., Larotonda, F. D., Hilliou, L., Gonçalves, M. P., & Sereno, A. M. (2008). Biodegradable Polymers Extracted from "Sargaço": Film Properties and Application to Edible Coating. *In Materials Science Forum* (Vol. 587, pp. 548-552). Trans Tech Publications Ltd.
78. Sreenikethanam, A., & Bajhaiya, A. (2021). Algae Based Bio-Plastics: Future of Green Economy.
79. Srinivasan, K. S. (1973). Phycologia Indica: (Icones of Indian Marine Algae) Vol. 2. Botanical Survey of India, Calcutta, 17.
80. Stöber, W., Fink, A., & Bohn, E. (1968). Controlled growth of monodisperse silica spheres in the micron size range. *Journal of colloid and interface scienc*e, 26(1), 62-69.
81. Tan, S. X., Andriyana, A., Ong, H. C., Lim, S., Pang, Y. L., & Ngoh, G. C. (2022). A Comprehensive Review on the Emerging Roles of Nanofillers and Plasticizers towards Sustainable Starch-Based Bioplastic Fabrication. *Polymers, 14*(4), 664.
82. Tran, T. N., Mai, B. T., Setti, C., & Athanassiou, A. (2020). Transparent bioplastic derived from CO2-based polymer functionalized with oregano waste extract toward active food packaging. *ACS applied materials & interfaces,* 12(41), 46667-46677.
83. Vaibhav, V., Vijayalakshmi, U., & Roopan, S. M. (2015). Agricultural waste as a source for the production of silica nanoparticles. *Spectrochimica acta* part A: Molecular and biomolecular spectroscopy, 139, 515-520.
84. Vieira, M. G. A., da Silva, M. A., dos Santos, L. O., & Beppu, M. M. (2011). Natural-based plasticizers and biopolymer films: A review. *European polymer journal, 47*(3), 254-263.
85. Wang, L. F., & Rhim, J. W. (2015). Preparation and application of agar/alginate/collagen ternary blend functional food packaging films. *International Journal of Biological Macromolecules,* 80, 460-468.
86. Weerasekera, A. C., Samarasinghe, K., de Zoysa, H. K. S., Bamunuarachchige, T. C., & Waisundara, V. Y. (2021). *Cinnamomum zeylanicum*: Morphology, Antioxidant Properties and Bioactive Compounds. In *Antioxidants-Benefits, Sources, Mechanisms of Action.* IntechOpen.

87. Wróblewska-Krepsztul, J., Rydzkowski, T., Bukowski, G., Szczypiński, M., Klepka, T., & Thakur, V. K. (2018). Recent progress in biodegradable polymers and nanocomposite-based packaging materials for sustainable environment. *International Journal of Polymer Analysis and Characterization*, *23*(4), 383-395.
88. Wu, N., Li, Y., & Lan, C. Q. (2011). Production and rheological studies of microalgal extracellular biopolymer from lactose using the green alga *Neochloris oleoabundans*. *Journal of Polymers and the Environment*, *19*(4), 935-942.
89. Wu, S., Lu, M., & Wang, S. (2017). Amylase-assisted extraction and antioxidant activity of polysaccharides from *Gracilaria lemaneiformis*. *3 Biotech*, 7(1), 1-5.
90. Wu, Y., Geng, F., Chang, P. R., Yu, J., & Ma, X. (2009). Effect of agar on the microstructure and performance of potato starch film. *Carbohydrate Polymers*, 76(2), 299-304.
91. Yan, S., Yin, J., Yang, Y., Dai, Z., Ma, J., & Chen, X. (2007). Surface-grafted silica linked with l-lactic acid oligomer: a novel nanofiller to improve the performance of biodegradable poly (l-lactide). *Polymer*, *48*(6), 1688-1694.
92. Youssef, A. M., Assem, F. M., Abdel-Aziz, M. E., Elaaser, M., Ibrahim, O. A., Mahmoud, M., & Abd El-Salam, M. H. (2019). Development of bionanocomposite materials and its use in coating of Ras cheese. *Food chemistry*, 270, 467-475.
93. Zhang, C., Show, P. L., & Ho, S. H. (2019). Progress and perspective on algal plastics–a critical review. *Bioresource technology*, *289*, 121700.